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ABSTRACTS

PLATFORM PRESENTATIONS

A Validated Method for the Simultaneous Determination of Δ^9-Tetrahydrocannabinol (THC), 11-Hydroxy- tetrahydrocannabinol and 11-Nor-9-carboxy- tetrahydrocannabinol in Human Plasma using Solid Phase Extraction and Gas Chromatography-Mass Spectrometry with Positive Chemical Ionization

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A fully validated, highly sensitive and specific method for the 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 plasma is presented. THC is rapidly oxidized to 11-OH-THC, an equipotent psychoactive metabolite, and further to the non-psychoactive THCCOOH. These metabolites generally undergo further biotransformation to glucuronide conjugates. Glucuronic acid forms either an ether bond or ester bond with the hydroxy or carboxy moieties, respectively. To characterize the total cannabinoid concentration in plasma, either alkaline hydrolysis, effective for the ester bond, or enzyme hydrolysis, effective for both bonds must be used to cleave the glucuronide conjugate. Quantitation was achieved by the addition of deuterated analogues for each analyte as internal standards. This method incorporates a16 h *Escherichia coli* (*E. coli*) β-glucuronidase (5000 units per 1.0 mL sample) hydrolysis step at 37°C to cleave glucuronic acid moieties to capture total analyte concentrations. The three analytes were eluted with a primary elution solvent (methylene chloride: 2-propanol: concentrated ammonium hydroxide, 80:20:2 by volume) and a second elution solvent of hexane: ethyl acetate (80:20 by volume) through a solid phase extraction column (Clean Screen ZSDAU020) using a vacuum manifold. Separation and quantification on a bench-top GC-MS (Agilent 6973N) was accomplished with a HP-5MS (30 m X 0.25 mm I.D., 0.25 μm film thickness) column with helium carrier gas at a flow rate of 1.0 mL/min and oven ramping from 120°C to 300°C. The MS was operated in the positive ionization mode: [$^2H_3$]-THC, *m/z* 390; THC, *m/z* 387; [$^2H_3$]-11-OH-THC, *m/z* 462; 11-OH-THC, *m/z* 459; [$^2H_3$]-THCCOOH, *m/z* 492; and THCCOOH, *m/z* 489. Methane (Grade 4.0, 99.99% pure) was used as the reactant gas at an apparent pressure of 1.0×10^{-4} torr in the ionization source. Limits of quantitation were 0.5, 0.5 and 1.0 for THC, 11-OH-THC and THCCOOH, with linearity ranging up to 50 ng/mL for THC and 11-OH-THC, and 100 ng/mL for THCCOOH. Absolute recoveries ranged from 67.3 to 83.5% for all three analytes. Intra-assay accuracy and precision ranged from 1.2 to 12.2% and 1.4 to 4.7%, respectively. Inter-assay accuracy and precision ranged from 1.4 to 12.2% and 3.1 to 7.3%, respectively. This method will be utilized in ongoing cannabinoid controlled administration studies and may be a useful analytical procedure for the fields of forensic toxicology and cannabinoid pharmacology.

Keywords: THC and metabolites, Plasma, Enzymatic hydrolysis assay

Herbal Products: Their Impact on Forensic Urine Drug Testing

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A cottage industry has developed to market products to substance abusers to assist them in circumventing positive forensic urine drug test reports. Many advertisements claim the inclusion of herbs and other supplements as active ingredients in the products. The objective of this work was to attempt to summarize currently available information concerning what herbs and supplements are reportedly used to "beat the drug test" and to determine their effectiveness claims and rationale for their alteration of test results. Very little information is available from classical scientific literature sources beyond herb and supplement use for various medical conditions and adverse reaction and potential drug interactions associated with their use. The information for this talk has been obtained through surfing the world-wide web and summarizes information concerning 13 products claiming the inclusion of herbs and/or other supplements which serve to confound the forensic drug test procedures to produce negative or no results. The effects of 25 of these commonly available over-the-counter herbs and supplements can be classified based upon their alleged in-vivo pharmacological action, e.g. diuretics, alteration of elimination kinetics of the drug used by changing urine color producing interference in the in-vitro testing procedures, or no rationale for effectiveness. The body of information available is based upon urine testing not hair or saliva testing.

Keywords: Regulated drug testing, Urine, Adulterants

The Detection of Drugs-of-Abuse in Liquid Perspiration

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Urinalysis drug testing is widely used in the criminal justice system and in the workplace. However, both the National Institute of Justice (NIJ) and the Substance Abuse and Mental Health Services Administration (SAMHSA) continue to explore new specimens to test and innovative testing methods. Sweat-patch testing is common in the criminal justice system, but its utility is limited because the volume of perspiration collected by the patch during wear is unknown which precludes meaningful (drug/mL of perspiration) reporting of results. Therefore, we undertook the research described below with the objective of determining the feasibility of collecting and testing liquid perspiration for the detection of abused drugs.

Two hundred and four paid volunteers were recruited at the Washington DC Pretrial Services Agency of inclusion in this IRB approved study. Written informed consent was obtained from each volunteer. Volunteers were assigned to two groups. Group A volunteers provided a single urine and sweat specimen and the specimens were screened. Group B subjects had two data collection sessions and the specimens were screened and confirmed for the tested drugs. During session 1, a sweat patch was applied to each volunteer, he/she provided a urine specimen and a sweat specimen was collected using the Macroduct® (an approved device for sweat collection). Volunteers were scheduled to return one week later for session 2 when the sweat patch was harvested and urine and liquid perspiration specimens were again collected. A brief questionnaire about their perceptions of the collection procedures was administered during each session.

The prevalence of amphetamine(s), cocaine and opiates was higher in sweat than in urine for both groups. More PCP and cannabinoids were detected in urine than sweat specimens in both groups. The sweat patch had the highest drug prevalence rate of the three specimens except more PCP and cannabinoids were detected in urine. The screening detection rates in urine and sweat, respectively from Group A were amphetamine, 1.2% and 13.6%; cocaine, 12.1% and 87% (screening failed); opiates, 7.5% and 23.5%; cannabinoids, 5.7% and 0% and PCP 4.2% and 3.5%. The average confirmed positive detection rates in urine, sweat and the sweat patch, respectively from Group B were amphetamine, 0%, 1.2% and 7.2%; cocaine, 11%, 26.9% and 38.6%; opiates 5.2%, 9.0% and 21.7%; cannabinoids, 6.8%, 0.6% and 1.2% and PCP 1.1%, 1.1% and 0%.

From this study, we concluded that sweat could be harvested (approximately 80 uL) in criminal justice settings and that common drugs of abuse and their metabolites are excreted in sweat at detectable concentrations. Given the appropriate cutoff concentrations, it is possible to detect drugs in the sweat and the sweat patch when not detected in the urine.

This study was supported by the National Institute of Justice through the National Institute of Standards and Technology (grant #60NANB9D0050).

Keywords: Sweat, Sweat patch, Urinalysis drug testing

Development of an Analytical Approach to the Specimens Collected from Victims of Sexual Assault

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Drug-facilitated sexual assault (DFSA) is a growing problem that has attracted the attention of the public through the news media. There are many anecdotal accounts of victims being given drugs without their consent to incapacitate them so they can be sexually assaulted. Recently, the Society of Forensic Toxicologists (SOFT) created a Sexual Assault Committee designed to address this issue. This committee prepared a list of drugs that could be, or have been, used in DFSA. The list comprises about 60 compounds, including illicit drugs, as well as prescription and over-the counter medications.

Samples of urine and hair of sexual assault victims from four sites across the United States are currently being received in our laboratory. A screening process has been developed to analyze these sexual assault samples for all of the drugs indicated by the Sexual Assault Committee. This screening methodology has been designed to minimize the amount of sample that is consumed and is quick, inexpensive and sensitive. We have been cognizant to the fact that in DFSA, the victim may have been given a single dose of a drug and that the amount of drug in the urine or hair will be low. Thus a screening process was needed to detect low quantities of drugs.

The samples were first screened for the "classic" drugs of abuse: cocaine, opiates, marijuana, PCP, barbiturates, ethanol, amphetamines, and benzodiazepines. This screening is performed using immunoassay and if a sample is positive, the drug is extracted from the urine and confirmed by GC/MS.

We then developed a method to screen for the other 30 drugs that do not have a readily available immunoassay. These 30 compounds are members of drug classes such as SSRI's, tricyclic antidepressants, muscle relaxants, and sedative-hypnotics. Each drug was first derivatized with BSTFA + 1% TMCS and ran separately on the GC/MS to determine retention time and which ions to scan for each drug. While not all of the compounds will derivatize with BSTFA + 1% TMCS, we ran each of the drugs with the derivatizing agent to mimic the extraction process employed. Knowing the ions and the retention times for each drug, the screening of the unknown patient samples can then proceed. Starting with two milliliters of urine, any glucuronide conjugates of the compounds were first enzymatically cleaved. Solid phase extraction using only one Clean Screen® column (UCT) per sample was then performed. This extraction process allows the recovery of acidic, basic and neutral drugs while only using one column. Following evaporation of the elution solvents, all of the samples were then derivatized with BSTFA + 1%TMCS to produce the corresponding derivatives. The samples were then screened by GC/MS in SIM to further increase the sensitivity of our method. Positive samples were then quantitated using a validated GC/MS method and a deuterated internal standard, if available.

Our laboratory has previously developed very sensitive methods for the analysis of three sexual-assaults drugs: clonazepam, flunitrazepam, and ketamine. NCI-GC/MS was employed for the detection of the parent compound and their metabolites following solid-phase extraction and derivatization. We have previously demonstrated that it is possible to detect these drugs after a single use, which is typical of a DFSA. We have also developed a different analysis for GHB and valproic acid, both small, acidic drugs that need a more specialized extraction method. Due to the very short half-life of GHB, we are expecting very small quantities of the drug in urine and believe that a more sensitive and specific method is needed to efficiently analyze for this drug.

Any toxicology laboratory that routinely receives sexual assault samples and wishes to determine if the victim may have been given a drug capable of incapacitating them can utilize this inexpensive and quick process.

Keywords: Drug-facilitated sexual Assault, "Date-Rape" drug analysis, GC/MS, Screening

Café Noir, Café Au Lait, or Just Caffeine?

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Caffeine (1,3,7-trimethylxanthine) is the world's most widely consumed drug with its main source found in coffee. Estimates of daily caffeine consumption in the United States in 1978 indicated that approximately 200 mg was consumed daily by adults over the age of 18 with coffee accounting for about 75% of the total caffeine ingested. Popularity of espresso and other specialty coffees has risen considerably over the last decade, especially for younger adults. The current study arose out of the concern that there is little awareness regarding the amount of caffeine present in these specialty coffees. Substantial variations in caffeine content arise from the variety of coffee drinks, their preparation (such as percolation, drip, or espresso) and from the geographical source of the coffee bean. Previous studies have been conducted to determine the mean value of caffeine content in regularly brewed coffee. However, there is a paucity of information on the caffeine content of specialty coffees.

Caffeine produces central nervous system stimulation and has been found to positively influence human performance. Further, it has been suggested that caffeine might improve road safety since driving simulation tests have found that caffeine use increases alertness and driving performance. While there are beneficial effects of caffeine ingestion, there may also be potentially harmful effects. There has been considerable study of the effects of caffeine on the cardiovascular system. In one study in which doses of 45-360 mg of caffeine were administered, both systolic and diastolic pressures increased, with a significant heart rate increase after the 360 mg caffeine dose. In another study, it was concluded that anxiety may be increased with doses of 300 mg or higher, while a separate study found increased anxiety with as little as 125 mg of caffeine.

We evaluated the caffeine content of twenty caffeinated and seven decaffeinated specialty coffee samples obtained from coffee shops in Severna Park and Bethesda, Maryland. Caffeine was isolated from the coffee by liquid/liquid extraction and analyzed by gas chromatography with nitrogen-phosphorous detection.

In this study, the seven coffees sold as decaffeinated were found to have caffeine concentrations less than the low point on the calibration curve (<17.7 mg/dose). There was a wide range in caffeine content present in caffeinated coffees ranging from 58 to 259 mg/dose. The mean (SD) caffeine content of the brewed specialty coffees was 188 (36) mg for a 16-oz cup. This would equate to a mean of approximately 59 mg of caffeine in a 5-oz cup of coffee as compared to an earlier report of 85 mg of caffeine (Graham, 1978). Although the amount of caffeine appears to be lower in this assessment, often larger volumes of coffee, and hence, increased amounts of caffeine, may be consumed at one time. Another notable find is the variation between brands of specialty coffees with a 16-oz cup of Big Bean coffee containing caffeine in the range of 148 to 245 mg and a 16-oz cup from Starbucks containing 259 mg of caffeine.

Reference

Graham DM. Caffeine-its identity, dietary sources, intake and biological effects. Nutr Rev 1978;36:97-102.

Keywords: Caffeine, Coffee, Gas chromatography

The use of the Plasma Metanephrine/Normetanephrine Ratio to Determine an Exogenous Epinephrine Administration in Suspected Epinephrine Poisonings

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Identification of intentional epinephrine poisoning has been dependent on epidemiological and circumstantial evidence. A scientific method that would distinguish between endogenous and exogenously administered epinephrine would provide information to support suspicions of epinephrine poisoning. We hypothesized that the exogenous administration of epinephrine to a mammal would result in an increase in the plasma ratio of the catecholamine metabolites, metanephrine/normetanephrine and that this ratio might be used to determine exogenous epinephrine administration. The present study investigated the temporal changes in plasma metanephrine/normetanephrine ratios following sub-lethal and lethal epinephrine administrations to a rabbit.

Three separate studies were performed using New Zealand White rabbits as an animal model. In the first study, 0.01 mg/kg epinephrine-HCl was administered to nine anesthetized rabbits. Blood samples were collected pre-administration and from 15-480 minutes post-administration. In the second study, pre-stress blood samples were collected from eight naïve rabbits that were subsequently subjected to immobilization stress for thirty minutes. Blood samples were collected at thirty minute intervals post-stress to 120 minutes post-stress. In the third experiment, 1.0 mg/kg epinephrine-HCl was administered to eight anesthetized rabbits. Pre-administration blood samples were collected and additional samples were collected at the time of death. Rabbits that survived the dose were euthanized, eight hours post-administration. An analytical method that combined extractive acetylation and high performance liquid chromatography (HPLC) / mass spectrometry (MS) was developed for the quantitative analysis of total plasma metanephrine and normetanephrine. The accuracy of the method ranged from 88-107 % for metanephrine and 84-95 % for normetanephrine. The limit of quantitation was 100 pg/mL and the upper limit of linearity was 10,000 pg/mL.

Using data obtained from the stress study, we were able to estimate maximal endogenous values for plasma metanephrine concentration and metanephrine/normetanephrine ratio. Rabbits receiving a sub-lethal dose of epinephrine produced plasma metanephrine concentrations that did not exceed this threshold concentration. The same group of rabbits produced metanephrine/normetanephrine ratios, however, that were greater than the threshold ratio for 180 min post-administration. Rabbits that died acutely from a lethal dose of epinephrine, as well as those that survived to 8 hrs following the lethal dose of epinephrine produced plasma metanephrine concentrations and metanephrine/normetanephrine ratios greater than the threshold values.

Analytical methodologies for the extraction and quantitation of total plasma metanephrine and normetanephrine by HPLC/MS/MS were developed. The plasma metanephrine/ normetanephrine ratio appeared to be a better parameter than plasma metanephrine

concentrations in distinguishing endogenous administrations of sub-lethal and lethal doses of epinephrine.

Keywords: Epinephrine, Poisoning, Metanephrine/Normetanephrine

Pharmacogenomics as an adjunct in death certification of amitriptyline, nortriptyline, clomipramine, and fluoxetine

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Pharmacogenomics, the study of the impact of heritable traits on pharmacology and toxicology, may serve as an adjunct for certifying amitriptyline (AMI), nortriptyline (NOR), clomipramine (CLO), and fluoxetine (FLU) fatalities. All drugs are metabolized by cytochrome P450 (CYP) 2D6, an enzyme encoded by a polymorphic gene. Although there are several different alleles that encode for a dysfunctional CYP2D6 enzyme, *CYP2D6*3, *4*, and **5* represent the most common variant alleles with a combined frequency of 95%. Individuals with these variant alleles are more susceptible to AMI, NOR, CLO, and FLU toxicity. By assessing the prevalence of *CYP2D6* polymorphisms and covariables, we hypothesized that AMI, NOR, CLO, and FLU fatalities may be partially due to poor drug metabolism caused by *CYP2D6* variant alleles.

From the Milwaukee County Medical Examiner's Office (MCMEO), a retrospective analysis of 7 AMI cases, 4 FLU cases, 1 NOR case and 1 CLO case was followed by genotyping of post-mortem blood samples for the variant alleles by real-time and conventional PCR. Institutional Review Board approval was obtained. Results from genotyping showed that 15.4% of all the cases studied were poor metabolizers (homozygous *4), while 23.1% of these cases were intermediate metabolizers (two heterozygous *4 and one heterozygous *5). The remaining 61.5% were all extensive metabolizers. In all cases, the whole blood concentrations by GC-MS for AMI, NOR, CLO, and FLU were well above the therapeutic range.

In conclusion, genotyping *CYP2D6* provided a more definitive interpretation of the AMI, NOR, CLO and FLU toxicities in a number of the cases. Therefore, pharmacogenomics may serve as an adjunct in death certification.

Keywords: Pharmacogenetics, Antidepressants, Fluoxetine, Amitriptyline, Nortriptyline, Clomipramine

Case Report: Metaxalone (Skelaxin ®) Related Death

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Metaxalone (Skelaxin ®) is a CNS depressant utilized in the treatment of acute skeletal muscle pain. The mechanism of action has not been established. It is thought that the mode of action is related to metaxalone's sedative properties. Approved by the Food and Drug Administration in 1962, metaxalone taken orally at a therapeutic dose of 800 mg has a peak plasma concentration of 4 mg/L.

Limited information has been published on metaxalone toxicity. Presently, there is only one reported death in which the causative agent(s) included metaxalone and limited therapeutic postmortem fluid and tissue concentrations have been reported.

This report involves history and toxicological findings of a fatal multi-drug overdose involving a 21-year-old woman found dead at home. The decedent was on multiple medications and had a history of meningomyelocele and a ventriculoperitoneal shunt. Her boyfriend reported 42 tablets of Lorcet® (hydrocodone/acetaminophen) missing. At autopsy, significant findings included a small thrombus in a lower lung lobe, vertebral scoliosis, resuscitative rib fractures, chronic gastritis, and pyelonephritis in both kidneys. No obvious pill content was noted in the gastric content. Heart and femoral blood, liver, urine, bile, vitreous and gastric contents were collected for toxicological analysis. Due to the limited information on metaxalone toxicity the pathologist reported the cause and manner of death as accidental acute hydrocodone intoxication. The following drugs and their respective concentrations were detected in heart blood by gas-liquid chromatography (GLC) or gas chromatography/mass spectrometry (GC/MS) techniques: metaxalone at 17 mg/L, acetaminophen at 190 mg/L, hydrocodone at 0.28 mg/L and diazepam, nordiazepam, amitriptyline and nortriptyline at 0.1 mg/L. The hydrocodone liver concentration was 0.74 mg/kg. Further toxicological analyses were negative for ethanol, carbon monoxide and common drugs of abuse.

Additional metaxalone concentrations were determined in body tissues and fluids by GLC with flame ionization detection following direct extraction and the method of standard addition. Qualitative identification of metaxalone was by electron impact gas chromatography/mass spectrometry. The following metaxalone concentrations: were determined: 19 mg/L (femoral blood) 67 mg/kg (liver), 1.1 mg/L (vitreous), 6.9 mg/L (urine), 42 mg/kg (bile) and 202 mg/kg (gastric).

Two other cases involving therapeutic concentrations of metaxalone were also analyzed and found to have heart blood concentrations of <0.75 mg/L and 1.9 mg/L which are consistent with therapeutic concentrations determined in living individuals.

Keywords: Metaxalone, Postmortem fluids and tissues, GLC, GC/MS

Evidence for Toxic Multiple Drug-Drug Interactions in Oxycodone Deaths

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Recent drug use surveys indicate that multiple drug abuse is pervasive throughout society, but often there is a tendency to attribute consequences of drug abuse to the drug "most likely" to be involved. This is frequently seen in fatality cases, particularly those involving opioids. However, it is difficult to determine the specific cause of death when multiple drugs were involved. At the same time, although enhanced toxicity of alcohol and other centrally-acting drugs with opioids has been well-established in animal studies, ethical considerations prevent well-controlled human studies.

We evaluated 1014 fatality cases involving oxycodone (OXC) for evidence of enhanced toxicity associated with multiple drug-drug interactions. Evaluated cases were previously classified by a standardized method into groups that distinguished cases unrelated to drug abuse from drug abuse cases, cases that involved only OXC from cases involving multiple drugs, drug-induced fatality cases from drug-related fatality cases, and cases involving OXC from cases in which OxyContin® (oxycodone HCl controlled-release) Tablets were identified. Our working hypothesis was that OXC in combination with other centrally-acting drugs is more toxic than in those cases involving solely OXC as evidenced by the finding of lower mean blood concentrations of OXC in multiple drug-induced deaths compared to single drug-induced deaths.

Assessment of blood levels of OXC determined by specific assay methodology (primarily GC-MS) in these cases provided the following means: OxyContin positive, multiple drug-induced cases, 0.93 μg/mL (N = 167), OxyContin negative, multiple drug-induced cases, 0.73 μg/mL (N = 579); OxyContin positive, single drug-induced cases, 1.55 μg/mL (N = 12), and OxyContin negative, single drug-induced cases, 1.70 μg/mL (N = 15). Overall, mean OXC concentration trends were as follows: single, drug-induced, drug abuse deaths > multiple, drug-induced, drug abuse deaths > drug-related, drug abuse deaths ≈ deaths unrelated to drug abuse; and OxyContin identified deaths ≈ OxyContin not identified deaths. Drug abuse patterns in the multiple drug-induced cases were complex. Over 135 drugs that were considered to be plausibly contributory to enhanced toxicity were identified in body fluids and tissues. Evaluation of mean OXC blood concentrations in cases which contained 1, 2, 3, 4, 5, and 6 or more contributory drugs in combination demonstrated consistently lower mean OXC concentrations than those cases in which OXC was the only drug identified. A smaller number of cases were available in the multiple drug-induced groups in which OXC was paired with only one other contributory drug. The overall mean OXC concentration for these cases was 0.61 μg/mL (N = 72) as compared to 1.64 μg/mL (N = 27) for the cases in the single drug-induced groups.

The consistent finding of lower mean OXC blood levels associated with multiple drug-induced fatalities supports the stated hypothesis that the combination of OXC with other centrally-active drugs is more toxic than when OXC was the only drug involved. It was concluded that in cases of multiple drug death fatalities, no single drug should be interpreted as the sole cause of death (COD). Rather, it is the unique combination of drugs, drug use patterns, and individual factors that must be taken into account in arriving at a valid COD statement.

Keywords: Oxycodone, Toxicity, Drug-drug interactions

Beta-Hydroxybutyrate: Abuse Substance or Endogenous Metabolite?

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Beta-Hydroxybutyrate (BHB) is considered to be an available analogue of GHB in the drug community, although there are no reports of its use. BHB is also a metabolite of fatty acids which is increased in diabetes, alcoholic ketoacidosis and starvation. BHB normal levels up to 30 mg/L are considered within normal range. The postmortem blood of males and females, ages ranging from 7 months to 76 years old were analyzed for BHB. The cases included in the study were chosen on the basis of age (n=16 children, 20 adults), diabetes (n=5), alcohol history and liver damage (n=3), the possibility of low caloric intake (n=7), and oral anomalies (n=2), as described in the case histories and preliminary autopsy report.

The blood from subjects with history of diabetes tended to have increased levels of BHB, with only one exception. Low chronic caloric intake does not appear to correlate with high levels of BHB. Hepatosteatosis and chronic alcohol consumption do not appear to correlate to BHB levels. The absence of acetone does not rule out high levels of BHB.

One of the cases arrived at the laboratory as a possible drug overdose with no other apparent cause of death. This 42 year old male was found in the back of a car with drug paraphernalia. Neither drugs of abuse nor prescription drugs were detected. The blood ethanol concentration was 0.03g/100mL. No acetone was detected. The vitreous glucose concentration was higher than 400 mg/dl. The BHB levels were 130 mg/L in both postmortem heart and peripheral blood, 81 mg/kg in liver and 130 mg in stomach contents.

A 12 month old female was found dead in her crib at home. She was considered small for her age (7.26 kg). There was suspicion of abuse and/or neglect. The BHB levels were: 200 mg/L in postmortem heart blood, 110 mg/L in peripheral blood, 87 mg/kg in liver and < 1 mg in stomach contents. There was strong family history of diabetes. No drugs of abuse, prescription drugs, alcohol or acetone were detected.

In both cases the cause of death was ruled as natural, possibly due to diabetic ketoacidosis. The origin of BHB appears to be endogenous rather than from consumption.

Keywords: Beta-Hydroxybutyrate, Diabetes, Ketoacidosis

A Short Series of Deaths Involving MDMA

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3,4-Methylenedioxymethamphetamine (MDMA, ecstasy) has appeared periodically in death investigation casework in Washington State. We reviewed sixteen cases and evaluated the significance of the drug concentrations. Of the sixteen cases, 5 were suicides, 4 homicides and 7 accidents. Six of the deaths were due to MDMA intoxication in whole or in part.

In the most clear-cut case, a 19 y.o. male was found dead at home. There was evidence of seizure activity with hands balled and wrists flexed in. He had a history of depression and had left a note of intent. MDMA and MDA were detected in peripheral blood at concentrations of 7.2 and 0.1 mg/L respectively. No other drugs were present. Normal recreational use of MDMA involves the ingestion of 50 – 150 mg, producing concentrations in the range 0.02 to 0.49mg/L (de la Torre et al, 2000). A tissue distribution study showed concentrations in the central blood to be about 30% higher for both the drug and its metabolite. The death was certified as acute MDMA intoxication.

In a second death attributed specifically to the drug, a 15 y.o. female who had been rumored to be experimenting with hallucinogens (LSD, mushrooms and ecstasy) was found dead. MDMA was the only drug detected, at a concentration of 0.87 mg/L in peripheral blood. The vitreous concentration was 0.02 mg/L, suggesting a somewhat acute ingestion, consistent with the absence of any metabolite. The death was certified as accidental; acute encephalopathy due to MDMA intoxication.

In other cases MDMA played a contributory or secondary role. These included a 54 y.o. male found dead with a blood MDMA concentration of 0.28 mg/L and ethanol concentration of 0.06g/100 mL. His death was found to be an accident and was ascribed to acute MDMA and ethanol intoxication. A 19 y.o. female ingested a large amount of cocaine, suffered a seizure, and was placed on life support. She also had a blood MDMA concentration of 1.28mg/L following her death several hours later. Death was certified as an accident due to cocaine, ethanol and MDMA. A 20 y.o male who was found dead in bed with a plastic bag placed loosely over his head had a blood MDMA concentration of 1.16 mg/L. His death was attributed to acute MDMA intoxication and asphyxiation. Finally a 28 y.o male died in police custody during an altercation. His blood methamphetamine concentration was 8.7mg/L and his MDMA concentration was 0.2 mg/L. This death was ascribed to acute methamphetamine and MDMA intoxication.

In the remaining cases, MDMA was an incidental finding. Seven cases (median age 22, range 21-49) were caused by suicidal or homicidal gunshot wounds, and had concentrations of between 0.16 and 4.23 mg/L (mean = 0.98, median = 0.30 mg/L). In two separate motor vehicle fatalities (ages 23 and 18) the MDMA concentrations were 2.62 and 0.60 mg/L in a driver and passenger respectively. The concentration in the driver significantly exceeds the highest level previously reported in a driver (2.14 mg/L, Crifasi and Long, 1996), and exceeds the range (0.025 – 0.58 mg/L, mean = 0.37 mg/L, median = 0.24 mg/L) reported in a series of 18 drug impaired drivers (Logan and Couper 2001).

MDMA is a sympathomimetic amine, which greatly enhances release of serotonin, producing euphoria and stimulation, with empathic effects promoting feelings of closeness and intimacy. In overdose, it has been linked to deaths resulting from serotonergic excess, with associated symptoms of sweating, hallucinations, loss of coordination, seizures, respiratory arrest, unconsciousness and death. The condition can be exacerbated by heat and dehydration. These deaths appear uncommon, but as seen here the drug may play other roles in the chain of events leading to death, including when combined with other CNS active drugs, suicide involving post-MDMA use depression, and driving impairment leading to a fatal collision.

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Keywords: MDMA, Ecstasy, Death investigation, Postmortem

Death Attributed to Chronic Cumulative Clozapine Toxicity

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Clozapine (CZ) is an atypical antipsychotic indicated for the management of schizophrenic patients. We present the first case of chronic cumulative CZ toxicity causing death reported in the Province of British Columbia.

A 47 year old Caucasian female was found unresponsive in bed, fully clothed, lying semi-prone on her right side. The deceased's medical history included schizophrenia, with no history of epilepsy. A full autopsy was performed approximately 29 hours after death. Significant findings included laceration of the right side of the tongue, scleral mucosal conjunctival petechiae noted in both eyes, and more confluent petechial hemorrhages on the head and upper torso. Internal examination revealed an empty urinary bladder. There was acute congestion of the viscera, and pulmonary edema. Specimens were collected for toxicological analysis.

The aortic blood specimen was initially subjected to a thorough qualitative analysis. Screening was performed for illicit drugs including morphine and cocaine by radioimmunoassay. Basic drugs were screened for by liquid-liquid extraction followed by GC-NPD and GC-MS electron impact detection. Acidic and neutral drugs were screened for by liquid-liquid extraction followed by HPLC-DAD. Volatiles were assayed by GC-FID. Qualitative analysis identified CZ, norCZ and quetiapine. The quetiapine concentration was 0.65 mg/L in blood (aorta) and is consistent with levels achieved therapeutically.

CZ and norCZ were assayed as follows: briefly, to 0.5 mL specimen 100 uL of 1 ug/mL levorphanol solution (internal standard) was added. The samples were extracted into 2 mL acetonitrile, vortexed for 5 minutes, followed by centrifugation. The supernatant (1 µL) was injected directly into an Agilent model 1100 liquid chromatograph coupled to an Agilent Model 1100 mass Spectrometer using a Zorbax SB-C8 2.1 x 50 mm 3.5 µm column (Agilent). Separation was achieved isocratically at 50% acetonitrile, 1.0 mmol/L ammonium acetate buffer solution with 0.1% trifluoroacetic anhydride with a constant flow rate of 0.20 mL/min. Detection was by SIM in electrospray mode. The concentration was measured by comparison of peak height ratios of CZ and norCZ to that of levorphanol against a standard curve. Linearity was observed up to 1.0 mg/L. Samples with concentrations exceeding the linearity were diluted.

The deceased was prescribed CZ 4x100 mg/day beginning 35 days (-35d) prior to death. Ante-mortem serum concentrations of CZ (and norCZ) were measured and determined to be: -28d 0.27 mg/L, at -21d 0.24 mg/L, at -17d 0.67 mg/L (0.48 mg/L), and -15d 0.60 mg/L (0.44 mg/L). During this time there was no change in the dose of CZ with which she appeared to be compliant, nor was there any change to concomitant medications. Analysis of available post-mortem biological fluids showed the following elevated concentrations of CZ (and norCZ): aortic blood 6.4 mg/L (6.4 mg/L); femoral blood 2.7 mg/L (1.6 mg/L); and vitreous fluid 0.27 mg/L (0.21 mg/L).

The gradual increases in the concentration of CZ and norCZ in ante-mortem serum samples without changes in dosage suggests the accumulation of the drug. Clozapine is metabolized primarily by CYP3A4, 1A2 and minor metabolic pathway by CYP2D6. There are

no significant variants of CYP 3A4 AND 1A2, and no tests for genetic polymorphisms or phenotypic determinations were done for CYP2D6. Post-mortem blood levels exceed what has been described as minimum lethal levels (1.2-13 mg/L), Additionally, at autopsy there was evidence of seizure activity, and post-mortem blood levels were above those where seizure activity has been described. The cause of death was ascribed to chronic, cumulative CZ toxicity.

Keywords: Clozapine, Toxicity, Chronic

Changes in Patterns of Drug and Alcohol Use in Fatally Injured Drivers in Washington State

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We previously reported on patterns of drug and alcohol use in fatally injured drivers in Washington State [Logan BK, Schwilke EW, Drug and alcohol use in fatally injured drivers in Washington State, J Forensic Sci. 1996 May;41(3):505-10]. We revisit that population here to examine how patterns have changed in the intervening nine years, by examining drug and alcohol data from drivers killed in crashes between February 1, 2001 and January 31, 2002. Blood and serum specimens from drivers who died within 4 hours of the traffic accident were collected from coroners and medical examiners offices from all 39 Washington counties. Of the 657 traffic related deaths in Washington State, 397 (60.4%) were drivers. Samples suitable for testing were received in 370 (93%) of these. Specimens were screened by immunoassay for cocaine metabolite, opiates, benzodiazepines, barbiturates, cannabinoids, amphetamines, PCP, propoxyphene, methadone, and tricyclic antidepressants using an Olympus AU400® EMIT analyzer. Basic drugs were confirmed by GC/MS following an n-butyl chloride extraction. Acidic and neutral drugs were confirmed by GC/FID and GC/MS following an XAD extraction at neutral pH. Benzoylecgonine and morphine were analyzed by GC/MS following a chloroform/isopropanol extraction at pH 9. Cannabinoids were confirmed by GC/MS following a hexane/ethyl acetate extraction at pH 4.5. Of the 370 cases analyzed, 277 (75%) were male and 93 (25%) were female. The average age for males was 38 (range 15 to 87), and the average age for females was 47 (range 16 to 91). Alcohol was detected above 0.01 g/100 mL in 41% of cases. The mean alcohol concentration for those cases was 0.17 g/100 mL (range 0.02 to 0.39 g/100 mL). Central nervous system (CNS) active drugs were detected in 144 (39%) of cases. CNS depressants including carisoprodol, diazepam, citalopram, hydrocodone, diphenhydramine, amitriptyline, and others were detected in 52 cases (14.1%) , cannabinoids were detected in 47 (12.7%) cases, CNS stimulants (cocaine and amphetamines) were detected in 36 (9.7%) cases, and narcotic analgesics (excluding morphine which is often administered iatrogenically in trauma cases) were detected in 12 (3.2%) cases. Drug and alcohol use continues to be a significant finding among fatally injured drivers. The data reveal that over the past decade, while alcohol use has declined, some drug use, notably methamphetamine, has increased significantly from 1.89% to 4.86% of fatally injured drivers between 1992 and 2002.

Keywords: Driving, Fatal crashes, Drug and alcohol use

Phencyclidine Findings in Drivers in Washington State

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The Washington State Toxicology laboratory has experienced an increase in the number of phencyclidine (PCP) positive driving cases in Washington State over the past six years. Since 1997, 88 persons have been arrested for driving under the influence of PCP. This number has increased from one driver in 1997 to 23 drivers in 2002 and as of July 2003, an additional 22 cases have been reported.

PCP is classified as a dissociative hallucinogen, formerly used as an anesthetic in humans but has had no legitimate medical use since 1978. The drug continues to be available through illicit manufacture however. Recreationally, PCP is used as a psychedelic or hallucinogen, often in combination with marijuana. PCP produces euphoria, calmness, feelings of strength and invulnerability, lethargy, disorientation, loss of coordination, distorted perceptions, impaired cognitive function, sedation, stupor and occasionally violent behavior. Peak effects occur between 1 and 30 minutes dependent upon the route of administration and gradually decline over 4 to 6 hours.

We reviewed toxicology and available case report information on 88 PCP-positive drivers. This population was predominantly male, (74%) with a mean age of 25 and a median age of 24 (range 16-44). Upon review of the cases, it was notable that there were a significant number of repeat offenders. Five individuals were arrested twice in the six year period, three had three arrests, and one female was arrested five times in three years for driving under the influence of PCP. The blood PCP concentrations quantified with a mean value of 0.037 mg/L and a median of 0.03 mg/L (range 0.02 – 0.10). Fifty-two cases (62%) were also positive for THC and/or carboxy THC which confirms that the most common co-ingested drug is marijuana. The second most commonly co-ingested drug was ethanol, with 11% positive (mean 0.07 g/100 mL, median 0.03 g/100 mL , range 0.01 – 0.28g/100mL).

Of those cases where we obtained information regarding the circumstances of the arrest (40), 20% were involved in a crash, and one individual was involved in 2 crashes, one of which was a vehicular homicide. In 27 cases we obtained the results of the Drug Recognition Expert (DRE) evaluation. All but two individual were noted to have slurred speech, four were slurred with fast and/or repetitive speech and the remaining were noted to have slow speech. Most were described as cooperative or withdrawn with two exceptions. One was described as agitated, and the other as exhibiting violent behavior. Twenty six of the 27 exhibited horizontal gaze nystagmus (HGN), 22 of which had all 6 clues present. Twenty of the 27 exhibited vertical gaze nystagmus (VGN). Fifty six percent had an elevated pulse rate with a mean 84 beats per minute, and 44% had elevated blood pressure. Twenty nine percent had elevated body temperature and 39% estimated 30 seconds as 39 seconds or longer. Ninety six percent performed poorly on the one leg stand, walk and turn. All exhibited marked sway in the Romberg balance test.

While the overall number of cases of PCP positive drivers is not a large portion of our DUI drug cases in Washington State, several factors are of concern. There has been a significant

increase in the number of PCP positive cases, there are a high percentage of repeat offenders, their intoxication can be severe, and the incidence of crash risk is significant.

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Keywords: PCP, Driving under the influence

Lorazepam and Driving Impairment

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Lorazepam (Ativan®) is a benzodiazepine frequently used to manage anxiety, pre-surgically, and as a sedative. Common side effects include sedation, dizziness, weakness, unsteadiness, and disorientation. Consequently lorazepam can have a significant effect on driving ability. We reviewed all positive lorazepam drug impaired driving cases submitted to the Washington State Toxicology Laboratory between January 1998 and May of 2003. The mean concentration found in the blood of these drivers (n=128) was 0.052 mg/L (std.dev.=0.06, median=0.03). Concentrations ranged from <0.01 to 0.39 mg/L. This population was 57% male, with a mean and median age of 39.5 years, (range 16-79). Seventy percent of these drivers tested positive for other drugs in addition to lorazepam that may have contributed to their impairment. We obtained Drug Recognition Expert (DRE) reports for fourteen of the remaining cases in which there were no other impairing drugs present. Lorazepam concentrations in these cases averaged 0.067 mg/L (median=0.04, range 0.01-0.32 mg/L). Six of the fourteen subjects had lorazepam prescriptions to treat anxiety, three were taking it to aid sleep, two to treat depression, and one to treat heroin withdrawal symptoms. Two individuals admitted to taking the pills from friends or family members.

The reasons for the stops or arrests were single-car accidents (n=6), erratic driving (n=4), two-car accidents (n=2), following too closely (n=1) and speeding (n=1). Despite the wide range of lorazepam concentrations, the subjects' behaviors were remarkably consistent. Speech was slow and/or slurred in all but one of the fourteen cases. Attitudes were cooperative in thirteen of the cases; the one exception was noted as being indifferent and argumentative. Coordination was poor and/or slow in all fourteen cases. The subjects had higher than normal pulses (mean 96.8, median 98, range 72-126). Blood pressures were unremarkable, and temperatures were on the low side of normal (mean 96.9°F, median 97.2°, range 97.9°-98.4°). There was a wide range in the subject's estimation of the passage of thirty seconds (mean= 36, range 13-90). Swaying 2-6 inches during the Romberg test was observed in all subjects (mean 3.29, median 2.5 inches). All subjects did poorly on the modified finger to nose test. There appeared to be no correlation between standardized field sobriety test (SFST) performance and the concentration of lorazepam detected. Horizontal Gaze Nystagmus (HGN) was observed in all drivers. Five of fourteen subjects had Vertical Nystagmus. All subjects performed poorly on the walk and turn and one leg stand tests.

This review of these subjects indicates that lorazepam is capable of causing significant impairment to driving and psychomotor abilities, independent of the concentration detected.

Keywords: Lorazepam, Driving under the influence, Field sobriety tests

Driving Under the Influence of Ephedrine

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The toxicology of over-the-counter products containing ephedrine has recently received considerable attention in the media. Ephedrine is found in over-the-counter (OTC) nasal decongestants and asthma medications (Primatene®), performance enhancers (BioLean®), and weight loss preparations (Fat Metabolizer®, Thermo-E®). It is a naturally occurring sympathomimetic amine with both peripheral and mild Central Nervous System (CNS) effects. Ephedrine is associated with increased risks of adverse events compared to other OTC products. Adverse events associated with ephedrine containing products include gastrointestinal symptoms, hypertension, heart palpitations, paranoid delusions and hallucinations,. Ephedrine has caused sudden death in abusers.

We present a case study of a 53-year-old female, cited for erratic driving, first in May of 2002 and again in May of 2003. Following the first arrest, she underwent a Drug Recognition Expert (DRE) evaluation. On the standardized field sobriety tests (SFST) she exhibited obvious body tremors during the Romberg Balance and the one-leg stand, visual examination demonstrated a lack of convergence, pulse was 108 BPM, blood pressure 172/104 mmHg. She was very talkative with slurred speech, and her movements were exaggerated. The DRE officer concluded that she was under the influence of a CNS stimulant. Following the second arrest she exhibited poor balance and could not follow instructions but refused to complete SFST. She later refused the DRE exam because she recognized the DRE officer from her 2002 arrest, and stated she did not like the way he treated her on the previous encounter. In both incidents she was arrested for driving under the influence of drugs, specifically a CNS stimulant. The subject reported taking ephedrine to stay awake, and that she had been taking extra doses recently.
Toxicological analysis revealed ephedrine concentration of 15mg/L in the 2002 incident, and 22 mg/L in 2003. Ephedrine concentrations were determined by butyl chloride extraction and GC/MS and GC/NPD analysis without derivatization. Subsequent stereospecific analysis (by reversed phase LCMS of underivatized drug) indicated that ephedrine, and not pseudoephedrine was present. The ephedrine concentrations in this subject were in the ranges associated with ephedrine fatalities in the literature. Impaired driving can be added to the list of ephedrine-related adverse events.

Keywords: Eephedrine, Driving impairment

Second Sample Analysis of Blood from DUID Arrests in the Commonwealth of Virginia; Demographics, Drug Findings, and Testing Scheme under the Revised Second Sample Testing Program

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In July 2001, the Commonwealth of Virginia changed the analytical testing process for the second blood sample from the accused in Driving Under the Influence (DUID) cases. The second sample was still collected at the same time the evidentiary sample for DUID cases was collected. However, now all screening and quantitative confirmation testing on the second sample would be performed by the Second Sample Laboratory (SSL), using a testing scheme established by the Division of Forensic Sciences (DFS). Screening was previously performed by the DFS, which sent a qualitative report to the SSL when testing was finished. The SSL then performed quantitative confirmation testing for the drugs identified by the DFS. Under the revised program, delays in testing were significantly reduced.

The testing scheme involved 3 levels of analysis. Level I, all samples were analyzed for alcohol. Results of samples with an alcohol concentration of 0.9 gm % or higher were sent to the courts and no further testing was performed on them. Level II, samples with an alcohol concentration less than 0.9 gm % were screened for 6 common drugs of abuse classes. All presumptive positives within the Level II were quantified, and confirmed by GC/MS. Results of a sample with at least one drug concentration in the level that exceeded a defined reporting limit were sent to the courts, no further testing was performed on them. Level III, all samples not exceeding Level I or II reporting criteria were screened by a base/neutral screen for common prescription and over the counter medications that may impair driving. All presumptive positives were quantified, and confirmed by GCMS before results were reported.

We present the demographics and drug findings of 2948 blood specimens submitted to the SSL for testing in the first 18 month of the revised testing program. The most commonly encountered drugs as percentage of total cases (their mode and range of blood concentrations) over the 18 month period are listed below. Males represented 77 % of those requesting second sample testing.

Drug	# Cases	% of Case	Mode	Range	Units
Blood Alcohol	1851	75.5	0.12	0.01 - 0.40	gm %
THC	345	12.0	1	<1 – 26	ng/ml
THC-Acid	442	15.0	24	2 - 280	ng/ml
Alprazolam	108	3.7	38	20 – 672	ng/ml
Diazepam	79	2.7	0.39	0.02 – 1.7	mg/L
Nordiazepam	91	3.1	0.16	0.05 – 2.1	mg/L
Cocaine	31	1.1	5	2 – 71	ng/ml
Benzoylecgonine	79	2.7	0.16	<0.10 – 3.2	mg/L
Butalbital	71	2.4	1.0	0.5 – 38.9	mg/L

Drug	# Cases	% of Case	Mode	Range	Units
Hydrocodone	44	1.5	20	20 – 202	ng/ml
Oxycodone	40	1.4	67	24 - >500	ng/ml
Carisoprodol	36	1.2	20	1 - 30	mg/L
Meprobamate	38	1.3	20	2.1 - 50	mg/L
Phencyclidine [PCP]	37	1.3	17	13 – 89	ng/ml
Morphine	32	1.1	30	20 - >500	ng/ml

Keywords: Drugs and driving, Drug abuse, Blood analysis

Effects of Frozen Storage on Nitrite Adulterated Urine Samples

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Nitrite is an effective adulterant used by illicit drug users to conceal drug test results. Methods for detecting nitrite at the screening level using automated analyzers include nitrite specific or oxidant reagents. Confirmation testing has been done using the automated analyzers along with nitrite specific dipsticks, Ion Chromatography (IC), Atomic Absorption (AA) and Capillary Electrophoresis (CE). This study evaluated the change from the automated analyzer/dipstick method to CE for confirmation of nitrite and the effect of freezing on nitrite stability.

Nitrite was measured in 10 uL urine specimens by Beckman Coulter Model P/ACE MDQ capillary electrophoresis instrument with sodium azide added as an internal standard. Nitrite mobility is measured by indirect ultraviolet absorption detected at 254 nm (mercury lamp) using an uncoated 50 um (i.d.) by 375 um (o.d.) by 60 cm (length) capillary. Quantitation is based on the peak area counts of the sample as compared to the peak area counts of a three point linear calibration curve. The limit of quantitation (LOQ) was established at 75 ug/mL and the upper limit of linearity (ULOL) at 10,000 ug/mL.

CE was used to retest ten samples that had been in frozen storage from 12 to 14 months and had initially tested positive on a Hitachi 747 analyzer but failed to confirm by nitrite dipstick. All ten samples resulted in a zero ng/mL quantitation for nitrite by CE.

Forty-six samples were initially tested by a Hitachi 747 analyzer and confirmed by a retest on the Hitachi 747 along with a nitrite dipstick test and stored frozen from 12 to 17 months. Of the 46 samples, there was an average loss of 28.96% from the original Hitachi 747 quantitation vs. the CE retest result. Nine of the 46 samples lost 50% or more of their original concentration while two samples tested slightly higher after storage. Seven of the 46 samples fell below the 500 ng/mL cutoff established by the Substance Abuse and Mental Health Services Administration (SAMHSA) for nitrite confirmation testing, but all 46 tested above the LOQ allowed by SAMHSA for the re-confirmation of positive samples.

Seventy-seven samples were screened by a Hitachi 747 analyzer and confirmed by CE and then frozen from 2 to 12 months. These samples lost an average of 18.9% from the original CE result. Twelve of the 77 samples lost 50% or more of the nitrite from their original test while 22 of the samples had slightly higher quantitations upon retest. Sixteen of the samples fell below the 500 ng/mL cutoff established by SAMHSA for nitrite confirmation but all tested positive above the LOQ.

This study validates that the original testing methods using nitrite dipsticks for confirmation of nitrite adulteration produced results consistent with samples confirmed by capillary electrophoresis. Frozen storage affects samples differently; however, it did not prevent the re-verification of nitrite adulterated samples at the LOQ level.

Keywords: Nitrite, Adulteration, Capillary electrophoresis

Comparison of Oxidant Reagents for the Purpose of Identifying Adulterated Samples

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Oxidant detection reagents from Axiom Diagnostics [1] (Test True Oxidant Assay), Dade Behring[2] (Ox Perfect Test), Kasey Inc[3] (Lark Oxidant Reagent), and Northwest Toxicology (NWT) Inc. (Oxidant Reagent, were evaluated to show their effectiveness in identifying samples that had been spiked with oxidizing agents as well as other substances known to interfere with analysis.

All calibrators and controls were made in the NWT Quality Control department. Axiom, Kasey Inc. and NWT Inc. reagent was calibrated using a NWT Inc. nitrite calibrator at 200 *u*g/mL. The Dade Behring reagent was calibrated using a NWT Inc. chromium calibrator at 20 *u*g/mL. Positive, low and negative controls were run with each calibration. Concentration of the controls were as follows: Positive nitrite = 250 *u*g/mL, Positive chromium= 25 *u*g/mL. Low nitrite= 150 *u*g/mL. Low chromium= 15 *u*g/mL. The negative control was certified blank urine.

Calibration was performed daily on the Hitachi 747 chemistry analyzer. Axiom, Kasey Inc. and the NWT Inc. reagent was calibrated with the 200*u*g/mL nitrite calibrator and the Dade Behring reagent was calibrated with the 20*u*g/mL chromium calibrator. Twenty-five solutions containing Iodine, Bleach, Chromium III, Chromium VI, Nitrite, Gluteraldehyde, Soap, Urine Luck 3.01, 6.0, 6.5 and 6.4, Instant Clean Additive, Klear Double Shot, Stealth, Stealth 51, Hydrofluoric Acid, Periodic Acid, Persulfate, Iodate, Iodic Acid, Cyanide, 5% and 25% Blood, Oxone and Silver Nitrate were analyzed once daily for 5 days using the Hitachi 747 automated chemistry analyzer.

Instrument parameters obtained from each reagent vendor were used in the evaluation. Conditions for calibration were consistent with the respective manufacturer's recommendations with the exception of the Lark reagent. The Kasey Inc. company did not have published parameters for the Hitachi 747 at the time of the evaluation. Parameters were adapted from the Hitachi 717 analyzer.

All four reagents tested positive for Urine Luck 3.01, Urine Luck 6.5, Chromium VI, Nitrite, Periodic Acid and Oxone. Only Axiom tested positive for Urine Luck 6.0, Urine Luck 6.4, Stealth, Iodate and Iodic Acid. Silver Nitrate triggered a positive response with the NWT Inc., Dade Behring and Kasey Inc. reagents. Blood at 5% concentration did not appear to interfere with any of the reagents. However a 25% Blood spike did cause positive results with the NWT Inc., Dade Behring and Kasey Inc. reagents. None of the reagents found the fluoride in the 125 *u*g/mL solution of Hydrofluoric acid. Persulfate triggered a positive response with Axiom, NWT Inc. and Kasey Inc. reagent. Cyanide and Chromium III did not trigger a positive result with any of the reagents. A result was interpreted as positive if it was $\geq$200 ug/mL with the Axiom, NWT Inc. or Kasey Inc. reagent which were calibrated with a 200 *u*g/mL nitrite calibrator, or $\geq$20 ug/mL with the Dade Behring reagent which was calibrated with a 20*u*g/mL chromium calibrator. Either is acceptable based on information obtained from the Federal Register/Vol.66 No.162 dated Tuesday August 21, 2001.

Overall the Axiom reagent performed best. It appeared to consistently find more adulterants that had been spiked in solution and was affected less by the addition of blood than the other reagent lines. The NWT Inc. reagent was second overall; however, it was observed that the NWT Inc. reagent appeared to affect the cuvettes on the Hitachi 747 casing cell errors to occur. Test results from the Kasey Inc. reagent were inconclusive due to the lack of published instrument parameters.

Keywords: Oxidant, Adulterated samples, Oxidizing agents, Reagent

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Controlled Oral Δ^9-Tetrahydrocannabinol Administration: Detection Rates and Times in Urine by CEDIA®, Syva® EMIT II, Microgenics® DRI and GC/MS

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The availability of cannabinoid-containing foodstuffs, cannabinoid-based therapeutics and continued abuse of oral cannabis require scientific data for the accurate interpretation of cannabinoid tests and for making reliable administrative drug testing policy. The NIDA Institutional Review Board approved this study and each subject gave informed consent. All subjects resided on the NIDA IRP research ward, under continuous medical surveillance. The study was designed as a randomized, double blind, double dummy, placebo-controlled within-subject protocol. The participants were dosed three times a day for five consecutive days followed by a ten-day washout period before the next dosing session. Three (N=7) healthy volunteers with a history of marijuana abuse ingested commercially available hemp seed oils of differing THC concentrations: 0, 9, 92, 347 μg/g, in liquid or capsule form, for total THC doses per day of: 0, 0.385, 0.472, and 14.8 mg; 7.5 mg of Dronabinol was used as a positive control. Dronabinol, Marinol®, is a synthetic THC therapeutic drug and was administered in 2.5 mg capsules. All urine specimens (N=4381) were collected from participants over the 10-week study and analyzed semi-quantitatively by CEDIA®, Syva® EMIT II, Microgenics® DRI and quantitatively by GC/MS. Detection rates, detection times, and immunoassays' sensitivities, specificities and efficiencies were determined. With the federally mandated 50 ng/mL immunoassay cutoff, and during ingestion of the two low doses typical of current hemp oil THC concentrations, mean detection rates were below 1%. The two high doses produced mean detection rates of 23 to 46% with intermittent positive tests up to 118 h. Maximum metabolite concentrations of 5.4 to 38.2 ng/mL followed the low doses and 19.0 to 436 ng/mL for the high doses. Emit II, DRI and CEDIA immunoassays had performance efficiencies of 92.8, 95.2 and 93.9%, respectively, but differed in their sensitivities. These data demonstrate that it is possible but unlikely for a urine specimen to test positive at the federally mandated cannabinoid cutoffs following manufactures' dosing recommendations for the ingestion of low THC concentration hemp oils. Urine tests have a high likelihood of being positive following Marinol therapy. The EMIT II and DRI assays had adequate sensitivity and specificity; the CEDIA assay failed to detect many true positive specimens.

Keywords: Marijuana, Cannabinoids, Hemp Oil, Urine, Marinol, Cannabis

Method for Detection of Cocaine Metabolite in Urine below Conventional Cutoff Concentrations

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Concurrent with the spread of the cocaine epidemic over the last two decades, a "cottage industry" has emerged that promotes the sale and use of *in vivo* and *in vitro* adulterants, substitution products and other paraphernalia that are designed to produce false negative urine testing results. To enable users to "beat the drug test", many *in vitro* products require ingestion of excessive amounts of water leading to production of highly dilute urine specimens. As a result, some individuals escape detection when specimens are tested at conventional cutoff concentrations.

The purpose of this study was to determine if enhanced screening methodology could be utilized to detect urine specimens that test negative by immunoassay at conventional cutoff concentrations, but contain benzoylecgonine (BZE) in amounts confirmable by GC-MS.

Six healthy males were administered approximately equipotent doses of cocaine by the IV, SM and IN routes of administration. Urine specimens were collected for a minimum of three days after drug administration and screened by immunoassay (EMIT and TDX, 300 ng/mL cutoff concentration). All specimens regardless of screening outcome were tested for BZE by GC-MS (LOQ $\approx$ 1 ng/mL).

Identification of BZE-positive urine specimens was significantly enhanced through the use of the "Zero Threshold Criteria Method", a method developed to differentiate false negatives from true negatives. The method relied on establishing a mean immunoassay response (MIR) baseline and variance (SD) for all GC-MS confirmed drug-free specimens. Arbitrary thresholds (MIR + 0.5 SD, MIR + 1 SD, MIR + 2 SD) were utilized to evaluate all specimens that initially tested negative at the conventional cutoff concentration. Specimens with response rates in the original screening assay that exceeded the MIR threshold and contained BZE concentrations at or above 40% GC-MS cutoff concentrations (40 ng/mL and 60 ng/mL) were identified as true positive specimens. Generally, optimal identification was achieved with EMIT using the MIR + 1 SD criteria and with TDX using the MIR + 2 SD criteria. With these criteria, up to 111 false negative specimens were redesignated as true positive specimens; this was in addition to the 208 true positives detected at recommended cutoff concentrations. This represents a 50% increase in positive detection rates through the use of enhanced screening criteria and confirmation at 40% cutoff concentrations.

Such methodology is recommended for further evaluation by drug testing programs for enhancement of positive detection rates and as an alternative to creatinine testing for dealing with dilute specimens that test negative by initial tests, but contain quantifiable amounts of drugs of abuse.

Keywords: Cocaine, Urine, False negatives

Production of Benzoylecgonine with a Wash and Extraction Technique for Removing External Contamination of Cocaine on Hair

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Hair is easily contaminated externally by exposure to the vapors of crack cocaine. An important part of hair testing is the ability to remove the majority of external contamination without converting cocaine to benzoylecgonine. Many laboratories rely upon the presence of cocaine metabolites to distinguish users from externally contaminated non-users, thus the production of benzoylecgonine during wash and extraction procedures may lead to erroneous interpretation of a positive result for cocaine.

The objective of this experiment was to determine if cocaine applied to the exterior of hair samples would produce benzoylecgonine during wash and extraction.

Hair samples which were previously assayed to be negative for cocaine and cocaine metabolites were exposed to pure cocaine by soaking the hair in a cocaine solution. Samples were washed for 5 minutes by percolating methanol through them, collecting the wash solutions, then repeating the wash for an additional 5 minutes with a second aliquot of methanol and collecting it. The washed hair samples were extracted in 0.1 N HCl at 75°C for 2 hours, then the extracts were purified by solid phase extraction. The wash solutions and acidic hair extracts were derivatized with hexafluoroisopropanol and pentafluoropropionic anhydride at 75° C for 15 minutes. The derivatized extracts were then analyzed for cocaine and cocaine metabolites by EI GC/MS. The ions monitored for cocaine were 182, 272 and 303; for cocaine d_3 they were 185 and 306. The ions monitored for benzoylecgonine were 300, 316 and 421; for benzoylecgonine d_3 they were 303 and 424. The limit of detection and limit of quantitation for cocaine was 50 pg/mg. The LOD and LOQ for benzoylecgonine was 10 pg/mg.

The extraction of unwashed hair produced benzoylecgonine from 0 to 0.6% of the total cocaine detected. The benzoylecgonine produced in the first wash was 0 to 1.3% of the total cocaine, and that of the second wash was 0 to 2.4% of the total cocaine.

In conclusion, when washing hair samples to remove external contamination a fraction of the external cocaine contamination may be converted to benzoylecgonine. Caution must be employed in interpreting the results of cocaine in hair as that of a user, based solely on the presence of benzoylecgonine as a metabolite.

Keywords: Hair, Cocaine, Wash, Crack

The Simultaneous Analysis of Amphetamines, Cocaine, Phencyclidine and Opiates in Oral Fluid by GC/MS-EI

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Amphetamines, cocaine, phencyclidine and opiates and their metabolites are incorporated into oral fluids from the blood stream and the oral mucosa. The limited volume of oral fluid collected can create problems for donor specimens that screen positive for multiple analytes.This method simultaneously extracts for phencyclidine (PCP), codeine, morphine, 6-acetylmorphine (6-AM), amphetamine, methamphetamine, methylenedioxyamphetamine (MDA), methylenedioxymethamphetamine (MDA), methylenedioxyethylamphetamine (MDEA) in a donor sample with a single extraction and multiple GC/MS-EI injections.

Approximately 1200 uL of buffered oral fluid is obtained from the Intercept™ collection device (Orasure). A 400 uL aliquot of this solution is adjusted to 2 mL using phosphate buffer and extracted for confirmation. The corresponding deuterated internal standards are added to donor and QC samples. The samples are extracted using Clean Screen® Extraction Columns by United Chemical Technologies, Inc (UCT). The solid phase extraction (SPE) columns are conditioned using methanol, water and phosphate buffer. The samples are applied to the SPE columns and washed using deionized water, 0.1M hydrochloric acid and methanol. The drugs are eluted using a solution of methylene chloride/isopropanol/ammonia hydroxide. Following evaporation, the samples are derivatized at 75°C for 15 ± 5 minutes using pentafluoropropanol (PFPOH) and pentafluoropropionic anhydride (PFPA). The derivatives are injected for analysis on an Agilent 5973 EI-MS/6890 GC using a DB-5MS, 12m x 0.20 mm x 0.33 µm capillary column. The GC program for amphetamines consists of an injector temperature of 200°C, an initial oven temperature of 70°C with a 0.5 minute hold, then ramping to 120°C at 100°/minute, 250°C at 10°/minute, then 50°/minute to 300°C. The GC program for the other analytes has an injector temperature of 250°C with identical temperature ramps.

The GC/MS method is a SIM procedure and the ions monitored for the analysis are listed below.

ANALYTE	QUANT ION	OTHER IONS	INTERNAL STANDARD	IS QUANT ION	OTHER IONS
Amphetamine	190	118	d-11 Amphetamine	194	128
Methamphetamine	204	160	d-14 Methamphetamine	211	163
MDA	135	162	d-5 MDA	136	167
MDMA	204	162	d-5 MDMA	208	163
MDEA	218	162	d-6 MDEA	224	165
Benzoylecgonine	300	421	d-3 Benzoylecgonine	303	424
Cocaine	182	303	d-3 Cocaine	185	306
PCP	200	242	d-5 PCP	205	246
Morphine	414	415	d-3 Morphine	417	418
Codeine	282	445	d-3 Codeine	285	448
6-AM	414	473	d-6 6AM	417	479

The cutoff of oral fluid samples is 50 ng/mL for amphetamine, methamphetamine, MDA, MDMA and MDEA, 8 ng/mL for cocaine and benzoylecgonine, 10 ng/mL PCP, 40 ng/mL for codeine and morphine and 4 ng/mL for 6-AM. The values obtained refer to the detection in oral fluid. Donor specimens collected from the Intercept™ device are calculated from a x3 dilution (400 uL of oral fluid with 800 uL of buffer). The validation results are listed below.

ANALYTE	LOD ng/mL	LOQ ng/mL	Precision at 40% Cutoff (CV)	Precision at 150% Cutoff (CV)	ULOL ng/mL
Amphetamine	2.5	2.5	2.93	4.85	500
Methamphetamine	2.5	2.5	5.69	6.1	500
MDA	10	10	1.07	4.22	500
MDMA	5	5	2.41	4.21	500
MDEA	10	10	3.76	7.52	500
Benzoylecgonine	0.8	0.8	0.82	4.07	200
Cocaine	0.8	0.8	7.5	4.95	200
PCP	0.5	1	5.69	7.78	125
Morphine	16	16	8.32	10.39	500
Codeine	2	2	6.82	6.9	500
6-AM	1.6	1.6	6.89	8.42	50

The results indicated that a single extraction of oral fluid enabled the analysis of 11 drugs using three GC/MS-EI injections with acceptable LOD/LOQ, precision and ULOL.

Keywords: Oral fluid, Simultaneous analysis, GC/MS-EI

Performance Characteristics of the Cozart® RapiScan Opiate Oral Fluid Drug Testing System in Comparison to the Cozart® Microplate EIA Opiate Oral Fluid Kit and GC/MS following Controlled Codeine Administration

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Oral fluid is of interest as an alternative matrix for drug testing in many different venues, including law enforcement, workplace drug testing, and drug treatment facilities. Oral fluid testing is non-invasive and specimens can be collected under supervision, reducing the opportunity for adulteration. Performance characteristics of the FDA-cleared, qualitative, Cozart® RapiScan Opiate Oral Fluid Drug Testing System (Opiate CRS) is compared to the semi-quantitative Cozart® Microplate EIA Opiate Oral Fluid Kit (Opiate ELISA) and also to gas chromatography/mass spectrometry (GC/MS). Different opiate cutoffs were evaluated, including 2.5 μg/L (GC/MS LOQ), 30 μg/L as currently used for forensic testing in the United Kingdom (UK) and the 40 μg/L cutoff proposed by the Substance Abuse and Mental Health Services Administration (SAMHSA).

Oral codeine doses of 60 mg/70kg and 120 mg/70kg were administered to subjects who signed informed consent to participate in an IRB approved research study and resided on the closed research ward throughout the study. The oral fluid specimens (N=1273) were analyzed for codeine (COD), norcodeine (NCOD), morphine (MOR) and normorphine (NMOR) by GC/MS with a LOQ of 2.5 μg/L for all analytes. MOR and NMOR were not detected in any sample; 26.5% of the specimens were positive for COD, and 13.7% were positive for NCOD.

Opiate CRS uses a preset, qualitative cutoff of 10 μg/L; diluting the specimens 1:3 with buffer yields an effective cutoff of 30 μg/L. Sensitivity, specificity, and efficiency of the Opiate CRS at a 30 μg/L Opiate ELISA cutoff were 98.6%, 98.1% and 98.2%, respectively. Similar performance characteristics were obtained for the 40 μg/L Opiate ELISA comparison (99.0% sensitivity, 96.2% specificity, and 96.6% efficiency).

Compared to the GC/MS LOQ, sensitivity, specificity and efficiency of Opiate CRS at the 30 μg/L cutoff were 66.8%, 99.3% and 90.7%. Increasing the GC/MS cutoff to the current UK level, yielded performance characteristics of 88.5% (sensitivity), 99.2% (specificity), and 97.1% (efficiency). At the proposed SAMSHA confirmation cutoff of 40 μg/L, sensitivity increased while specificity and efficiency decreased slightly (91.3% sensitivity, 98.9% specificity, and 97.5% efficiency).

Oral fluid is an advantageous matrix for detecting drugs of abuse. CRS, with a preset 30 μg/L cutoff, is a sufficiently sensitive, specific and efficient device for oral fluid opiate analysis, performing similarly to the Opiate ELISA at the same cutoff, and having performance characteristics >91% when compared to GC/MS at the proposed SAMHSA cutoff.

Keywords: Codeine, Oral fluid, SAMHSA cutoff

Cozart® RapiScan Oral Fluid Drug Testing System: An Evaluation of Sensitivity, Specificity, and Efficiency for Cocaine Detection Compared to ELISA and GC/MS following Controlled Cocaine Administration

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Oral fluid has become a widely accepted and advantageous alternative matrix for drugs of abuse detection. Due to the advantages of oral fluid as a testing matrix, on-site testing devices have been developed to screen oral fluid for drugs of abuse, including cocaine and metabolites. The purpose of this study was evaluation of the usefulness of the Cozart® RapiScan Oral Fluid Drug Testing System (CRS) as an on-site screening tool for the detection of cocaine in oral fluid.

The performance of CRS was evaluated in comparison to Cozart® Microplate EIA Cocaine Oral Fluid Kit (COC ELISA) and GC/MS at cutoffs proposed by SAMHSA, cutoffs currently utilized in the United Kingdom (UK), as well as the lowest calibrator of the COC ELISA and the limit of quantitation of the GC/MS method. Eighteen healthy volunteers with a history of cocaine use provided informed consent to participate in a controlled cocaine administration protocol. Oral fluid samples (n= 1271) were collected prior to and following the subcutaneous administration of three low doses (75 mg/70 kg) of cocaine hydrochloride within 7 days, and after a 3-week interval, three high doses (150 mg/70 kg) within 7 days.

CRS provides a qualitative screen at a pre-set cutoff of 30 µg/L. Sensitivity, specificity, and efficiency for CRS (30 µg/L) as compared to COC ELISA with a cutoff of 30 µg/L were 92.1%, 91.8%, and 92.0%. The comparison of CRS (30 µg/L) to the 8 µg/L proposed SAMHSA confirmation cutoffs for cocaine and/or benzoylecgonine exhibited a sensitivity of 82.7%, a specificity of 94.5%, and an efficiency of 87.6%. The addition of ecgonine methyl ester as a target analyte at this cutoff had less than a 1% effect on the performance characteristics of the assay; ecgonine methyl ester was present with concentrations of cocaine and benzoylecgonine above the respective cutoffs in all but six specimens. At cutoffs in use in the UK, 30 µg/L CRS screen and 15 µg/L GC/MS cutoffs for cocaine, benzoylecgonine, and/or ecgonine methyl ester, sensitivity, specificity, and efficiency were 89.4%, 92.2%, and 90.7%, respectively. Alternative cutoffs can be programmed into the CRS software and for this study an alternative CRS cutoff of 20 µg/L was evaluated. Sensitivity, specificity, and efficiency of CRS (20 µg/L) at the proposed SAMHSA confirmation cutoffs were 89.9%, 89.7%, and 89.8%, respectively.

Cozart® RapiScan exhibited performance similar to the COC ELISA assay for the detection of cocaine exposure and suitable sensitivity and specificity at all cutoffs, including those proposed by SAMHSA. In this sample set, improved assay sensitivity and efficiency were achieved by utilizing the CRS with the alternative screening cutoff.

Keywords: Cocaine, Oral fluid, SAMHSA cutoffs

Comparison of Commercially Available ELISA Kits for the Analysis of THC-COOH in Hair

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It was the goal of these experiments to provide information on the selectivity and sensitivity of commercially available ELISA kits for the routine analysis of hair specimens. It is not the object of the authors to recommend any particular manufacturers product, but rather to provide this information to assist others in selecting the product that is right for their particular needs. The kits of four major manufacturers (OraSure[3], Immunalysis[4], Neogen[5], and International Diagnostic Systems[6]) were challenged with identical spiked samples over a broad range of concentrations (Hair (n = 18) from 0 to 500 pg/mg; Urine (n=16) from 0 to 2000 ng/mL; Water (n= 14) from 0 to 2000 ng/mL), and also with common interfering compounds from the Toxi-Disk library. Hair samples were extracted, dried and re-suspended with phosphate buffer, prior to fortification with THC-COOH. A single set of controls was prepared daily for each matrix to reduce bias. Triplicate analysis was performed and the results were evaluated to determine the point of maximum slope, and minimum acceptable cutoff of each ELISA plate. Additionally time course was plotted by measuring each plate at three time intervals (t = 0, 15, 30 min) post reaction termination. These simple experiments revealed startling differences between the plates tested. It was revealed, that while all of the plates tested produced similar selectivity, large differences were present on sensitivity and in slope about the cutoff. Though most of the plates analyzed exhibited detection levels suitable for hair analysis (IDS, Immunalysis, and OraSure) the Neogen kit produced results more consistent with urine ranges. Further only two of the kits tested (IDS, and Immunalysis) were capable of giving adequate separation about our proposed 1 pg/mg screening cutoff. These data suggest that great care should be taken in the selection of ELISA products, so that the laboratories needs for sensitivity are properly satisfied.

Keywords: ELISA, THC-COOH, THCA, Screening

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Simultaneous Analysis of Morphine, Codeine, Oxymorphone, Hydromorphone, 6-Acetylmorphine, Oxycodone, Hydrocodone and Heroin in Hair and Oral Fluid

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Hair and oral fluid have been becoming increasingly useful matrices for the determination of drug use in the forensic toxicology laboratories. The objective of this study was to analyze morphine, codeine, oxymorphone, hydromorphone, 6-acetylmorphine (6-AM), oxycodone, hydrocodone and heroin in hair and oral fluid, simultaneously, using solid phase extraction and gas chromatography-mass spectrometry (GC-MS) with electron impact mode (EI) and selected ion monitoring (SIM).

One hundred milligram of hair was collected. The collected hair was cut into small fragments that were blended. Eight hundred to 1000 µl of oral fluid was collected from the donor by insertion of absorptive collectors. Four hundred microliter of the collected oral fluid was used for the extraction. Twenty milligram of hair samples were pretreated and incubated with 2 ml of methanol at 75^{O}C for two hours after adding all the controls to the control samples and deuterated d_3-optiate combined internal standard to all specimens including controls. The opiates in the sample were derivatized with 10% methoxylamine to stabilize the keto functional group. All samples were extracted using solid phase extraction with CLEAN SCREEN® CSDAU column from United Chemical Technology, Inc. Each column was conditioned with 2 ml of elution solvent (methylene chloride/isopropanol/ammonium hydroxide, 80:20:1.8, v/v), then 3 ml of methanol followed by 2 mL of 0.1 M phosphate buffer, pH 6. Each sample was poured into the corresponding extraction column. The sample was allowed to flow through the column at a rate of 1 to 2 ml/min. Each column was then washed with deionized water (2 ml), acetate buffer (pH=4, 2ml), hexane (1 ml) and methanol (3 ml). The drugs were eluted with elution solvent after the columns were dried under nitrogen stream for 5 min. The eluted drugs were dried and the dried extracts were derivatized with 25 µL ethyl acetate and 25 µL of N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) with 1% trimethylchloroslane (TMCS). The derivatized extracts were transferred to the auto-sampler vials. One microliter of the derivatized extracts was analyzed by GC-MS in EI mode equipped with J&W DB-17MS, 15 m x 0.25 mm x 0.25 µm capillary column.

By adding methoxylamine, the synthetic opiates(hydrocodone, hydromorphone, oxycodone and oxymorphone) are converted to a single oxime derivative product and eliminate the interference from the synthetic opiates . Therefore, it was able to separate the synthetic opiates from codeine and morphine. However, without adding methoxylamine, the use of trimethylsilyl (TMS) produces a multiple TMS derivates forms of the synthetic opiates due to the keto functional group. The enolization of keto group in the synthetic opiates hinders accurate quantitation and can cause interference in the identification and quantitation of morphine and codeine.

The cutoff of hair samples is 200 pg/mg while the cutoff of oral fluid is 4 ng/ml for 6-AM and 40 ng/ml the other opiates. The validation results are listed below.

	LOD	LOQ	Precision at 40% Cutoff	Precision at 150% Cutoff	Upper limit of linearity
Hair	(pg/mg)	(pg/mg)	%CV	%CV	(pg/mg)
Morphine	60	60	9.26	13.69	2500
Codeine	60	60	9.39	1.69	2500
Oxymorphone	60	60	4.13	6.08	2500
Hydromorphone	60	60	6.05	3.68	5000
6-AM	20	60	2.42	7.03	2500
Oxycodone	60	60	7.53	3.3	5000
Hydrocodone	60	60	8.99	4.92	5000
Heroin	60	60	10.44	5.18	5000
Oral Fluid	(ng/ml)	(ng/ml)	%CV	%CV	(ng/ml)
Morphine	10	10	5.99	2.16	800
Codeine	4	4	4.69	2.46	800
Oxymorphone	4	10	7.35	2.04	800
Hydromorphone	4	10	6.17	5.71	800
6-AM	0.4	1	5.58	5.97	800
Oxycodone	4	10	6.97	2.71	800
Hydrocodone	4	4	6.83	1.92	800

The results indicated that a single methoxime derivative product is produced using methoxylamine and provides separation of the opiates by GC-MS.

Keywords: Hair, Oral fluid, Opiates

Evaluation of Immunalysis® ELISA Assays for the Detection of Drugs of Abuse in Postmortem Bile and Urine

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The availability of a particular specimen for immunoassay screening is not always guaranteed for postmortem toxicological analysis. The Wayne County Medical Examiner's office (WCMEO) had been screening for drugs of abuse in urine by EMIT® for many years. However, when urine is not available bile becomes the specimen of choice for screening for drugs of abuse. Direct screening of bile using EMIT® technology is not possible. Therefore the use of the Immunalysis® ELISA assays for use in urine and bile matrices was evaluated.

The EMIT® assays and their respective cutoff concentrations were opiates (300 ng/mL), benzoylecgonine (300 ng/mL), amphetamine class assay (polycolonal) (1000 ng/mL), barbiturates (300 ng/mL), benzodiazepines (300 ng/mL) and phencyclidine (25 ng/mL). For the Immunalysis® assays the same cutoff and assays were used except that the methamphetamine assay (cutoff concentration, 300 ng/mL) was substituted for the amphetamine class assay.

The Immunalysis® assays were performed using Tecan® equipment. Pipetting was performed on a Miniprep 75 using a 1:75 specimen dilution. Plates were washed with a Columbus II plate washer and read using a Spectra II plate reader. The EMIT® assays were run on an ETS analyzer according to the manufacturer's instructions.

The precision of the ELISA assays were determined by assaying multiple negative (N=18), cutoff (N=12), above threshold (2 times the cutoff concentration, N=17) and below threshold (half the cutoff concentration, N=17) calibrators and controls interspersed throughout a single plate. The average %CV across the plate ranged from 6.2 to 14.4% for urine and 7.2 to 13.3% for bile, depending on the assay. These CV's appear to reflect the variation in ELISA assays and are not a result of pipetting imprecision as the Miniprep 75 demonstrated a CV of only 0.11% for the pipetting of the sample and diluent (N=64). To compensate for the higher CV's obtained with ELISA compared with traditional immunoassays, it was decided to calibrate using the mean of duplicate negative and cutoff calibrators. In practice, this approach proved satisfactory with an overall control failure rate of 2.2% for 1800 assayed controls.

208 urine specimens were selected for analysis by EMIT® and ELISA. For the opiate, benzoylecgonine, barbiturate and benzodiazepine assays the majority of positive and negative specimens were in agreement by both assays and most discrepancies could be readily explained by variation about the cutoff or relative cross-reactivity. The number of positive cases for methamphetamine and phencyclidine were too small for assay comparison.

To evaluate the application of the Immunalysis® assays to the bile matrix calibrators and controls were prepared using pooled negative bile. Although there were slight differences in the absorbance values for the two matrices at a given drug concentration, they were minimal and bile above and below threshold controls met acceptance criteria based on either the bile or urine cutoff calibrator. Consequently it was decided that urine or bile matrices could be assayed on the same plate using urine for assay calibration with urine-based controls interspersed throughout the plate. At the end of each plate, negative bile, and bile controls at the cutoff, 50% below and 100% above the cutoff were assayed. To determine acceptability for bile specimens, the bile-

based controls were compared against the bile cutoff calibrator, however, the decision to confirm a presumptive positive bile sample was based on the urine calibrator absorbance for simplicity. This approach was used in a study of 500 sequential bile samples.

The confirmation rates for either urine or bile specimens which screened positive by ELISA were comparable. The opiate, benzoylecgonine and barbiturate assays demonstrated confirmation rates at or near 100%. The benzodiazepine confirmation rate ranged from 67% (bile) to 89% (urine). However, the WCMEO does not confirm all benzodiazepines to which the ELISA assay has cross-reactivity. There were no methamphetamine positive cases and only one positive PCP case. There was a tendency for the methamphetamine assay to exhibit positive or elevated results when pseudoephedrine was present. The PCP assay exhibited positive for results when dextromethorphan was present on six occasions however this appeared to be lot specific.

Keywords: ELISA, Urine, Bile, Drugs of abuse

Multiplexed CYP 2D6 *3 *4 and *5 Polymorphism - Real Time PCR for Application in Forensic Pathology/Toxicology Methamphetamine/MDMA Cases

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Pharmacogenomics may be used as an adjunct for certifying drug toxicity. In particular, the identification of a mutation in the gene, which expresses the CYP450 2D6 enzyme, may be used as an adjunct for certifying the cause of death involving certain synthetic drugs of abuse. The CYP450 2D6 is a polymorphic gene (*3, *4, and *5 are considered the major variances) containing as many as 50 alleles. 5-10 % of the Caucasian population contains one or more of these alleles, while 17 % of some ethnic populations may be affected. The prevalence for poor metabolism is expressed in subjects who are homozygous for *3, or *4, or *5, or heterozygous for any two of these mutant alleles. Individuals who are homozygous for the wild type will have functional enzyme activity, while those who are heterozygous will have some variation in their enzyme activity. Genotypic association studies of an individual's drug metabolism may establish that individual's pharmacogenetic profile. This study used whole blood source purified genomic DNA from seven deceased individuals who tested positive for methamphetamine and/or 3,4-methylenedioxymethamphetamine (MDMA), both of which are substrates for CYP450 2D6. The three alleles *3, *4, and *5, were multiplexed using allele specific amplification with SYBER-green ® detection. Summaries of the results are as follows: Six of the seven cases were male with a median age of 40 (range 22-43). Six of the cases had toxicology results confirmed for methamphetamine use with a median blood concentration of 0.68 mg/L (range 0.04-1.41). Two of the seven cases were confirmed positive for MDMA with a blood concentration ranging from 0.27-0.92 mg/L. The genotyping results revealed the following: Six of the seven cases involved Caucasian individuals. Four of the seven individuals were genotyped as heterozygous for both the *3 and *4 variance, while the remaining three were determined to be wild type. All seven individuals contained the *4 variance, while one individual was identified as *4/unknown.

The implementation of this molecular assay in combination with the toxicology assays will enable the toxicologist to establish a pharmacogenetic association with specific drugs metabolized by this pathway.

Keywords: Multiplex, PCR, CYP450 2D6, Methamphetamine, MDMA

The Redistribution of Psychiatric Drugs in Postmortem Cases

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Psychiatric drugs, including selective serotonin reuptake inhibitors (SSRIs), serotonin/noradrenaline reuptake inhibitors (SNARIs), and atypical antipsychotics, comprise one of the most diverse drug categories. However, their high volumes of distribution (up to 28 L/kg), protein binding (0.27-0.99), and lipophilicity (logP = 2.91-5.45) suggest redistribution is likely for most drugs in this class. Findings from previous studies of individual psychiatric drugs support this theory (Table 1), but provide insufficient data to draw conclusions regarding their redistribution as a class. This makes it difficult to interpret postmortem drug concentrations, particularly when the postmortem interval is long.

We assessed the redistribution of five SSRIs (citalopram, fluoxetine, fluvoxamine, paroxetine, and sertraline), one SNARI (venlafaxine), and one atypical antipsychotic (risperidone), as well as their associated metabolites, by analyzing blood specimens collected from heart and femoral sites. Since the possibility exists for drugs in the stomach contents to diffuse into the liver or centrally-collected blood, this data was compared to drug concentrations measured in liver and stomach contents, when such specimens were available. Specimens from 13 cases were extracted with n-butyl chloride and analyzed via liquid chromatography-mass spectrometry (LC-MS), using atmospheric pressure electrospray ionization (APESI) operated in positive mode. LC-MS analysis was performed on an Agilent 1100 Series HPLC configured with a G1946A mass selective detector (MSD). Chromatographic separation was achieved using a Zorbax Extend-C_{18} column from Agilent (2.1 x 150 mm, 5 μm particle size), operated at 21 °C pumping at 0.25 mL/min for 40 min, with an isocratic mobile phase of 0.05M ammonia/methanol/ THF (32.5:67.0:0.5) at pH 10.0. To validate this method, accuracy and precision data were obtained by performing replicate analyses of blank blood specimens spiked with either a low (0.075 mg/L) or high (1.0 mg/L) standard of each drug. Five replicates of each spiking level were analyzed. Within- and between-day coefficients of variation were all below 14%. Accuracy ranged from 78-104% for all drugs.

There were not enough specimens in which blood from both sampling sites was available to assess the drugs individually for significance of concentration differences (concentrations shown in Table 1). However, heart blood concentrations were significantly higher (34 %, on average) than those measured in femoral blood when results from all drugs were included together ($p<0.05$). Heart:femoral blood concentration ratios ranged from 0.50-6.2, although they averaged between 2-3:1. With the exception of norfluoxetine in one case, the mean metabolite concentration ratios were similar to those of their parent drugs. Three cases accounted for the highest heart:femoral blood concentration ratios. No significant difference in concentration was observed for citalopram, sertraline, or venlafaxine in blood collected from heart versus femoral regions. Possible reasons for the observed redistribution include diffusion from solid tissues or gastric contents to centrally collected blood, taking blood from a femoral site without first ligating the vessel, or differences in specimen haematocrit. Based on a comparison of data from liver, gastric contents, and heart and femoral blood specimens, it appears that the most likely

explanation for the observed redistribution is diffusion of drug from solid tissues and organs into centrally-collected blood.

Table 1. Heart:Femoral Concentration Ratios for Selected Psychiatric Drugs Reported in Previous Studies and in the Present Work*.

Compound	Heart:Femoral Ratio	Compound	Heart:Femoral Ratio
Previous Studies			
Citalopram (Fu et al, 2000)	1.3	Sertraline (Jaff, 1997)	1.5-1.9
Fluoxetine (Jaffe, 1997)	1.2 (0.79-1.3)	Sertraline (Levine et al, 1994)	0.56-1.36 (0.55-1.77)
Fluoxetine (Rohrig and Prouty, 1989)	3.5 (2.6)	Sertraline (Logan et al, 1994)	1.29 (1.10)
Fluoxetine (Roettger, 1990)	4.6 (1.5)	Venlafaxine (Jaff, 1997)	2.0-3.0
Fluvoxamine (Kunsman et al, 1999)	3.1	Venlafaxine (Levine et al, 1996)	1.83 (2.11)
Paroxetine (Jaff, 1997)	0.83-1.5	Venlafaxine (Parsons et al, 1996)	1.31-1.76 (0.79)
Paroxetine (Vermeulen, 1998)	1.28		
This Study			
Citalopram	1.1	Sertraline	1.0-4.8 (0.76-2.2)
Fluoxetine	6.2 (33)	Venlafaxine	0.90-5.5 (0.80-2.6)
Fluvoxamine	0.79	Risperidone	0.50-5.0 (1.3-2.0)
Paroxetine	1.1-4.4		

*Metabolite ratios in parentheses.

Keywords: Postmortem redistribution, Psychiatric drugs, LC-MS

Fatal Nicotine Intoxication Resulting from the Ingestion of "Ayahuasca"

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A case report is described involving the unexpected death of a 71 year old native female who was partaking in a holistic healing ritual involving the ingestion of "ayahuasca". "Ayahuasca" is a beverage that has been used for centuries, in South America, for medicorelegious purposes.The woody vine *Banisteriopsis caapi* forms the basis of this beverage with other plant materials usually added to obtain a "tea" that imparts a variety of psychoactive effects.In this case, *Banisteriopsis caapi* was mixed with tobacco leaves to prepare the "ayahuasca" brew.

The ritual took place over 3 days and involved the ingestion of the "ayahuasca" brew followed by self-induced vomiting. Over the course of the 3 days, the health of the female deteriorated significantly. It is believed that she did not vomit following the ingestion of the brew. On the third day she was apparently given an enema of the "ayahuasca "brew, following which she collapsed and died a short time later. An autopsy revealed no anatomical cause of death. Heart and femoral blood and a sample of the brew were submitted for analysis.

A screen for drugs of abuse, alcohol and common prescription basic drugs was performed on the blood using a combination of IA, GC-FID, GC-NPD and GC-MS techniques. Analysis of the blood revealed only the presence of nicotine. Quantification of the nicotine was performed by GC-NPD utilizing a standard basic drug liquid-liquid extraction procedure.Heart and femoral blood nicotine concentrations of 1900 and 710 ng/mL, respectively, were found. A GC-MS screen of the "ayahuasca" brew revealed the presence of nicotine, harmine and harmaline.

The detected heart and femoral blood nicotine concentrations are well above nicotine concentrations associated with the recreational use of tobacco products. Nicotine levels in habitual smokers average around 20 ng/mL.The deceased was a non- smoker.

The cause of death was attributed to acute nicotine intoxication. To the author's knowledge there are no other reports in the literature of accidental fatalities resulting from the ingestion of a nicotine-containing solution as part of a holistic healing ritual. A review of the literature revealed very few case reports of fatalities resulting from nicotine intoxication. Nicotine blood concentrations ranging from 11,000 to 63,000 ng/mL have been reported in cases involving the suicidal ingestion of a nicotine containing solution. Heart and femoral blood nicotine concentrations of 1,400 and 460 ng/mL, respectively, were detected in a female who was found dead with 18 nicotine transdermal patches on her body and a plastic bag taped over her nose and mouth.

Keywords: Nicotine, Intoxication, Ayahuasca

A Comparative Solid-Phase Extraction Study for the Simultaneous Determination of Fluoxetine, Amitriptyline, Nortriptyline, Trimipramine, Maprotiline, Clomipramine and Trazodone in Whole Blood by Capillary Gas-Liquid Chromatography with Nitrogen-Phosphorus Detection

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In systematic toxicological analysis (STA) one of the main purposes is screening analysis. Antidepressants are an important class of drugs in forensic and clinical cases. They are widely used for the treatment of a variety of depressive states and other psychiatric disorders. An increase in antidepressants intoxication led to the development of reliable analytical methods for their analysis. A comparative study for the simultaneous GC resolution and detection of seven antidepressants fluoxetine, amitriptyline, nortriptyline, trimipramine, maprotiline, clomipramine and trazodone in whole blood at concentration levels of 100-2000 ng/mL was developed. This method allows toxicological screening as well as therapeutic drug monitoring of these antidepressants. A comparative and validation study using two solid-phase extraction (SPE) columns, Chem Elut and Bond Elut Certify, were developed regarding their recovery, precision, sensitivity and matrix purification efficiency. The first procedure consists of the employment of diatomaceous earth, Chem Elut columns, based on the principle of liquid-solid absorption extraction. The second focuses on the use of Bond Elut Certify columns, a mixed SPE: reversed-phase and cation exchange sorbent, more recently developed in the market. Each procedure required 2.0 mL of whole blood extraction and injection into a gas chromatograph equipped with a methylsilicone capillary column and a nitrogen-phosphorus detector. Prazepam was used as chromatographic standard. Both procedures provided extracts free of chromatographic interferences in the areas corresponding to the retention time of the studied compounds. Recoveries of the compounds using Chem Elut columns at 500 ng/mL were in the range 30-50% with intra-assay and inter-assay precisions of less than 9 and 17%, respectively. Limits of detection (LODs) and limits of quantitation (LOQs) ranged from 13 to 146 ng/mL and from 44 to 485 ng/mL, respectively. Recoveries of the compounds using Bond Elut Certify columns at 500 ng/mL were in the range 59-84% with intra-assay and inter-assay precisions of less than 8% and 11%, respectively. LODs and LOQs ranged from 8 to 67 ng/mL and from 25 to 223 ng/mL, respectively. An excellent linearity was observed with both extraction procedures from the LOQs up to 2000 ng/mL. Higher recoveries, cleaner extracts, better sensitivity, better precision and less solvent consumption and disposal were achieved for the screening of these antidepressants with the use of the mixed SPE, Bond Elut Certify comparing with Chem Elut columns.

Keywords: Antidepressants, Whole blood, Solid-phase extraction, Nitrogen-phosphorus detection.

Urinary Pharmacokinetics of Methamphetamine and Its Metabolite, Amphetamine Following Controlled Oral Administration to Humans

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Methamphetamine (METH) is widely abused for its euphoric effects throughout the world. It also is a DEA Schedule II drug with limited therapeutic use for the treatment of attention deficit hyperactivity disorder, obesity, and narcolepsy. The objectives of this study were to characterize the urinary excretion pattern and urinary pharmacokinetics of METH and its major metabolite, amphetamine (AMP), following controlled oral administration of METH.

This Institutional Review Board approved study was conducted on a closed research unit over 10 weeks. Participants provided informed consent and were paid for their participation. Participants (n=8) were administered four daily 10 mg (low) oral doses of the sustained release form of (d)-METH hydrochloride within 7 days. After a 4-week interval, five of the eight participants also received four daily 20 mg (high) oral doses. Urine specimens were collected *ad libitum* prior to and for up to 8 days following the last dose and were stored at -20°C until analysis. METH and AMP were isolated by solid phase extraction followed by gas chromatography/positive chemical ionization-mass spectrometry.

Maximum urinary excretion rates ranged from 403 - 4,919 μg/h for METH and 59.0 - 735.2 μg/h for AMP across drug regimens with no relationship between dose and excretion rate. After the last dose, the mean peak METH excretion rate was 774 ± 438 μg/h (range 208-1560 μg/h) and occurred at a mean time of 16.6 ± 15.8 h (range 0.7 - 48 h), while mean peak AMP excretion rate was 231 ± 252 μg/h (range 26-735 μg/h) at a mean of 24.2 ± 17.3 h (range 1.3-51.8 h). The mean % of dose excreted in the urine as total METH and AMP was 57.5 ± 21.7% for the low dose and 40.9 ± 8.5% for the high dose, possibly due to saturation of an active transport system for renal excretion. Mean terminal elimination half-lives in urine were 22.6 ± 7.2 h and 25.1 ± 6.0 h for METH and 19.8 h and 22.0 h for AMP at the low and high doses, respectively. Urine METH and AMP concentrations exceeded the method's limit of quantitation (2.5μg/L) for up to 8 days. The mean % ratio of AMP/METH based on the area under the urinary excretion curve (AURC) for the first 24 h after the first dose was 13.4 ± 6.5%; however, the mean % ratio of AMP/METH by AURC for the first 24 h after the last dose increased to 22.7 ± 16.1 %. The mean % ratio of AMP/METH based on AURC from the time of the last dose to the time when the drug was no longer detectable was 35.7 ± 26.6%.

METH and AMP had similar mean urinary terminal elimination half-lives of 23.6 h for METH and 20.7 h for AMP across doses. The mean % ratios of AMP/METH AURC were increased over time from 13.4 to 35.7%.

Keywords: Methamphetamine, Urinary pharmacokinetics, Terminal elimination half-life

Evaluation of the DrugCheck® 9 On-Site Immunoassay Test Cup According to a Standard Method Validation Protocol

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There is currently no standard method evaluation protocol for "point-of-care" (POC) drug testing devices. We evaluated the DrugCheck® 9 cup, a qualitative visually read, competitive binding, immunoassay cup that measures 9 analytes, amphetamine, methamphetamine, carboxy – THC, cocaine metabolite, PCP, opiates, benzodiazepines, and barbiturates and tricyclic antidepressants. The study was performed according to the recent National Laboratory Certification Program (NLCP) guidelines for validating a laboratory-based immunoassay. The study included a linearity challenge with 5 replicates at concentrations 0, 25%, 50%, 75%, 100%, 125% and 150% of the cutoff and also determination of the limit of detection (LOD). At 75% of the cutoff, all replicates of each analyte were positive with the exception of morphine. At 50% of the cutoff, all replicates for barbiturates, cocaine metabolite, methamphetamine, and tricyclic antidepressants were positive, while all replicates at 50 % of the cutoff for benzodiazepines, opiates, and Carboxy-THC were negative. Amphetamine and PCP were mixed (2 positive and 3 negative) at 50% of the cutoff. Only barbiturates were positive at 25% of the cutoff, while all of the remaining analytes were negative for all 5 replicates. All analytes were negative for all replicates in drug free urine. All replicates above the cutoff were positive. Interference (specificity) studies were included to evaluate the cross reactivity of common structural analogs or others purported to interfere with the assay. Ephedrine, pseudoephedrine, chloroquine, diphenhydramine, phenylpropanolamine methylphenidate. D-methamphetamine and L-methamphetamine, MDMA, MDEA produced no interference with the amphetamine assay, indicating a very specific antibody to amphetamine. Only phentermine and MDA showed a positive result at 1000 ng/mL. The same group of sympathomimetic amines (including phentermine) showed no interference with the methamphetamine assay. MDMA produced a positive at 1000 ng/mL. Oxycodone, oxymorphone, tramadol, meperidine, nor-meperidine, nor-morphine, nor-codeine, and buprenorphine showed no interference at 50,000 ng/mL in the opiate assay. Codeine produced a positive result at 300 ng/mL (the cutoff), while hydromorphone and hydrocodone produced a positive result at 5000 ng/mL.

Out of 136 donor parallel comparisons with the CEDIA® laboratory immunoassay and GC/MS (50 Negatives and 86 Positives) there were 133 out of 136 in agreement (97.8%). There was agreement for all analytes except for 2 opiates which were shown to have very low levels of total morphine by GC/MS at 346 and 351 ng/mL, respectively, and also one specimen that was positive for benzodiazepines which approached the LOD of the GC/MS assay. These results indicate a high degree of correlation, greater sensitivity with extended linearity below the cutoff when compared to the laboratory based immunoassay, and improved specificity over previously evaluated POC testing devices.

Keywords: On-Site, Point-of-Care, DrugCheck

ABSTRACTS

POSTER PRESENTATIONS

P1

Analysis of Caffeine and Metabolites by Liquid Chromatography-Mass Spectrometry

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In recent years, the utilization of liquid chromatography-mass spectrometry (LC-MS) to analyze drugs and poisons has increased. LC-MS has been especially useful to analyze compounds that have poor gas chromatographic characteristics. An LC-MS method was developed to analyze caffeine (CAF) and its metabolites paraxanthine (PAX), theobromine (THB), and theophylline (THP). 7-(ß-Hydroxypropyl) theophylline (7HT) was used as the internal standard (800 ng/mL 7HT). The method used liquid-liquid extraction with a 5 mL extraction volume. A 1 mL volume of 0.5 M sodium acetate (pH 6.5) and a 5 mL volume of ethyl acetate was added to each extraction tube. After mixing 30 minutes on a laboratory shaker, the tubes were centrifuged 10 minutes at 2000 rpm. The organic layer was collected into clean, separate 13 x 100 culture tubes. The organic layer was evaporated to dryness under a stream of air by using a TurboVap evaporator (Zymark). Following evaporation, the extracts were reconstituted with 0.1 % formic acid: methanol (97:3). The extracts were analyzed using an Agilient 1100 LC-MSD. The LC column used was a Luna, C-18, 5 µm, 250 X 2 mm (Phenomenex). The mobile phase consisted of 0.1 % formic acid: methanol (75:25) at a flow rate of 0.25 mL/minute. Electrospray ionization was employed. Selected ion monitoring was used to monitor the ions at m/z: 181 (PAX, THB, THP), 195 (CAF), and 239 (7HT). The chromatographic conditions permitted excellent analyte peak separation with relative retention times of 0.37 (THB), 0.53 (PAX), 0.60 (THP), 0.89 (CAF), and 1.00 (7HT). Calibration curves were run from 30 ng/mL to 5000 ng/mL. In house control samples were prepared with blank horse serum. Intra-assay and inter-assay accuracy and precision were evaluated at 40 ng/mL, 800 ng/mL, and 3000 ng/mL. For THB, THP, and CAF, the intra-assay and inter-assay coefficient of variances (CV) were less than 8 %. For PAX, the intra-assay CVs were within 8 %, but the inter-assay CVs were 12-16 %. In the inter-assay accuracy evaluation, the analyte concentrations were within 7 % of target. This method was used to analyze mouse plasma and brain samples for a research study. Because of the increased use and misuse of dietary supplements that contain caffeine, effective methods for the analysis of caffeine and metabolites are important. (Supported by NIDA Contract No. DA-6-7052.)

Keywords: LC-MS, Caffeine, Metabolites

P2

Gas Chromatographic/Mass Spectromatric Confirmation of Beta-Blockers

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Pilots who have successfully controlled their hypertension with medications, diet, and/or exercise can be medically certified to fly an aircraft. Currently, approximately 8% of active pilots have been designated as "hypertensive with medication." Beta-blockers, such as atenolol, metoprolol, and propranol, are commonly prescribed for the treatment of hypertension. The Federal Aviation Administration (FAA) closely monitors those pilots who use these medications. The FAA's Toxicology and Accident Research Laboratory receives and analyzes postmortem biological samples from pilots involved in fatal civil aviation accidents. Over the past 10 years, the toxicology laboratory has identified 52 fatal-pilots in which beta-blockers were detected. Atenolol was found in 30 pilots, metoprolol in 16 pilots, and propranol in 6 pilots. This distribution mirrors the prescription frequency reported in the top 200 most-prescribed drugs. Periodically, toxicological results suggest the presence of both atenolol and metoprolol. However, there is no medical history in these cases supporting the simultaneous prescription and use of both drugs. It is also unusual for a patient to be prescribed atenolol and metoprolol together. Therefore, further examination of these cases was undertaken. Atenolol, metoprolol, and propranol, with their possible metabolites, were isolated from the selected specimens using Varian's Bond Elut® solid phase extraction for basic and neutral compounds, derivatized with pentafluoropropionic anhydride (PFPA), and analyzed by the gas chromatography/mass spectrometry (GC/MS). Given their chemical and structural similarities, when these drugs and their metabolites are derivatized with PFPA, their MS spectra are nearly identical. All of the PFPA derivatives have baseline resolution on the GC, with the exception of a metoprolol metabolite, which co-elutes with atenolol. This metabolite could be misidentified as atenolol. There are 3 primary fragments (366, 408, and 202 m/z) found with all of the 3 derivatized beta-blockers and the interfering metabolite. The atenolol mass spectrum was found to contain 3 unique fragments (244, 172, and 132 m/z). Metoprolol produced 2 unique fragments at 559 and 107 m/z. The mass spectrum of propranol contained 4 unique fragments at 551, 183, 144, and 127 m/z. The metoprolol metabolite that co-eluted with atenolol had 2 unique fragments at 557 and 149 m/z. The distinctive fragments chosen were further validated for their uniqueness using ThermoFinnigan HighChem Mass Frontier™, a computer program that predicts logical mass fragments. Several unique mass fragments reported here can be used to ensure the positive identification of the 3 common beta-blockers and co-eluting metoprolol metabolite. Therefore, these ions can be used for differentiating and simultaneously analyzing these beta-blockers in biological samples.

Keywords: Beta-blockers, Gas Chromatographic/Mass Spectrometric Analysis; Aviation toxicology

P3

Multicenter Evaluation of the Roche OnLine® TDM Phenytoin Assay on Roche/Hitachi Analyzer Systems

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Phenytoin (diphenylhydantoin or Dilantin®) is one of the most prescribed anti-convulsant drugs for the treatment of epilepsy. Because this drug has a narrow therapeutic index, monitoring of drug levels in serum is essential to ensure effective therapy and to avoid overdose, which can result in nystagmus, ataxia, respiratory distress, and convulsions.

The analytical performance of a new, Roche homogeneous microparticle immunoassay for Phenytoin was evaluated at four sites – two in Europe and two in the Unites States. Intra- and interassay imprecision, lower detection limit, control recovery, and linearity were assessed. Further, the assay method was compared to the CEDIA Phenytoin assay on Roche/Hitachi 917 and MODULAR P analyzers, Abbott FPIA Phenytoin assay on the Abbott AxSYM analyzer, and the Roche FP assay on INTEGRA 800. Serum vs. sodium heparin plasma comparison was also performed.

OnLine Phenytoin intra-assay imprecision showed SDs ≤0.44 µg/mL for concentrations up to 10 µg/mL and CVs ≤3.36 for concentrations >10 µg/mL. Interassay imprecision showed SDs ≤0.42 µg/mL for concentrations up to 10 µg/mL and CVs ≤5.50 for concentrations >10 µg/mL. Analytical sensitivity (lower detection limit) to 0.54 µg/mL and linearity to the 40.0 µg/mL were observed. Roche COBAS FP control materials recovered within range at all sites and competitor control materials recovered within manufacturers' ranges with one exception.

Passing/Bablok regression analysis was used to assess method comparison. All comparisons demonstrated close agreement between competitive methods and were well within acceptable therapeutic limits as noted in the table below:

	Methodology/Instrument x	y	N	Slope	Intercept	Correlation Coefficient
Site 1	CEDIA MOD P	OnLine MOD P	280	0.968	1.201	0.990
Site 2	CEDIA 917	OnLine 917	159	0.958	1.704	0.990
	Abbott AxSYM	OnLine 917	162	1.011	-0.690	0.967
Site 3	CEDIA MOD P	OnLine MOD P	194	0.906	0.836	0.990
	COBAS FP	OnLine MOD P	194	0.957	0.420	0.994
	Abbott AxSYM	OnLine MOD P	194	0.943	-0.893	0.983
Site 4	CEDIA 917	OnLine 917	222	0.987	0.871	0.985
	Abbott AxSYM	OnLine 917	223	0.941	-0.083	0.992

Comparison of serum vs. sodium heparin plasma produced this regression equation: $y = 1.000x + 0.200$, $r=0.992$ ($n=50$). The OnLine Phenytoin TDM reagent met or exceeded analytical specifications and all clinically relevant performance criteria in this evaluation.

Keywords: Phenytoin, Automated method, Multicenter evaluation

P4

Simultaneous Determination of Buprenorphine, Norbuprenorphine, Buprenorphine-3-Glucuronide and Norbuprenorphine-3-Glucuronide in Human Plasma by Liquid Chromatography – Electrospray ionization - Tandem Mass Spectrometry

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Buprenorphine is used for treatment of pain and as a substitution therapy for opioid dependence. Buprenorphine is primarily metabolized by N-dealkylation to norbuprenorphine; both buprenorphine and norbuprenorphine are glucuronidated. To more fully understand the metabolism and pharmacokinetics of buprenorphine, a sensitive method is needed for simultaneous determination of buprenorphine (BUP), norbuprenorphine (NBUP), buprenorphine-3-glucuronide (BUPG) and norbuprenorphine-3-glucuronide (NBUPG). With provision of BUPG from the National Institute on Drug Abuse and the recent synthesis of NBUPG by ElSohly Laboratories, we have now been able to develop a method that employs solid phase extraction (SPE) followed by liquid chromatography -electrospray ionization -tandem mass spectrometry (LC-ESI-MS/MS). One milliliter of plasma was fortified with buprenorphine-d_4, norbuprenorphine-d_3 and morphine-3-glucuronide-d_3 as internal standards. Ammonium carbonate buffer (pH 9) was added to plasma samples and extracted using C18 SPE cartridges. SPE columns were washed with buffer and water, dried, and then eluted with methanol. Extracts were evaporated to dryness and reconstituted with 65 µL of mobile phase. LC-ESI-MS/MS analyses were carried out using a TSQ-Quantum tandem mass spectrometer. A Metasil basic 2.0 x 100 mm, 3-µm column was employed for LC separation. Solvent A was water with 0.1 % formic acid; solvent B was acetonitrile with 0.1% formic acid. A linear gradient from 95 to 70% A over 15 minutes with 0.25 mL/ min. flow rate was used. For BUPG, NBUPG and morphine-3-glucuronide-d_3 the molecular ions of m/z 644, 590 and 465 were selected at Q1 and underwent collision-induced dissociation in Q2 with selection of the respective product ions of m/z 468, 414 and 289 at Q3. For BUP, NBUP and their respective internal standards the molecular ions at 468, 414, 472 and 417 were selected at Q1, and underwent collision-induced dissociation at Q2 and with the respective product ions of 396, 101, 400 and 101 measured at Q3. The method is linear from 0.1 to 50 ng/mL, and is currently undergoing validation experiments. Preliminary specificity experiments that assess peak area ratios in plasma containing only internal standards to those in samples fortified to the lower limit of quantitation (LLOQ) support the ability to establish the LLOQ at 0.1 ng/mL for all analytes except norbuprenorphine, where a slightly higher LLOQ may be needed. (Supported in part by a grant from NIDA, R01-DA-10100).

Keywords: Tandem mass spectrometry, Glucuronide quantitation, Buprenorphine

P5

Static Headspace Analysis of Alcohol and Common Abused Inhalants in Blood Using Dual-Column Gas Chromatography

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Static headspace analysis is routinely used in forensics laboratories for blood alcohol analysis and detection of abused inhalants. Like the blood alcohols, industrial solvents can be detected using static headspace analysis. The experimental configuration was a Teledyne-Tekmar 7000 static headspace analyzer and HP 6890 gas chromatograph with dual FID detectors. The instrument conditions are listed in table 1.

Tekmar 7000	**Agilent 6890**
Equilibrate: 10 min at 70°C	Injector: 200°C, split 1:10
Mix: 0.5 min at level 5	Oven: isothermal 40°C for 5 mins
Pressurize: 0.2 min @ 10psi	(ramp @ 35°C/min to 230°C for cleanup)
Loop fill: 0.2 min	FID: 250°C, He makeup gas
Inject: 0.5 min	Carrier gas: He at 10 ml/min
Temperature (line and valve): 85°C	Columns: Restek BAC-1/BAC-2 30mX0.53mmX3.0µm

Table 1. Instrument conditions for Tekmar 7000 and Agilent 6890.

For blood alcohol, reproducibility was determined by evaluating the response factors for six sample replicates at the 0.02% level. Since different legal limits for ethanol exist for different age groups, a calibration curve needs to be generated to cover this range of limits. A five-point calibration curve was prepared covering the blood alcohol concentration range 0.008% - 0.16%. The following compounds were analyzed: methanol, acetaldehyde, ethanol, isopropanol and acetone. The Relative Standard Deviations (RSD) were less than 4% and the linearity (R^2) of the calibrations were 0.998 and greater.

Industrial solvents are detectable in blood when abused as inhalants. The compounds typically found in industrial solvents consist of polars and nonpolars. The nonpolar compounds analyzed in this study were methylene chloride, chloroform, 111-trichloroethane, benzene, trichloroethene, toluene, 112-TCA, m,p-xylene, 1112-PCA, 1122-PCA and o-xylene. The polar compounds were diethlylether, acetonitrile, MTBE and MEK. Since the nonpolar compounds can be detected at much lower levels, they were separated from the polar compounds. Reproducibility was determined by evaluating the response factors for six sample replicates at the 10ppm level for polars and the 800ppb level for nonpolars. For all compounds the RSD was below 17%. Each compound was also calibrated over a broad range of 0.5ppm–100ppm using a 10 point calibration curve with correlations (R^2) greater than 0.99.

In conclusion, the analysis of blood alcohols is easily quantified and confirmed using the Teledyne-Tekmar 7000 headspace analyzer with dual FIDs and complementary column phases. For both types of analysis the results yielded excellent reproducibility and linearity, making the system very versatile.

Keywords: Headspace, Inhalants, Blood-alcohol

P6

Ephedrine Toxicity and Psychiatric Consequences: Case Reports and Literature Review

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Native Chinese physicians have used ma huang for over 5000 years. The active alkaloid, ephedrine, was isolated in the late 1800's and it sympathomimetic actions were elucidated in 1930. Psychosis related to ephedrine abuse has been known since the 1930's and reported in the German literature since the 1940's with two cases of ephedrine psychosis first noted in the British literature in 1968. Symptoms in general would arise from chronic use of ephedrine for treatment of medical conditions including asthma and abuse of ephedrine for its stimulant effect. Its accelerated use in the 1990's led to many reports of medical and psychiatric complications. Recent reports of the medical side effect profile of ephederine are well documented: insomnia, nervousness, tremor, headaches, kidney stones, hypertension, seizures, arrhythmias, heart attack, stroke and death. Psychosis and mania have been thought to be the primary psychiatric manifestations of ephedrine toxicity due to CNS stimulant actions. Specific to ephedrine is a psychosis characteristic of no prior psychiatric history, acute onset of positive psychiatric symptoms, paranoid ideations, clear sensorium, and variable mood disturbance. Three case reports of patients presenting for psychiatric evaluation associated with ephedrine containing weight loss products are presented. *Case I* involved a 38 y.o. female who presented on a request for protective custody initiated by a family member for evaluation of mental status changes for four days prior to evaluation. She had no prior history of psychiatric illness or suicidal behavior. She had been taking escalating doses of an ephedrine containing herbal weight-loss product (Xenadrine RFA-1, Cytodyne Technologies), had poor food intake, was drinking coffee and smoking heavily. It was anecdotally reported that she was so impaired that she could have put her infant child in the oven. *Case II* involved a 45 y.o. employed male referred for a mental health evaluation as he was in a custody dispute. On two single occasions, two years apart he used corporal punishment to discipline his child and his dangerousness toward his child was questioned. He had no prior history of psychiatric treatment for mental illness or substance abuse. Close scrutiny of his medical history revealed that he was taking an herbal dietary product (AM-300, Advantage Marketing Services) containing ephedrine and caffeine for weight control. *Case III* involved a 37 y.o. female with a history of depression and anxiety dating back to her teens. She also had a history of polysubstance abuse, having used primarily cocaine, ecstasy and GHB previously and reported being "clean" for 20 months. She stated that she took an ephedrine containing weight-loss product (Metabolife, Metabolife, International) three to four tablets at once and drank a lot of coffee to remain awake. She reported being very anxious and nervous during the day. Although Case III has a history of depression, anxiety and substance abuse these three cases provide instances of a plausible link between herbal weight-loss agent use and ephedrine psychosis. A similar picture occurs in amphetamine psychosis.

Keywords: Ephedrine, Behavioral toxicology, Psychosis

P7

Stability of Urine Riboflavin Determined by High Performance Liquid Chromatography

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Riboflavin has become a compound commonly used to monitor drug compliance, in which urine riboflavin is typically measured at concentrations above 1,000 ng/mL. It is also a component of many dietary supplements. Its presence at high concentrations in a forensic sample would be indicative of prescribed drug or supplement use in many cases. A direct analysis of urinary riboflavin by high performance liquid chromatography (HPLC) with fluorescence detection is described. The method was used to study the stability of urinary riboflavin under various conditions. A volume of 5 μL urine was directly injected onto a C18 reverse-phase column and riboflavin was separated under isocratic conditions and detected by a fluorescence detector with the excitation and emission wavelengths set at 450 and 530 nm, respectively. The concentration of riboflavin was quantitated using calibration standards over the range of 10 to 5,000 ng/mL. The lower limit of quantification was 10 ng/mL. When intra-assay precision and accuracy were evaluated at concentrations of 10, 40, 400 and 4,000 ng/mL, mean calculated concentrations did not deviate more than 9.9% from the target and coefficient of variance (CVs) did not exceed 3.3%. When inter-assay precision and accuracy were evaluated at the same concentrations, mean calculated concentrations did not deviate more than 11.2% from the target and CVs did not exceed 9.1%. When exposed to fluorescent light at room temperature, riboflavin stored in amber glass vials decreased by 4% in 24 hours and 9% in 72 hours while riboflavin stored in clear glass vials decreased by 52% in 24 hours and by 81% in 72 hours. Indeed, riboflavin-free urine was prepared by exposing the urine to the natural light for one week. Urinary riboflavin was stable up to six months when stored at either 4°C or –20°C protected from light exposure. Without sample preparation and with only 5 minutes of run time for each injection, this method is simple and rapid, so it is suitable for large scale investigation of medication compliance and for other forensic or clinical samples where riboflavin concentrations may be of interest. (Supported by N01DA-7-8074)

Keywords: Riboflavin, HPLC, Stability.

Comparison of Δ^9-Tetrahydrocannabinol, 11-Hydroxy- tetrahydrocannabinol and 11-Nor-9-carboxy- tetrahydrocannabinol Concentrations in Human Plasma following *Escherichia coli* β-glucuronidase Hydrolysis

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The purpose of this study is to collect preliminary data on the effect of β-glucuronidase on the concentration of Δ^9-tetrahydrocannabinol (THC), 11-hydroxy-THC (11-OH-THC) and 11-nor-9-carboxy-THC (THCCOOH) in plasma. THC is rapidly oxidized to 11-OH-THC, an equipotent psychoactive metabolite, and further to the non-psychoactive THCCOOH. These metabolites generally undergo further biotransformation to glucuronide conjugates. Glucuronic acid forms either an ether bond or ester bond with the hydroxy or carboxy moieties, respectively. Kemp et al. reported increases in THC and 11-OH-THC in urine following *Escherichia coli* (*E. coli*) β-glucuronidase hydrolysis. To date it is unclear what percentage of drug is glucuronide bound in plasma and whether there are differences following different routes of drug administration. In the current study, plasma specimens (n=10) collected from two individuals participating in a controlled oral THC administration study, were analyzed for total THC, 11-OH-THC and THCCOOH concentrations with and without an *E. coli* β-glucuronidase hydrolysis. The hydrolysis step utilized 5000 units of β-glucuronidase/mL of plasma, Type IX-A bacterial from E. coli, with incubation at 37° C for 16 h. Excess protein was precipitated with acetonitrile, samples were centrifuged, the supernatant diluted with 2N sodium acetate (pH 4.0) prior to analysis with our plasma cannabinoid method (See Gustafson SOFT Abstract 2003). Extracts were derivatized with BSTFA(1%TMCS) and analyzed by positive chemical ionization GC-MS in the selected ion monitoring mode. Preliminary data showed statistically significant ($p \leq 0.05$) increases of 40% and 42% for 11-OH-THC and THCCOOH concentrations, respectively, in hydrolyzed specimens. Enzyme hydrolysis increased THC concentrations the least by a mean of 16 ± 19%. This method will be utilized in characterizing the percent free and bound cannabinoids in plasma and whole blood in ongoing cannabinoid controlled administration studies.

Keywords: Tetrahydrocannabinol, 11-hydroxy-tetrahydrocannabinol, Plasma, β-glucuronidase

P9

Driver Behavior, Responses and Performance with Blood Alcohol Concentration (BAC) greater than 0.30g/100mL

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During 1999 and 2000 the Washington State Toxicology Laboratory received 3282 requests for blood alcohol analysis (not including drug testing) in DUI cases. Of these, 165 cases had BAC ≥0.30 g/100 mL. These cases were interesting from several aspects, including the subject's level of consciousness, their involvement in accidents, their ability to understand their situation or respond to questions, their appearance, their driving, and their ability to perform field sobriety tests. The mean BAC of this population was 0.33 g/100 mL (median 0.33g/100 mL, range 0.30-0.50). They were predominantly males (81%) with a mean age of 42 years (median 41, range 24-75). Of 165 cases testing greater than or equal to 0.30g/100 mL, we reviewed police reports of 111 cases to evaluate Standard Field Sobriety Test (SFST) performance, the reason for the traffic stops and other pertinent information. SFSTs consisted of Horizontal Gaze Nystagmus (HGN), Walk and Turn (WAT), and One Leg Stand (OLS).

All subjects exhibited signs of intoxication such as, odor of intoxicants, bloodshot watery eyes, slurred speech and very poor coordination. A number of subjects fell while exiting the vehicle. In all cases, the subjects performed poorly in SFSTs, but the performance did vary. HGN was attempted in 30 cases (27%), while only 16 (14%) attempted the WAT and OLS tests. In most cases, field sobriety tests were not administered because the arresting officer determined that the subject was too intoxicated and could not perform them safely.

Seventy-seven drivers (69%) were involved in traffic accidents, about half of which were identified as single car accidents. Typical accidents involved striking parked cars, telephone poles, pedestrians, and one case a vehicle was struck by a train while trying to cross a level crossing in spite of warning lights. Approximately one quarter of the drivers were unconscious or passed out behind the wheel when encountered by the officer. Many more were asleep in their vehicles or fell asleep during the arrest process. Twenty-four subjects were noted to be driving with a suspended license.

This survey shows that while drivers with BAC 0.30g/100 mL or greater are severely impaired, many are conscious and able to operate their motor vehicles, converse, and engage in goal directed activities like fleeing the scene. Some subjects were able to attempt field sobriety tests, although they uniformly performed poorly. The HGN test was shown to be useful in assessing these subjects for CNS depression, when they were unable to safely perform other divided attention sobriety tests. Once aroused, most subjects were capable of communication to some extent and could follow simple instructions. Some could perform (albeit poorly) tests of psychomotor skills. Many subjects were communicative and engaged in purposeful goal directed behavior including shopping and robbery in addition to driving, and several tried to flee the scene following accidents. The data supports the view that tolerance to alcohol can result in subjects with very high BAC's being oriented, ambulatory and active, capable of communication, and retaining short term memory at concentrations that would be lethal or incapacitating in others.

Keywords: Driving Under the Influence, Alcohol, Standard Field Sobriety Tests

P10

Drug Diversion Testing at a Major Medical Center

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Medical institutions have dealt with drug abuse by health care workers through pre-employment urine drug testing and/or "for cause" drug testing after an incident has occurred that may or may not have affected patient care. However, such programs seldom, if ever address issues of drug diversion. In 1998, the pharmacy and toxicology laboratory at the Medical College of Virginia Hospitals and Physicians at Virginia Commonwealth University Health Systems intiated a proactive program to address the issue of in-hospital diversion of pharmaceuticals. Each month over 100 samples are collected from various dispensing pharmacies within the medical center. Approximately, two thirds of the samples are collected for quality control of in-house compounded pharmaceuticals. One third of the samples are from post-use waste solutions or "for cause" testing. High Pressure Liquid Chromatography and Ultraviolet Spectrophotometry procedures are routinely applied for quantitative analysis of pharmaceutical preparations for opiates, synthetic opiates, local anesthetics and midazolam. On occasion, gas chromatography (GC) or GC-mass spectrometry may be used to identify other specific drugs or diluents that may have been added to diverted solutions. Over the time course of this program for the "post-use waste" samples, most incidences of "questionable diversion" results were from fentanyl preparations. Of the "for cause" samples, morphine and fentanyl were the most commonly diverted drugs. Additionally, the laboratory collects "for cause" specimens and performs forensic urine drug testing. This type of program reduces incidences of drug abuse that may affect patient care, as well as other risk management issues. Specific case examples, including instances in which drug abuse was disclosed by initial diversion testing, have resulted in "for cause'' urine drug testing.

Keywords: Drug diversion, Drug abuse, Opiates, Fentanyl

P11

The Regional Distribution of Phenothiazine Antipsychotics in Postmortem Brain from Schizophrenic Subjects

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Antipsychotic drugs are likely to target specific brain regions for their therapeutic effects, although there is little data in the literature to show the extent to which this occurs in humans. The limited published data suggests most antipsychotics partition preferentially into the frontal cortex, midbrain, and caudate-putamen, and that a degree of differential partitioning between the left and right brain hemispheres occurs. How a drug partitions in the brain postmortem could have significant implications for our understanding of how different antipsychotics work and how they produce any toxic outcomes. From a toxicological standpoint, it is desirable to sample the most appropriate region, if in fact such a region can be established. This region may allow an improved interpretation of possible toxic reactions to such drugs and may also reveal whether antipsychotic drugs follow a general trend in brain distribution.

We undertook a study to address whether phenothiazine antipsychotics partition preferentially to particular brain regions in schizophrenics and whether any trends could be observed in any such partitioning. Additionally, the extent of correlation of blood-to-regional brain concentrations was examined. Brain regions selected for their known involvement in schizophrenia were collected at autopsy from 22 confirmed schizophrenic subjects. Antipsychotic drug concentrations were determined in these brain regions and compared for inter-subject variability. Specimens were homogenized, extracted with n-butyl chloride, and analyzed via liquid chromatography-mass spectrometry (LC-MS), using atmospheric pressure electrospray ionization (APESI) operated in positive mode. LC-MS analysis was performed on an Agilent 1100 Series HPLC configured with a G1946A mass selective detector (MSD). Chromatographic separation was achieved using a Zorbax Extend-C_{18} column from Agilent (2.1 x 150 mm, 5 µm particle size), operated at 21 °C pumping at 0.25 mL/min for 40 min, with an isocratic mobile phase of 0.05M ammonia/methanol/THF (32.5:67.0:0.5) at pH 10.0. To validate this method, accuracy and precision data were obtained by performing replicate analyses of blank blood specimens spiked with either a low (0.075 mg/L) or high (1.0 mg/L) standard of each drug. Five replicates of each spiking level were analyzed. Within- and between-day coefficients of variation were all at or below 10%. Accuracy ranged from 82-105% for all drugs.

There were no cases in which haloperidol or the depot drug flupenthixol were detected, likely due to the low dosages required for therapeutic effect. These cases were not included for comparison between subjects. A great deal of inter-subject variability in brain distribution was observed, and cannot be accounted for by assay variability. Interestingly, the regional brain distribution of thioridazine and its metabolites was dependent on overall concentrations in brain. Generally speaking, the highest thioridazine and chlorpromazine concentrations were found in caudate putamen, while the lowest were found in cerebellum. The lowest concentrations of both trifluoperazine and fluphenazine, however, were detected in caudate putamen. Trifluoperazine was found at highest absolute concentrations in occipital cortex, while the highest concentrations of fluphenazine sulfoxide were found in grey matter from the frontal cortex.

If regional drug concentrations determined in this study were normalized for those observed in cerebellum, three distinct patterns of distribution were observed, corresponding to different structural features of each type of phenothiazine. Chlorpromazine and thioridazine, both associated with high affinity for dopamine receptors, were detected at highest concentration in caudate putamen, a region known to have a high concentration of such receptors. However, fluphenazine sulfoxide and trifluoperazine, both associated with relatively lower dopaminergic activity, were found at highest concentration in occipital cortex, a region with a relatively low concentration of dopamine receptors.

Keywords: Phenothiazine, Antipsychotics, Schizophrenia, Postmortem

P12

Unusual Distribution of Methamphetamine in a Fatality

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Unusual distributions of methamphetamine and amphetamine were observed in an individual who sustained two gunshot wounds to the head. The cause of death was determined as gunshot wounds to the head and the manner of death was homicide. The toxicological findings are not typical of postmortem methamphetamine distributions and suggest that the results may be an artifact of autopsy sampling.

Methamphetamine and amphetamine were determined using solid phase extraction (Polychrom Clin II) and gas chromatography/mass spectrometry (GC/MS) of acetylated derivatives using acetic anhydride. Acquisition was in the selective ion monitoring mode and deuterated internal standards were used throughout. The assay had a limit of detection of 0.005 mg/L in blood. R^2 values in the linear range (0.005 – 1.000 mg/L) were 0.999 for both drugs. Intraassay CVs were 2.4% and 4.0% respectively using a commercial whole blood QC (Utak Laboratories) fortified with 0.100 mg/L methamphetamine and amphetamine respectively. Methamphetamine, amphetamine and pseudoephedrine were the only drugs detected in the blood. No alcohol was present. However, methamphetamine and amphetamine in femoral blood were elevated compared to heart blood. Quantitative analysis was not performed on pseudoephedrine; however, quantitation of methamphetamine and amphetamine was conducted on all tissues retrieved at autopsy. Methamphetamine concentrations in heart blood, femoral blood, vitreous fluid and urine were 1.3, 41, 2.1 and 45 mg/L respectively. Amphetamine concentrations in heart blood, femoral blood, vitreous fluid and urine showed a similar trend: 0.24, 5.08, 0.28 and 5.4 mg/L. Repeat analysis confirmed the results. Femoral blood concentrations were very much higher than expected. In a series of 20 fatalities heart/femoral blood concentration ratios of methamphetamine averaged 2.1 (Barnhart et. al, 1998) and urine/blood ratios in a fatality were 14 (Baselt, 2000). Urine/blood ratios for amphetamine in a single fatality were 28 (Baselt, 2000). Rationalization for the unusual toxicology findings, including autopsy sampling issues and potential anatomic sources of contamination are discussed.

Keywords: Methamphetamine, Postmortem, Distribution

P13

Fatal Cold Medication Intoxication in an Infant

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The case history and toxicological findings of an infant fatality are presented. In brief, a two-month old infant suffering from a cold was found unresponsive in her crib and pronounced at the scene by emergency personnel. An autopsy by the medical examiner revealed pulmonary edema, however there were no gross abnormalities of any organs including the heart and brain, and no evidence of traumatic injuries. Blood, gastric contents, liver specimens, and two baby bottles found at the scene were sent for toxicological analysis.

Although the mother reportedly fed the baby a bottle containing water and a small amount of Tylenol, the only drugs found in the infant and in one of the baby bottles were pseudoephedrine, brompheniramine, and dextromethorphan. Concentrations of brompheniramine and dextromethorphan were measured in both postmortem blood and liver specimens using gas chromatography equipped with a nitrogen-phosphorous detector (GC/NPD). Brompheniramine and dextromethorphan were 0.40mg/L and 0.50mg/L, respectively in the blood sample and 0.16mg/kg and 0.57mg/kg in the liver sample. The concentration of pseudoephedrine in blood and liver specimens was measured using gas chromatography-mass spectrometry (GC/MS) and was determined to be 14.4mg/L in the blood and 16mg/kg in the liver. The amount of total brompheniramine, dextromethorphan, and pseudoephedrine remaining in one of the baby bottles was 1.4mg, 9.4mg, and 40mg, respectively, far exceeding the recommended dosage for each of these drugs.

Although there are limitations in interpreting postmortem drug levels in blood, especially in the context of limited data on children, the values of brompheniramine, dextromethorphan, and pseudoephedrine in the infant as well as in the baby bottle are elevated. Therefore, taking into consideration the age of the infant, the negative autopsy findings, and the level of drug found in the infant, the cause of death was listed as multiple drug toxicity. The manner of death has not yet been classified.

Keywords: Brompheniramine, Dextromethorphan, Pseudoephedrine, Postmortem toxicology, Pediatric toxicology

P14

The Distribution of Sevoflurane in a Sevoflurane Induced Death

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The distribution of sevoflurane (fluoromethyl 2,2,2,-trifluoro-1-(trifluoromethyl) ethyl ether) in femoral blood, urine, liver, kidney, vitreous fluid, and tracheal aspirate is presented from a subject with a sevoflurane induced death. Sevoflurane is a nonflammable general anesthetic administered by inhalation of vaporized liquid (1). Although general inhalation anesthetics have the potential to be fatal if not properly administered, the incidence of abuse is minute in comparison to other illicit drugs (2). Currently, there are no citations in the literature defining the body distribution of sevoflurane in a sevoflurane induced death. The decedent was found lying in a bed with an oxygen mask containing a gauze pad secured to his face. Three empty bottles and one partially full bottle of Ultane™ (sevoflurane) were found with the body in addition to two pill boxes containing a variety of prescription and non-prescription drugs. Serum, urine and gastric contents from the deceased were screened for numerous drugs and metabolites using a combination of thin layer chromatographic, colorimetric and immunoassay techniques. Analysis of biological specimens from the deceased revealed the presence of: amphetamine, caffeine, pseudoephedrine, nicotine, nicotine metabolite, and valproic acid. Amphetamine and valproic acid quantitative analysis revealed 7.02 μg/mL of amphetamine in the urine and 0.275 μg/mL of amphetamine in the blood and 60.8 μg/mL of valproic acid in the serum. The sevoflurane working standard was prepared by two initial serial dilutions of Ultane (Abbott Laboratories, Abbott Park, IL.) into chilled (20 °C) dimethyl sulfoxide, followed by a final dilution into chilled (0 °C) 18 MΩ water. Sevoflurane concentrations were determined by head space gas chromatography with flame ionization detection and revealed concentrations of 26.2 μg/mL in the blood, 105.2 μg/mL in the urine, 31.9 μg/mL in the tracheal aspirate, 30.8 μg/kg in the liver, and 12.8 μg/kg in the kidney. The decedent had pathologies consistent with respiratory suppression including pulmonary atelectasis, pulmonary edema, and neck vein distention. The official cause of death was respiratory suppression by sevoflurane and the manner of death was unclear.

Keywords: Sevoflurane, Fatal, Distribution

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P15

Interpreting Postmortem Tricyclic Antidepressant Levels in Vitreous Humor

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Tricyclic antidepressants (TCAs) are a group of drugs which can be lethal in overdose concentrations or when combined with monoamine oxidase inhibitors (MAOIs). They are known to exhibit significant postmortem redistribution and, consequently, interpreting TCA levels in postmortem blood samples can often be difficult. As concentrations of analytes in vitreous humor remain relatively stable after death, the measurement of TCA levels in vitreous humor may be helpful in the postmortem investigation of TCA overdose. In addition, vitreous humor is sometimes the only suitable postmortem specimen available in a death investigation. At present, most of the interpretable data on TCAs is available only on blood or tissues and not on vitreous humor. For correct interpretation of vitreous humor TCA levels, an understanding of the relationship between vitreous humor and peripheral and central blood is necessary.

In this study we measured amitriptyline and/or nortriptyline in vitreous humor, and cardiac and peripheral blood in 22 postmortem cases using a liquid-liquid extraction followed by high performance liquid chromatography with ultraviolet detection. Intra-run coefficient of variations (CV) for amitriptyline at levels 150 ng/mL and 600 ng/mL were 1.3 and 1.7 respectively; for nortriptyline the CVs were 2.3 and 1.1 respectively. Inter-run CVs at various levels were less than 10. Peripheral blood/vitreous humor (PB/VF), cardiac blood/vitreous humor (CB/VF) and cardiac blood/peripheral blood (CB/PB) ratios for amitriptyline and nortriptyline were calculated. The average (standard deviation) ratios of amitriptyline for PB/VF, CB/VF and CB/PB were 7.2(3.7), 10.9(3.9), and 2.1(1.2) respectively. The average (standard deviation) ratios of nortriptyline for PB/VF, CB/VF and CB/PB were 4.3(3.2), 5.6(4.9), and 3.8(7.0) respectively. The coefficients of correlations (r) were also calculated. For amitriptyline r values of PB/VF, CB/VF and CB/PB were 0.7, 0.9, and 0.8 respectively. For nortriptyline r values of PB/VF, CB/VF and CB/PB were 0.6, 0.8, and 0.8 respectively. The results indicate that there are significant concentration correlations between PB/VF, CB/VF and CB/PB for TCAs. These correlations may allow the investigating pathologist and toxicologist to use vitreous humor TCA levels to estimate peripheral and central levels at the time of death or validate the findings from other collections sites.

Keywords: Vitreous humor, Tricyclic antidepressants, Postmortem

P16

Distribution of Cocaine and Benzoylecgonine in Postmortem Casework

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Interpretation of postmortem cocaine concentrations is hampered by drug stability as well as site dependent postmortem changes in drug concentration. In this study cocaine and benzoylecgonine (BE) were quantitatively determined in the femoral vein, femoral artery, right ventricle, left ventricle and pooled blood (inferior vena cava). Quantitative analysis was performed using solid phase extraction and gas chromatography mass spectrometry. Intra-assay CVs for cocaine and BE were 6.2% and 5.2% respectively. Inter-assay variability for cocaine was 15%. Samples were collected in gray-top tubes containing sodium fluoride and potassium oxalate. Cocaine was not stabilized by acidification. Instead, samples were collected in accordance with protocols utilized for routine casework (refrigeration at 4° C prior to analysis-approximately 3-4 days).

In a series of 23 cocaine-involved cases, postmortem BE concentrations in the femoral vein were 0.04-12 mg/L (median 0.54mg/L, and SD 2.94). Of these, 14 cases had detectable cocaine concentrations that ranged from 0.01-4.8 mg/L (median 0.11 and SD 1.25). The remaining samples were negative or below the limit of detection of the assay (0.01 mg/L).

Cocaine ratio concentrations in the femoral artery, right ventricle, left ventricle and pooled blood averaged 1.03 (SD 0.90), 0.61 (SD 0.70), 0.58 (SD 0.65), and 0.70 (SD 0.78) of the concentration in the femoral vein. The corresponding range was 0.34 to 2.50 between sites. Both the mean and SD supports site dependent variability. BE ratio concentrations in the femoral artery, right ventricle, left ventricle and pooled blood were more consistent, averaging 1.07 (SD 0.40), 1.18 (SD 0.45), 1.00 (SD 0.34), and 1.04 (SD 0.32) of the concentration in the femoral vein. Yet, the BE concentration ratios ranged from 0.34 to 2.48 between sites. However, mean and SD values did not support the variability of site dependence. The variations were not concentration dependent but appeared to be random in nature.

This study characterizes site dependent differences in drug concentrations and highlights the difficulty involved in postmortem drug interpretation which is largely compounded by drug stability issues resulting from the postmortem interval.

Keywords: Cocaine, Postmortem, Blood

P17

Validation of the Cozart® Microplate ELISA for the Detection of Methadone in Hair

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The purpose of this study was to determine the performance characteristics of the Cozart® Methadone Microplate ELISA assay for the detection of methadone in hair samples. Hair samples (N=110) were collected from volunteers (N=46) with a history of drug use and from drug related deaths (N=64). The hair samples (approximately 20 mg) were prepared for microplate screening by sonicating in methanol and then incubating overnight at 60°C. The methanol extract was evaporated to dryness and reconstituted in ELISA negative calibrator. 25µL of calibrator (0, 50, 100, 200, 300, 500 and 1000pg/mg), control and sample were assayed in duplicate according to manufacturers instructions for the Cozart® Methadone ELISA – Forensic Application Kit.

For GC-MS analysis, deuterated internal standard mixture (methadone-d3 and EDDP-d3) and 0.1M HCl were added to approximately 20 mg of sample, or spiked blank hair and sonicated for 1 hour. The pH was adjusted to neutral and methadone and its primary metabolite EDDP were analysed by GC-MS following solid-phase extraction using Bond Elut Certify columns and pH7.4 phosphate buffer (0.1M). 36.4% (N=40) hair samples were confirmed positive for methadone, concentrations ranged from 0.10 – 8.3 ng/mg (methadone) and 0.1 – 1.2 ng/mg (EDDP).

There are currently no approved or proposed guidelines for methadone cut-offs in hair, therefore the performance of the Cozart® Methadone Microplate ELISA was assessed at various cut-offs (100, 200, 300 and 500pg/mg) in comparison to the GC-MS as the reference method. Using a GC-MS cut-off of 0.1ng/mg for both methadone and EDDP, the optimum screening cut-off was 200pg/mg with a sensitivity of 97.5%, specificity of 100% and an accuracy of 99.1%.

Keywords: Methadone, hair, ELISA

P18

Simultaneous Assay for Nicotine, Nornicotine, Cotinine, Norcotinine, and 3-Hydroxycotinine in Oral Fluid by SPE and GC/MS/EI

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The fate of nicotine (NIC) in man has not been thoroughly established due to its extensive metabolism, but nornicotine (NNIC), cotinine (COT), norcotinine (NCOT), and 3-hydroxycotinine (3OHCOT) have been identified as major metabolites. Some of these metabolites also are found as minor components in tobacco leaf. A simultaneous assay for multiple NIC components has been difficult to achieve due to ineffective isolation techniques and the different physicochemical properties of NIC and metabolites. 200-mg Clean-Thru® DAU solid phase extraction columns (UCT, Bristol, PA) were used to extract NIC and metabolites from oral fluid. The extraction conditions were as follows: add deuterated internal standards (d3-NIC (Sigma, St. Louis, MO), d3-COT (Cerilliant, Round Rock, TX), and d3-3OHCOT (TRC, North York, Ontario, Canada)) and sodium acetate buffer (pH 5.5, 2N) to 1-mL samples; condition the columns with elution solvent, methanol, water, and buffer; after the samples are introduced to the column, wash with water, 0.2 N HCl, and acetonitrile; and elute with methylene chloride: 2-propanol: ammonium hydroxide (80:20:2). Extracts were carefully concentrated under a stream of nitrogen just to dryness, derivatized with BSTFA (with 1% TMCS), and analyzed by GC/MS/EI in the SIM mode. The following ions for each analyte were monitored in the following elution order (quantitative ions are indicated in parenthesis) for the derivatized analytes: d3-NIC, m/z (87), 136; NIC, m/z (84), 133, 162; NNIC, m/z (142), 205, 220; d3-COT, m/z (101), 124; COT, m/z (98), 121, 176; NCOT, m/z 118, (219), 234; d3-3OHCOT, m/z 147, (252); and 3OHCOT, m/z 144, (249), 264. Extraction efficiencies were ≥90% for all analytes. Correlation coefficients of the calibration curves were ≥0.98. Responses were linear across a concentration range of 2.5-100 ng of drug/mL of oral fluid (2.5, 5, 10, 25, 50, and 100 ng/mL). The limit of quantitation (LOQ) is 2.5 ng/mL for all analytes. Oral fluid blanks fortified with potential drug interferences and analyzed as described above showed no interference with NIC, NNIC, COT, NCOT, 3OHCOT, and the deuterated internal standards. This assay is applicable to pharmacokinetic studies of NIC and its metabolites in oral fluid. The method could accommodate the addition of other metabolites and possible biomarkers. Further refinement of this assay should make it applicable for analysis of NIC and its metabolites in other biological matrices.

Keywords: Nicotine and metabolites, Simultaneous assay, SPE, GC/MS

P19

Determination of Five Opium-Related Compounds in Human Hair by LC/MS.

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Human hair may be used as a biomarker in epidemiological studies. Analysis of hair has the potential for retrospective detection of exposure to drugs, such as Opium. Opium, derived from *Papaver somniferum*, contains at least 5 principal alkaloids. These include: morphine (MOR), codeine (COD), thebaine (THEB), noscapine (NOS) and papaverine (PAP). This report describes a sensitive and specific method for the detection of these compounds in human hair. High performance liquid chromatography (HPLC) – electrospray ionization (ESI) – mass spectrometry (MS) was for both qualitative and quantitative determination of these and other opioid compounds. Norcodeine (norCOD), normorphine (norMOR) and 6-monoacetylmorphine (6-MAM) were also included in the analysis for differentiation of opium vs heroin use. A calibration curve (0.2 ng/mg to 50.0 ng/mg) and controls (0.3, 2.0 and 10.0 ng/mg) were prepared by fortification of drug-free human hair (20 mg) with analytes. Deuterated internal standards (COD-d3; MOR-d3; 6-MAM-d3; 2.5 ng/mg each) were then added, followed by the addition of 1 mL acetonitrile. Samples were incubated overnight at room temperature in a shaking water bath. The following day, the pH was adjusted to greater than 9.0 with concentrated ammonium hydroxide. Four mLs of n-butyl chloride:acetonitrile (4:1; v/v) was added and samples extracted 1 hr by gentle rocking. After centrifugation at approx. 2250 rpm, the organic phase was collected and evaporated to dryness under a gentle stream of air (10 psi). Extracts were reconstituted in 0.1% formic acid:methanol (90:10; v/v) and analyzed on a Hewlett-Packard series 1100 LC-mass selective detector (MSD). A YMC ODS -AQ™ (S 3μ 2.0 x 50mm Cartridge) column was used to achieve chromatographic separation. Data was quantitated on Hewlett Packard® ChemStation using quadratic curve fits and $1/X^2$ weighting. Coefficients of determination (r^2) were greater than 0.99 for all analytes. The lower limit of quantitation (LLOQ) was determined to be 0.2 ng/mg, the lower level of detection was determined to be 0.1 ng/mg. Intra- and inter-assay precision ranged from 1.59 – 8.29% and 2.67 – 16.21%, respectively. Accuracy, expressed as a percent of the theoretical weighed-in target concentration, was determined to be within 15% of target concentration for all analytes. Sample extracts were also determined to be stable for up to 7 days when stored in mobile phase at -20° C. This method was applied to the analysis of 34 authentic human hair specimens obtained from possible opium users. Seven hair specimens were determined to be positive for two or more compounds. When positive, specimens contained MOR, COD, THEB, PAP, and NOS. These specimens are considered to have been from individuals exposed to opium. 6-MAM was not detected in any hair specimen. This work was supported by NIDA grant no. DA09096.

Keywords: Opium, LC/MS, Hair

P20

Comparison of Three Assays for Specimen Validity Testing for Urine Creatinine

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Effective May 28, 2003, the National Laboratory Certification Program (NLCP) required all certified laboratories to certify that their laboratory have an experimentally determined and properly validated limit of detection (LOD) and limit of quantitation (LOQ) for creatinine that is 1.0 mg/dL or less. Under the NLCP program, creatinine determinations are used for Specimen Validity Testing. Our objective was to compare three different methods for creatinine determination using the newly published NLCP guidelines.

Three methods were validated using the NLCP guidelines on an Hitachi 717 autoanalyzer. Method 1 was our current urine creatinine method (3 µL SV, 250 µL R1, 50 µL R2). Method 2 was a serum creatinine method (15 µL SV, 150 µL R1, 150 µL R2). Method 3 was the same as method 2, except the sample volume was 10 µL. All methods utilized the modified Jaffe reaction, using commercially available reagents (Sciteck Diagnostics, Inc.).

For method 1, we determined an LOD of 0.5 mg/dL, LOQ of 2.0 mg/dL, and an upper limit of linearity (ULOL) of 400 mg/dL. For method 2, we determined an LOD and LOQ of 0.2 mg/dL with an ULOL of 200 mg/dL. For method 3, we determined an LOD of 0.2 mg/dL, LOQ of 0.5 mg/dL, and an ULOL of 200 mg/dL. Precision for method 1 was 4.5% at 23.0 mg/dL and 8.6% at 1.4 mg/dL. Precision for method 2 was 1.5% at 23.0 mg/dL and 3.4% at 1.4 mg/dL. Precision for method 3 was 1.8% at 23.0 mg/dL and 4.8% at 1.4 mg/dL.

Urine creatinine methods on an autoanalyzer instrument are designed to target concentrations greater than 50.0 mg/dL, with an ULOL of 400 mg/dL or greater. Serum creatinine methods target concentrations between 0.5 and 20.0 mg/dL. The NLCP guidelines effective May 28, 2003 require laboratories to accurately quantitate creatinine concentrations less than 5.0 mg/dL. Therefore, our laboratory decided to use a serum creatinine method for measuring creatinine in urine. We chose method 2, as it demonstrated the lowest LOD/LOQ value, at 0.2 mg/dL. Although the ULOL for this method is 200 mg/dL, the quantitation of creatinine in urine at this upper range is not as critical for Specimen Validity Testing as is accurately quantitating creatinine at very low concentrations.

Keywords: NCLP, Creatinine, Specimen Validity testing

P21

A Multicenter Evaluation of Roche ONLINE® DAT II Methadone, Cocaine, and Cannabinoid Assays

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Second generation, monoclonal methadone, cocaine, and cannabinoid reagents were applied to the Roche Hitachi and COBAS® Integra analyzer families, and were evaluated in five laboratories in Europe and North America. DAT II methodology is kinetic interaction of microparticles in a solution (KIMS) as measured by changes in light transmission. In the absence of sample drug, soluble drug conjugates bind to antibody-bound microparticles, causing the formation of particle aggregates. As aggregation proceeds in the absence of sample drug, the absorbance increases. The objective of the evaluation protocol followed by all reporting laboratories included imprecision, accuracy, calibration stability, method comparison, and performance of an inter-laboratory survey.

Methadone content can be determined qualitatively or semiquantitatively at a cutoff of 300 ng/mL. Intra- and inter-assay precision CVs from 1.6 to 5.3 % and 2.2 to 10.8 % respectively were obtained. Between-day (2.1 to 9.2 %) as well as total CVs (2.9 to 11.1 %) fulfilled the target specifications at the levels tested (150–1500 ng/mL). Median recovery of calibrator and control assigned target values ranged from 91 to 105 %. The calibration was stable for at least 28 days.

Cocaine content can be determined qualitatively or semiquantitatively at cutoff levels of 150 or 300 ng/mL. Intra- and inter-assay CVs from 0.9 to 8.3 % and 1.5 to 8.8 % respectively were obtained. Between-day (1.2 to 11.0 %) as well as total CVs (1.1 to 10.6 %) were obtained at all levels tested (75–3000 ng/mL). Median recovery of calibrator and control assigned target values ranged from 89 to 103 % for all levels tested (75, 113, 150, 188, 225, 300 and 375 ng/mL). One laboratory reported a lower recovery of 85 % for the 75 ng/mL control. Median recovery of the 3000 ng/mL control was elevated in all laboratories. The calibration was stable for at least 21 days.

Cannabinoid content can be determined at cutoff levels of 20, 50 or 100 ng/mL. Intra- and inter-assay CVs from 2.4 to 7.5 % and 3.6 to 10.4 % respectively were obtained. Between-day (2.8 to 13.2 %) as well as total CVs (4.2 to 10.1%) were also obtained. Median recovery of calibrator and control assigned target values ranged from 90 to 107 % for cutoff 20 ng/mL, and from 90 to 122 % for cutoff 50 ng/mL, respectively. The calibration was stable for at least 21 days.

Agreement between DAT II assays and routine immunoassays with GC/MS was calculated for all confirmed positive and all negative urine specimens. Greater than 95 % agreement with GC/MS is observed in all laboratories. A panel of 50 frozen urine specimens characterized for the presence of the evaluated drugs was distributed to all participants. All samples were classified correctly by the new assays in all reporting laboratories.

We conclude that the DAT II technology demonstrates good technical performance and is well suited for routine drug of abuse urine screening.

Keywords: Methadone, Cocaine, Cannabinoids, Immunoassay

P22

Urine Adulteration Testing and Stability Studies for Chromium VI by HPLC

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Historically attempts have been made to defeat the testing for drugs of abuse by adding chemical compounds to the urine samples to "mask" the presence of drugs. As early as 1998 a strong oxidant, pyridinium chlorochromate (PCC), was marketed commercially as an additive to urine samples. Procedures for detecting pyridine by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS) were soon developed by some drug testing laboratories. The companies producing this adulterant quickly changed to other hexavalent chromium based compounds including potassium dichromate and chromium trioxide. A method for detecting the hexavalent chromium, while distinguishing it from the trivalent chromium found in human urine, was required.

The objectives of this study were to develop a fast, efficient, scientifically defensible method to test for chromium VI in urine without the possibility of chromium III compound interference and to evaluate the stability of chromium VI in urine after freezer storage.

The urine samples were treated with 1,5-diphenylcarbazide (DPC) to provide a color reaction, with strychnine added as an internal standard, and then tested by High Performance Liquid Chromatography (HPLC) for chromium VI. The sample was carried through a Prodigy 5 µ ODS (2) column in a 20 mM ammonium acetate: 5.0% v/v acetonitrile: 0.33% v/v acetic acid mobile phase and the absorbance of the violet to purple color change was measured at a wavelength of 540 nm. A single point calibration at the cutoff concentration of 20 µg/mL was used affording a linear range of 2 to150 µg/mL.

The accuracy and precision of this method were evaluated, and coefficients of variation were determined to be lower than 6.6%. This method meets all Substance Abuse and Mental Health Services Administration (SAMHSA) validation requirements for testing urine for chromium adulteration. The DPC does not react to solutions with chromium III compounds added and none of the other common components of human urine interfere with this procedure.

Retest values for routine samples that had been stored frozen for 12 to 15 months were lower than original test values, with the samples losing an average of 12.6 µg/mL or approximately 20% of the initial quantitation. Of the 59 samples that confirmed positive, 76% (45 samples) reconfirmed at or above the cutoff of 20 µg/mL and 98% (58 samples) reconfirmed at or above the limit of quantitation (LOQ) of 2 µg/mL. One sample that originally tested positive at 28.3 µg/mL retested at 0 µg/mL.

HPLC is a fast and efficient method for testing for chromium VI without the disadvantage of detecting the chromium III inherent in human urine. It is reasonably stable when frozen and retests at LOQ can be expected to reconfirm.

Keywords: Adulteration, Chromium, HPLC

P23

Evaluation of the Cedia® 6-Acetylmorphine Immunoassay with Urine Specimens from a Criminal Justice Drug Testing Program

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Detection of drug use is the primary goal of a forensic urine drug testing program in the criminal justice system. Opiate immunoassays have been utilized to screen for heroin use, with the knowledge that individuals ingesting codeine, morphine and other opioids will test positive, based upon differing cross reactivities of the various immunoassays. Therefore, an immunoassay with high specificity for 6-acetylmorphine (6-AM) would have the potential of detecting heroin (illicit drug) use while decreasing the number of positive samples due to legitimate analgesic use.

This study evaluated the use of the Microgenics™ Cedia® enzyme immunoassay to detect 6-AM in a set of urine specimens (N=525). These samples were obtained from individuals on probation who were subject to drug testing to monitor compliance with the non drug use departmental policy. This assay is a homogenous enzyme assay for use with human urine, with a cutoff concentration of 10 ng/mL for 6-AM. According to the manufacturer, the following concentrations (ng/mL) produced negative results: heroin HCl 80; codeine 500,000; morphine 9,000; hydromorphone 10,000; and oxycodone 400,000. All specimens which screened positive were confirmed by SPE GC/MS for 6-AM, morphine, codeine, dihydrocodeine, hydromorphone, hydrocodone, and oxycodone using a four point calibration and nalorphine internal standard. Furthermore, the immunoassay was challenged with standard solutions of opioids assayed in triplicate under blind conditions [morphine 500-100,000; codeine 500-20,000; oxycodone 200-80,000; hydromorphone 100-10,000; and 6-AM 1-500, ng/mL].

The response values of the EIA for the urine specimens ranged Δ5-212. 6-AM was confirmed in 517/525 (98%) of the urine specimens using a confirmation cutoff of 5 ng/mL. 6-AM concentrations ranged from 7-16,923 ng/mL. All confirmed 6-AM specimens also contained morphine in concentrations ranging from 8-222,427 ng/mL. Codeine was the most common additional drug detected (8-64,915 ng/mL). Of the 8 specimens which screened positive by EIA (with response values ranging from Δ 38-103), but were not confirmed, 2 contained 6-AM at concentrations below the cutoff (3, 4 ng/mL). In addition, 5 contained oxycodone (20-54,000 ng/mL) and one contained dihydrocodeine (378 ng/mL) and hydrocodone (10,294 ng/mL). When challenged with standard drug concentrations, the EIA correctly identified drug free urine, and produced positive results [lowest concentration (ng/mL) which produced a positive result] with morphine at 10,000; oxycodone 61,000; codeine>20,000; hydromorphone 10,000 and 6-AM 10.

In conclusion, the Cedia® 6-AM immunoassay produced a 98% confirmation rate when challenged with 525 urine specimens from a criminal justice drug testing program. Potential users of the EIA should be aware that high concentrations of other opioids (e.g. oxycodone) may produce positive screening results.

Keywords: Urine drug testing, Immunoassay, 6-Acetylmorphine

P24

Abuse of Methylenedioxymethamphetamine in Taiwan — Analytical Approaches and Analytes Distribution in Antemortem and Postmortem Specimens

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With increasing requests for the analysis of various specimens related to fatal and non-fatal abuse of methylenedioxymethamphetamine (ecstasy, MDMA), the toxicology laboratory of the Institute of Forensic Medicine has established appropriate protocols for the analysis of MDMA and related compounds in hair, urine, and various postmortem specimens.

Analytical protocols included extraction, derivatization, and GC-MS using deuterated analogs as internal standards. Analytical data include (a) postmortem distribution of MDMA and MDA in heart blood, gastric content, urine, and bile in 14 cases; (b) other drugs found in the heart blood from these 14 fatal cases; and (c) the concentrations of MDMA and MDA in 25 antemortem urine and several hair specimens. Data shown in Table 1 are compared to those reported in the literature. The MDA/MDMA concentration ratio observed in hair specimens appear to be higher than those found in other specimens. Compared to other commonly abused drugs, e.g., cocaine and heroin, the metabolite/parent drug concentration ratio (MDA/MDMA) in hair is significantly higher than the ratios in other specimens, such as urine and blood. This observation is consistent with the relative drug/metabolite incorporation rates reported for cocaine/benzoyl-ecgonine, tetrahydrocannabinol/tetrahydrocannabinoic acid, and MDMA/MDA [8].

Table 1. Highest MDMA level and MDA/MDMA ratio in ante- and postmortem specimens reported from our laboratory

Specimen	Highest MDMA concentration[a]	MDA/MDMA ratio observed			Literature reference
		Range	Mean	Std dev	
Urine (n = 10)	67.115	0.011–0.174	0.061	0.050	[1]
Bile (n = 3)	16.021	<0.001–0.063	0.033	0.027	[2]
Gastric (n = 9)	40.515	<0.001–0.463	0.094	0.154	[3]
Heart blood (n = 10)	4.971	<0.002–0.205	0.082	0.060	[4,5]
Hair (n = 6)[b]	59.91	0.128–0.211	0.160	0.032	[6,7]
Urine (n = 23)[b]	34.454	0.018–0.228	0.101	0.052	

[a] In ng/mg for hair; in μg/mL for other specimens.
[b] Antemortem specimens.

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Keywords: MDA, MDMA, Postmortem distribution

P25

Urine Adulteration Testing for Iodine by HPLC

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Iodine/Iodic acid is one of several new compounds being used by illicit drug users to conceal drug test results. These compounds are easily detected at screening levels using a Hitachi 747 automated analyzer and commercially available oxidant reagents. Confirmation is complicated for Atomic Absorption (AA) and Inductively Coupled Plasma (ICP) analysis as they measure total iodide (I^-) and iodine (I_2) concentrations. Since iodide is a natural component of human urine, this study focused on developing an iodine specific method.

Samples producing positive oxidant results undergo a triage procedure to determine the potential source of adulteration (nitrite, chromium, iodine, bleach, etc.). Iodine adulterated samples are typically darker brown than normal urine samples and have a medicinal smell. This information is recorded and a drop of the sample is placed on plain copy paper and allowed to sit for approximately one minute. Samples containing iodine react with the starch in the sizing of the paper to produce a violet to purple spot. Two compounds that interfere with the High Performance Liquid Chromatography (HPLC) confirmation, thiosulfate and persulfate, do not react with the plain paper starch test.

Iodine (I_2) is then measured in 10 µL urine specimens by a procedure which detects the color change resulting from the combining of the specimen with a citric acid buffer and Leuco Crystal Violet (LCV) [4,4'4"-methylidynetris (N,N-dimethylaniline)]. The violet to purple color change is measured at a wavelength of 540 nm. Strychnine was added to each sample to serve as an internal standard. The sample is carried through a Prodigy 5µ ODS(2) 150 x 4.6 mm column to the HPLC detector in a 1.28% v/v cetyltrimethylammonium chloride: 11.27 mM dibasic sodium phosphate: 20% v/v acetonitrile mobile phase. Quantitation is based on the peak height of the sample as compared to the peak height of a single point calibrator. The cutoff for this method is 100 µg/mL with a dynamic linear range of 30 to 600 µg/mL.

This method was validated to the same linearity, precision and interference requirements that the Substance Abuse and Mental Health Services Administration (SAMHSA) has for drugs of abuse testing. The coefficient of variation between precision batches for this method was determined to be 6.24% and the citric acid/LCV reaction does not respond with iodide, iodate or any of the other common components of human urine.

The combination of oxidant testing by automated analyzer, intermediate bench testing and HPLC analysis provides a scientifically defensible procedure for the analysis and quantitation of iodine/iodic acid adulteration of human urine.

Keywords: Adulteration, Iodine, HPLC

P26

Ethanol Origin in Postmortem Urine: The LC/MS Determination of Serotonin Metabolites

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Specimens from fatal aviation accident victims are submitted to the Federal Aviation Administration (FAA) Civil Aerospace Medical Institute (CAMI) for toxicological analysis. During toxicological evaluations, ethanol analysis is performed on all cases. Care must be taken when interpreting a positive ethanol result due to the potential for postmortem ethanol formation. Several indicators of postmortem ethanol formation exist; however, none are completely reliable. The consumption of ethanol has been shown to alter the concentration of two major serotonin metabolites, 5-hydroxytryptophol (5-HTOL) and 5-hydroxyindole-3-acetic acid (5-HIAA). While the 5-HTOL/5-HIAA ratio is normally very low, previous studies using live subjects have demonstrated that the urinary 5-HTOL/5-HIAA ratio is significantly elevated for 11-19 hours after acute ethanol ingestion. The 5-HTOL/5-HIAA ratio is not affected by the microbial formation of ethanol. This methodology has not been routinely utilized for the determination of ethanol origin in forensic samples due to the difficulty of the analysis of these two compounds. Until now two different analytical techniques were required to perform this analysis.

In our study we investigated the 5-HTOL/5-HIAA ratio as a potential indicator of ethanol origin in postmortem urine samples. Our laboratory developed and validated a method for the simultaneous determination of 5-HTOL and 5-HIAA in forensic urine samples using a simple liquid/liquid extraction in combination with LC/MS/MS and LC/MS/MS/MS. The liquid/liquid extraction involved buffering the urine specimens to pH 6.00 followed by the addition of a saturated NaCl solution. Ethyl Acetate was added to isolate the compounds of interest, which were then derivitized with BSTFA-1% TMS. The LC was operated in an isocratic mode using a mobile phase composed of 80:20 methanol:50 mM formate buffer pH 5.00. The extraction employed provided an average recovery of approximately 80% for both compounds. The LC/MS method proved highly selective and sensitive, having an LOD of 0.1 ng/mL for both compounds. The accuracy and precision was also very good. Utilizing our LC/MS method we examined the 5-HTOL/5-HIAA ratio in 21 ethanol-negative and 23 true ethanol-positive postmortem urine specimens. We found that all ethanol-negative specimens had 5-HTOL/5-HIAA ratios below 15 pmol/nmol, a previously established antemortem urine cutoff for recent ethanol ingestion. All ethanol-positive urine samples had 5-HTOL/5-HIAA ratios above 15 pmol/nmol. These results validated the antemortem cutoff for use with postmortem urine specimens. This method is currently being used to examine cases suspected of containing postmortem ethanol.

Keywords: Postmortem ethanol, LC/MS, Serotonin

P27

The Detection of Δ^9-Tetrahydrocannabinol in Whole Blood, Plasma, and Liver Homogenates and the Detection of 11-nor-Δ^9-Tetrahydrocannabinol-9-carboxylic acid in Urine using Disposable Pipette Extraction

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The detection of Δ^9-Tetrahydrocannabinol (Δ^9-THC) in whole blood has long been a tedious and time-consuming extraction process. The detection of Δ^9-THC in blood is very important in human performance toxicology in order to better correlate recent drug use with impaired performance. However, the short half life of THC in the blood makes it difficult to detect THC in many cases of suspected drug impairment.

Disposable Pipette Extraction (DPX) has become a very valuable method of extracting Δ^9-THC in whole blood, plasma, and liver homogenates and 11-nor-9-carboxy-Δ9-Tetrahydrocannabinol (11-COOH- Δ^9-THC) in urine. DPX also has the additional capability of extracting the hydroxy and carboxy metabolites of Δ^9-THC in whole blood. Specimen volume requirements are low with only 0.75mL of blood, plasma, or liver homogenate and 0.2mL of urine. The DPX method only takes 2-3 minutes per sample compared to hours using conventional solid phase extraction or liquid-liquid extraction techniques. Reduction in solvent usage and solvent waste is also another added benefit. Only 2.6mL of solvent is needed per sample to extract Δ^9-THC from whole blood, plasma, and liver homogenates; and approximately 1.3mL of solvent is needed per sample to extract 11-COOH- Δ^9-THC from urine. Prior to using the DPX method of extraction, this laboratory used a conventional liquid-liquid extraction that required approximately 11.25mL and 10.30mL of solvent for blood and urine, respectively.

The DPX pipette tip contains a frit near the tapered opening. The loosely packed sorbent material is held in the tip between the frit and the plug placed at the pipette end of the tip. The loose packing of the sorbent allows sufficient mixing of sample with the sorbent material. Urine samples are hydrolyzed prior to extraction of 11-COOH- Δ^9-THC. Whole blood, plasma, and liver homogenates (supernatant only) undergo protein precipitation prior to extraction of Δ^9-THC. The supernatant of the blood, plasma, and liver homogenate samples are transferred to a disposable borosilicate culture tube, 2mL of de-ionized water is added and the sample is vortex mixed. The hydrolyzed urine is treated with 300µL of 50% glacial acetic acid in water and vortex mixed. The sample is drawn into the DPX pipette tip and allowed to mix for 1 minute. The sample is then sent to waste and the sorbent containing the drug is washed with solvent. The Δ^9-THC or 11-COOH- Δ^9-THC is eluted from the sorbent material and transferred to a 1.0mL silanized conical reaction vial. The final elution solvent is then dried down under a gentle stream of nitrogen at 40°C.

BSTFA with 1% TMS, and ethyl acetate (1:1) is added to the reaction vial. The vial is capped, vortex mixed and heated to 90°C for 30 minutes. After the vial cools, the final extract is analyzed in the selected ion monitoring (SIM) mode using an Agilent 6890 GC equipped with a 5793 MSD, 2µL is injected under a specific temperature program onto a 30 m DB5-MS column having 0.25 mm ID and 0.25 µm film thickness.

Method evaluation and validation has been performed in terms of limit of detection (LOD), linearity, precision, accuracy, specificity, interferences and percent recovery. The DPX method was also compared to our existing method, which was provided by United Chemical Technologies, Inc. Current LOD for Δ^9-THC in plasma and 11-COOH- Δ^9-THC in urine is 0.001µg/mL and 0.010µg/mL, respectively. The LOD was determined by the ability to reliably measure 0.001 and 0.010 µg/mL in fortified blood and urine specimens with inter and intra precision coefficients of variation (CV's) of 10% or less. The intra-run CV for Δ^9-THC in plasma was 5.4 % based on ten replicate analysis of a Utak control with a weighed in value of 0.025 µg/mL. For six replicate analyses of this same control the inter-run CV for Δ^9-THC in plasma was 6.7 %. Only inter-run precision was evaluated for 11-COOH- Δ^9-THC in urine. The CV obtained was 8.5% based on six replicate analyses of a Utak control with a weighed in value of 0.019 µg/mL.

Results were confirmed when all three major masses for the silyl derivatives of Δ^9-THC and 11-COOH- Δ^9-THC were recovered. Selected ion masses monitored for Δ^9-THC were 386.4, 371.2, and 303.2 with 389.4 selected for Δ^9-THC -D3. 11-COOH- Δ^9-THC masses monitored were 371.2, 473.3, and 488.3 with mass 374.2 for 11-COOH- Δ^9-THC -D3. The calibration curve for Δ^9-THC was found to be linear up to at least 0.100µg/mL with an R^2 value of 0.9992. The 11-COOH- Δ^9-THC calibration curve was found to be linear up to at least 1.0µg/mL with an R^2 value of 0.9999. Percent recovery of Δ^9-THC in plasma was 43%, which was determined using the average of four different un-extracted methanolic calibrators ranging from 0.005 to 0.075µg/mL compared to 4 extracted, protein-precipitated calibrators, with exclusion of an internal standard. Under identical extraction conditions percent recovery of 11-COOH- Δ^9-THC in urine was 47% using 3 different calibrators ranging from 0.05 to 0.75µg/mL.

Current demographics involving motor vehicle and industrial accidents from both human performance and postmortem toxicology cases obtained from the western third of the state of Ohio will be discussed in terms of THC alone versus THC in combination with other drugs including ethanol.

Keywords: Disposable Pipette Extraction (DPX), Cannabinoids, GC/MS

P28

Analysis of Keto Opioids using Solid Phase Extraction and Gas Chromatography Mass Spectrometry

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Hydrocodone, hydromorphone, oxycodone and oxymorphone were analyzed using solid phase extraction (SPE) and gas chromatograph/mass spectrometry (GCMS). Following protein precipitation, Cerex Polychrom Clin II SPE columns (SPEware, San Pedro, CA) were used to isolate opiates from blood. The columns contain a polymeric phase that does not require conditioning. Following addition of blood or urine (1 mL) to the column, samples were washed with deionized water, 0.1M hydrochloric acid, methanol and ethyl acetate. After the columns were dried under full vacuum for 5 minutes, drugs were eluted using a mixture of methylene chloride/isopropanol (80:20) containing 2% concentrated ammonia solution. Following evaporation of the solvent, extracts were derivatized with acetic anhydride and analyzed using an HP 5973 MSD using selective ion monitoring and deuterated internal standards. Quantitation ions for hydrocodone, hydromorphone, oxycodone and oxymorphone were 299, 285, 357 and 343 (m/z) respectively.

The limit of detection (LOD) was defined as the lowest concentration of drug that produced a signal to noise ratio of 3:1 or more and ion ratios within acceptable limits (±20%). The limit of quantitation (LOQ) was defined as the lowest concentration of drug that produced a signal to noise ratio of 10:1 with ion ratios within acceptable limits and calculated concentration within 20% of the target concentration. LODs for hydrocodone, hydromorphone, oxycodone and oxymorphone were 5ng/mL in blood and 10 ng/mL in urine. LOQs in blood for hydrocodone and hydromorphone were 5 ng/mL and 25 ng/mL for oxycodone and oxymorphone. External whole blood controls indicated an overall accuracy of 105%. Calculated concentrations of internal controls that were fortified with drugs at a concentration unknown to the analyst, yielded values ranging from 98—109% of the target concentration in blood, and 88-113% of the target concentration in urine. Calibrations were linear to 2500 ng/mL and R^2 values were 0.999 or greater. Intraassay CVs in blood were 5.2-5.7% at 300 ng/mL and 6.5-13.3% at 30 ng/mL. This assay is routinely used for quantitative analysis of keto opioids in antemortem and postmortem casework samples.

Keywords: Oxycodone, Hydrocodone, GCMS, SPE

Determination of Nalmefene in Plasma by High Performance Liquid Chromatography- Electrospray Ionization -Tandem Mass Spectrometry

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Nalmefene is an opioid antagonist used in the treatment of alcoholism and opioid overdose. A highly sensitive method was developed to measure nalmefene in human and rabbit plasma. Nalbuphine was used as internal standard. 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 Finnigan model TSQ7000 Thermo Quest triple-stage quadrupole. Quadrupole 1 was set to pass only ions at m/z 340 and 358 that correspond to the MH^+ ions of nalmefene and nalbuphine. The MH^+ ions were caused to undergo collision induced dissociation in quadrupole 2 that produced product ions at m/z 322 and 340 respectively, which were then monitored selectively by quadrupole 3. The calibration range was from 0.1 to 100 ng/mL with the calibration curve constructed as quadratic with $1/X^2$ weighting. Specificity for nalmefene was determined from analysis of blank plasma fortified with internal standard only (3 replicates) and with LLOQ concentration (0.1 ng/mL) (1 replicate) in six different lots of plasma. The primary evaluation was to compare the mean peak area ratio of any signal at the retention time of nalmefene 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 2.09 to 5.29 with a mean of 3.21% for human plasma and from 4.08 to 6.63 with a mean of 5.55% for rabbit plasma. Intra-run accuracy of the LLOQ was within 10.0% of target with intra-run precision within 13.6%. Intra- and inter-run precision and accuracy were also evaluated at 0.3, 35 and 75 ng/mL. The intra-run accuracy was within 10.0% of target with intra-run precision within 6.6%. The inter-run accuracy was within 8.0% of target with inter-run precision within 13%. Nalmefene was stable in human and rabbit plasma up to 24 hours at room temperature and in human plasma after 3 freeze-thaw cycles. The mean extraction efficiency for nalmefene was 80.0%. Application of the method to rabbit plasma samples following intravenous injection of nalmefene was also performed. The mean area under the curve for 0 to 24 hours was 1116 $(ng)(mL)^{-1}(h)$ and the mean plasma clearance was calculated as 75.2 $(mL)(min)^{-1}(kg)^{-1}$. The results of these studies show that nalmefene can be quantified using liquid/liquid extraction and LC-ESI-MS/MS from 1-mL aliquots of human or rabbit plasma. The method is very sensitive for pharmacokinetic studies and other clinical applications. (Portions of this research were funded by a subcontract from Biotek, Inc. who were funded by Contract No. N44-AA-12003 with the National Institute on Alcohol Abuse and Alcoholism.)

Keywords: Nalmefene, HPLC-ESI-MS/MS, Plasma

P30

Simultaneous Quantification of Opiates, Methamphetamine, Cocaine, and Metabolites in Skin by Positive Chemical Ionization Gas Chromatography/Mass Spectrometry

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We have developed and validated a positive chemical ionization gas chromatography mass spectrometric (GC/PCI-MS) method to simultaneously quantify opiates, methamphetamine, cocaine, and metabolites in skin. Three mm skin punch biopsy samples (containing epidermis, dermis and fat tissue) were homogenized (Kinematica Polytron) with 1 mL methanol and sonicated for 15 to 30 min at room temperature. Methanol was evaporated under nitrogen at 40°C until dryness. Extracts were reconstituted with 3 mL 2.0 M sodium acetate pH 4.0. After mixing and centrifugation, supernatants were decanted onto preconditioned solid phase extraction columns (Clean Screen® ZSDAU020, UCT). After washing with water, 0.2 N HCl, and methanol, analytes of interest were eluted with methanol: isopropanol: ammonium hydroxide (80:20:2, v:v:v). After evaporation, extracts were derivatized with MTBSTFA and BSTFA and analyzed using an Agilent 6890 GC interfaced with an Agilent 5973 mass selective detector operated in SIM mode. Calibration curves (2.5 – 100 ng/skin biopsy) for morphine, codeine, 6-acetyl morphine, methamphetamine and amphetamine, cocaine, norcocaine, benzoylecgonine, cocaethylene, norcocaethylene, and anhydroecgonine methyl ester exhibited correlation coefficients > 0.999. Deuterated analogs were used as internal standards for most compounds, with the exception of anhydroecgonine methyl ester and norcocaethylene, in which a closely related deuterated analyte was employed. Within and between-run precision were calculated at 6, 30 and 60 ng/skin biopsy with coefficients of variation less than 11%. Accuracies at the same concentrations were ±10% of target for all analytes, except morphine (±18%). Extraction efficiencies at 10 and 100 ng/skin biopsy were 90 to 122%. Twenty-one potential interfering compounds were spiked (1,000 ng/skin biopsy) into low quality control samples (6 ng/skin biopsy) to evaluate specificity, and all quantified within ±20% of target, except for norcocaethylene that exhibited interference from clonidine. Analyte stability was determined by subjecting low and high quality control samples to three freeze-thaw cycles. Comparisons of drug concentrations to routine controls were reported as ±11% of target for all analytes, except for anhydroecgonine methyl ester (±33%). We present a rugged, sensitive and specific method to simultaneously and accurately quantify opiates, methamphetamine, cocaine and metabolites in a biological matrix that may be useful for medical examiner/coroner laboratories utilizing GC/PCI-MS.

Keywords: Positive chemical ionization, Fat tissue, Skin

P31

The Presence of N-Methyl-1-(1-(1,4 cyclohexadienyl))-2-propanamine, a Birch Reduction Product, in Methamphetamine Positive Toxicology Samples

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Methamphetamine can be clandestinely manufactured using several different chemical synthesis methods. One such method is the Birch Reduction, which employs an alkali metal dissolved in liquid ammonia to reduce ephedrine or pseudoephedrine to methamphetamine. The reaction is rapid, and is complete in about an hour. A reaction byproduct resulting from over-reduction of the methamphetamine product is N-methyl-1-(1-(1,4 cyclohexadienyl))-2-propanamine, also called the "Birch reduction product". This is sometimes referred to by forensic chemists as the "150 compound" due to the presence in the mass spectrum of mass 150 m/z as the molecular ion, rather than 148 m/z as is the case with methamphetamine. Reaction conditions, including the use of an alcohol to terminate or quench the reaction, can affect the amount of the byproduct formed. In some cases it may be as much as 50% or more of the total reaction product.

The over-reduction product has been observed in both blood and urine samples submitted to the Washington State Toxicology Laboratory. It is not known to be a methamphetamine metabolite, and therefore its detection would suggest ingestion of methamphetamine prepared via an alkali metal reduction as opposed to one of the other popular illicit syntheses.

All cases testing positive for methamphetamine over the course of twelve months were retrospectively reviewed for the presence of this reduction byproduct. It was found to be present in blood and/or urine in approximately 10% of methamphetamine positive cases. The amount relative to methamphetamine varied considerably but was typically between 1 and 10% of the size of the methamphetamine peak.

The mammalian toxicology of this compound is not known, however other cyclohexadiene species readily form free radicals, and may have cytotoxic properties.

Keywords: Clandestine synthesis, Methamphetamine, Ephedrine

P32

Performance of a Microtiter Plate ELISA for Screening of Postmortem Blood for Cocaine and Metabolites

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The object of this study was to evaluate the suitability of the Neogen® Corporation microtiter plate enzyme-linked immunoassay (ELISA) for cocaine and metabolites for screening of post-mortem blood. Sixty-five post mortem whole blood specimens were obtained from drug-involved deaths, which had been screened and confirmed positive for cocaine and/or benzoylecgonine. Fifty-eight negative specimens were obtained from non-cocaine-involved deaths. The negative specimens were tested initially by ELISA and base extractions followed by GC/NPD and GC/MS to ensure the negative specimens were truly negative for Cocaine. Specimens were tested using the Neogen Cocaine/Benzoylecgonine (BE) microtiter plate ELISA assay. No matrix effects were found for whole blood in this assay. The effect of dilutions of the whole blood specimens of 1:5 through 1:50 was studied. A dilution of 1:5 was chosen to correspond to that used for other Neogen microtiter plate assays for drugs in whole blood.

True positive, true negatives, false positives and false negatives were determined and graphed for the ELISA results against GC/MS, gas chromatography-NPD and case histories. From these graphs and the ROC curves the optimal cutoff for the Neogen Cocaine/BE ELISA was found to be 5 ng/ml benzoylecgonine equivalents at a 1:5 dilution. The optimum cutoff for a 1:50 dilution was 50 ng/ml benzoylecgonine equivalents. The Neogen Cocaine/BE ELISA had a sensitivity of 93.8% ± 2.9% and a specificity of 96.6% ± 2.4% vs GC/MS at a cutoff of 5 ng/ml ml benzoylecgonine equivalents (1:5 dilution) and a sensitivity of 100% ± 0.5 % and specificity of 98.3% ± 1.7% vs GC/MS at a 50 ng/ml benzoylecgonine equivalents cutoff (1:50 dilution).

Keywords: ELISA, Cocaine, Postmortem blood

P33

An Unusual Loxapine Intoxication

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A case is presented of a 53-year-old white male with a history of dementia and Pick's disease that was a resident of a mental institution. He reportedly had a seizure, arrested and died. Remarkable autopsy findings included severe coronary artery disease with a previous myocardial infarction.

Subsequent investigation suggested an unauthorized use of loxapine. As a result, comprehensive toxicological analyses were performed. The heart blood was negative for methanol, ethanol, acetone and isopropanol at a cutoff of 0.01 g/dL. Comprehensive testing was performed on the urine, including color tests for acetaminophen, ethchlorvynol and salicylate, an acid neutral drug screen by gas chromatography-nitrogen phosphorous detector, an alkaline drug screen by gas chromatography-nitrogen phosphorous detector and morphine by radioimmunoassay. The urine screen identified metoprolol, loxapine, and amoxapine. These substances were confirmed by full scan electron ionization gas chromatography/mass spectrometry. No metoprolol was identified in the blood at a limit of quantitation of 0.1 mg/L. Loxapine and amoxapine were quantitated by gas chromatography-nitrogen phosphorous detector with the following results:

Specimen	Concentrations	
	Loxapine	Amoxapine
Heart blood (mg/L)	42	0.6
Peripheral blood (mg/L)	68	0.9
Liver (mg/kg)	29	2.5
Kidney (mg/kg)	32	2.5
Bile (mg/L)	43	4.6
Urine (mg/L)	5.9	1.2

The heart blood loxapine concentration was approximately five times greater than the highest previously reported loxapine intoxication case. The low concentration of amoxapine in relation to loxapine in all specimens is consistent with an acute ingestion of a large amount of drug. One surprising finding was the fact that the liver concentration of loxapine was lower than the blood concentrations. This was unusual in that tricyclic antidepressants have significantly higher liver concentrations than blood concentrations. The medical examiner ruled that the cause of death was loxapine intoxication and the manner of death was undetermined.

Keywords: Loxapine, Intoxication, Postmortem

P34

Stability of ethanol in postmortem blood and vitreous humor in long-term refrigerated storage

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Ethanol concentration in postmortem blood and vitreous humor samples collected at the Miami-Dade Medical Examiner Department over five to six years ago were reexamined to assess whether vitreous humor is a more reliable specimen for the analysis of ethanol in samples stored long-term.

Initial analyses were performed in 1996 and 1997 on a Sigma 2000 Gas Chromatograph with a HS100 headspace autosampler (PE). The columns used were DB-Wax and DB-1 wide bore, 30 m columns. The samples were incubated for twelve minutes at 55°C before sampling and analysis. The samples were re-analyzed by headspace gas chromatography with flame-ionization detection. Each sample was incubated for 15 minutes at 60°C before sampling and analyzing. The gas chromatograph is calibrated before every run, and controls are placed every tenth position to ensure that the results are consistent and reliable. Precision of the procedure, established by repeated analysis of the control (n=20), was 0.154 mean, 0.002 standard deviation, and 1.5% CV.

Vitreous humor samples, stored in 10 mL gray top Vacutainer tubes, for prolonged storage were more stable than postmortem blood samples stored in 50 mL polypropylene tubes. The average change in 50 mL polypropylene tubes containing blood was 0.06 gm/dL (35 % loss). On the other hand, vitreous humor samples collected in 10 mL Gray Top Vacutainer tubes yielded an average change of 0.01 gm/dL (6.1% loss). The percent of ethanol loss in vitreous humor and blood samples was independent of the initial concentration of ethanol. In addition, the amount of ethanol lost in postmortem vitreous humor and blood due to vaporization into headspace was negligible compared to the amount of ethanol lost due to oxidation in the postmortem blood as illustrated by the average change for ethanol in postmortem blood as opposed to the average change for vitreous humor.

Vitreous humor is an acceptable alternative to blood when performing ethanol analysis on samples stored under refrigeration. Opening and sealing of postmortem blood has a minor effect on the ability to reproduce viable data as compared to the significant losses in ethanol concentration caused by oxidation in the postmortem blood. Vitreous humor, however is unaffected by oxidation because it lacks hemoglobin. This is extremely important factor if ethanol analysis needs to be repeated at a later date. If blood is to be reanalyzed after prolonged storage, a filled gray top Vacutainer tube will give more reliable results than blood stored in a large tube with a large headspace.

Keywords: Ethanol, Vitreous humor, Postmortem, Headspace gas chromatography

P35

Death Following Long Term Fluoxetine Use

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Fluoxetine (Prozac®), a selective serotonin reuptake inhibitor (SSRI), is a widely prescribed anti-depressant which has been clinically used in the United States since 1987. Fluoxetine is approved for the treatment of depression, compulsive-obsessive disorder, bulimia nervosa and anxiety. The therapeutic index of fluoxetine is very high, making it a relatively safe drug. Common adverse side effects associated with fluoxetine use include diarrhea, nausea, drowsiness, headaches, and insomnia. More serious side effects include syndrome of inappropriate secretion of antidiuretic hormone (SIADH) and serotonin syndrome. Heart disease may be exacerbated due to vasoconstriction from excess platelet 5HT.

Fluoxetine is metabolized to the active metabolite, norfluoxetine. The half-life of the parent compound (1 to 3 days) and the half-life of norfluoxetine is even longer (7 to 15 days). Cytochrome P450 is involved in fluoxetine metabolism but there is conflicting information in the literature as to which CYPs are involved. Several in vitro studies indicate that is CYP2D6 plays a major role in this pathway with additional contribution of CYP2C9 and CYP3A4. However, another study implicates CYP2C9 as the principal pathway, with possible contribution from CYP2C19 and CYP3A4 and negligible CYP2D6 contribution. Both fluoxetine and norfluoxetine are potent inhibitors of CYP2D6 and moderate inhibitors of CYP3A4. Because there is both metabolism by and inhibition of CYP's, the parent and the active metabolite exhibit auto-inhibition causing a non-linear relationship between dose and concentration. Steady-state therapeutic concentrations are 0.03 to 0.47 mg/L and 0.02 to 0.47 mg/L for fluoxetine and norfluoxetine, respectively. Deaths have been attributed to fluoxetine with concentrations in excess on 1.0 mg/L.

We present a case study of a 56 year-old female, found dead at home, lying across her bed in a supine position, feet touching the floor. She was overlying her deceased four month old granddaughter. The cause of death of the infant was asphyxia due to suffocation. Autopsy results from the grandmother indicated no significant anatomical cause of death. Focal severe atherosclerosis was noted in one coronary artery with no other significant occlusions and there were mild fatty changes to the liver (steatosis). The decedent's medication list revealed fluoxetine-HCl (40 mg daily for approximately a year) for treatment of depression and chronic pain disorder. A few days prior to her death her fluoxetine dose was increased to 60 mg daily. She also was using fentanyl transdermal patches (Duragesic® TTS) for her chronic back pain. Prior to her death, she reportedly suffered from dizziness with lethargy and malaise.

Basic drugs were screened by GCMS without derivatization following BuCl extraction. The results are shown in the following table. The gastric contained a total of 40mg of

fluoxetine. Peripheral blood fentanyl concentrations were elevated perhaps due to the drug-drug interaction of fentanyl with fluoxetine.

Tissue	Fluoxetine (mg/L)	Norfluoxetine (mg/L)	Fentanyl (mg/L)
Blood (peripheral)	3.92	4.48	0.02
Blood (central)	21.36	14.10	0.03
Liver	4.70	4.40	None detected
Bile	14.13	12.31	0.04
Brain	16.19	15.03	None detected
Gastric	24.29 mg total	2.21 mg total	<0.01

Based on history and reconciling the pill count with the date of prescription, the decedent had apparently been taking her medication as prescribed (40 mg/day increased to 60 mg/day), there is no clear explanation for these remarkably high concentrations of parent drug and metabolite, more usually associated with chronic or acute overdose. In contrast to other reports, this decedent's peripheral blood parent:metabolite ratio, (0.88) was consistent with the therapeutic ratio (mean 0.88) than the ratio in overdoses (mean 4.17). This may reflect a slow accumulation rather than an acute ingestion. The central to peripheral blood ratios were 5.45 which is consistent with post-mortem redistribution. Although her blood concentrations were consistent with concentrations reported in fatalities, the liver and brain concentrations were considerably lower than in those reports. The death was attributed to a combined fluoxetine and fentanyl overdose, and the manner of death was probable accident.

Keywords; Fluoxetine, postmortem, death investigation

P36

Association of Citalopram with Deaths in Northern Ontario: Comparison of Cases with the Literature

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The selective serotonin reuptake inhibitor citalopram (Celexa®) is one of the most frequently prescribed antidepressants world-wide. This is due to its selective mode of action, less severe adverse effects compared to other antidepressants, and high safety margin. Limited data is available concerning adverse drug interactions for citalopram, which is attributable, in part, to its novelty in North America.

A review of forensic cases in Northern Ontario from 1998 to 2003 demonstrated a number of citalopram-related fatalities (n = 14). The citalopram concentrations detected in these fatalities were in the range of 0.017 to 0.620 mg/100 mL (mean 0.169 mg/100 mL, median 0.049 mg/100 mL). This range is in good agreement with levels found previously in the literature.

In examining the literature and Northern Ontario findings, it was determined that there is as a strong association of citalopram with other frequently prescribed and over-the-counter medications in many of these deaths. Sedatives (45%) and analgesics (42%) were commonly found in the literature and in Northern Ontario (54% and 36%, respectively) in citalopram-associated deaths. Other drugs that were frequently present include antidepressants (approximately 20% in both literature and Northern Ontario) and drugs of abuse or ethanol (27% in the literature, 36% in Northern Ontario). In the reviewed literature (n = 64), citalopram concentrations were lower in the polydrug-related fatalities (mean of 0.123 mg/100 mL; median 0.044 mg/100 mL; range of 0.009 to 0.481 mg/100 mL; n = 17) than in citalopram-alone situations (mean of 0.338 mg/100 mL; median 0.087 mg/100 mL; range of 0.080 to 1.10 mg/100 mL; n = 4). This is opposite to what was determined in the reviewed forensic cases as citalopram concentrations in polydrug-related deaths were higher (mean of 0.185 mg/100 mL, median of 0.100 mg/100 mL, range 0.026 to 0.620 mg/100 mL, n = 11) than those in citalopram only-related cases (mean of 0.102, median of 0.049, range of 0.017 to 0.240 mg/100 mL, n = 3). This difference may be related to the cause and manner of death.

These data suggest a need for further research into polydrug interactions when citalopram is prescribed. Specifically, research should address possible synergistic toxicity of various citalopram concentrations when administered with other drugs that also act by altering central nervous system neurotransmitter levels.

Keywords: Citalopram, Post-mortem, Northern Ontario

P37

Increased Incidence of Gabapentin and Baclofen in Postmortem Casework, both Alone and In Combination with Other Drugs

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Gabapentin (Neurontin®), is an analog of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA), and is used clinically as an antiepileptic and more recently in the management of chronic pain. Gabapentin is not metabolized and once absorbed, is completely eliminated by renal excretion. Gabapentin has been compared to GHB for its sedative and social effects. It has been cited, by the Erowid Internet site, that "recreational value definitely exists" due to the social enhancements of joy and increased confidence.

Baclofen (Atrofen®, Lioresal®), is also a GABA analog which is used to treat spasticity associated with multiple sclerosis. Recently, however, baclofen has been used in the treatment of alcohol, amphetamine, and heroin addiction. Baclofen elimination is primarily renal with 85% excreted as unchanged drug.

Currently, two different methods for extracting both gabapentin and baclofen are being reviewed at the Montgomery County Coroner's Office (MCCO) along with recent postmortem case demographics involving both gabapentin and baclofen.

Several methods are available for the detection of gabapentin and baclofen. Three specifically are GC/MS, TLC and HPLC methods. The HPLC method utilized by the MCCO involves derivitazation with trinitrobenzene sulfonic acid (TNBSA), to create the chromophor necessary for detection in the UV/VIS region. In using the HPLC method to analyze for gabapentin and baclofen, one analyte serves as an internal standard for the other. Other internal standards are being evaluated which can be used for both analytes. The analysis is performed with either a liquid-liquid extraction or solid phase extraction (SPE) using 0.5mL of specimen (blood, urine or liver homogenate). Calibrators are in concentrations of 5, 25, and 100ug/mL for gabapentin and 0.5, 2.5, and 10ug/mL for baclofen. In the liquid-liquid extraction baclofen and gabapentin are extracted from biological samples with acetone, which is then evaporated to dryness. In the SPE extraction, the eluate is evaporated to dryness. In both extractions the residue is derivatized for one hour at room temperature in an aqueous solution of trinitrobenzene sulfonic acid (TNBSA). In the purification step, acetic acid is added to form a precipitate of the analytes. This precipitate is then dissolved in HPLC mobile phase (1:1 Acetonitrile: pH 4.7 Acetate buffer) and injected on the HPLC. An Agilent 1100 Series HPLC with an Eclipse XDB-C8, 150mm length, 4.6mm diameter, 5µ particle size column is used for analysis.

The MCCO has seen an increase in positive cases for both drugs, especially gabapentin. Many of these cases involve multiple drug use including diazepam, methadone, oxycodone, fentanyl, morphine, and hydrocodone. According to the PDR, impaired renal function or co-administration of morphine or hydrocodone with gabapentin causes an increase in the AUC for gabapentin by as much as 44%. In addition to blood and urine, other biological matrices, including cerebral spinal fluid, brain, vitreous, and bile, were analyzed in cases that had gabapentin concentrations of >10µg/ml in blood.

Keywords: Gabapentin, Baclofen, HPLC

P38

Stability of Cocaine, Heroin and Metabolites in Oral fluid collected with the Intercept® Collector

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There is increasing interest in the use of oral fluids for detection of drugs of abuse in the workplace and as a possible substitute for blood in driving under the influence cases. Oral fluids often have higher concentrations of parent drugs that are detectable for longer periods of time than in urine and blood. This increased window of detection of the parent compounds can help provide evidence as to the time of use of the drug. However, some of these parent compounds such as Cocaine and Heroin are less stable then their corresponding metabolites. In this study, Cocaine, Benzoylecgonine (BE), Heroin, 6-monoaceytlmorphine (MAM) and Morphine were spiked into oral fluid samples collected with the Intercept® device from volunteers. The Intercept® collector utilizes a collection pad impregnated with dried salts that increase osmotic pressure, facilitating increased collection efficiency. Following collection, the pad is stored in a preservative solution. The pooled spiked volunteer samples were incubated at -20°C, 4°C, room temperature (RT) and 37°C for 14 days. The samples were then extracted by solid phase extraction (SPE) using the Zymark RapidTrace SPE Workstation. The SPE extraction used Bond Elute Certify® extraction columns that were washed with methanol, deionized water and 0.1 M pH 6 phosphate buffer. Samples were applied followed by rinsing with 1M acetic acid and methanol. The drugs were extracted with 78/20/2 (methylene chloride/isopropanol/ammonium hydroxide), evaporated to dryness, derivatized with BSTFA+1%TCMS and then analyzed by GC/MS/MS using the Varian 1200 GC/MS/MS.

The stability of Cocaine and Heroin spiked into oral fluid samples collected with the Intercept® collector was tested during the extraction procedure. In addition, Cocaine, Benzoylecgonine (BE), Heroin, 6-monoaceytlmorphine (MAM) and Morphine were spiked into oral fluid samples collected with the Intercept® device at 20ng/mL and tested on day 0,1,2,3,7,10 and 14 at the four different temperatures. Cocaine was more stable then Heroin and we found no degradation of Cocaine during the actual analysis, whereas Heroin showed a conversion to MAM of up to 10% over the 3 hour time period to sequentially extract 10 samples on the RapidTrace Workstation. We compared our method to previous publications which utilized several changes to reduce this conversion of Heroin to MAM and found that the previously published methods produced no measurable loss of Heroin over the same time period using the RapidTrace Workstation. For the temperature stability study, Heroin also showed the quickest decline out of all of the analytes tested with a complete conversion to MAM in 1 day at 37°C. Cocaine was next with all converted to BE by 7 days at 37°C. MAM showed a decline of 50% at 37°C by 14 days. Morphine and BE remained relatively stable during the entire 14 day testing period at all temperatures. Reduction of temperature did slow down the breakdown of Heroin, Cocaine and MAM. Cocaine and MAM were stable the entire 14 days at 4°C, but Heroin required –20°C to maintain stability for the 14 day time period tested.

This is the first reported stability data of Cocaine, Heroin and metabolites in oral fluid collected with the Intercept® collector. The stability of Cocaine was similar to that of blood as reported in the literature.

Keywords: Heroin, Stability, Oral fluid

P39

Opiate Prevalence in Oral Fluid

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This study was undertaken to identify and quantitate some typical opiate class drugs in oral fluid specimens of a large workplace population. All oral fluid specimens were collected by the Intercept collection device. Screening was performed by Intercept micro-plate EIA, OraSure ® technologies, for the presence of opiates. Screen positive specimens (>/=10 ng/mL morphine equivalents) were confirmed by GC/MS/MS initially for the presence of codeine and morphine. Morphine positive specimens were confirmed for the presence of 6-acetylmorphine. Specimens that did not contain codeine or morphine above assay limit of quantitation (LOQ, 5 ng/mL) were then confirmed for the presence of hydrocodone, hydromorphone and oxycodone.

A total of 23,829 oral fluid specimens were screened for the presence of opiates. One hundred ninety eight specimens (0.8%) were presumptive positive by micro-plate screening. Of the screen positive specimens, 29 had a presence of codeine (0.1%) and 15 had a presence of morphine (0.06%). Three specimens contained both codeine and morphine. Eleven specimens were analyzed for 6-am. Fifty-five percent confirmed positive for the presence of 6-am. Three specimens had insufficient volume to complete confirmation testing.

The mean concentration of the codeine present specimens was 71 ng/mL with a high value of 857 ng/mL. The mean concentration of morphine present specimens was 113 ng/mL with a high value of 1205 ng/mL. The 6-am present specimens had a mean concentration of 14.6 ng/mL with a high value of 43.4 ng/mL.

The specimens that did not confirm above assay LOQ for the presence of codeine or morphine were extracted a second time and confirmed for the presence of hydrocodone, hydromorphone and oxycodone. One hundred forty nine specimens were taken through the second confirmation procedure. One hundred twenty eight (0.54%) showed the presence of hydrocodone at or above the assay LOQ (5.0 ng/mL). None of the specimens, however, contained hydromorphone. Five of the hydrocodone present specimens were positive for oxycodone.

The mean concentration of the hydrocodone present specimens was 48 ng/mL with a high value of 471 ng/mL. The five oxycodone positive specimens had a mean concentration of 148 ng/mL with a high value of 269 ng/mL.

An evaluation of almost 24,000 oral fluid specimens showed an overall positive screening rate of 0.8%. This is the same screen positive rate for opiates of 104,173 non-federally mandated urine samples tested during the same time period by Lab*One* (0.8%). Overall, a small number of oral fluid specimens had a presence for codeine and/or morphine with the majority of specimens containing hydrocodone.

Keywords: Oral fluid, Opiates, Workplace

P40

An ONLINE® DAT II Immunoassay for the Detection of Cannabinoid Metabolites in Urine

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An ONLINE® Generation II immunoassay has been developed for the determination of Cannabinoid Metabolites in urine and has been applied to the Roche/Hitachi and INTEGRA® families of analyzers. This two-reagent system utilizes a THC-specific monoclonal antibody. The extent of the agglutination of antibody-coated microparticles with drug-polymer conjugate is monitored. This new assay offers a broad dynamic range of 0-300 ng/mL with 20, 50 and 100 ng/mL cutoff concentrations. Both semi-quantitative and qualitative applications of the assay on the Roche/Hitachi 917 are examined below and are representative of the performance obtained on all Roche/Hitachi analyzers to which his assay has been applied.

Studies demonstrate that the new immunoassay displays a low background, with a clinical sensitivity (mean + 2 S.D.) of 4.8, 2.6 and 0.8 ng/mL for 20, 50 and 100 ng/ml cutoffs respectively. A method comparison of 100 clinical samples that were screened negative by the EMIT® immunoassay yield negative sample results with the ONLINE THC II assay, and 52 GC/MS confirmed clinically positive samples result in positive values with the new assay system. A 5-day precision and recovery study of control samples across the range of the curve was performed for all three cutoffs. The median semi-quantitative intra-assay %CV of the 10, 15, 20, 25, and 37.5 ng/mL controls (n=20) for the 20-cutoff assay on Roche/Hitachi 917 are 6.3%, 4.8%, 3.6%, 3.5%, and 3.3%, respectively. The Inter-assay precision (n=100) of the same levels ranged from 3.6 to 4.7%. The median semi-quantitative intra-assay %CV of the 25, 37.5, 50, 62.5, and 200 ng/mL controls (n=20) for the 50-cutoff assay on Roche/Hitachi 917 are 5.2%, 4.0%, 4.0%, 3.9%, and 4.4%, respectively. The Inter-assay precision of the same levels ranged in value from 4.1 to 6.3%. The median semi-quantitative intra-assay %CV of the 50, 75, 100, 125, and 200 ng/mL controls (n=20) for the 100-cutoff assay are 4.5%, 3.9%, 4.4%, 3.0%, and 2.4%, respectively. The Inter-assay precision of the same levels ranged from 2.7 to 5.1%. Comparable results have been obtained on the INTEGRA platform.

The antibody used in this assay was developed against Δ9-11-nor-9-carboxy-tetrhydrocannabinol (COOH-THC); however, retains broad cross-reactivity to many cannabinoid metabolites. Studies demonstrate extended reagent stability and higher throughput in comparison to the OnLine Gen I THC assay and come ready to use with no reagent reconstitution required. In summary, the ONLINE® DAT II assay for cannabinoids produces reliable results as demonstrated by the above performance characteristics and is well suited for routine drugs-of-abuse screening.

Keywords: THC, Cannabinoid, ONLINE, Immunoassay

P41

A Modified Method for the Liquid-Liquid Extraction and GC/MS Analysis of Amphetamine/Methamphetamine from Human Urine in a SAMHSA Certified Drug Testing Laboratory

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In order to produce forensically accurate and reproducible results in a competitive, cost-effective, and time conserving manner, we took an existing method for the liquid-liquid extraction of amphetamine/methamphetamine from urine and made several significant modifications. The extraction solvent composition was modified from (35:15:40:10) toluene:ethyl acetate:hexane:methanol to (25:25:50) toluene:ethyl acetate:hexane. The internal standard concentration was increased from 333 ng/mL to 1333 ng/mL. The derivatizing reagent was changed from MBTFA to PFPA this enabled the elimination of a second method (utilizing sodium periodate) to analyze specimens containing phenethylamines. Adjustments to the GC oven ramping program, and a change in ions monitored were made to achieve optimal peak resolution for the analytes of interest. The above changes had a positive impact on the amphetamine/methamphetamine confirmation procedure in a high volume laboratory setting. The (ULOL) increased from 8000 ng/mL to 15,000 ng/mL and the LOD/LOQ was 50 ng/mL for both analytes allowing for accurate quantitation of high concentration samples, and a reduction in the number of potential carryover issues. Precision studies at 500 ng/mL produced a CV of 2.6% for amphetamine and 3.9% for methamphetamine. Changes with the extraction solvent, derivatizing reagent, and GC oven ramp eliminated potential contribution from interfering phenethylamines such as ephedrine and pseudoephedrine by shifting their peaks to higher elution temperatures. Interference studies verified this conclusion. The original confirmation procedure included an additional extraction method utilizing sodium periodate for samples that contained potentially interfering phenethylamines. This new procedure enabled the laboratory to eliminate that additional step.

Keywords: Methyl alcohol, Derivatization, Amphetamine/Methamphetamine

P42

Use of a Novel Large Volume Splitless Injection Technique and Sequential Full Scan/SIM for Simultaneous Screening and Confirmation of Toxicological Specimens

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Toxicology specimens present unique analytical challenges that include complex matrices, time constraints, complex sample preparation, and the need for solid scientific practices upon which to base the results. Historically, gas chromatography and mass spectrometry (GC/MS) has played a crucial role in the forensic toxicology laboratory. A novel injection technique coupled with a sequential full scan/SIM acquisition can expand that role and consequently increase the value of GC/MS as an analytical tool. By combining an injection technique that allows injection of up to 35 uL of sample with a sequential full scan/SIM acquisition, the forensic scientist can achieve several objectives with a single injection. SIM analysis enables confirmation of pre-screened presumptive positives, while the presence of full scan data allows investigation into other compounds of interest that may be present in a sample.

A number of biological matrices, including urine and oral fluid, were analyzed using a quadrupole mass spectrometer coupled to a gas chromatograph equipped with a split/splitless injector configured for large volume splitless injection (LVSI). A 5 m precolumn connected to a 30 m Rtx-5MS (Restek Corporation, Bellefonte, PA) 0.25 mm x 0.25 um column was used for LVSI. For comparison, a 15 m column of the same phase (Rtx-5MS, Restek Corp.) was used for standard splitless injection. Custom libraries were created containing common drugs of abuse in derivatized and underivatized form. Acquisition methods appropriate to target compounds were created, and LVSI was optimized using the accompanying software tool. This facilitated development of the oven method according to solvent selection.

With LVSI, sample preparation was minimized for underivatized samples; derivatized samples were reconstituted with larger volumes of solvent than those prepared for standard injection. The larger injection volume also provided increased sensitivity, enabling lower detection limits where desired or for finding compounds of interest present at low concentrations.

Emphasis was placed on cocaine and metabolites in urine and oral fluid, and extraction methods were appropriate for the target compounds. The resulting data provided confirmatory, quantitative results for the target compounds as well as library searchable full-scan spectra for evaluating unknown components. The custom library contained standards ranging across a broad spectrum of acidic, basic, and neutral drugs. The limit of detection using a standard splitless injection technique was 30 ng/mL for benzoylecgonine (BE) in urine, and this LOD was decreased to 300 pg/mL using an injection volume of 35 uL. The correlation coefficient for BE in urine using the SIM data was 0.991, calibrated from 300 pg/mL to 1200 ng/mL. Run times using LVSI were longer than those using the standard technique, due to use of a longer column and a required period of isothermal oven temperature. These longer run times were offset by the decreased detection limits achievable with LVSI. Overall, the use of LVSI coupled with sequential full scan/SIM GC/MS provided a valuable tool for the forensic scientist.

Keywords: Large volume splitless injection, GC-MS, Quantitation, Sequential full scan/SIM

P43

An Uncertainty Budget for the Measurement of Ethanol in Blood by Head-Space Gas Chromatography

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An uncertainty budget for the measurement of ethanol in blood (BAC) by head-space gas chromatography was constructed based on the principles in the Guide for the Expression of Uncertainty of Measurement (GUM). The uncertainty budget, covering the analytical range up to an ethanol concentration of 3.00 per mille (0.317g/100mL), included analytical uncertainty components, traceability uncertainty components, and matrix effects, respectively. The combined analytical uncertainty was estimated from duplicate measurements of real samples, and validated with results on aqueous control samples. The combined analytical uncertainty included contributions from variation between columns, injection, repeatability of analytical signals, statistical uncertainty of the calibration function etc. The traceability uncertainty was estimated in a sub-budget based on information about the calibrator and about the preparation of the aqueous standards. The interindividual variability of the hematocrit was also considered an uncertainty component, and its contribution was estimated. Depending on concentration the relative standard uncertainty varied from 1.6% to 5.0%. The analytical uncertainty was the dominating uncertainty component, accounting for around 90% of the variance.

Keywords: Uncertainty budget, BAC, Head-space gas chromatography

P44

The Detection of Cocaine Metabolite in Urine with an ONLINE® DAT II Immunoassay

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ONLINE® COCAINE II is a homogeneous immunoassay for the detection of cocaine metabolite in urine at concentrations ranging from 0-5000 ng/mL. The assay system utilizes the KIMS technology and consists of two liquid, ready-to-use reagents. The R1 reagent contains a drug-polymer conjugate with an accelerant, and the R2 reagent contains a monoclonal, benzoylecgonine-specific antibody that is covalently bound to polystyrene microparticles. Studies summarized herein include evaluations of the 150 and 300 ng/mL cutoff assays on the Roche/Hitachi and COBAS INTEGRA® clinical analyzers.

Within run precision studies have been performed by running 20 replicates of benzoylecgonine controls in the semi-quantitative mode on the Hitachi 917. The %CV results of the 75, 113, 150, 188, 225, 300, 375, and 3000 ng/mL controls are 3.8%, 2.6%, 1.6%, 1.9%, 1.7%, 1.3%, 1.0%, and 1.2%, respectively. The respective %CV results for the Integra 700 assay are 7.5%, 4.4%, 3.8%, 2.8%, 2.7%, 2.3%, 2.0%, and 2.3%. The inter-assay precision (n=20 reps x 5 days, with 5 calibrations) on the same controls range from 1.4% to 5.2% on the Roche/Hitachi 917 and from 3.4% to 9.2% on the COBAS INTEGRA® 700. Both the 150 and 300 ng/mL assays are contained in one semi-quantitative curve with six calibrators. The clinical sensitivity of the assay, performed by running 100 clinically negative samples (mean $\pm$ 2 S.D.), is 32.6 ng/mL on the Hitachi 917 and 27.8 on the Integra 700. Testing of 50 GC/MS confirmed clinically positive samples results in positive values with the ONLINE® COCAINE II assay. Screening of samples that challenge the 150 ng/mL cutoff (113-188 ng/mL) and the 300 ng/mL cutoff (225-402) all give the correct qualitative response. The assay is specific for benzoylecgonine with cross-reactivity of less than 2% to cocaine, 0.5% to cocaethylene, and 0.1% to ecgonine, ecgonine methyl ester, and norcocaine. After stressing reagents at 35° for 10 weeks, performance is equivalent to unstressed reagents, demonstrating >18 months shelf life. The above studies demonstrate that the ONLINE® COCAINE II assay provides an accurate and reliable urine screening method for the detection of cocaine use.

Keywords: Cocaine, ONLINE®, Immunoassay

P45

Effect of Benzodiazepines on the In Vitro Metabolism of Buprenorphine in Human Liver Microsomes

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Buprenorphine is a partial mu agonist. A sublingual formulation is used as an anti-abuse medication in opioid dependent individuals, while an intravenous formulation is used for treatment of pain. In human liver microsomes (HLM) the depletion of buprenorphine exceeds the formation of norbuprenorphine by N-dealkylation, suggesting other pathways of metabolism exist. Both buprenorphine depletion and N-dealkylation are performed to a large extent by cytochrome P450 3A4. Cytochrome P450 3A4 is a common site for drug interactions due to inhibition of the enzyme. As an initial study on the in vitro inhibition of buprenorphine metabolism, we focused on benzodiazepines because of their established adverse interaction with buprenorphine.

Inhibitors may or may not be metabolically-activated, the former are more potent when preincubated with HLM. Benzodiazepines (or benzodiazepine metabolite) at therapeutic concentrations were added just before initiation of the reaction with the NADPH-generating system, with buprenorphine (50 nM) added at this time or after a 15 minute pre-incubation. The experiments were performed in HLM from 3 different donors, each in duplicate. Buprenorphine and norbuprenorphine concentrations were determined by liquid chromatography-mass spectrometry.

While no benzodiazepine was a potent inhibitor at the concentrations tested, a number caused significant reductions from controls and may warrant further study. The following hypothesis will be tested when selecting candidates for further study. Compounds that produced a significant inhibition with 15 minute preincubation that was greater the inhibition produced with 0 minute preincubation will be further studied for metabolism-activated inhibition. The following benzodiazepines (concentration tested in nM) met this first criteria: clonazepam (100), 3-hydroxy-7-acetamido-clonazepam (100), 7-acetamido-nitrazepam (100), triazolam (100), α-hydroxy-triazolam (100), demoxepam (2500), and midazolam (500). Compounds that did not show a preincubation time dependence, but produced greater than 15% inhibition will be further studied for non-metabolic dependent inhibition. Flunitrazepam ((100), 7-amino-flunitrazepam ((100), 7-amino-nitrazepam (100), alprazolam (100), chlordiazepoxide (2500), norchlordiazepoxide (2500), temazepam (2500), oxazepam (2500), nordiazepam (500), estazolam (500) and lorazepam (500) all met this latter criteria. Norflunitrazepam (100), nitrazepam (500), α-hydroxy-alprazolam (100), flurazepam (100) and diazepam (2500) did not meet either criteria.

Benzodiazepines have only a modest capacity to inhibit buprenorphine metabolism at therapeutic concentrations. Further studies are needed to determine if any of these act thru metabolically-activated mechanisms, particularly as suicidal substrates; the latter may be capable of greater inhibition with more prolonged exposure to the enzyme. (Supported by R01 DA10100)

Keywords: Buprenorphine, Benzodiazepines, In vitro inhibition

P46

Metallic poisoning case: Interpretation of Results

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The toxicological analyses of biological samples pertaining to metallic poisoning cases usually result from surrounding environmental contamination. Forensic analyses in such cases lead to a considerable amount of variability in expert opinions. Sample preparation and the analysis of the metallic elements are well standardized; the techniques commonly used being atomic absorption spectrophotometry (AAS), Inductively Coupled Plasma atomic emission spectroscopy (ICP-AES) and Neutron Activation Analysis (NAA). The amount of sample, the time in transit and analysis of the sample does not lead to loss of the element. The hazardous toxic metals and metal compounds used in industry are potential causes of metallic poisoning, referred to in the present scenarios. The use of the metallic element/compounds used in the particular industry and their possible toxic effects should be studied before arriving at the conclusion. As most elements have an essential trace role for normal body function, metal concentrations need to be evaluated carefully in that context. In this presentation, three cases are discussed; ingestion of copper sulphate; a suspected poisoning case with chromium detected; a deceased individual found near a zinc sulphate tank. Analytical results from ICP-AES in case of metallic poisoning are presented. The measurement wavelengths used were as follows: Zn l213.856 nm, Cr l205.552 nm, Cu l324.754 nm. The ICP results of the elements present in the exhibits (viscera: stomach, small intestine, liver, spleen, kidney) were compared. To ascertain whether the metal concentrations were toxic, a comparative study of three cases in the same area was carried on visceral organs (stomach and small intestine, liver, spleen, kidney) to assess the normal background levels of the elements and to assist in interpreting the analytical results. The paper discuses the case study and the methodology adopted in solving it.

	Stomach and small intestine (ppm)	Liver (ppm)	Spleen (ppm)	Kidney (ppm)
Case 1 - Zn	51	68	89	70
- Cr	5	6	8	10
- Cu	7873	7500	7032	7298
Case 2 - Zn	55	60	77	84
- Cr	139	150	145	130
- Cu	1200	1360	1350	1250
Case 3 - Zn	297	280	260	240
- Cr	6	7	3	5
- Cu	1600	1700	1648	1768
Blank – Zn (48)				
- Cr (4)				
- Cu (153)				

Keywords: ICP-AES, Environmental metallic contaminants, Forensic toxicology

P47

Complexometric Determination of Diazepam

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The benzodiazepine group of drugs which includes diazepam, are commonly used as sedatives for inducing sleep, for insomnia patients, and patients suffering from pain. Diazepam is misused by mixing with eatables like biscuits, soft drinks, and food items during train journey in order to sedate a person, so as to steal their belongings. In the recent past, instances of mixing diazepam in biscuits and soft drinks have been reported by law enforcement agencies. Therefore it was necessary to develop a simple and rapid method for the qualitative and quantitative analysis of diazepam. Complexometric determination of diazepam was carried using Dragendorff reagent (DD). Dragendorff reagent forms complexes with nitrogenous bases. It is orange in color and forms a reddish pink color with diazepam in chloroform. Different standard concentrations of diazepam were prepared in chloroform and the UV absorbance characteristics of the spectra of the complex with DD was found to be linear. The absorbance maxima wavelength range of diazepam is 227-230 nm and in the DD complex is 536-540 nm. The stoichiometric complex that was formed with diazepam was used to estimate diazepam quantitatively. The method developed can be used for routine analysis of diazepam in forensic crime cases or modified as a post derivative reagent for HPLC analysis of diazepam.

Keywords: Diazepam, Complexing reagent, UV