Abstracts
Platform Presentations

S1 Mother's Little Helper: Analysis of Common Benzodiazepines by LC/MS

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Benzodiazepines are a class of psychoactive drugs prescribed for relief of anxiety and depression. The primary objective was to develop a method to quickly extract and resolve 25 common benzodiazepines and metabolites in urine specimens by liquid chromatography mass spectrometry (LC/MS). In addition, the laboratory aimed to quantify five benzodiazepines commonly analyzed for in pain management patients. Benzodiazepines were extracted from 2 mL of alkalinized urine with 2 mL of ethyl acetate. Benzodiazepines were separated by liquid chromatography using a Zorbax phenyl column with a mobile phase of 40% 10 mM ammonium formate in methanol. Quantitation was performed using selected ion monitoring mode on a Waters ZMD mass spectrometer. The linear range of the assay for lorazepam, oxazepam, nordiazepam and temazepam was 50-1000 ng/mL and 20-250 ng/mL for α-hydroxy-alprazolam. Precision and recovery tests yielded coefficients of variation less than 12 and percent recoveries of 96-136%, for all analytes. The method developed is simple, fast, inexpensive and robust for the extraction, separation, identification and quantification of benzodiazepines and metabolites in urine.

Keywords: **Benzodiazepines, LC/MS, Urine**

S2 A Direct Comparison of GC/MS and LC/MS/MS Analysis of Urine for Benzoylecgonine, Morphine, Codeine, and 6-Acetylmorphine

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On November 25, 2008, the U.S. Department of Health and Human Services posted a final notice in the Federal Register authorizing the use of liquid chromatography/tandem mass spectrometry (LC/MS/MS) and other technologies in federally regulated workplace drug testing (WPDT) programs. These rules will become effective in May 2010. To support this change, it is essential to explicitly demonstrate that LC/MS/MS as a technology can produce results at least as valid as gas chromatography/mass spectrometry (GC/MS), the long-accepted standard in confirmatory analytical technologies for drugs of abuse and currently the only confirmatory method allowed for use in support of federally regulated workplace drug testing programs.

A series of manufactured control urine samples (n= 10 for each analyte) containing benzoylecgonine, morphine, codeine, and 6-acetylmorphine at concentrations ranging from 10% to 2000% of federal cutoffs were analyzed with replication by five federally regulated laboratories using GC/MS (5 replicate analyses per lab) and at RTI International using LC/MS/MS (10 replicate analyses). Interference samples as described in the *National Laboratory Certification Program 2009 Manual* were also analyzed by both GC/MS and LC/MS/MS. In addition, matrix effects were assessed for LC/MS/MS, and both analytical technologies were used to analyze previously confirmed urine specimens of WPDT origin (n= 60).

Results indicated that LC/MS/MS analysis produced results at least as precise, accurate, and specific as GC/MS for the analytes investigated in this study. Matrix effects, while evident, could be controlled by the use of matrix-matched controls and calibrators with deuterated internal standards. LC/MS/MS data parameters such as retention time and product ion ratios were highly reproducible.

Keywords: **GC/MS, LC/MS/MS, Urine**

S3 Comparing the Efficacy of Sweat Testing to Urine and Oral Fluid Opiate Analysis in Methadone-Maintained Pregnant Women

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Methadone is the only recommended pharmacotherapy in the US for opioid-dependent pregnant women. Drug relapse to heroin is reduced, and obstetrical and neonatal outcomes are improved with methadone given in the context of comprehensive care. Currently, illicit opioid use in most treatment programs is monitored with urine tests; oral fluid and/or sweat analyses may be effective alternatives.

The efficacy of sweat, urine and oral fluid testing were compared to identify opioid consumption among 15 methadone-maintained women throughout gestation. Gas or liquid chromatography (tandem) mass spectrometry quantified heroin, 6-acetylmorphine (6AM), 6-acetylcodeine (6AC), morphine, codeine, normorphine, norcodeine and morphine and codeine glucuronides. Results were evaluated at our analytical limits of quantification (LOQ) for all analytes and proposed cutoffs for SAMHSA-mandated analytes morphine, codeine, and 6AM. When present, heroin, 6AM and/or 6AC documented heroin use. Furthermore, comparisons were based on the number of urine or oral fluid specimens collected while wearing a single sweat patch for approximately one week (range 3 – 14 days): 1 or ≥2 specimens per patch. Forty-seven sweat patches had 1 and 53 patches ≥2 urine specimens collected during the wear period. Thirty-one patches had 1 associated oral fluid specimen and 89 had ≥2.

At the method LOQ for any biomarker, 55-70% agreement was observed between urine and sweat, and 40-55% for oral fluid and sweat. Agreement substantially improved for both matrices (~90%) at the SAMHSA cutoff, as most specimens were negative. Urine and oral fluid more often contained an opiate biomarker ≥LOQ, as compared to sweat. Limiting results to morphine, codeine or 6AM ≥LOQ, sweat testing was equally sensitive as a single urine or oral fluid specimen; however, if multiple urine or oral fluid specimens were obtained, they better identified opiate consumption. At SAMHSA cutoffs, sweat testing detected more positive specimens than a single urine or oral fluid test, and similar numbers when multiple matched oral fluid and urine specimens were available.

The ability of each specimen type to clearly identify heroin abuse also was evaluated. 87% of opiate-positive sweat patches, 62% of oral fluid specimens, and only 6% of urine specimens specifically identified heroin biomarkers ≥LOQ. These are some of the first data directly comparing the efficacy of simultaneously collected sweat, oral fluid and urine specimens in detecting relapse in opioid-dependent patients. These data will help inform the decisions of drug-treatment programs in selecting the most appropriate drug monitoring system for their clinical needs.

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Keywords: **Oral Fluid, Sweat, Methadone**

S4 Determination of β-Hydroxybutyrate (BHB) in Blood and Urine using Gas-Chromatography-Mass Spectrometry (GC-MS)

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β-hydroxybutyrate (BHB) is considered as a potential biomarker for alcoholic ketoacidosis (AKA). A robust and sensitive method was developed and validated for the quantitative determination of BHB in post-mortem blood and urine using deuterated gamma-hydroxybutyrate (GHB-d6) as an internal standard. Samples were analysed by gas chromatography-mass spectrometry (GC-MS) following liquid-liquid extraction and silyl derivatization. The limits of detection and lower limits of quantification in blood and urine were 2 and 7 mg/L and 2 and 6 mg/L respectively. The inter-day and intra-day precision was measured by coefficients of variation (CV%) for blood and urine and ranged from 1.0 to 12.4% for quality control samples spiked at 50 and 300 mg/L. The linear range of 50-500 mg/L resulted in an average correlation of $R2 > 0.99$ and the average extraction recovery in blood and urine was equal to or greater than 82% and 59% respectively. BHB remains stable in blood spiked at a concentration of 300 mg/L for 15 days when stored within a refrigerator (2-5 °C). Post-mortem blood and urine samples were analysed using the validated method for cases where the deceased had a history of chronic alcohol abuse to establish the use of BHB as a potential marker of alcoholic ketoacidosis.

Keywords: **β-Hydroxybutyrate, GC/MS**

S5 A Two-Dimensional Gas Chromatography/Electron-Impact Mass Spectrometry Method for the Simultaneous Detection of Cannabidiol, Δ^9-Tetrahydrocannabinol (THC), 11-Hydroxy-THC (11-OH-THC) and 11-Nor-9-Carboxy-THC (THCCOOH)

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Background: Cannabis sativa contains over sixty cannabinoids, including Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD). The ratio of THC:CBD in illicit cannabis in the US is approximately 20:1. Sativex®, a whole-plant cannabinoid extract oromucosal spray, contains THC and CBD in a ratio of nearly 1:1. Sativex® was approved for neuropathic pain in multiple sclerosis (MS) in Canada and will be evaluated in Phase III trials in the U.S. for cancer pain analgesia.

Experimental: Chromatographic separation of analyte from matrix was accomplished using a primary ZB-50 column (30 m x 0.25 mm x 0.25 μm; Phenomenex) and a secondary DB-1ms column (15 m x 0.25 mm x 0.25 μm; Agilent Technologies) connected in series via a microfluidic Deans switch in an Agilent 6890 gas chromatograph. Derivatized extracts (4 uL) were injected into the front inlet operated in pulsed splitless operation mode. Heart cuts (0.3-0.4 min) were made surrounding each analyte peak and flow was diverted to the secondary column. An air-cooled cryogenic focusing trap was positioned at the head of the secondary column and "trapped" or condensed analyte "cuts" at 100°C. Cryotrap temperature was increased at 700°C/min to 275°C and analytes were released for chromatographic separation on a secondary column. Final detection was accomplished with an Agilent 5973 mass selective detector operated in electron impact mode.

Results: Linearity was assessed with least squares regression with an applied 1/x weighting factor. Linear dynamic ranges were 0.25-25 ng/mL for CBD and THC and 0.125-25 for 11-OH-THC and THCCOOH. To quantify elevated concentrations, a high THCCOOH calibration curve (25-75 ng/mL) was constructed by modifying injection parameters. All calibrators were within 15% of target. Coefficients of determination were >0.990 for all calibration curves. Extraction efficiencies were >74.4, 73.7, 84.7 and 78.9 for CBD, THC, 11-OH-THC and THCCOOH, respectively. No exogenous interferences were observed when analyzing over-the-counter medications, other illicit drugs, and drug metabolites. Ten blank plasma pools were evaluated for endogenous interferences and had no quantifiable analyte peaks at method LOQ. Analytes were stable in fortified plasma for 3 freeze/thaw cycles, when stored at 4°C or room temperature for 16h.

Conclusion: We report a sensitive chromatographic method for simultaneous quantification of CBD, THC, 11-OH-THC, and THC-COOH in human plasma. This method is effective for monitoring the therapeutic use of Sativex® and the detection of plasma cannabinoids in forensic investigations.

This research was supported by the Intramural Research Program, NIH, National Institute on Drug Abuse.

Keywords: **THC, Cannabidiol, Cannabinoids, Plasma, Two-Dimensional Chromatography**

S6 Effect of SPE and Derivatization Conditions on GC/MS and LC/MS/MS Detection Sensitivity of Multi-Functional Ractopamine

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Ractopamine belongs to Beta-agonist compounds and has been often used as growth-promoting agent to improving the feed efficiency and enhance lean meat to fat ratio in many animal species. It is a forbidden molecule as feed additive in most countries because of the many reported collective intoxication outbreaks in humans. Unlike most commonly abused drugs, ractopamine is a multi-functional organic compound with high boiling point and poor thermal/chemical stability as it contains phenol, hydroxyl and secondary amine moieties. Due to these properties, ractopamine is not suitable for GC/MS analysis without derivatization. So far, a lot of chromatography analytical methods with detection limit around 5ng/ml have been reported, but little effort have been paid to achieve the desired detection sensitivity below 1ng/ml (or 1ng/g) to meet the requirements of zero tolerance policy. Due to its poor solubility, it is very difficult to derivatize the ractopamine HCl salt with BSTFA without neutralizing it first with base. After optimizing the SPE and derivation conditions, a highly sensitive GC/MS method with detection limit at 0.5ng/ml has been developed. Interestingly, the sample treatment procedure, derivation solvent composition, heating temperature and heating time have significant effect on the detection sensitivity of ractopamine HCl salt, but have little effect on structurally related but more soluble isoxuprine HCl salt used as internal standard. By neutralization of ractopamine HCl salt first with ammonium hydroxide and adding organic solvent such as ethyl acetate into BSTFA derivatization reaction, and followed by heating it at 85^{o}C for at least 45minutes, the ultra detection limit can be easily improved from less than 5ng/ml to 0.5ng/ml. We also examined the LC/MS analysis of ractopamine HCl salt and free ractopamine, the detection sensitivity between ractominine HCl.salt and free ractomine is almost identical at 0.1ng/ml as there is no derivatization step involved and both ractominine HCl.salt and free ractomine are readily soluble in injection solvent MeOH. Both GC/MS and LC/MS methods are able to detect free ractopamine in ractopamine positive urines up to 6 days after the withdrawal of the drug. This will greatly improve the detection window of ractopamine in urine because after the withdrawal of the drug for 2 days the concentration of ractopmine and its metabolites could be below 5ng/ml.

Keywords: **Derivatization, SPE, GC/MS, LC/MS/MS**

S7 Postmortem Study of Fentanyl

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Fentanyl is a potent opioid used by patients for chronic pain from cancer, arthritis and some fractures. One of the major side effects for fentanyl is respiratory depression, which may lead to death. Postmortem toxicologists have the ability to assist the Medical Examiner with the interpretation of fentanyl concentrations and aid in the decision on the appropriate cause and manner of death. A study was conducted to evaluate fentanyl stability through reanalysis, evaluate vitreous humor as a comparable specimen to blood for interpretation purposes, and measure fentanyl concentration in gastric contents as an indicator of oral route of administration.

The analysis of fentanyl from postmortem specimens (3-mL or g sample size) consisted of a basic liquid-liquid extraction followed by confirmation and quantitation on a GC/MS. Central and femoral blood specimens were preserved with sodium fluoride, whereas all other specimens such as liver, bile, urine, gastric contents, and vitreous contained no preservative; all specimens were maintained at a refrigeration temperature.

The fentanyl stability study consisted of the reanalysis of 153 specimens (central and femoral blood, vitreous, liver, urine, and bile) that ranged from 1-43.6 months from date of original analysis; 74% of the specimens had fentanyl concentrations that were within 20% of the original analysis.

Fentanyl concentrations were measured in 41 vitreous humor specimens with 35 having a corresponding central blood, 28 having a femoral blood specimen, and 20 having a liver specimen for comparison. The vitreous fentanyl concentration averaged 18.4 ng/mL (2.5-101 ng/mL), with a median of 12.0 ng/mL and a standard deviation of 18.4. It was determine that vitreous humor/femoral blood ratio had an average ratio of 0.96 and the median of 0.90, which made a good correlation.

A total of 27 cases had gastric contents submitted and one had a history of fentanyl oral ingestion. The total fentanyl amount measured in the one gastric contents of known oral fentanyl was 3403.14 ug. For the remaining cases with gastric contents, the total fentanyl amount averaged 89.19 ug (0-1,118.14 ug) and a median of 4.27 ug.

Fentanyl is a frequently used medication that can pose challenges for the interpretation of postmortem concentrations and cause/manner of death. This study exemplified that fentanyl was stable over a wide array of specimens 74% of the time. The vitreous humor specimen had good correlation with the femoral blood and could be used for analysis or interpretation in cases where central or femoral blood was not available. The study also demonstrated that Fentanyl concentration in excess of ~3,400 ug may be indicative of oral ingestion.

Keywords: **Fentanyl, GC/MS, Portmortem**

S8 The Contribution of Hepatic CYP26A1 to All-Trans-Retinoic Acid Clearance

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Retinoic acid (RA) is the biologically active form of vitamin A. RA can exist as three different isomers: all-trans RA (at-RA), 9-cis-RA, and 13-cis-RA. The main source of RA in humans, is via synthesis from dietary precursors, however at-RA or tretinoin (Vesanoid®) and 13-cis-RA or isotretinoin (Accutane®) are administered clinically. Tretinoin is used to induce cytodifferentiation and decrease proliferation of acute promyelocytic leukemia whereas isotretinoin is used in the treatment of severe nodular acne that has been unresponsive to other forms of treatment. Both too much and too little RA has been shown to be detrimental for health in both developing embryos and adults. Auto-feedback induction of RA clearance during RA therapy is a significant clinical challenge. It is generally believed that the decreased peak plasma concentrations and AUCs observed after repeated dosing of RA is due to induction of metabolic enzymes. Additionally, metabolic drug interactions have been reported during RA therapy.

Several P450s are shown in the literature to metabolize at-RA. CYP2C8 was reported to be the most efficient P450 at metabolizing at-RA to 4-OH-RA, with intrinsic clearance values ranging from 6.1 – 1410 μL/min/nmol P450. CYP3A4 and CYP2C9 showed similar kinetic parameters and were the second most efficient hepatic P450s, with intrinsic clearance values ranging from 2.5- 20 uL/min/nmolP450. The large variability in the numbers reported was likely due to variable experimental conditions. A newer P450, CYP26A1 is now proposed to be the primary RA hydroxylase in the body. We recently expressed and performed a biochemical characterization of this enzyme. We demonstrated that the intrinsic clearance of at-RA by CYP26A1 is more than 800-fold higher than the clearances previously reported by others for CYP2C8.

The goal of this study was to determine the relative contribution of CYP26A1 to the clearance of at-RA, using in-vitro to in-vivo extrapolation. We screened a panel of P450s using baculovirus-infected insect cells (supersomes^{TM}) at appropriate protein concentrations to determine the enzymes that can metabolize at-RA. While not as efficient as CYP26A1, CYP2C8, CYP3A4, CYP3A5, and CYP3A7 all participated in the metabolism of at-RA. We then performed incubations to accurately determine the kinetic parameters for these enzymes.

Using protein quantification of CYP2C8, CYP3A4, CYP3A5, and CYP26A1 and kinetic parameter data, we predicted the contribution of CYP26A1 and other P450s, in hepatic clearance of at-RA.

Based on this preliminary data, it appears that even when CYP26A1 is expressed in low amounts, it is still be the primary enzyme responsible for clearing endogenous at-RA from the liver. However, when at-RA is administered endogenously, CYP26A1 will become saturated and the contribution of other P450s will increase.

Keywords: **Retinoic Acid, CYP26A1**

S9 In Vitro Stability of Salvinorin-A in Human Blood at Various Temperatures and Time

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An increase in abuse of Salvinorin-A, a psychoactive compound, has led to higher demands for the establishment of proper collection and storage techniques of biological specimens containing this compound. Stability of Salvinorin-A is established by storage at refrigerated and room temperatures in human blood samples with and without esterase inhibition. Samples are extracted using solid phase extraction followed by quantitation by Liquid Chromatography Mass Spectrometry. Refrigerated temperatures (5°C) showed the least degradation (8%) as compared to room temperature (23%) when stored in serum for one week. Plasma showed the most degradation of Salvinorin-A, at 58% (25°C), whereas concentrations in serum decreased significantly less, at 23% (25°C) over one week. Collection in tubes containing sodium fluoride, an esterase inhibitor showed the most stability (12%) as compared to serum (23%) and plasma (26%) over two weeks. Therefore, Salvinorin-A is most stable in human blood samples collected in tubes containing sodium fluoride stored at a refrigerated temperature.

Keywords: **Forensic Science, Salvinorin-A, Esterase Inhibition**

S10 11-Nor-Δ^9-Tetrahydrocannabinol-9-Carboxylic Acid (THCA) and 11-Nor-Δ^9-Tetrahydrocannabivarin-9-Carboxylic Acid (THCVA) in Urine - The Problem of Adsorption to Surfaces Such As Glass

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The analysis for 11-Nor-Δ^9-Tetrahydrocannabinol-9-Carboxylic Acid (THCA) in urine is commonly used to determine if an individual is using marihuana or a Δ^9-tetrahydrocannabinol (THC) formulation such as Marinol. The compound Δ^9-tetrahydrocannabivarin is also present in marihuana. Its major urinary metabolite is 11-Nor-Δ^9-Tetrahydrocannabivarin-9-Carboxylic Acid (THCVA). Analysis for THCVA in urine is helpful to verify marihuana use versus THC formulation use. Adsorption of THCA to surfaces such as glass and plastic that result in analytical inaccuracies have been reported. Whether this is an issue with THCVA is not yet known. Our laboratory noted that in-house prepared urine controls frequently had measured THCA concentrations below the target while the THCVA was usually closer to target. These in-house controls were prepared by adding THCA/THCVA working solutions and blank human urine to a separate glass volumetric flask for each control [3 (L), 20 (M), and 80 ng/mL (H)]. After mixing, each control was transferred to a separate beaker to facilitate the subsequent transfer of 1 mL aliquots to separate glass tubes. The tubes were stored frozen until use. For the analysis, a liquid-liquid extraction procedure, HFIP/TFAA derivatization and GC-MS negative chemical ionization were employed. The assay used a 1 mL volume and calibrators ranged from 1 to 100 ng/mL. For these controls (2 runs, total n=6), the mean THCA concentrations (% of target in parentheses) for the controls were L: 2.18 ng/mL (72.6 %), M: 17.1 ng/mL (85.6 %), H: 72.3 (90.4 %); the mean THCVA concentrations were L: 2.95 ng/mL (98.3 %), M: 21.1 ng/mL (105.4 %), H: 84.5 ng/mL (105.6 %). In an attempt to minimize adsorptive losses, the in-house control preparation was modified by transferring the accurate volume of blank urine and THCA/THCVA working solutions to a separate glass beaker for each L, M, and H controls. Aliquots (1 mL) of the control were then transferred to separate glass tubes and stored frozen. The modified control preparation procedure eliminated one step that had the potential for adsorptive losses. In the controls made by the modified procedure (9 runs, n=24), the mean THCA concentrations were L: 2.94 ng/mL (98.0 %), M: 19.3 ng/mL (96.5 %), H: 73.1 ng/mL (91.3 %) and the mean THCVA concentrations were L: 2.90 ng/mL (96.7 %), M: 18.2 ng/mL (91.0 %), H: 72.1 ng/mL (90.1 %). Analysis of 200 ng/mL dilution controls (DC) that were stored as 1 mL or 0.1 mL volumes also showed the importance of reducing procedural steps where adsorption can occur. For 1 mL stored DC, 0.1 mL of the DC was transferred to the extraction tube at assay time and the THCA was only 66.5 % of target while the THCVA was 103.9 % of target. For the 0.1 mL stored DC, the storage tube was assayed directly without an additional transfer step and the THCA was 111.0 % of target while the THCVA was 104.1 % of target. By eliminating transfer steps in the control preparation, potential adsorptive losses were reduced and THCA concentrations in the controls were closer to target. It appears that THCVA was not impacted by adsorption. Since THCVA has a C_3H_7 side chain and THCA has a C_5H_{11} side chain, the more lipophillic nature of THCA could account for its affinity to surfaces such as glass.

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Keywords: **Cannabinoids, Metabolites, Adsorption**

S11 Methadone and Cocaine Related Deaths in Massachusetts

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In the U.S. for the past several years there has been an increase in the number of cases positive for methadone [MTD] in medico-legal death investigations. Death following co-ingestion of other CNS depressants typically results in lower blood MTD concentrations than when administered alone. The mechanism of death in these instances is probably respiratory depression. MTD is reported to increase the length of the QT interval resulting in torsade de pointes. The objective of the current study was to evaluate MTD related deaths in the presence of another drug known to increase the QT interval, namely cocaine [COC].

All 2008 cases from the Office of the Chief Medical Examiner, State of Massachusetts, were reviewed. A total of 230 cases were identified for inclusion based on confirmation of MTD in either postmortem blood (central or peripheral) or antemortem blood/serum. Blood specimens were screened for MTD by ELISA (50 ng/mL cut-off calibrator) and/or gas chromatography/mass spectrometry [GC/MS] (approximate LOD 25 ng/mL) and subsequently quantitated and confirmed by solid phase extraction [SPE] followed by GC/MS-SIM using a deuterated internal standard. A six point calibration curve was generated in the linear range 50-1000 ng/mL. Blood specimens were screened for benzoylecgonine [BE] by ELISA (50 ng/mL cut-off calibrator) and/or COC by GC/MS (approximate LOD 50 ng/mL) and subsequently quantitated and confirmed by SPE extraction followed by GC/MS-SIM for COC, BE and cocaethylene [CE] using deuterated internal standards. A six point calibration curve was generated for each analyte in the linear range 50-2000 ng/mL.

The decedents ranged in age from preterm fetus to 76 years, with an average age of 43. The majority (69%) were male. Blood concentrations of MTD ranged from <25 ng/mL to 3,917 ng/mL with an average concentration of 650 ng/mL (N=219). The range and mean concentrations of MTD were similar for central (mean=667 ng/mL, N=73) and peripheral blood (mean =648 ng/mL, N=132). There was a statistically significant ($p<0.05$) difference in mean blood concentrations between males (mean=556 ng/mL, N=148) and females (mean=848 ng/mL, N=69).

The average concentration of MTD in COC/CE/BE positive cases was 498 ng/mL (N=67), which was statistically significantly lower ($p<0.05$) than cases without COC/CE/BE (mean= 715 ng/mL, N=163). For cocaine and metabolite positive cases, MTD concentrations in females (N=18) and males (N=49) were not statistically different ($p=0.24$). The presence of active (COC or CE) or inactive drug (BE) did not result in significant differences in MTD concentrations (mean, 566 and 469 ng/mL, respectively). These data suggest there may be a pharmacodynamic interaction between methadone and cocaine which warrants further investigation.

Keywords: **Methadone, Cocaine, QT Interval**

S12 Blood Cannabinoids Levels and Effects on Cognitive Performance and Behavior as a Function of Joint Smoking Procedure and Inhalation Technique

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Studies on the effects of cannabis smoking on cognitive and behavioral performance in humans are influenced by the great diversity by which the subjects prepare and smoke cannabis joints. In toxicological studies, joints are generally smoked according to a paced procedure, with a fixed duration of inhalation, breath-holding and interpuff interval. This should provide a greater dosing consistency across subjects and reduce the risks of under- or over-dosage.

We present a case report involved in a placebo-controlled, double blind cannabis smoking administration study in which one subject out of 22 had smoked a joint (11% THC) at 2 separate sessions, once as usual according to the same protocol as the other volunteers, a second time according to an imposed and more carefully controlled procedure. This male subject, aged 26, was an occasional cannabis smoker (about 2 joints per month) since 3-4 years.

Resulting in a very low THC blood concentration (highest level: 3.3 ng/ml), the first joint had been puffed without inhaling ("crapoter"). In the second session in which keeping the smoke in the mouth without inhaling was not possible, the whole blood THC concentration measured by GC-MS/MS reached a maximum value of 94.6 ng/ml.

"Crapoter" or smoking the placebo did not alter the heart pulse rate while a 43%-increase was observed after effective smoking. A strong, moderate, and inexistent feeling of "high" and of intoxication were felt after effective or ineffective inhalation of the cannabis joint, or of the placebo, respectively. A similar decrease or lack of change was observed in the self-assessment of the capability to drive and the willingness to drive under various fictitious circumstances.

Surprisingly, a slight decrease in critical task performance was noticed after inefficient smoking only. Quantification of the performance by measuring the precision of the behavioral responses in a fMRI dual tracking task with distractors (i.e. % of time of correct tracking and reaction times to distractors) showed a decrement in the performance after inefficient and efficient smoking compared to the control condition.

By comparing BOLD responses between the efficient THC inhalation and placebo condition, we found an activation pattern comparable to the same results in the whole smoking group. The same comparison between the inefficient inhalation and the placebo condition failed to reveal the same differences. However, robust differences were mainly observed in a statistical group analysis.

These results show that standardization of the cannabis smoking procedure is difficult and that self-report of cannabis usage, for epidemiological investigations remains unconvincing unless confirmed by cannabinoids quantification.

Keywords: **Cannabis Smoking Procedure, Blood Concentrations, Cognitive Performances**

S13 Development of a Novel Homogeneous Enzyme Immunoassay Method for the Detection of Carisoprodol and Meprobamate in Urine

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Carisoprodol (Soma®/isopropylmeprobarmate) and meprobamate are widely prescribed musculoskeletal pain relief drugs and are frequently identified drugs associated with DUI cases. Carisoprodol has a short elimination half –life of 1- 3 hours; however its major active metabolite, meprobromate has a longer elimination half –life of 6 -17 hours. For that reason it is important for an immunoassay to detect both compounds. Carisoprodol is administrated in 340mg/per dose that yields less than 1% carisoprodol and about 4.7% of meprobamate in a 24 hour urine sample. On the other hand, meprobamate can be administered in much larger dosages (up to 2.4g/per day) that will yield much higher concentrations (20-176 μg/ml) in urine.

Since the concentrations of meprobamate in urine are up to 10 times higher than carisoprodol, this newly developed homogeneous enzyme immunoassay (HEIA) can be used to measure both carisoprodol and meprobamate with a single assay despite the lower cross reactivity (20%) for meprobamate . Intra-assay coefficient of variation (CV%) for the assay is less than 10%. Furthermore, this homogeneous assay was also validated with a total of 70 urine samples that had been quantitatively analyzed by gas chromatography/mass spectrometry (GC/MS) with concentrations ranging from 0 to 20,000ng/mL for both drugs. The accuracy was found to be 100% when the immunoassay cutoff concentration was set at 100ng/mL for carisoprodol and meprobamate.

		Gas chromatography/Mass spectrometry (GC/MS)	
		Positive	Negative
HEIA	Positive	38	0
	Negative	0	32

A high throughput and highly reliable HEIA has been developed for the detection of carisoprodol and meprobamate in human urine. It has been validated by comparison with GC/MS. This is the first report of a homogeneous immunoassay for carisoprodol and meprobamate.

Keywords: **Carisoprodol; Meprobamate; Enzyme Immunoassay**

S14 The QuEChERS Approach: Evaluation of a Novel Sample Preparation Method for Analysis of Multiple Drug Classes in Urine

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Although screening methods for multiple drug classes by LC-MS/MS are gaining popularity, GC-MS instrumentation is still very prevalent due to lower instrument cost, established methods, and ease-of-use. One of the main advantages of LC-MS/MS analyses is the ease of sample preparation, which often requires little to no time. A new sample preparation method called QuEChERS is rapidly gaining popularity in the food safety market because it allows for fast, straightforward sample preparation of a wide variety of matrices for a broad spectrum of analytes. QuEChERS is a two-step procedure consisting of a simple shake extraction followed by a dispersive solid phase extraction (dSPE) cleanup of extract, which is then ready for GC-MS and/or LC-MS/MS analysis. The objective of this project was to evaluate the QuEChERS approach for high-throughput GC-MS screening of multiple drug classes in biological matrices.

Urine samples were spiked with several classes of abused drugs including cannabinoids, cocaine, amphetamines, opiates, benzodiazepines, and opioids. The spiking mixture included both parent compounds and metabolites. Samples were deconjugated with potassium hydroxide. The samples were then extracted with acetonitrile solvent and a mixture of $MgSO_4$ and NaCl. After extraction, samples were cleaned up using different combinations of dSPE sorbents including C18, PSA, and graphitized carbon black (GCB). After cleanup, samples were concentrated and analyzed. Derivatization methods were also explored.

The use of dSPE sorbents does effect an acceptable cleanup, however PSA gave reduced recoveries for compounds capable of hydrogen bonding, such as carboxy-THC. C18 removed many hydrophobic interferences, but it was found that GCB effected the best overall extract cleanup while preserving analytes of interest. The use of toluene improved analyte recoveries when performing GCB cleanup. The LOD for derivatized cannabinoids was estimated to be between 2.5 and 10ng/mL, and linearity was 0.9994 over a range of 2.5 – 100ng/mL.

The QuEChERS approach has applicability to multiple drug classes that allows for screening analysis of biological samples with one sample preparation method.

Keywords: **GC-MS, Screening, dSPE**

S15 Simultaneous Determination of Ethyl Glucuronide and Ethyl Sulfate in Urine by HPLC-MS/MS

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Testing of urine samples for alcohol metabolites such as ethyl glucuronide (EtG) and ethyl sulfate (EtS) has recently grown in popularity due to the fact that these compounds can indicate alcohol ingestion for much longer than conventional tests for ethanol. EtG and EtS are both hydrophilic compounds that are difficult to retain on reversed-phase HPLC columns. Many methods for the analysis of these compounds use long columns with highly aqueous mobile phases. Lack of retention and a highly aqueous mobile phase both play a role in reducing MS sensitivity. The objective of this project was to develop an RP-HPLC method to adequately retain both EtG and EtS on a short column. The use of a shorter column allows for faster run times due to reduced re-equilibration time. Adequate retention reduces the chance for ion suppression from co-eluting matrix components and allows for the use of a mobile phase containing more organic solvent, which improves LC-MS/MS sensitivity.

The method developed to analyze EtG and EtS in urine used dihexylammonium acetate (DHAA), a commercially-available, volatile, LC-MS-compatible ion-pairing reagent and methanolic mobile phase. The use of a similar ion-pairing reagent has been previously documented for environmental LC-MS/MS methods. Mobile phases A and B consisted of 5mM DHAA in water and methanol, respectively. A simple step gradient was used to elute the compounds of interest as well as eliminate buildup of matrix components in the LC column. A 5µm Ultra II Biphenyl 50mm x 2.1mm column was used for this work. Initial exploratory work was performed on a Shimadzu LCMS-2010, while validation work was performed on an API-3200 QTRAP™ LC-MS/MS system. Both instruments were operated in ESI(-) mode. Two transitions were monitored for both EtG (221/75, 221/85) and EtS (125/80, 125/97). Quantitation for both compounds was accomplished using deuterated internal standards. Transitions for creatinine were also monitored. A simple 10:1 dilution of urine was used for sample preparation.

EtG and EtS were well-separated from most matrix components and exhibited good retention with k' values of 2.75 and 4.0, respectively. The total run time for the method, including re-equilibration, was 7.5 min. Validation experiments included LOD/LOQ, ion-suppression, specificity, linearity, precision, and accuracy.

The method presented here allows for fast, quantitative, simultaneous analysis of EtG and EtS in urine samples.

Keywords: **Ethyl Glucuronide, Ethyl Sulfate, LC-MS/MS**

S16 Forensic Multi-Target Screening and General Unknown Screening on an LC/MS/MS System with Automatic Library Searching for Compound Identification

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Objective: To develop LC/MS/MS methods for targeted and non-targeted drug screening to detect pharmaceutical and illicit drugs in biological forensic samples

Methods: Both multi-target screening (MTS) and general unknown screening (GUS) utilized a hybrid triple quadrupole/linear ion trap LC/MS/MS system. Information dependent acquisition (IDA) was utilized to detect signals of interest and automatically acquire full scan MS/MS spectra of these signals. These spectra were then searched against a library for compound identification and confirmation.

For the MTS method, the survey scan consisted of a table of MRM transitions corresponding to the target analytes. The high selectivity and sensitivity of MRM, combined with the sensitive ion trap full scan MS/MS spectra, allowed screening for hundreds of drugs at low concentrations. The use of the *Scheduled MRM*™ algorithm further enhances screening capabilities and increases the number of analytes that can be included in a single method by acquiring data only around the retention time of interest.

The use of non-targeted GUS methods has continued to increase. GUS screening utilized a full scan ion trap MS spectrum as a survey scan. Sensitive full scan MS/MS spectra of intense peaks in the survey scan were acquired and searched against a library for compound identification and confirmation. Dynamic background subtraction was utilized for more intelligent selection of signals submitted for MS/MS acquisition by minimizing acquisition of MS/MS spectra of background interferences.

Urine samples known to be positive for drugs were analyzed using both methods. Sample preparation consisted of hydrolysis and a 10x dilution with mobile phase prior to injection for LC/MS/MS analysis. Data was acquired and processed using Cliquid® Software. Results using the two methods were compared.

Results: Results showed that both methods had their advantages and limitations. The MTS method, as expected, had much better sensitivity due to the selectivity of the MRM survey scan. This method had the ability to detect most drugs with concentrations in the low ng/mL range. The GUS method had the advantage of not using retention time, molecular weight, or MRM transitions for data acquisition of compound identification. However, because a full scan MS spectrum has may more interferences than an MRM scan, sensitivity was in the mid-/high- ng/mL range.

Conclusion: Targeted and untargeted screening methods have their advantages and limitations. Because of their sensitivity, targeted methods are most often utilized for drug screening. Non-targeted approaches do not rely on retention time or molecular weight information so have the capability to detect unexpected compounds that may be present.

Keywords: **LC/MSMS Screening**

S17 Quetiapine (Seroquel®) and Tricyclic Antidepressants by Solid Phase Extraction Liquid Chromatography-Electrospray Ionization Mass Spectrometry with an Emphasis on the Postmortem Distribution of Quetiapine

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Demographics of the drugs detected in toxicology cases in the MCCO service area commonly involve a combination of tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs), and the antipsychotic drug quetiapine. A method was developed to simultaneously confirm and quantify these drugs. This method uses solid phase extraction (SPE) with detection by liquid chromatography-electrospray ionization mass spectrometry (LC/MS). Sertraline, norsertraline, paroxetine, fluoxetine, norfluoxetine, venlafaxine, norvenlafaxine, amitriptyline, nortriptyline, cyclobenzaprine, imipramine, desipramine, doxepin, nordoxepin, quetiapine, and quetiapine metabolites are confirmed and measured using this method. Trimipramine, protriptyline, fluvoxamine, thioridazine, and clomipramine can also be measured using this method. Linearity was established for all drugs from 0.05 to 1.5µg/mL with a lower limit of quantification of 0.05µg/mL. The extraction consisted of treating each sample with 2mL of pH6.0 phosphate buffer, paroxetine-D6 internal standard, and applying the mixture to SPE columns. Samples are eluted, concentrated, reconstituted, and injected on an Agilent 1100 Series LC/MS using single ion monitoring (SIM). The correlation coefficients for the calibration curves are 0.99 or greater. Routinely, femoral blood is analyzed for the confirmation of these drugs; however when femoral blood is not available, an alternate blood source along with liver is analyzed. In addition, with limited postmortem distribution data available for quetiapine, all matrices were analyzed where quetiapine was detected in a gas chromatography/mass spectrometry basic drug screen. For each case, femoral blood, heart blood, vitreous fluid, cerebral spinal fluid, urine, brain, and bile were analyzed when available. In particular, one case ruled as acute quetiapine intoxication had a femoral blood level of 6.5µg/mL, heart blood level of 14.5µg/mL, and brain and liver levels of 224 and 200µg/g, respectively. Quetiapine was also quantified in cerebral spinal fluid, bile, and urine for this case. This is the second case, to our knowledge, that cited quetiapine toxicity as the sole cause of death. Other cases included cite multiple drug intoxication as the manner of death and have femoral blood concentrations ranging from 0.1-1.6µg/mL. Liver concentrations from these cases range from 1-8µg/g. A distribution study of nine cases along with case history will be presented. As more positive cases arise they will be incorporated into the presentation.

Keywords: **Antidepressants, Quetiapine Distribution, LC/MS**

S18 Completely Automated LC/MS/MS Analyses of Drugs and Metabolites Using Disposable Pipette Extraction

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Chromatographic analysis (confirmation) of drugs from biological specimens requires sample preparation, which is generally tedious and time consuming. Conventional solid-phase extraction (SPE) can be automated, but the SPE extraction methods generally require multiple steps due to the need for conditioning and wash steps. Hence, most automated SPE methods focus on "off-line" applications where extractions of multiple samples are processed simultaneously.

For "on-line" extractions, it is desirable to perform extractions in a short time frame so that the extraction of one sample can be processed during the chromatographic analysis of the previous sample of a sequence. In this manner, samples could be loaded onto the sample tray of an automated sampler without any additional handling of evidence.

Recently, Disposable Pipette Extraction (DPX) has been found to be a rapid SPE method because it does not require conditioning steps. The sorbent is loosely contained inside the pipette tip, and therefore sample solutions are mixed with the sorbent to provide efficient extractions without concerns of channeling or solution flow rates. With automated DPX using a GERSTEL MPS instrument, the extractions can be performed in about 3-7 minutes, depending on the method. Hence, extractions are completed within chromatographic run times, providing high-throughput analysis "one sample at a time".

In this study, the DPX method used cation exchange (CX), reversed phase (RP) and weak anion exchange (WAX) mechanisms to extract basic, acidic and neutral drugs. In addition, this presentation demonstrates the use of sample collection tips (SC-Tips) that are used for rapid automated sample preparation of biological specimens. The SC-Tips contain sorbent material to adsorb and remove sample matrix components including salts and proteins. The advantage of the SC-Tips is that the extractions are comprehensive and take less than 1 minute to perform.

Recoveries and %RSDs for over 40 drugs are shown using various DPX products and SC-Tips, with most recoveries and %RSDs being greater than 70% and less than 10%, respectively. The drugs include opiates (morphine, codeine, 6-MAM) and opioids (fentanyl), benzodiazepines (diazepam, alprazolam, clonazepam), barbiturates (butalbital, secobarbital), stimulants (amphetamine, methampehtamine, MDMA, cocaine), analgesics (propoxyphene, tramadol), hallucinogens (PCP, THC), and muscle relaxants (carisoprodol, meprobamate). Validation studies include at least 20 replicates for each drug, calibration plots, and analyses of commercial quality control samples.

Applications for analysis of whole blood and urine are presented.

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

S19 Targeted Drug Screening Using Ultrafast HPLC Coupled with High-Definition Accurate-Mass TOF-MS

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A method to detect 55 drugs and metabolites in serum/plasma specimens with minimal sample preparation and a six-minute analysis time was developed using ultrafast HPLC coupled with an Agilent 6230 accurate-mass time-of-flight mass spectrometer (TOF-MS) equipped with an Agilent Jet Stream Technology ESI. The ability to detect individual analytes eliminates issues with cross-reactivity and the need to perform multiple tests per sample to cover multiple drug classes that exists with immunoassay. The target compounds for the method include stimulants (sympathomimetic amines and cocaine), natural and synthetic opioids, propoxyphene, buprenorphine, meperidine, tramadol, barbiturates, benzodiazepines, PCP, their major metabolites and carboxy-THC.

The method utilizes ultrafast HPLC on an Agilent 1200SL system using an Agilent Zorbax EclipsePlus C_{18} (2.1 mm x 100 mm, 1.8 µm particles) Rapid Resolution High Throughput column, at 0.5mL/min, more than twice the previous theoretical optimum for 2.1 mm columns. The >12,000 resolution of the TOF-MS allows extraction of EICs having only a $\pm$ 20 ppm mass width to locate low-pg quantities of the target compound with sensitivity comparable to SIM on a single quadrupole instrument. A background-subtracted spectrum is automatically obtained from each peak, and the target compound is identified by: retention time (calibrated with standards), mass error for the primary adduct, and mass score (a weighted average of scores for mass agreement, correct isotope spacing, and correct isotope ratios for the target molecule). Criteria have been developed to identify a reliable presumptive positive; these criteria can be 'tuned' for more specificity or for more general detection tolerating a higher false positive rate.

Sample preparation is a simple precipitation of 0.25 mL of serum or plasma with 0.3 mL of acetonitrile, followed by centrifugation, evaporation and reconstitution in LC mobile phase. The method was validated by analyzing both patient specimens and drug-free plasma fortified with the target analytes. The fortified concentrations were values of common ELISA cutoffs (see Table 1). Detection limits were typically low-pg on column. The availability of accurate-mass TOF-MS and ultrafast HPLC have provided a fast, accurate method for screening serum/plasma specimens for 55 commonly abused and/or prescribed drugs.

Table 1. Cutoffs for each drug class

Drug Class	cutoff (ng/mL)	Drug Class	cutoff (ng/mL)
amphetamines and club drugs	20	PCP	10
cocaine	20	cannabinoids	20
fentanyl	2.5	methadone	20
buprenorphine	5	propoxyphene	20
meperidine, tramadol	5	benzodiazepines	50
opioids	20	barbiturates	50

Keywords: **Drug Screen, TOF-MS, Plasma**

S20 Roche DAT Immunoassay: Sensitivity and Specificity Testing for Amphetamines, Cocaine, and Opiates in Oral Fluid

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Laboratory testing of oral fluid for drugs of abuse continues to expand in the workplace, legal, treatment, and health settings. In this study, we assessed recently developed homogeneous screening assays (Roche DAT assays) for amphetamines, cocaine metabolite, methamphetamines and opiates in oral fluid on two Roche Modular P instruments. Assay parameters were as per the manufacturer. Control samples at -25% and +25% of cutoff were utilized to assess precision and accuracy. Sensitivity, specificity, and agreement of the Roche DAT assays compared to LC/MS/MS was assessed by analysis of oral fluid specimens collected from 994 subjects enrolled in a drug treatment, or probation and parole drug testing program. An additional 180 research specimens from Kroll Laboratories also were analyzed for amphetamine and methamphetamine.

Precision was calculated as the %CV of replicates of the control samples, also analyzed on two instruments. The results are as follows: amphetamines, 3.4 – 5.2%; cocaine metabolite, 4.9 – 7.3%; methamphetamine, 4.9 – 6.7%; opiates, 3.4 – 5.2%. The accuracy was acceptable: all -25% control sample results gave a negative result and all +25% control sample results gave a positive result.

Sensitivity, specificity, and agreement were calculated by comparison of the semi-quantitative immunoassay (IA) result to the quantitative LC/MS/MS result at the respective cutoff concentration. A specimen was designated as a true positive (TP) as follows:

Drug	IA value, ng/mL	LC/MS/MS value, ng/mL
Amphetamine	≥ 40	Amphetamine or MDA ≥ 40
Methamphetamine	≥ 40	Methamphetamine or MDMA or MDEA ≥ 40
Cocaine Metabolite	≥ 3	Benzoylecgonine ≥ 2
Opiates	≥ 10	Codeine and/or morphine ≥ 10

Sensitivity, specificity, and agreement were acceptable. The results are as follows:

	Amphetamine	Methamphetamine	BZE	Opiates
Sensitivity TP/(TP+FN) x 100	94.4	94.7	94.9	98.2
Specificity TN/(TN+FP) x 100	98.8	96.3	99.4	95.7
Agreement (TP+TN)/(TP+TN+FP+FN) x 100	98.3	96.1	97.9	96.0

The performance of the Roche DAT assays suggests that these new homogeneous screening assays will be an attractive alternative to existing more labor-intensive EIAs.

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

S21 Abuse of Prescription Drugs Among Health Professionals

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The objective of this presentation is to shed some light on the problem of prescription drug abuse among health professionals. The presentation will include definition of drug abuse, dependence and addiction. Prevalence of, triggers to, manifestations of and behavioral and physical characteristics of drug dependence, will be discussed. Seven criteria for diagnosis of drug dependence as identified by the American Psychiatrist Association (APA) will be listed. Treatment approaches and strategies to prevent relapse will also be discussed. Forensic toxicologists have the expertise to educate healthcare students about the pitfalls and dangers of drug abuse in the workplace with the purpose of preparing them for the potential of such problem. Forensic toxicology experts can help law enforcement agencies with interpretation of results of biological sample analysis.

Keywords: **Manifestations, Drug Abuse, Dependence**

S22 High Throughput Simultaneous Analysis of Buprenorphine, Methadone, Cocaine, Opiates, Nicotine, and Metabolites in Oral Fluid by Liquid Chromatography Tandem Mass Spectrometry

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Objective: A method for the simultaneous determination of buprenorphine (Bup), norbuprenorphine (NBup), methadone, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), cocaine, benzoylecgonine (BE), ecgonine methyl ester (EME), anhydroecgonine methyl ester (AEME), morphine, codeine, 6-acetylmorphine (6AM), heroin, 6-acetylcodeine (6AC), nicotine, cotinine, and *trans*-3'-hydroxycotinine (OH-cotinine) in 0.25mL of oral fluid (OF) was developed and fully validated using LCMSMS. This method was applied to oral fluid specimens collected with the Salivette® oral fluid collection device from opioid-dependent Bup-maintained pregnant women.

Method: 0.8mL cold acetonitrile was added into wells of a 96-well Isolute PPT+ protein precipitation plate. 0.25mL OF, previously fortified with 30µL internal standard mixture (deuterated analogs of all analytes), was added to the well, and allowed to stand for approximately 10 min. Vacuum was applied to filter the sample for 10 min. The filtrate was evaporated to dryness under nitrogen at 45ºC, and reconstituted in 100µL of 0.1% formic acid in water. Reverse-phase separation was achieved with a Synergi Polar column (2.1x75mm, 4µm) in 16 min under gradient conditions. Mobile phase consisted of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile. Quantification was achieved by multiple reaction monitoring: 468.3>396.2, 414.3 Bup; 414.3>340.1, 326.0 NBup; 310.3>265.2, 223.2 methadone; 278.2>234.1, 249.2 EDDP; 304.3>182.3, 82.2 cocaine; 290.2>168.3, 105.1 BE; 200.2>182.2, 82.2 EME; 182.2>122.0, 150.1 AEME; 286.3>152.0, 165.2 morphine; 300.3>151.9, 165.1 codeine; 328.2>165.2, 152.2 6AM; 370.1>268.2, 328.1 heroin; 342.2>225.2, 282.2 6AC; 163.2>132.2, 80.1 nicotine; 177.2>80.1, 98.1 cotinine; and 193.2>80.0, 134.0 OH-cotinine. Validation parameters included linearity, limits of detection (LOD) and quantification (LOQ), endogenous (10 unique oral fluids) and exogenous (28 licit and illicit drugs) interferences, carryover, and stability. Intra-, inter-day, and total imprecision (n=20), analytical recovery (n=20), extraction efficiency (n=5), process efficiency (n=5), and matrix effect (n=10) were evaluated at low (0.3 and 1.5ng/mL), medium (30ng/mL), and high (300ng/mL) concentrations. OF specimens (n=472) from 9 opioid-dependent Bup-maintained pregnant women were obtained three times per week throughout pregnancy.

Results: The assay was linear from 0.5 or 1.0, depending on the analyte, to 500ng/mL. Intra, inter-day and total imprecision were <13.2%, and analytical recovery was 92.4-114.3%. Extraction efficiencies were >77.3%, and process efficiencies >45.4%. Although ion suppression was detected for EME, cocaine, morphine, 6AC, and heroin (<56.4%), and enhancement for BE and nicotine (<315.5%); deuterated internal standards compensated for these effects. The method was sensitive (LOD 0.2-0.8ng/mL) and specific (no interferences) except that HMA interfered with AEME. No carryover was detected, and all analytes were stable under different storage conditions (24h room T, 72h 4ºC, 3 freeze/thaw cycles) except cocaine, 6AC, and heroin (22.2-96.9% loss). 85% of 472 OF specimens were positive for Bup, 15% for methadone, 44% for cocaine, 17% for opiates, and 86% for tobacco biomarkers.

Conclusion: This method permits a fast and simultaneous quantification of 16 drugs and metabolites in OF, with good selectivity and sensitivity. OF is a good alternative matrix to monitor drug use in opiate-dependent patients.

Supported by the Intramural Research Program, National Institute on Drug Abuse, NIH.

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

S23 Results from a North American Oral Fluid Proficiency Testing Program

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Introduction and Objective: From early 2000 to August 2006, the Substance Abuse and Mental Health Services Administration (SAMHSA) through the National Laboratory Certification Program (NLCP) sponsored an oral fluid (OF) Pilot Proficiency Testing (PT) Program to assess testing capabilities for this matrix. In December 2007, RTI provided an opportunity for the 16 Pilot PT Program participants and other laboratories to continue in an independent OF PT program. Currently, 28 laboratories participate in RTI's North American OF PT program, which provides for initial testing and confirmatory quantitative assessments of 26 drug analytes. RTI also manages a separate but similar OF PT program for 12 laboratories in the European Union that participate in the Driving under the Influence of Drugs, Alcohol and Medicines (DRUID) program. Data presented here are for the North American program.

Methods: OF samples were prepared in a synthetic OF matrix. Each sample was formulated to contain 3 to 5 analytes. Three mL of neat OF was dispensed into a 4 mL silanized amber vial, capped with a Teflon-lined cap and frozen until shipment. A total of 5 sets with 5 samples in each set were shipped between December 2007 and April 2009. There were multiple challenges for each drug analyte over the 5 sets. Laboratories were instructed to analyze the OF samples as neat OF or to dilute the OF with collection tube buffer using the same dilution factor as their OF collection device, and to not use the collection pad. Laboratories used a mass spectrometry-based confirmatory test to identify and quantitate both directed analytes and analytes positive by initial testing (using the kit manufacturer's cutoff). Reported analyte concentrations were corrected for dilutions to provide the concentration for the neat OF shipped to the laboratory. Samples were expected to be tested and electronically reported to RTI within 10 days after receipt.

Results: Of the total 109 analyte challenges, 69 challenges for 23 drug analytes were reported by at least 5 laboratories. Fewer than 5 laboratories reported results for zopiclone, zolpidem, and flunitrazepam. The table presents the results reported for the lowest concentration challenges for the 23 analytes. Analyte values more than 50% from the group mean were not included in the statistics. The table also shows the corresponding % positive initial test results for the drug class of each analyte (Note: some samples included more than one analyte of the same class of drugs).

Analyte	Target ng/mL	Confirmatory Test			Initial Test		Analyte	Target ng/mL	Confirmatory Test			Initial Test	
		Mean ng/mL	% CV	n	% Pos	n			Mean ng/mL	% CV	n	% Pos	n
THC	4.0	4.0	21.1	17	75	24	Methadone	30	27	16.7	11	78	14
Morphine	40	41.2	15.8	20	100	24	Ethanol	0.025 g/dL	0.026 g/dL	15.5	6	75	4
Codeine	60	63.9	14.1	21									
6-AM	8	7.7	18.2	20	NA		Diazepam	15	16.0	15.2	11	94	17
Cocaine	30	33.6	24.7	18	83	23	Lorazepam	3	3.0	25.9	6		
PCP	15	15.1	9.1	20	100	20	Nordiazepam	5	4.7	13.2	12	59	17
BZE	30	32.9	17.5	21	88	25	Clonazepam	2	2.2	19.3	5		
MDMA	75	76.5	14.5	22	83	24	Alprazolam	6	7.2	22.9	6	93	14
Methamp	75	81.0	12.1	21	83	24	Oxazepam	10	10.5	35.9	6		
MDEA	75	80.9	9.1	16	17	24	Hydrocodone	80	82.2	18.8	11	100	24
MDA	75	75.9	18.1	21			Hydromorph	80	85.9	12.1	10		
Amphet	75	87.2	11.2	21	64	22	Oxycodone	80	85.7	14.5	9		

Conclusions: The group means were within 20% of the target values for all 23 analytes. The group coefficient of variation (%CV) was ≤20% for 18 of the 23 analytes. Only 2 of the challenges presented in the table, and 8 of the 69 analyte challenges, had an ideal variance (% CV) of <10%. A lack of a uniform cutoff or sensitivity of the initial test assays accounted for the relatively low positive rates.

Keywords: **Proficiency Testing, Oral Fluid**

S24 Cannabinoid Concentrations in Oral Fluid after Controlled Around the Clock Oral Synthetic THC (Marinol®) Administration

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Oral fluid offers a good alternative to plasma or urine in clinical and forensic settings since oral fluid can be collected non-invasively while under direct observation. In addition, in some cases, oral fluid concentrations may reflect plasma drug concentrations, providing a better estimate of drug intoxication than urine. Distribution of drugs into oral fluid is complex and incompletely characterized, especially for cannabinoids, due to the lack of controlled drug administration studies. We administered round the clock oral dronabinol, synthetic Δ^9-tetrahydrocannabinol (THC) (Marinol®) approved for the treatment of nausea and vomiting following cancer chemotherapy and for HIV-related wasting disease, to study the disposition of THC and metabolites in oral fluid. Oral fluid specimens (n=440) were obtained prior to, during and after multiple 20 mg dronabinol doses with the Quantisal™ collection device. Participants (n=10) with a history of cannabis use provided written informed consent to participate in this IRB-approved study. Each participant received 37 THC doses while residing on the closed clinical research unit for 8 days. Specimens were extracted with multiple elution solvents from a single SPE column. Analyte separation was achieved with two-dimensional gas chromatography mass spectrometry (2D-GCMS) utilizing electron impact (EI) for THC, 11-hydroxy-THC (11-OH-THC), cannabidiol (CBD) and cannabinol (CBN), and negative chemical ionization (NCI) for 11-nor-9-carboxy-THC (THCCOOH). Linear ranges were 0.5-50ng/mL, with the exception of CBN (1-50ng/mL) and THCCOOH (7.5-500pg/mL). Intra- and inter-assay imprecision, expressed as percent RSD, were 0.3-6.6% and analytical recovery was within ±13.8% of target. THCCOOH was the primary analyte detected in 432 specimens (98.2%), with concentrations up to 982pg/mL. THCCOOH concentrations increased as the study progressed. In contrast, 11-OH-THC was not identified in any specimen; CBD and CBN were found in 3 and 8 specimens, respectively, with maximum concentrations of 2.1 and 13ng/mL. THC was present in only 21% of specimens (n=91), with the highest concentrations observed near admission to the secure unit, prior to dronabinol administration. THC appeared to be from previously self-administered smoked cannabis. There was large inter-subject variability. Median THC and THCCOOH concentrations at admission were 3.3 ng/mL (range 0.5-293.2ng/mL) and 23.6pg/mL (range none detected-176.8pg/mL, only one subject negative for THCCOOH), respectively. THC, CBD and CBN were not identified in oral fluid after 37 oral dronabinol doses. This fully validated oral fluid cannabinoid method quantified THC, 11-OH-THC, THCCOOH, CBD, and CBN disposition in oral fluid after oral THC administration.

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Keywords: **Oral Fluid, Dronabinol, Cannabinoids**

S25 Development and Validation of a Novel Homogeneous Immunoassay for Detection of Fentanyl in Urine

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Fentanyl is an extremely potent synthetic opioid that is widely used for chronic pain treatment. This drug is highly addictive and prone to abuse. The objective of this project was to develop a high throughput homogeneous enzyme immunoassay (HEIA) for rapid detection of fentanyl in human urine. We started with an in-house antibody that was capable of measuring fentanyl at low concentrations. We now have a highly sensitive HEIA for fentanyl in urine which has been challenged with authentic human urine samples that have been confirmed positive or negative for fentanyl/norfentanyl by LC-MS/MS.

This qualitative assay has a detection limit that was determined to be 0.5 ng/mL. We chose a cut-off concentration of 2 ng/mL for urine. The intra-day (n = 15) and inter-day (n =60) rate precision of the assays were less than 10% CV. No interferences from structurally unrelated and commonly ingested drugs were observed at a concentration of 10,000 ng/mL.

A total of 93 LC-MS/MS verified urine specimens (61 positive and 32 negative samples) were analyzed by HEIA. Sixty-one tested positive and twenty-nine tested negative by HEIA as listed in the following table. Three samples that tested borderline positive by the HEIA were negative by LC-MS/MS at a 1 ng/ml cut-off.

		LC-MS/MS	
		Positive	Negative
HEIA	Negative	0	29
	Positive	61	3

In summary, a high throughput, highly sensitive HEIA has been developed for the detection of fentanyl in human urine which correlates well with LC-MS/MS. This is the first report of a homogeneous immunoassay for fentanyl.

Keywords: **Fentanyl, Enzyme Immunoassay, Validation**

S26 Development of a Generic Monoclonal Antibody Against Tricyclic Antidepressants

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Tricyclic antidepressants (TCAs) are indicated for the treatment of clinical depression, neuropathic pain, nocturnal enuresis and attention deficit hyperactivity disorder (ADHD). Whilst therapeutic drug monitoring (TDM) of TCAs is well established in the treatment of depression, TCA overdose remains the most common cause of death from prescription drugs. We report the development of a monoclonal antibody presenting a broad specificity profile, which will be of value in developing more effective immunoassays for both TDM and toxicological applications.

Sheep were immunized with nortryptyline conjugated to bovine thyroglobulin (BTG) as a carrier. Lymphocytes were then collected and fused with heteromyeloma cells. Supernatants from the resulting hybridomas were screened for the presence of generic TCA antibody using ELISA based assays. Positive hybridomas were cloned to produce stable monoclonal hybridomas. The antibodies were purified and evaluated by competitive ELISA to determine their specificity for a range of TCAs.

Initial evaluation shows significant generic TCA recognition of the antibody for nordoxepin HCl, imipramine HCl, chlorpromazine HCl, desipramine, norchlorpromazine, doxepin HCl, promazine, dothiepin, lofepramine, norclomipramine, amitryptiline HCl, nortriptyline, protriptyline, cyclobenzapine, trimipramine (% cross-reactivity ranging 42.96-325.56 relative to 100% dothiepin). Sensitivity expressed as IC50 were <1.5ng/ml (protriptyline, amitriptyline HCl desipramine, promazine, cyclobenzapine and trimipramine); <3.0ng/ml (nortriptyline, lofepramine, dothiepin, doxepin HCl, imipramine HCl, norchlorpromazine); 4.04ng/ml for norclomipramine, 6.51ng/ml for chlorpromazine HCl and 6.82ng/ml for nordoxepin HCl.

This generic monoclonal antibody exhibits high sensitivity and specificity for a wide range of TCAs and their metabolites and represents an improvement for use in developing more effective immunoassays applicable to TDM and toxicology fields.

Keywords: **Tricyclic Antidepressants, Generic Monoclonal Antibody, Immunoassays**

S27 A Novel Solution for Improving Instrumental Productivity in High Throughput Labs

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Obejctives: The purpose of this study was to evaluate a simple duplexing upgrade option for the existing LC/MS/MS systems for improving instrumental throughput in high volume labs. Feasibility of running two columns in parallel using an additional set of LC pumps and two flow diverter valves (switching valves) to divert the column flow into the mass spectrometer is evaluated.

Methods: The analytical capabilities of liquid chromatography tandem mass spectrometry for sensitive and highly selective determination of target compounds in complex biological samples makes it well suited for high-throughput analysis. We report a fast method for the analysis of ethyl glucuronide and ethyl sulfate via multiple reaction monitoring (MRM) liquid chromatography/mass spectrometry using two chromatographic columns in parallel. The analytes were extracted from human urine samples along with their deuterium-labeled internal standards by simple protein precipitation and centrifugation for 2 minutes. Analysis was achieved by using one rack changer (Shimadzu Prominence) and one autosampler (SIL 20AC, Shimadzu) coupled to two sets of LC pumps (LC-20AD, Shimadzu), two flow diverter valves mounted in a column oven (CTO 20AC, Shimadzu), two chromatographic columns (Synergi Polar - RP 4µ Minibore, 150 x 2mm) and one tandem mass spectrometer (API 3200, Applied Biosystems) operated in the turbo ion spray mode with negative ion detection. Through electronic switching valves, the autosampler was synchronized with the mass spectrometer so that injections were made as soon as the mass spectrometer was ready to collect data. While one column was sending the eluent to the mass spectrometer, the other column was being washed and equilibrated for the next injection, thereby eliminating the need for extra time for washing and equilibrating the column post elution. Chromatographic integrity, response and relative retention times for the various analytes were relatively constant between the two columns. Analysis time was reduced to half (2 min) by using two columns in parallel. The results show acceptable precision and accuracy and demonstrate the feasibility of using two columns in parallel with tandem mass spectrometry for high-throughput analysis of biological samples containing multiple analytes.

Results and Conclusions: The use of duplexing technology has the potential to increase productivity by replacing the instrument time wasted in chromatographic column cleanup and equilibration with mass spectrometer data acquisition. Sample throughput in our lab was doubled by utilizing this duplexing option with minimal expense. The procedure was applied to authentic urine specimens and all analytes were successfully detected in the 2 minute run time utilized. The limit of detection for EtG and EtS was 25 ng/mL and 10 ng/mL respectively. The limit of quantitation was 50 ng/mL for EtG and 25 ng/mL for EtS. Precision was within 5% and quantitative accuracy was over 94% for all analytes. The method was linear up to 500,000 ng/mL for EtG and up to 100,000 ng/mL for EtS. No carryover was noticed up to 500,000 ng/mL for both EtG and EtS. These results demonstrate the feasibility of using parallel analysis to reduce the time required for sample analysis in high throughput labs, without investing in costly multiplexing options. This system can easily be utilized for other applications. T

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Keywords: **Urine, Ethyl Glucuronide, Ethyl Sulfate, Duplexing**

S28 Differences Between Elution Profiles of MDA, MDMA, MDEA, Amphetamine, Methamphetamine and their Corresponding Deuterated Analogues Using Solid Phase Extraction Cartridges

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The views expressed in this article are those of the authors and do not necessarily reflect the official policy of the Department of the Navy or the U.S. Government.

When performing quantitative analysis of a drug, an internal standard is chosen that has physical and chemical properties that closely resemble the drug. The internal standard of choice is the deuterated form of the drug where one or more hydrogen atoms are replaced with deuterium. The deuterated form of the drug is expected to mimic the drug throughout the analytical process, although separation of the two is sometimes observed with the use of gas chromatography. This study evaluates the potential for partial separation of the drug from the deuterated internal standard using CEREX® Polycrom™ CLIN II solid phase extraction cartridges. The cation exchange cartridges evaluated have a 35 or 50 mg sorbent bed containing 10 µm particles of modified highly cross-linked divinylbenzene. The drugs were eluted from the columns with 0.5 mL increments with an organic solvent containing 2% ammonium hydroxide.

GC/MS results showed that undeuterated drug was eluted off of the solid phase extraction cartridge slightly earlier than the corresponding deuterated drug. This effect was greater for MDMA and methamphetamine compared to the other drugs studied. The drugs were eluted in 0.5 mL increments. On a 6 mL column with a 50 mg sorbent bed the first 1.0 mL of eluate contained 8% of the total MDMA collected, but only 5% of MDMA-D5. For d-methamphetamine the first 1.0 mL contained 23% of the d-methamphetamine, but only 14% of the d-amphetamine-D14. The MDMA/MDMA-D_5 ratios (expected value of 1.00) for the successive 0.5 mL eluates were: na, 1.68, 1.28, 0.78 and 0.54. The d-methamphetamne/d-methamphetamine-D_{14} ratios (expected value = 0.40) for the 0.5 mL eluates were: 0.65, 0.67, 0.55, 0.39, 0.32, 0.26, 0.23, 0.20, 0.19 and 0.20. Use of a 3 mL column with a 35 mg sorbent bed reduced the amount of eluate needed to remove the drug from the column for MDA, MDMA and MDEA.

Due to the differences between the affinity of a drug and its deuterated internal standard to SPE columns, extraction procedures should add sufficient elution volume to provide adequate recovery of drug and internal standard to assure accurate and consistent quantification of results.

Keywords: **Extraction, Amphetamines, Internal Standards**

S29 An Analysis of Paired Hair-Urine Drug Test Results Over a 5½ Year Period

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Background: Hair drug testing detects a pattern of repetitive drug use, typically over a period of up to 90 days, but not recent or initiation of drug use. In contrast, urine drug testing detects recent drug use, typically over a period of up to three days, but not long-term use. Some drug testing programs – e.g. workplace, rehabilitation, family/social services – call for the collection of paired hair and urine specimens, for a more comprehensive detection of drug use than through hair or urine alone. To date, there have been no large published data sets comparing positive prevalence rates in paired hair and urine specimens.

Methods: Drug test results from more than 193K paired hair-urine specimens, submitted for routine testing, over a 5½ year period (Jan. 2004 thru Jun 2009) were compared. Approximately 80% of the specimens in the data set were workplace related, with the remainder from family/social services or court-ordered tests. Urine specimens were screened using enzyme immunoassay – Syva EMIT® or Microgenics DRI® (Dade Behring, Cupertino, CA; and Microgenics, Fremont, CA, respectively) – and confirmed using GC/MS. Hair specimens were screened by Quest Diagnostics HairCheck-DT systems – which use enzyme-linked immunosorbent assay (ELISA) technology – and confirmed using GC/MS or GC/MS/MS.

Results: The analysis indicates that the overall positivity rate in hair is – as may be expected owing to the longer window for detection of use – higher than the positivity rate in urine (12.6% vs. 7.6%), with marked differences between drugs. Generally, hair has a higher positivity rate – especially for methamphetamine (5.9% vs. 1.8%) and cocaine (4.8% vs. 0.65%). However, no difference was observed in the overall detection rates of marijuana metabolite (3.4% vs. 3.4%) or phencyclidine (0.05% vs. 0.05%). While a single substance, opiates, showed higher positivity rates in urine than in hair, cutoff differences – ~20% were tested at a lower cutoff – and the use of additional confirmation analytes may complicate a direct comparison between hair and urine positivity. Table 1 illustrates the positivity rates (as a percentage of all positives) for specimens that were positive only in urine, only in hair, and positive in both urine and hair.

Table 1.	Urine Pos Only	Hair Pos Only	Hair & Urine Pos
Overall	18.4%	51.0%	30.6%
Amphetamines	9.6%	67.6%	22.7%
Methamphetamine Only	5.9%	70.8%	23.4%
Cocaine/Metabolites	2.1%	86.7%	11.2%
Opiates	64.6%	19.8%	15.7%
Phencyclidine	29.9%	30.6%	39.6%
Marijuana Metabolite	30.8%	30.5%	38.7%

Conclusions: The data from these paired – collected simultaneously from the same donor – specimens suggest that hair and urine drug tests, when used together, provide a comprehensive look at drug use patterns. Hair specimens provide a longer window for detection than do urine specimens—up to 90 days versus up to three days. As a result, higher hair positivity rates are not unexpected. While hair affords the ability to detect a pattern of use over time of select substances, urine testing provides a recent view of use that hair may not, and allows for testing of a broader range of substances. When used together, hair and urine testing serve as complements and provide information about an individual's drug use that neither test alone provides.

Keywords: **Hair, Urine, Workplace Drug Testing**

S30 Case Report: A Dead Child –Methadone Analyses in Alternative Matrices

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A 2 year-old child was reported dead by the mother who had called emergency services the evening before. A post-mortem examination was carried out and samples taken including femoral blood (small volume), heart blood, stomach content, vitreous humour and hair.
Immunoassay screening of the heart blood sample gave a high positive result for methadone.

There was insufficient femoral blood sample for methadone quantification. Quantifications were performed on heart blood and vitreous humour. Further samples were requested including muscle and liver. Methadone quantification was performed on 4 muscle samples and liver and the amount measured in the stomach content. High levels of methadone were detected in all samples with the exception of stomach content where only a small amount was detected.

A defence of ingestion via breast milk was put forward as an explanation for the results by the mother's legal team.

Interpretation of these results is discussed.

Segmental analysis of hair was performed and regular ingestion of methadone, heroin and cocaine was demonstrated in the deceased. A similar pattern of use was shown in the mother's hair.

Methadone poisoning was given as the cause of death.

Keywords: **Methadone, Child, Matrices**

S31 Heroin Metabolite Concentrations in Medulla Oblongata and Cervical Medulla After Acute Heroin Administration to Rats

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Background: Among forensic pathologists and toxicologists, it is well known that the blood morphine concentration varies substantially in heroin overdose deaths. Since there is no biomarker of opiate tolerance, the blood concentrations *per se* cannot be used to determine the toxic effects. We hypothesized that the concentration of heroin metabolites in medulla oblongata (OBL) comprising respiratory control centers is a more adequate specimen for quantification of heroin metabolites, and that cervical medulla (CM) could be a substitute, should OBL be used for more specific assays. We investigated concentrations of morphine, 6-acetylmorphine (6-mam), morphine-3β-D-glucuronide (m3G), and morphine-6β-D-glucuronide (m6G) in OBL and CM in rats given heroin after various pre-treatments. The aims were to see if rats died at a certain concentration of metabolites in the brain stem regardless of dose, if pretreatments changed the survival rate and if heroin metabolite concentrations correlated in OBL and CM.

Methods: Male Sprauge-Dawley rats weighing 250-300 g were randomized into three different treatment groups; i.p injections of heroin-HCl (4 mg/kg), amphetamine sulphate (7 mg/kg) or 0.9% NaCl were given twice daily for three days to each group. At 14-17 hours after the last i.p injection, the animals were given an i.v. injection of 22, 30 or 38 mg of heroin-HCl. The animals were monitored for 30 minutes, and were then decapitated if death had not already occurred. The brain was dissected, and select regions were immediately frozen and stored in Eppendorf tubes at -20°C until analysis.

Brain samples (20-60 mg) were homogenized in 1 mL MilliQ water and 10 µL of internal standard (20 µg/mL) of deuterated analogs to all analytes was added. After centrifugation at 14,000 rpm the supernatant was filtered through a 0.22 µm Millipore filter and transferred to a vial. A 4 µL aliquot was injected into an LC-MS-MS system with an HSS T3 column (2.1x50 mm 1.8u particles) and an Acquity UPLC coupled to an Applied Biosystems 4000 mass spectrometer.

Results: An i.v. dose of 22 mg/kg of heroin caused only two spontaneous deaths. The higher doses caused more deaths. The mortality was slightly higher among rats receiving 38 mg/kg than those receiving 30 mg/kg. Rats pretreated with heroin for only three days had a lower mortality upon i.v. heroin injection than NaCl or amphetamine pretreated rats. Amphetamine pretreatment did not affect the mortality. Mean concentrations of morphine and 6-mam in OBL and CM were very similar in rats dying spontaneously, approx. 71 and 4 ng/mg tissue, respectively, among rats given 30 mg/kg of heroin i.v. The corresponding average concentrations in spontaneously dying rats that had received the 38 mg/kg dose were 77 and 6 ng/mg tissue.

Conclusions: In this experimental setting, we showed that a short pretreatment with heroin reduced mortality, whereas amphetamine pretreatment had no influence on the outcome of the acute i.v. heroin injection. Further, the concentrations of heroin metabolites in OBL and CM were similar, and varied moderately in each specimen regardless of i.v. dose administered, among rats dying spontaneously. The results suggest that either OBL or CM levels of heroin metabolites could be a better measure than whole blood in the diagnosis of heroin overdose. Interestingly, rats pretreated with heroin that died spontaneously after i.v. injection showed similar levels of heroin metabolites in OBL and CM as rats pretreated with NaCl or amphetamine.

Keywords: **Heroin Overdose, Brain, Morphine-Glucuronides, Animal**

S32 Importance of Nor-Metabolites in Urine Testing for Synthetic Opioids

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Opioids are important therapeutic agents available to patients with moderate to severe pain. The synthetic opioids, buprenorphine, fentanyl, meperidine, methadone, and propoxyphene have been utilized for decades as analgesics. One of the major biotransformation pathways of these drugs occurs through N-demethylation by cytochrome P450 enzymes leading to the formation of nor-metabolites (NM). NMs may be pharmacologically active and contribute to adverse events associated with opioid analgesic use. In addition, NMs generally exhibit longer half-lives than the parent drug leading to accumulation with prolonged use. As part of continuing research efforts to improve monitoring programs of chronic pain patients undergoing opioid treatment, we evaluated the prevalence and relative abundance of NMs of buprenorphine, fentanyl, meperidine, methadone, and propoxyphene in patients' urine specimens. The study was approved by an Institutional Review Board. Approximately 2600 urine specimens from pain patients undergoing treatment for chronic pain were tested by liquid chromatography (LC) tandem mass spectrometry (MS) for fentanyl, meperidine, methadone, propoxyphene and their respective nor-metabolites. In addition, a subset of 151 specimens was tested for buprenorphine and norbuprenorphine. Cutoff concentrations, ng/mL, were as follows: buprenorphine, 1; fentanyl, 1; meperidine, 100; methadone, 200; and propoxyphene, 200. Cutoff concentrations for NMs were equal to the parent drug with the exception of norbuprenorphine (2.5 ng/mL). Limit of quantitations (LOQs) typically were 50% of the cutoff concentrations. The percentage of positive specimens that contained parent drug (only) in excess of the cutoff concentration, parent drug in combination with NM, and NM (only) is shown in the Table. The median concentration ratio of NM to drug (D) is also listed for specimens that contained both analytes. The percentage of positive specimens containing only

Drug	#Positives (D + NM)	%Positive D	%Positive D & NM	%Positive NM	Median Ratio (NM/D)
Buprenorphine	61	0	32.8	67.2	21.4
Fentanyl	279	42.7	31.2	26.2	7.7
Meperidine	18	77.8	5.6	16.7	N/A
Methadone	134	19.4	70.9	9.7	1.6
Propoxyphene	202	30.7	22.8	46.5	22.5

NM ranged from approximately 10% to 67%. Likely explanations for specimens containing only NM are: 1) specimens were collected at a later time after drug administration relative to those specimens containing parent drug; 2) metabolism was increased due to genetic polymorphisms or drug interactions; and 3) the longer half-lives of the NMs contributed to their accumulation. The authors conclude that these results establish the importance of including synthetic opioid NMs in urine test panels for monitoring pain patients.

Keywords: **Opioids, Nor-Metabolites, Urine Tests**

S33 Detection of Driving Under the Influence of Cocaine in a Hit and Run Accident Based on the Analysis of Blood Smears on the Deployed Airbag Using LC/MS/MS Technology

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Background: In a hit and run accident, it may be difficult or impossible to prove drug or alcohol impairment on the part of the driver. Through the use of LC/MS/MS technology, blood smears or spatters from the vehicle may be analyzed for the presence of drugs and inferences made about the condition of the driver at the time of the accident.

Objective: To describe the screening and quantitative analysis of cocaine and cocaine metabolites in blood residue on a deployed airbag.

Case History: A disabled vehicle stopped along the side of an expressway with its emergency lights flashing was struck from behind by a car. The driver of the car, an off-duty police officer seen running from the scene, turned himself in 8 hours later claiming no memory of the events due to a head injury. The DA requested that we test swabs taken of blood spatter from the car dashboard and the deployed airbag for the presence of cocaine.

Methods and Results: Initial screening for cocaine was performed on 6 swabs of blood spatter taken from the car. The volume of blood absorbed onto each swab was estimated and the swab was eluted into 10 times that volume of 0.1 M PO_4 buffer and analyzed using a Cocaine/BE Elisa assay (Immunalysis). Two of the swabs gave presumptive positive results, one swab was negative and three results were categorized as inconclusive (absorbance between that of a negative control and the lowest concentration standard). The package containing the airbag was then opened and after sampling the airbag for DNA, 5 samples of the airbag containing apparent blood contamination and 2 "control" samples with no visible blood contamination were taken for analysis. Airbag samples were eluted into a mixture of saline/water/pH 6 PO_4 buffer (1:4:2), extracted by SPE, derivatized with BSTFA/TMCS and analyzed by GC/MS for cocaine (coc), methylecgonine (ME), benzoylecgonine (BE) and cocaethylene (CE) using deuterated internal standards. All 5 airbag samples were positive for coc and two were positive for BE. One airbag control was negative, the other was positive for coc near the LOD of the method. Because we felt that the sensitivity of the GC/MS method was insufficient, a quantitative method was developed using LC/MS/MS. Four additional airbag swatches were taken along with 4 more "control" samples. They were eluted and extracted as before, then analyzed on an Applied Biosystems 3200 Q-trap with electrospray ionization interface operated in MRM mode. Two transitions were monitored for each of the 4 drugs. Coc and BE were detected in all 4 airbag samples in amounts ranging from 4.8-14 and 2.9-9.9 ng/sample respectively (BE:coc ratio range 0.60-0.71). Coc was detected in all 4 control swatches (0.43-1.1 ng/sample). No BE was detected in any of the controls. All samples were negative for ME and CE. The officer was eventually convicted of DUI cocaine. Interpretation of the case results will be discussed.

Conclusion: The sensitivity of LC/MS/MS technology allows the analysis of novel samples contaminated with very small quantities of biological fluids for the presence of drugs. This sensitivity can be a valuable tool in the prosecution of hit and run accidents.

Keywords: **Cocaine, LC/MS/MS, Airbag**

S34 Comparison of the Alcotest 7410 with the Intoxilyzer 5000 and Breathalyzer 900/900A

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A comparison of an alcohol breath test result from an Alcotest 7410 screening device was conducted with a result from another breath sample provided within two minutes prior into an Intoxilyzer 5000 or Breathalyzer 900/900A instrument under forensic laboratory conditions.

Alcotest 7410 screening devices (n=2) analyzed breath samples from 675 human alcohol-dosed subjects provided within 2 minutes of a prior test using an Intoxilyzer 5000 instrument (n=7) on 522 subjects, or Breathalyzer 900/900A (n=10) on 153 subjects. The Alcotest 7410 devices were calibrated daily using "100" mg/210 L forensic ethanol standard solutions: the median calibration check result was 100 mg/210 L (n=195, s.d.=2.4, range 94 to 106). Prior calibration check results of the device did not differ by more than 6 mg/210 L. Calibration checks on the Intoxilyzer 5000 used aqueous standards from 10 to 280 mg/210 L, including a blank: the median result for the 100-standard was 102 mg/210 L (n=1091, s.d.=2.0, range: 95 to 111). Similar calibration checks of the Breathalyzer had a median of 100 mg/210 L (n=395, s.d.=1.7, range: 94 to 110). Breath volumes were measured using a Spirobank G or Wright spirometer.

The regression of Alcotest 7410 result against Intoxilyzer 5000 was $y=0.913x + 4.125$ ($R^2 = 0.952$, n=3436), and $y=0.834x + 2.101$ ($R^2=0.885$, n=1309) against the Breathalyzer 900/900A. The median Alcotest result was 65 mg/210L (range: 5 to 204).

Instrument results were grouped in ±20 mg/210 L at 20, 40, 50, 60, 80, 100 and 120 mg/210 L. The sensitivity (TP/(TP+FN)) of result for the device against the instruments decreased with concentration, while specificity (TN/(TN+FP)) slightly increased.

Test results where the device exceeded the instrument by more than 10 mg/210L were scrutinized. Results were excluded when: (a) the volume of breath with the Intoxilyzer 5000 were under one standard deviation less than the mean (n=13) for that subject, or (b) the duration of exhalation with the Breathalyzer 900/900A were under one standard deviation less than the mean (n=4) for that subject. After these exclusions, 2.1% (n=73) of sample results from the device for 7.6% (n=40) subjects, with 4.0% (n=21) of subjects more than once, exceeded the Intoxilyzer 5000 result by more than 10 mg/210 L. For the Breathalyzer, 0.7% (n=9) of samples from the device for 3.9% (n=6) subjects, with 1.9% (n=3) of subjects more than once, exceeded by more than 10 mg/210L. These results were independent of concentration and 93.9% (n=77) were from subjects in the elimination phase. No diagnostic characteristic for these subjects was identified. Two instances were found, once in two subjects, for which the device result exceeded the instrument by 39 and 29 mg/210 L, with an impromptu result (≤1 minute) from the second device that exceed by 23 and 7 mg/210 dL, respectively. Both subjects were confirmed in the elimination phase of alcohol, and their drinking of alcohol beverages (wine, vodka) had ceased 47 minutes prior for each of these cases.

The Alcotest 7410 devices used in this study over 87 months tend to underestimate the result from the evidentiary Intoxilyzer 5000 and Breathalyzer instruments examined, and if representative of law enforcement practice, such devices may provide more forensic benefit to some drivers under investigation rather than an undue analytical result.

Keywords: **Alcotest 7410, Validation, Forensic**

S35 Driving Under the Influence (DUI/DUID) in South Florida--Most Commonly Encountered Drugs in DUI/DUID Casework

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After attending this presentation, attendees will become aware of the most commonly detected drugs seen in DUI/DUID cases analyzed in the Palm Beach County Sheriff's Office Forensic Sciences Division (PBSO-FSD) in Palm Beach County, Florida. The lab serves approximately 1.3 million residents of the county spread over 1974 square miles. This presentation will be an overview of the most commonly encountered drugs detected in DUI cases analyzed at the PBSO-FSD. This review will cover drugs detected in 1173 DUI cases since 2007. Case samples will be reviewed and quantitive values in blood will be listed when available.

This presentation will impact the forensic community and/or humanity through the presentation of the most frequently encountered drugs in south Florida. This data may be used by other labs analyzing DUI cases to make changes to their analytical approach or screening protocols. The top ten drug classes detected in blood and urine specimens will be reviewed in detail. Mention will be made of Florida's status as a pill mill haven and attempts to correct this issue. Mention will also be made of Florida's DUI law and its effect on the ability to prosecute cases involving impaired drivers.

Methods: DUI blood cases are first subjected to a quantitative ethanol analysis by headspace gas chromatography. Depending on the ethanol result and the case history, testing may end or continue on for drug testing. Drug testing begins with enzyme linked immunosorbent assays (ELISA) for the following drugs or drug classes utilizing the cut-offs listed (ng/mL): amphetamine (50), methamphetamine (50), barbiturates (100), benzodiazepines (50), carisoprodol (1000), cocaine/metabolite (50), methadone (50), opiates (50), oxycodone/oxymorphone (50), and cannabinoids (10). DUI urine cases are first subjected to screening by ELISA for the following drugs or drug classes utilizing the cut-offs listed (ng/mL): barbiturates (200), benzodiazepines (100), carisoprodol (1000), cocaine/metabolite (300), opiates (200), oxycodone/oxymorphone (200), and cannabinoids (50). All blood and urine samples are also screened by GC/MS for drugs not covered by ELISA screening. Cases screening negative for all classes by ELISA and by GC/MS may be subjected to additional testing depending on the amount of sample and the case history. Additional analyses can include but are not limited to: basic, acidic, and neutral screens, benzodiazepine screens, and GHB screens by GC/MS. All positive screening results are subjected to confirmation by GC/MS and bloods are quantitated when possible by gas chromatography or GC/MS.

Results: The most commonly encountered drugs by specimen type are listed by class in the Table 1. Drug occurrences are listed because most cases involve multiple drug/ethanol findings. The data can be further broken down into occurrences of each drug individually. Benzodiazepine occurrences were alprazolam 248 , α-OH alprazolam 116, diazepam 34, nordiazepam 58, oxazepam 57, temazepam 55, clonazepam 5, 7-amino clonazepam 24, lorazepam 20, midazolam 4, chloridiazepoxide 1, and desalkylflurazepam 1. Opioid occurrences were oxycodone 161, methadone 77, EDDP 49, hydrocodone 53, morphine 27, codeine 23, propoxyphene 10, norpropoxyphene 9, heroin 5, 6- MAM 5, methorphan 9, tramadol 8, fentanyl 3, hydromorphone 2, and meperidine 1. Muscle relaxant occurences were carisoprodol 57, meprobamate 63, cyclobenzaprine 14, orphenadrine 1, and methocarbamol 1. Antidepressant occurrences were citalopram 27, fluoxetine 19, sertraline 12, venlafaxine 11, trazodone 10, amitriptyline 8, nortriptyline 7, buproprion 5, paroxetine 3, mirtazapine 2, doxepin 1, and desipramine 1. Antihistamine occurrences were diphenhydramine 42, chlorpheniramine 8, doxylamine 8, pheniramine 7, promethazine 6, and hydroxyzine 5. Hypnotic occurrences were zolpidem 28, zopiclone 1, and GHB 1. Amphetamine occurrences were amphetamine 15, methamphetamine 8, MDMA 4, MDA 3, and phentermine 3. Barbiturate occurrences were butalbital 17 and phenobarbital 6. The data for all drugs reported quantitatively more than 10 times was evaluated statistically to determine the range, mean, and mode for the reported data. The results of this evaluation is summarized in Table 2.

Table 1

	Number of Occurrences			
Drug Class	**Blood**	**Rank**	**Urine**	**Rank**
Ethanol	537	1	N/A	N/A
Benzodiazepines	134	2	491	1
Cocaine	118	3	156	3
Opiates	88	4	346	2
Cannabinoids	63	5	122	4
Muscle Relaxants	42	6	94	5
Antidepressants	19	7	94	5
Antihistamines	15	8	64	6
Hypnotics	9	9	21	8
Amphetamines	5	10	28	7

Table 2

Drug	**Range (ng/mL)**	**Mean (ng/mL)**	**Mode (ng/mL)**
Alprazolam	10 - 437	105	55
Diazepam	50 - 1000	258	50
Nordiazepam	64 - 692	258	232
Oxycodone	50 - 395	195	191
Methadone	64 - 574	244	207
Carisoprodol	500 - 11000	4488	4213
Meprobamate	1300 - 33000	12247	12000
Zolpidem	119 - 853	424	416

Summary: The data demonstrates that a great majority of the drugs responsible for DUI cases in the state of Florida are prescription medications. Illicit substances account for less than 20 percent of the drugs identified in DUI cases. The prescription medications were confirmed in blood specimens at levels ranging from the low therapeutic level well into the toxic ranges for some drugs. The majority of cases were positive for multiple prescription drugs, often from multiple drug classes, further emphasizing the prevalence of polydrug use in drug impaired drivers. The growing number of mostly unregulated pain management clinics has given Florida the status as a prescription "pill mill" haven. These so-called pill mills are a major source of the most commonly abused prescription drugs not only in Florida but also across the country. During Florida's last legislative session, lawmakers took a positive step toward getting this issue under control by joining 38 other states that have created a prescription drug monitoring system. The new law requires that Florida track prescriptions of controlled substances designated as Schedule II, III, and IV. This law is a step in the right direction although it is too early to tell the true impact of this tracking system.

Keywords: **DUI, DUID, Florida**

S36 It is Not Your Grandma's Marijuana

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The National Institute on Drug Abuse (NIDA) and the Office of National Drug Control Policy (ONDCP) reported a 1.5-fold increase in the delta-9-tetrahydrocannabinol (THC) content of street cannabis seizures from1997-2001 versus 2002-2006. This study was conducted to compare the changes, over those years, in blood and urine cannabinoid concentrations with the potency of THC reported in the cannabis plant. Cannabinoids were screened using radioimmunoassay (RIA) for blood and fluorescence polarization immunoassay (FPIA) for urine and confirmed using GC/MS. A total of 95 individuals were found to be using cannabis from a total number of 2769 (3.4%) individuals tested over the period 1997 through 2006. Other impairing drugs were found in 39% of the cannabinoids-positive individuals. The mean concentration of THC in blood for 1997-2001 was 2.7 ng/mL; for 2002-2006, it was 7.2 ng/mL, a 2.7-fold increase in the mean THC concentration of specimens from aviation fatalities, compared to a 1.5-fold increase in cannabis potency reported by the NIDA and ONDCP. The mean age for cannabis users was 42 years (range 18-72) for aviation fatalities. For all blood and urine specimens tested from aviation fatalities, the mean age of the individuals with negative test results was 50 years (range 14-92). More than half of the fatalities tested were 50 years or older, whereas, 80% of the positive cannabis users were under 50. As indicated by these findings, members of the transportation industry, government regulators, and the general public should be made aware of the increased potential for impairment from the use of high potency cannabis currently available and being used.

Keywords: **Marijuana, Drugs, Potency, Impairment, Transportation**

S37 The Application of F-SPE and Fast LC-MS/MS for the Analysis of THC/Carboxy-THC in Drugs and Driving Cases

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Abstract: In this oral presentation, attendees will learn about fluorous solid phase extraction (F-SPE) using a novel sorbent (Heptadecafluorotetrahydrodecyl (C10H4F17)) to efficiently isolate and quantify THC and Carboxy-THC from whole blood samples taken in drugs and driving cases.

Method:F-SPE was performed in hydrophobic mode after samples of whole blood (1 mL) were precipitated with acetonitrile (2 mL) and evaporated to 200µL. The samples were applied to the F-SPE in 5 mL of aqueous aqueous phosphate buffer (pH 7). The sorbent was washed with deionized water, phosphate buffer (pH 7) (3 mL of each, respectively), and dried under full vacuum. Each F-SPE column was eluted with 3 mL of a solvent consisting of ethyl acetate/ hexane (50:50 3 mL) containing 2% acetic acid. The eluates were collected, evaporated to dryness and dissolved in mobile phase (50 µL) for analysis by fast LC-MS/MS in positive/ negative MRM mode. Data is presented for MRM's of THC, THC-D3, Carboxy-THC, and Carboxy-THC-D3, respectively.

Liquid chromatography was performed in gradient mode employing a 50 x 2.1 mm C18 analytical column and a mobile phase consisting of acetontitrile and 0.1% aqueous formic acid. The gradient was programmed to run from 50% to 90% acetonitrile in 4.5 minutes and equilibrate. The total run time for each analysis was less than 5 minutes.

Results: The limits of detection/ quantification for this method were determined to be 0.1 ng/ mL and 0.25 ng/ mL, respectively. The method was found to be linear from 0.25 ng/ mL to 50 ng/ mL ($r2>0.999$). Matrix Effects and Ion Suppression were not found to be significant. Recoveries of the individual cannabinoids were found to be greater than 85%. In this presentation, the results of authentic drugs/driving case samples (0.4-2.8 ng/ mL (THC), 1.9-38.0 ng/mL (Carboxy-THC)) that were analyzed by F-SPE and fast LC-MS/MS are shown. The relationship between this novel method and previous SPE methodologies are discussed in terms of their chemistries to demonstrate the effectiveness of this technique.

Conclusion: The use of F-SPE and fast LC-MS/MS will be advantageous analysts in the field of drug/driving toxicology as it will serve to reduce the turnaround times for the analysis of these cannabinoids. This method also lowers the levels of detection/ quantification for reporting the results of THC/ Carboxy-THC in drug related driving cases.

Keywords: **THC, LC-MS/MS, F-SPE**

S38 Two Counts 1ST Degree Reckless Homicide with Alcohol, Fluoxetine, Venlafaxine and Olanzapine

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The case study that will be presented involves a high profile case where two recent high school graduates were killed. Their vehicle was hit broadside by a Porsche Boxster that ran a red light at a high rate of speed. The female driver of the Porsche was injured, preventing the administration of standardized field sobriety tests. A blood specimen collected two hours after the crash had an alcohol concentration of 0.072 g/100 mL, fluoxetine (330 ng/mL), norfluoxetine (130 ng/mL), venlafaxine (410 ng/mL), norvenlafaxine (100 ng/mL) and olanzapine (20 ng/mL).

The defendant had been drinking alcoholic beverages at a bar prior to the crash. Detailed drinking information was available from a video surveillance tape showing that she ordered four drinks within 1 ¾ hours. She left the bar 37 minutes before the crash. This information was used to perform retrograde extrapolation of the alcohol concentration back to the time of the crash.

Testimony regarding the drugs detected, their combined effect with alcohol and possible driving impairment is extremely complex. Working with the prosecutor in this area proved to be the greatest challenge, and provided some surprises during the trial. The full case will be presented with emphasis given to drug interpretation, pharmacokinetics, prosecutor preparation and the twists and turns that occurred during this serious case.

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

S39 Butalbital and Driving Impairment

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Butalbital (Fiorinal, Fioricet) is a short to intermediate acting barbiturate used in the treatment of tension headaches and off-label for the treatment of migraines. The sedative hypnotic effects of butalbital make this drug potentially a high-risk medication for drivers, and it is frequently noted as being the most commonly encountered barbiturate in driving populations.

Twenty-one drivers arrested for driving under the influence, who later tested positive for butalbital were evaluated by drug recognition expert (DRE) officers, and their driving pattern, field sobriety test performance, DRE evaluations scores, and toxicology results evaluated.

Driving behaviors included erratic lane travel, no headlights, weaving, driving on the shoulder or curb, striking parked vehicle, hits and rub, slow speeds, stopping in intersection, driving off the road, collisions and near collisions, striking fixed objects, driving into oncoming traffic, failing to stop at traffic signals. Overall the driving patterns were consistent with marked psychomotor intoxication, with obvious problems in gross motor vehicle control.

There was a general concentration dependent decline in performance in field sobriety tests. The subjects generally had an appearance similar to alcohol intoxication, but no or insufficient alcohol to account for their appearance. Symptoms noted by the officers included generally cooperative attitude, poor to very poor coordination, a concentration dependent increase in speech problems, with slow, slurred, thick-tongued speech being very common. Subjects commonly could not perform or performed very poorly on field sobriety tests having marked balance problems, and sway. Subjects also displayed horizontal gaze nystagmus, and at concentrations above therapeutic routinely displayed vertical gaze nystagmus also.

Butalbital concentrations ranged from 1 to 30.3mg/L, with a mean and median of 16mg/L. Eight of the 21 subjects had concentrations within the recognized therapeutic range of 1-10mg/L, although these frequently had other drugs present including codeine, and nortriptyline, the concentrations were low or therapeutic. Alcohol was present in only two cases at low concentrations (0.02g/100mL).

Butalbital has a side effect profile of drowsiness, sedation, dizziness, and feelings of intoxication. Consequently, it should be used with caution by drivers, or others in safety sensitive occupations.

Keywords: **Butalbital, Driving, DRE**

S40 Prevalence of Gabapentin in Impaired Driving Cases in Washington State from 2003- 2007

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Gabapentin (Neurontin®) is an antiepileptic drug commonly prescribed for pain treatment. In the past 15 years indications for gabapentin have been increasing even though the complete mechanism of action is unknown[1]. Side effects include somnolence, dizziness, ataxia, nystagmus, and fatigue. This study examined all driving cases submitted to the Washington State Toxicology Laboratory between January 2003 and December 2007 (n=23,479). Gabapentin quantitation was performed using GC/MS. The concentrations of gabapentin in blood from impaired driving cases (n=137) ranged from <2.0 to 24.7 mg/L, with a mean of 8.4 ± 5.4 mg/L and a median of 7.0 mg/L. The driving population was 50% male with a mean age of 43.0 ± 10.9 years (range 23-73). Of the cases studied, only 7% were positive for gabapentin alone with the remaining 93% indicative of polydrug use. Drug Recognition Expert reports were examined from four cases where the only drug detected likely to be causing impairment was gabapentin. These reports demonstrated that subjects may exhibit psychophysical indicators of a central nervous system depressant (horizontal gaze nystagmus, poor performance on standardized field sobriety tests) with clinical indicators (dilated pupils, low body temperature, elevated pulse and blood pressure) that are not consistent with a depressant.

[1]A. Mack. *J Manag Care Pharm*. 9(6): 559-68 (2003).

Keywords: **Gabapentin, Driving, Impairment**

Abstracts
Poster Presentations

P1 Immunoassay for the Detection of Amphetamines** in Oral Fluid on Automated Clinical Analyzers

Davina Ananias*[1], Glenda Soriano[1], Howard Woods[1], Yuhong Zhao[1], Kathryn Yoder[1], Justin Stall[1], Kristen Blum[2], Dean Fritch[2], Keith Kardos[2], and Steven Lee[2]. [1]Roche Diagnostics, Indianapolis, IN, USA; [2]OraSure Technologies, Inc., Bethlehem, PA, USA

A homogeneous immunoassay for the semi-quantitative and qualitative detection of amphetamines in oral fluid on automated clinical analyzers is presented. The use of oral fluid as a test matrix has risen in popularity as an alternate test matrix to urine for measuring drugs of abuse. It is advantageous due to its non-invasive nature and ease of collection and is less readily adulterated. The automated immunoassay utilizes the KIMS technology (Kinetic Interaction of Microparticles in Solution) with liquid reagents. The two-reagent system consists of a reagent containing an amphetamine monoclonal antibody that is covalently linked to carboxy-modified polystyrene microparticles, with a drug-polymer conjugate in solution as the second reagent. The amphetamines oral fluid assay utilizes a cutoff concentration of 40 ng/mL when using the Intercept® Oral Specimen Collection Device from OraSure Technologies, Inc. (OTI). The cutoff is the diluted cutoff in the Intercept device. The range of the assay is 0-320 ng/mL.

When run in a semi-quantitative mode based on a six-point calibration with d-amphetamine on Roche/Hitachi Modular P and Olympus AU5400® analyzers, control samples at the cutoff and ±25% of the cutoff showed mean recoveries of 30.1 ng/mL (100%), 40.2 ng/mL (100%), and 50.1 ng/mL (100%). Within-run precision (n=21) showed %CV values of 2.6%, 3.3%, and 2.0%, respectively, for these calibrator and control levels. Analytical specificity testing showed cross-reactivites of 84% to MDA, 0.22% to d-methamphetamine, 3.7% to l-amphetamine, 115% to PMA, 0.28% to MDMA, and <0.1% to pseudoephedrine; with no interference from a panel of 59 structurally unrelated drug compounds tested in the assay at a concentration of 10,000 ng/mL. Further studies showed no interference over a sample pH range of 4.5 to 8.5 and no positive or negative interference from a panel of potential food, dental product, and endogenous interferents. A set of 150 clinical samples (50 positive for d-amphetamine and/or MDA at concentrations of 46-2000 ng/mL) showed 100% agreement with LC/MS/MS when the assay was run in both qualitative and semi-quantitative mode. LOD/LLOQ for Methamphetamine, MDMA, Amphetamine and MDA was 2.5/5 ng/mL of diluted oral fluid. In conclusion, the assay was shown to produce accurate and reliable results and is well suited for routine screening of amphetamines in oral fluids on automated systems.

**These assays are currently in development and have not been approved for use in the US by the FDA.

Keywords: **Amphetamines, Oral Fluid, Automation**

P2 Immunoassay for the Detection of Cocaine** in Oral Fluid on Automated Clinical Analyzers

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A homogeneous immunoassay for the semi-quantitative and qualitative detection of cocaine metabolite in oral fluid on automated clinical analyzers is presented. The use of oral fluid as a test matrix has risen in popularity as an alternate test matrix to urine for measuring drugs of abuse. It is advantageous due to its non-invasive nature and ease of collection and is less readily adulterated. The automated immunoassay utilizes the KIMS technology (Kinetic Interaction of Microparticles in Solution) with liquid reagents. The two-reagent system consists of a reagent containing a benzoylecgonine monoclonal antibody that is covalently linked to carboxy-modified polystyrene microparticles, with a drug-polymer conjugate in solution as the second reagent. The cocaine oral fluid assay utilizes a cutoff concentration of 3 ng/mL when using the Intercept® Oral Specimen Collection Device from OraSure Technologies, Inc. (OTI). The cutoff is the diluted cutoff in the Intercept device. The range of the assay is 0-24 ng/mL.

When run in a semi-quantitative mode based on a six-point calibration with benzoylecgonine on Roche/Hitachi Modular P and Olympus AU5400® analyzers, control samples at the cutoff and ±25% of the cutoff showed mean recoveries of 2.34 ng/mL (104%), 3.11 ng/mL (104%), and 3.82 ng/mL (102%). Within-run precision (n=21) showed %CV values of 6.2%, 4.7%, and 4.4%, respectively, for these calibrator and control levels. Analytical specificity testing showed cross-reactivites of <0.5% to cocaine, cocaethylene, ecogonine, and ecgonine methyl ester; with no interference from a panel of 68 structurally unrelated drug compounds tested in the assay at a concentration of 10,000 ng/mL. Further studies showed no interference over a sample pH range of 5.0 to 8.5 and no positive or negative interference from a panel of potential food, dental product, and endogenous interferents. A set of 150 clinical samples (50 positive for benzoylecgonine and/or cocaine at concentrations of 3.25-4970 ng/mL) showed 100% agreement with LC/MS/MS when the assay was run in both qualitative and semi-quantitative mode. The LC/MS/MS LOD/LOQ for benzoylecgonine and cocaine was 0.4/0.8 ng/mL of diluted oral fluid. In conclusion, the assay was shown to produce accurate and reliable results and is well suited for routine screening of cocaine in oral fluids on automated systems.

**These assays are currently in development and have not been approved for use in the US by the FDA.

Keywords: **Cocaine, Oral Fluid, Automation**

P3 Immunoassay for the Detection of Methamphetamine** in Oral Fluid on Automated Clinical Analyzers

Davina Ananias*[1], Yuhong Zhao[1], Daniel Hoch[1], Howard Woods[1], Kathryn Yoder[1], Justin Stall[1], Kristen Blum[2], Dean Fritch[2], Keith Kardos[2], and Steven Lee[2]. [1]Roche Diagnostics, Indianapolis, IN, USA; [2]OraSure Technologies, Inc., Bethlehem, PA, USA

A homogeneous immunoassay for the semi-quantitative and qualitative detection of methamphetamine/MDMA in oral fluid on automated clinical analyzers is presented. The use of oral fluid as a test matrix has risen in popularity as an alternate test matrix to urine for measuring drugs of abuse. It is advantageous due to its non-invasive nature and ease of collection and is less readily adulterated. The automated immunoassay utilizes the KIMS technology (Kinetic Interaction of Microparticles in Solution) with liquid reagents. The two-reagent system consists of a reagent containing monoclonal antibodies that are covalently linked to carboxy-modified polystyrene microparticles, with drug-polymer conjugates in solution as the second reagent. The methamphetamine oral fluid assay utilizes a cutoff concentration of 40 ng/mL when using the Intercept® Oral Specimen Collection Device from OraSure Technologies, Inc. (OTI). The cutoff is the diluted cutoff in the Intercept device. The range of the assay is 0-320 ng/mL.

When run in a semi-quantitative mode based on a six-point calibration with d-methamphetamine on Roche/Hitachi Modular P and Olympus AU5400® analyzers, control samples at the cutoff and ±25% of the cutoff showed mean recoveries of 32.0 ng/mL (107%), 42.7 ng/mL (107%), and 52.1 ng/mL (104%). Within-run precision (n=21) showed %CV values of 5.8%, 6.1%, and 3.7%, respectively, for these calibrator and control levels. Analytical specificity testing showed cross-reactivites of 143% to MDMA, 5.3% to d-amphetamine, 78% to MDEA, 58% to MBDB, and <0.2% to pseudoephedrine; with no interference from a panel of 60 structurally unrelated drug compounds tested in the assay at a concentration of 10,000 ng/mL. Further studies showed no interference over a sample pH range of 4.5 to 8.5 and no positive or negative interference from a panel of potential food, dental product, and endogenous interferents. A set of 150 clinical samples (50 positive for d-methamphetamine, MDMA, or MDEA at concentrations of 51-1440 ng/mL) showed 100% agreement with LC/MS/MS when the assay was run in both qualitative and semi-quantitative mode. The LC/MS/MS LOD/LOQ for Methamphetamine, MDMA, Amphetamine and MDA was 2.5/5 ng/mL of diluted oral fluid. In conclusion, the assay was shown to produce accurate and reliable results and is well suited for routine screening of methamphetamine in oral fluids on automated systems.

**These assays are currently in development and have not been approved for use in the US by the FDA.

Keywords: **Methamphetamine, MDMA, Oral Fluid**

P4 Immunoassay for the Detection of Opiates** in Oral Fluid on Automated Clinical Analyzers

Davina Ananias*[1], Daniel Hoch[1], Christopher Lawrence [1], Yuhong Zhao[1], Kathryn Yoder[1], Justin Stall[1], Kristen Blum[2], Dean Fritch[2], Keith Kardos[2], and Steven Lee[2] . [1]Roche Diagnostics, Indianapolis, IN, USA; [2]OraSure Technologies, Inc., Bethlehem, PA, USA

A homogeneous immunoassay for the semi-quantitative and qualitative detection of opiates in oral fluid on automated clinical analyzers is presented. The use of oral fluid as a test matrix has risen in popularity as an alternate test matrix to urine for measuring drugs of abuse. It is advantageous due to its non-invasive nature and ease of collection and is less readily adulterated. The automated immunoassay utilizes the KIMS technology (Kinetic Interaction of Microparticles in Solution) with liquid reagents. The two-reagent system consists of a reagent containing morphine monoclonal antibody that is covalently linked to carboxy-modified polystyrene microparticles, with a drug-polymer conjugate in solution as the second reagent. The opiates oral fluid assay utilizes a cutoff concentration of 10 ng/mL when using the Intercept® Oral Specimen Collection Device from OraSure Technologies, Inc. (OTI). The cutoff is the diluted cutoff in the Intercept device. The range of the assay is 0-80 ng/mL.

When run in a semi-quantitative mode based on a six-point calibration on Roche/Hitachi Modular P and Olympus AU5400® analyzers, control samples at the cutoff and ±25% of the cutoff showed mean recoveries of 7.9 ng/mL (105%), 10.3 ng/mL (103%), and 12.7 ng/mL (102%). Within-run precision (n=21) showed %CV values of 4.7%, 3.0%, and 3.9%, respectively, for these calibrator and control levels. Analytical specificity testing showed cross-reactivites of 150% to codeine, 93% to 6-acetylmorphine, 27% to hydrocodone, and 22% to hydromorphone, with no interference from a panel of 63 structurally unrelated drug compounds tested in the assay at a concentration of 10,000 ng/mL. Further studies showed no interference over a sample pH range of 4.5 to 8.5 and no positive or negative interference from a panel of potential food, dental product, and endogenous interferents. A set of 150 clinical samples (50 positive for morphine and/or codeine with concentrations of 10.1-1330 ng/mL morphine) showed 100% agreement with LC/MS/MS when the assay was run in both qualitative and semi-quantitative mode. The LC/MS/MS LOD/LLOQ for morphine and codeine was 2/4 ng/mL of diluted oral fluid. In conclusion, the assay was shown to produce accurate and reliable results and is well suited for routine screening of opiates in oral fluids on automated systems.

**These assays are currently in development and have not been approved for use in the US by the FDA.

Keywords: **Opiates, Oral Fluid, Automation**

P5 Immunoassay for the Detection of PCP** in Oral Fluid on Automated Clinical Analyzers

Davina Ananias*[1], Christopher West[1], Christopher Lawrence[1], Kathryn Yoder[1], Justin Stall[1], Kristen Blum[2], Dean Fritch[2], Keith Kardos[2], and Steven Lee[2]. [1]Roche Diagnostics, Indianapolis, IN, USA; [2]OraSure Technologies, Inc., Bethlehem, PA, USA

A homogeneous immunoassay for the semi-quantitative and qualitative detection of phencyclidine in oral fluid on automated clinical analyzers is presented. The use of oral fluid as a test matrix has risen in popularity as an alternate test matrix to urine for measuring drugs of abuse. It is advantageous due to its non-invasive nature and ease of collection and is less readily adulterated. The automated immunoassay utilizes the KIMS technology (Kinetic Interaction of Microparticles in Solution) with liquid reagents. The two-reagent system consists of a reagent containing a phencyclidine monoclonal antibody that is covalently linked to carboxy-modified polystyrene microparticles, with a drug-polymer conjugate in solution as the second reagent. The PCP oral fluid assay utilizes a cutoff concentration of 2 ng/mL when using the Intercept® Oral Specimen Collection Device from OraSure Technologies, Inc. (OTI). The cutoff is the diluted cutoff in the Intercept device. The range of the assay is 0-16 ng/mL.

When run in a semi-quantitative mode based on a six-point calibration with PCP on Roche/Hitachi Modular P and Olympus AU5400® analyzers, control samples at the cutoff and ±25% of the cutoff showed mean recoveries of 1.41 ng/mL (94%), 1.89 ng/mL (95%), and 2.38 ng/mL (95%). Within-run precision (n=21) showed %CV values of 5.7%, 4.5%, and 2.2%, respectively, for these calibrator and control levels. Analytical specificity testing showed cross-reactivites of 1.2% to dextromethorphan and <0.3% to ketamine, with no interference from a panel of 65 structurally unrelated drug compounds tested in the assay at a concentration of 10,000 ng/mL. Further studies showed no interference over a sample pH range of 4.0 to 8.5 and no positive or negative interference from a panel of potential food, dental product, and endogenous interferents. A set of 150 clinical samples (50 positive for PCP, combination of native and spiked samples with concentrations from 3-29 ng/mL) showed 100% agreement with LC/MS/MS when the assay was run in both qualitative and semi-quantitative mode. LOD/LLOQ for PCP was 0.2/0.4 ng/mL of diluted oral fluid. In conclusion, the assay was shown to produce accurate and reliable results and is well suited for routine screening of PCP in oral fluids on automated systems.

**These assays are currently in development and have not been approved for use in the US by the FDA.

Keywords: **PCP, Oral Fluid, Automation**

P6 Identification and Quantitation of Amphetamines, Cocaine, Opiates, and Phencyclidine in Oral Fluid by Liquid Chromatography-Tandem Mass Spectrometry

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Analytical methods for measuring multiple licit and illicit drugs and metabolites in oral fluid require high sensitivity, specificity and accuracy. With the limited volume available for testing, comprehensive methodology is needed for simultaneous measurement of multiple analytes in a single aliquot. This report describes the validation of a semi-automated method for the simultaneous extraction, identification and quantitation of 21 analytes in oral fluid. The target compounds (cutoff concentration, ng/mL) included amphetamine (12.5), methamphetamine (12.5), 3,4-methylenedioxymethamphetamine (MDMA) (12.5), 3,4-methylenedioxy-amphetamine (MDA) (12.5), 3,4-methylenedioxyethylamphetamine (MDEA) (12.5), pseudoephedrine (12.5), cocaine (2), benzoylecgonine (2), codeine (10), norcodeine (10), 6-acetylcodeine (5), morphine (10), 6-acetylmorphine (1), hydrocodone (10), norhydrocodone (10), dihydrocodeine (10), hydromorphone (10), oxycodone (10), noroxycodone (10), oxymorphone (10), and phencyclidine (1). Oral fluid specimens were collected with the Intercept device and extracted by SPE. Drug recovery from the Intercept device averaged 84.3% and SPE extraction efficiency averaged 91.2% for the 21 analytes. Drug analysis was performed by liquid chromatography-tandem mass spectrometry (LC-MS-MS) in the positive electrospray mode using ratios of qualifying product ions within $\pm$ 25% of calibration standards. The inter-assay precision for the controls at $\pm$ 25% of the cutoff concentrations ranged from 2.5 to 16.6%. Matrix ion suppression ranged from -57 to 8%. The limit of quantitation ranged from 0.4 to 5 ng/mL using 0.2 mL of diluted oral fluid sample. Application of the method was demonstrated by testing oral fluid specimens from drug abuse treatment patients. Thirty-nine patients tested positive for various combinations of licit and illicit drugs. Nine patients tested positive for "opiate" derivatives (morphine, codeine, 6-AM and 6-AC). All 9 patients were positive for 6-AM and 3 were positive only for 6-AM. In conclusion, this validated method is suitable for simultaneous measurement of 21 licit and illicit drugs and metabolites in oral fluid.

Keywords: **Oral Fluid, LC-MS-MS, Drugs of Abuse**

P7 Sampling and Preparing Natural Oral Fluid for Quantitative Determination of Buprenorphine and Metabolites

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Oral fluid as an alternate specimen for monitoring drug use has recently become a popular topic of study. In our view, none of the reported methods can effectively produce *representative* specimens with *definite sizes* for *quantitative* determination of drugs of interest. This study was designed to develop a collection and processing approach that may reproducibly prepare natural oral fluid specimens for quantitative drug analysis.

Oral fluid was collected by naturally flowing through a straw into a 15-mL collection tube, without stimulating its secretion by an agent or other means. Specimens were heated and centrifuged to remove forth and potential contaminations. These processes were found effective in: (a) preparing specimens that can be accurately determined in sample size, without affecting the composition of buprenorphine (B) and its metabolites; and (b) achieving quantitative recoveries of B and norbuprenorphine (NB). Utilizing a sample size of 500 μL, the derivatization and GC-MS protocols achieved 3 and 5 ng/mL as the method's limits of detection and quantitation. This approach was applied to the analysis of clinical specimens, collected from patients under a substitution therapy program to better understand the distribution and correlation (if any) of B and its metabolites in oral fluid, blood, and urine. Preliminary data derived from the analysis of oral fluid and urine specimens collected at the same time from six subjects are shown in Table 1 (time of last dose unknown).

Table 1. Concentrations (ng/mL) of B and NB found in oral fluid and urine (collected at the same time) from six patients under B-treatment (time of last dose unknown)

Sample	Analyte	1	2	3	4	5	6
Oral fluid	B	16.8	361.4	ND[b]	ND	1.1[a]	ND
	NB	1.6[a]	15.5	ND	ND	ND	ND
Urine	B	148.9	381.7	16.4	32.1	27.1	6.3
	NB	152.2	930.2	57.3	167.3	187.0	40.3

[a] The presence of ions designating the analytes in these specimens are credible; however, these data are lower than the limits of detection and quantitation established for the assay. They are listed for reference only. [b] Not detected.

These preliminary data reflect the validity of the oral fluid collection and treatment processes and the relative concentrations of B and NB in these specimens. Adaptation of the LC-MSMS methodology for achieving lower detection and quantitation levels is currently under investigation. Further study will also include the following parameters to facilitate pharmacokinetic interpretation of the observed data: (a) exact dose and time of drug intact; and (b) specimens collected at multiple time points.

Keywords: **Oral Fluid, Buprenorphine, Substitution Therapy**

P8 Disposition of Oxycodone in Oral Fluid

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The objective of this research was to provide data on the incidence, concentrations and relative abundances of oxycodone and its metabolites in oral fluid obtained from a controlled Oxycontin dosing experiment. Such information may guide the interpretation of oral fluid testing results.

There is an active interest in oral fluid as an alternative matrix for monitoring drug use. Drugs and metabolites passively diffuse into oral fluid from plasma across a concentration gradient via the salivary glands. Limited data on the disposition of drugs into oral fluid is available. Oxycodone is of particular interest, given its prevalence and the widespread, persistent abuse of prescription opiates. Oral fluid is a promising drug-testing matrix because it is relatively clean, readily accessible for sampling, non-invasive and inexpensive. The application of more sensitive, advanced technologies like liquid chromatography tandem mass spectrometry (LC/MS/MS) can offset the small sample volumes and low detection limits associated with this matrix.

Twelve subjects were orally administered Oxycontin (80 mg), every 12 hours, and naltrexone (50 mg), every 24 hours, for four days. Once steady state was established, the Quantisal™ device was used to collect oral fluid specimens on Day 3 and 4 at the six hour post-dose time-point. The samples were analyzed quantitatively by LC/MS/MS, following sample pre-concentration by mixed-mode solid phase extraction. There was no interference from naltrexone with the LC/MS/MS method. In a separate experiment, a randomly selected set of samples (n=8) underwent acid hydrolysis, prior to extraction, to assess the presence of oxycodone and oxymorphone glucuronides in oral fluid.

Oxycodone, noroxycodone and oxymorphone quantifications were based on an eight-point calibration curve; linear range of 5-1000 ng/mL (R^2>0.99). Limits of quantification (LOQ) were 5 ng/mL. Interday precision (n=5) and intraday precision (n=15) did not exceed 10% coefficient of variation. Accuracy was within 15% of the target. Noroxycodone (43-228 ng/mL) was detected in all oral fluid specimens and always in conjunction with oxycodone (172-1180 ng/mL). Mean relative abundances were 20% (noroxycodone) and 80% (oxycodone). Mean concentrations of 115 ng/mL (noroxycodone) and 418 ng/mL (oxycodone) were well above recommended Substance Abuse Mental Health Services Administration (SAMHSA) oral fluid cutoffs (40 ng/mL). Only trace amounts, below the LOQ, were detected for oxymorphone, even after hydrolysis. The observed metabolite ratios are consistent with previous pharmacokinetic studies, which indicated N-demethylation as the major oxidative pathway for oxycodone, as compared to O-demethylation. There was no significant difference in the oxycodone concentrations with and without hydrolysis; $t(7)= 0.863$, $p = 0.417$ (95% confidence level).

The parent drug, oxycodone, is the dominant species present in oral fluid following ingestion. Noroxycodone is the major metabolite present, in tandem, at lower concentrations than oxycodone. Noroxycodone can be a reliable biomarker to substantiate oxycodone use. Glucuronides of oxycodone and oxymorphone were not detected in oral fluid in significant concentrations. Therefore, hydrolysis prior to quantitative analysis of oral fluid for oxycodone and its metabolites appears to be unnecessary.

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

P9 Comparison of Oral Fluid Proficiency Specimens Utilizing Homogeneous Enzyme Immunoassay (HEIA) and Enzyme Linked Immunosorbent Assay (ELISA)

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Proficiency samples have often been used to challenge both screening and confirmatory laboratory procedures. With the increased sensitivity of rapid, simple high throughput screening platforms using liquid reagents, Homogeneous Enzyme Immunoassay (HEIA) has begun to increase in popularity as a screening choice for oral fluid, which to date has been carried out using enzyme linked immunosorbent assays (ELISA). The objective of the research was to determine whether HEIA proficiency data has a strong correlation to ELISA data collected over a span of 5 testing cycles.

Neat oral fluid proficiency samples received from January 2008 to May 2009 were all reanalyzed in June 2009 by both HEIA and ELISA. Proficiency samples were stored at 4°C in their original amber glass containers and were not sampled again until June 2009. All samples (N=22) and calibrators were diluted 1 + 3 with Quantisal™ buffer to achieve concentration values recommended for their corresponding tests. All samples were analyzed for the following tests; Cannabinoids (THC), cocaine (COC), amphetamine (AMP), methamphetamine (METH), opiates (OPI), phencyclidine (PCP), benzodiazepines (BZP), methadone (MTD), and oxycodone (OXYC) with cut off concentrations at 4, 20, 50, 50, 40, 10, 10, 50, 25ng/mL respectively; the only exceptions being THC HEIA at 8ng/mL and OXYC HEIA at 40ng/mL.

For OPI, PCP, MTD, and OXYC all 22 samples had 100% correlation (POS/NEG). METH at 95% correlation with the one sample missed by HEIA was found to have 48ng/mL of methamphetamine, just below the cut off concentration. The AMP correlation was 91% with HEIA not picking up two samples containing only MDA, though minor inhibition was seen in the raw data. In this case there are different antibodies used and the cross-reactivity for MDA differs between the two kits by 178% for ELISA and 40% for HEIA. The BZP correlation was 91% with one sample containing 11ng/mL oxazepam and the second sample containing only 2.1ng/mL alprazolam. The lowest correlation, as expected, was with COC and THC (86% correlation). The three samples that did not agree all fell below 4ng/mL. As with the amphetamine antibody the cocaine HEIA and ELISA antibodies do differ slightly with respect to cross-reactivities. Two more were found positive by ELISA but negative with HEIA. In all cases where POS/NEG data did not correlate inhibition was seen in the raw data for HEIA and ELISA within +/- 20% of cut-off concentration.

In conclusion with 207 individual tests performed, the overall correlation between HEIA and ELISA using proficiency samples was 94%. Routine liquid reagent chemistry analyzers and ELISA platforms show a high degree of qualitative correlation. Discrepant results can be attributed to different cut-off concentrations, or variation in the antibodies used for screening.

Keywords: **HEIA, ELISA, Oral Fluid**

P10 Stability of Tetrahydrocannabinol (THC) in Oral Fluid Proficiency Samples Over Eighteen Months

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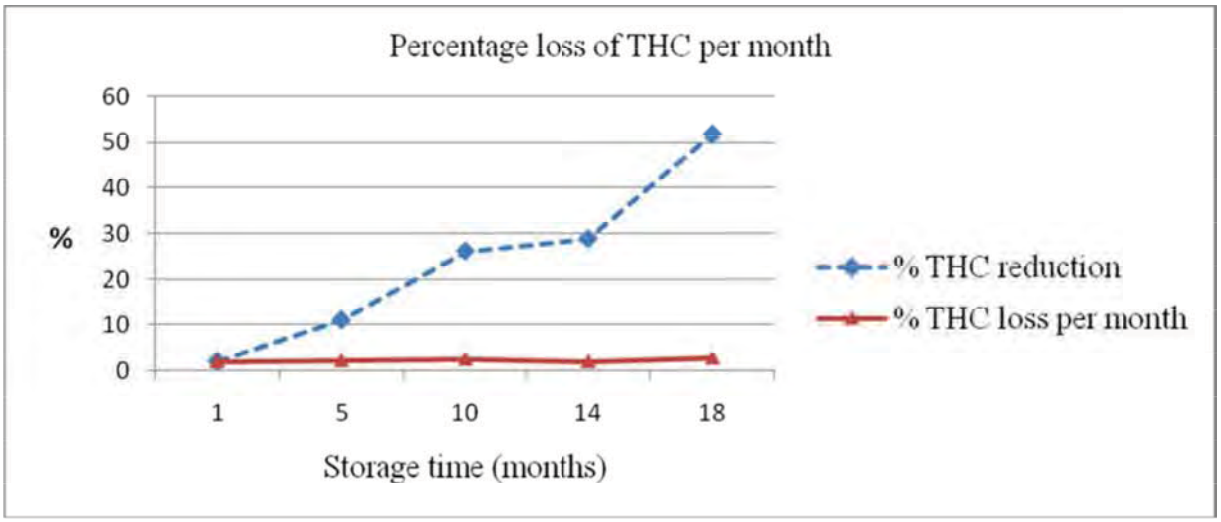
Objective: Using proficiency samples of known concentration received between January 2008 and May 2009, the stability of THC in oral fluid was determined by GC/MS data comparison.

Methods: Neat oral fluid proficiency samples (3mL) were received from RTI International. The neat oral fluid was stored in 4mL amber glass bottles at 4°C until ready for analysis. ELISA immunoassay screening and GC/MS quantitation were carried out initially upon receipt of the specimens. Samples were not re-analyzed until June 2009. All samples (N=8) and calibrators were diluted 1+3 with Quantisal™ buffer prior to analysis, however, samples were not stored Quantisal™ buffer.

Results: Of the 25 proficiency samples 8 originally tested positive for THC. Original reported results were all with in +/- 20% of expected concentrations, based on target values provided by RTI, with concentrations ranging from 3 to 20ng/mL. Samples that were reanalyzed were stored for various lengths of time 18, 14, 10, 5, and 1 month increments. All samples showed some amount of drug loss. After 18 months the percentage loss of THC was 51% and after 1 month, only a 2% loss was observed. With the exception of one outlier the percentage loss per month when calculated for each sample was very consistent, with a range from 1.7% to 2.8%. The one outlier had an original concentration of 8ng/mL and after 1 month of storage the concentration had fallen to 6.3ng/mL (21% loss). However, on average a loss of 2.2% can be expected per month of storage.

Summary: THC in neat oral fluid degrades at a consistent rate of approximately 2.2% per month when stored in glass bottles at 4°C. In comparison, data collected in 2008 from oral fluid samples stored in the Quantisal™ buffer showed an average loss of 1.6% per month over 9 months (reported at SOFT 2008).



Keywords: **THC, Oral Fluid, GC/MS**

P11 Conversion of Cocaine to Benzoylecgonine in Oral Fluid Proficiency Samples Over Eighteen Months

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Objective: The stability of cocaine and benzoylecgonine in oral fluid proficiency specimens received between January 2008 and May 2009 was investigated by comparison of the originally reported results with re-analyzed specimens using GC/MS.

Methods: Neat oral fluid proficiency samples (3mL, pH 7.0) were received from RTI International, NC via overnight delivery using a Thermo Chill insulator package. The specimens were stored in glass amber bottles at 4°C until ready for analysis. ELISA immunoassay screening and GC/MS analysis were initially performed upon receipt of the proficiency sets, and then the specimens were returned to refrigerated storage. A second analysis of all proficiency samples forming the basis of the cocaine (COC) to benzoylecgonine (BZE) conversion data comparison was performed in June 2009 using GC/MS analysis. Aliquots of the proficiency samples (1mL; n=10), calibrators (n=5) and controls (positive and negative in each batch) were diluted 1+3 with Quantisal™ buffer prior to analysis. Briefly, specimens, calibrators and controls were extracted using mixed mode solid phase columns and derivatized with trifluoroethanol and heptafluorobutyric anhydride in methylene chloride.

Results: Five specimens were originally positive in our laboratory for BZE alone by both ELISA and GC/MS. When reanalyzed, all of those samples were within +/- 10% of their original value over the entire 18 month range. Four samples were originally positive for cocaine only; upon re-analysis the cocaine concentration in all specimens had decreased, with those stored for 18 months showing the highest loss. BZE was present in all four of those specimens, showing conversion of parent cocaine to BZE over time. One sample, which was originally positive for COC (33ng/mL) and BZE (46ng/mL) showed a reduction in cocaine concentration to 22ng/mL and an increase in BZE concentration to 58ng/mL over a 5 month storage period.

Storage time (months)	*Original concentration (ng/mL)*		*Re-analysis concentration (ng/mL)*	
	Cocaine	**BZE**	**Cocaine**	**BZE**
1	0	32	0	34
5	33	46	22	58
5	0	89	0	89
10	0	63	0	66
10	40	0	13	27
14	0	24	0	24
14	0	40	0	42
14	28	0	6	26
18	58	0	8	50
18	37	0	0	34

Summary: Cocaine was converted to benzoylecgonine in neat oral fluid proficiency samples stored at 4°C, while benzoylecgonine in oral fluid remained stable. This has implications for the interpretation of oral fluid test results if specimens are re-tested after some time. Further studies will focus on storage of cocaine in transportation buffer supplied with oral fluid collection devices in an effort to improve the stability of cocaine.

Keywords: **Oral Fluid, Cocaine, Stability**

P12 Semi-Quantitative Determination of Tramadol in Oral Fluid Utilizing Homogeneous Enzyme Immunoassay (HEIA): Comparison with Enzyme Linked Immunosorbent Assay (ELISA)

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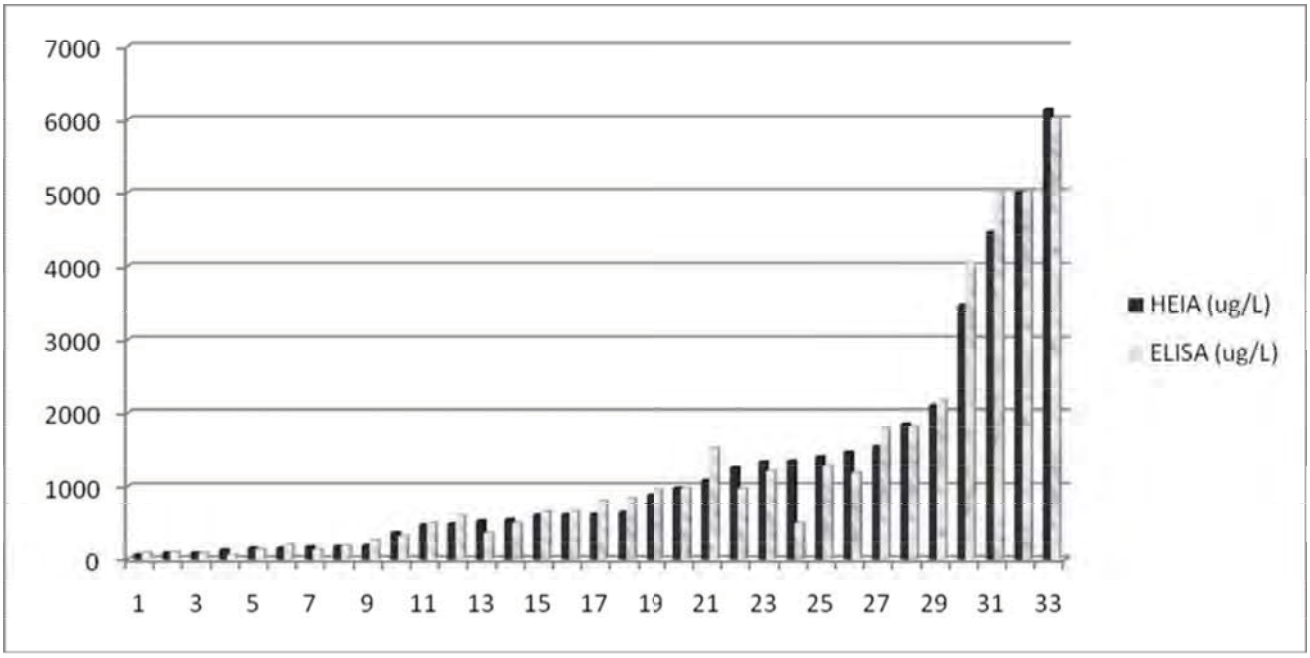
Tramadol is a CNS depressant and analgesic used widely in the treatment of moderate to severe pain. The objective of the research was to develop a rapid semi-quantitative method for the screening of samples for tramadol in oral fluid and to determine whether semi-quantitative data collected with HEIA has a strong correlation with routine immunoassay, ELISA.

Oral fluid samples collected using the Quantisal™ devices were screened at a cut-off concentration of 50ug/L using both ELISA and HEIA (Olympus AU400e). Calibrators for ELISA and HEIA were diluted 1:4 with Quantisal™ buffer to achieve neat oral fluid concentration. Calibration standards at 25, 50, 100, 200, 500ug/L were analyzed for both HEIA and ELISA. Inter-day precision was performed over 2 runs per day for 20 days with 2 replicates (n=80). The coefficient of variation (CV) at control levels of 150ug/L and 250ug/L were 0.65% and 0.64% respectively for HEIA; 5.35% and 4.05% for ELISA. For ELISA, the linearity was r^2=0.9794 on a semi-quantitative curve. HEIA utilized a multipoint polygonal calibration.

A total of 53 specimens were analyzed. 20 specimens were negative by both assays; 33 previously found to be positive using GC/MS at a cut-off of 50ug/L were positive by both assays giving a specificity and sensitivity of 100%. Samples outside the upper limit of linearity were diluted 1:10 or 1:100 and re-run to fit within the standard linear range. Tramadol can be determined in oral fluid specimens using semi-quantitative screening modes on routine chemistry analyzers or ELISA platforms. Both assays were rapid and simple to operate, showing a high degree of quantitative correlation.



Keywords: **Tramadol, Oral Fluid, Enzyme Immunoassay**

P13 Stability of 6-Acetylmorphine in Oral Fluid Proficiency Samples Over Eighteen Months

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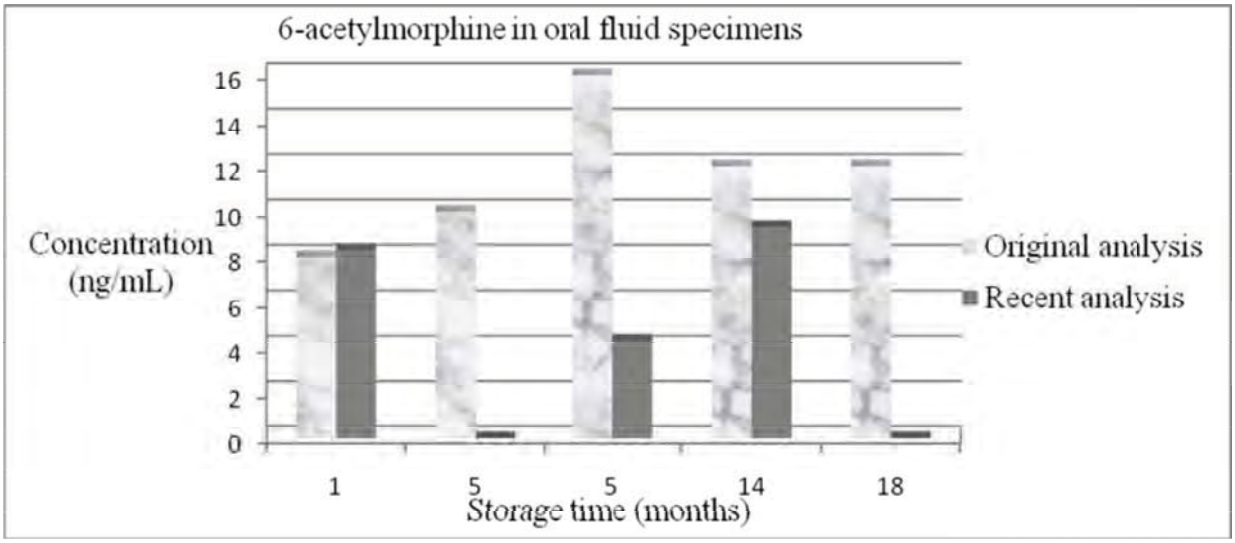
Objective: Using proficiency samples of known concentration received between January 2008 and May 2009, the stability of codeine (COD), morphine (MOR), and 6-acetylmorphine (6-AM) in oral fluid was determined by GC/MS and the results compared to originally reported values.

Methods: Neat oral fluid proficiency samples (3mL, pH 7.0) were received from RTI International, NC as part of the proficiency scheme. The neat oral fluid was stored in amber glass bottles at 4°C until ready for analysis. ELISA immunoassay screening and GC/MS were initially carried out upon receipt of the specimens. Samples were not re-analyzed until June 2009. All samples (N=5) and calibrators were diluted 1+3 with Quantisal™ buffer prior to analysis but were stored in the original containers.

Results: Of the 25 proficiency samples 6 tested positive for opiates. One other sample testing positive for oxycodone, hydrocodone and hydromorphone is not discussed here. Originally reported results were all with in +/- 20% of expected concentrations, as targeted by RTI, at concentrations ranging from 8 to 16ng/mL. Samples that were reanalyzed were stored for various lengths of time 18, 14, 5, and 1 month increments. All samples, when reanalyzed for codeine and morphine showed no loss of drug and were well within +/- 10% of original values. However, 6-AM demonstrated a significant loss ranging from 100% for a sample stored for 18 months to no measurable loss in a sample stored for one month; there was no consistent rate of loss per month. One sample originally had a concentration of 12ng/mL showed no 6-AM remaining after 18 months of storage; and another at 10ng/mL originally also showed no 6-AM remaining after 5 months of storage. An initial hypothesis that the loss may occur quickly in the first 5 months was disproved by analysis of one sample with an original concentration of 12ng/mL showing a concentration of 9ng/mL after 14 months of storage (1.6% loss per month).

Summary: The stability of 6-AM in neat oral fluid should be studied using a much larger pool of samples and at a greater number of time intervals. However, 6-AM has been shown to degrade rapidly with the majority of the drug lost within the first 5 months.



Keywords: **Oral Fluid, 6-acetylmorphine, Stability**

P14 Enzyme Immunoassay Validation for Detection of Cannabinoids in Oral Fluid

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Oral fluid (OF) is gaining prominence as an alternative matrix for monitoring drugs of abuse in workplace, criminal justice and driving under the influence of drugs (DUID) programs. Validation of screening techniques for Δ-9-tetrahydrocannabinol (THC) in oral fluid is important for characterizing assay performance and limitations.

The protocol was approved by the NIDA Institutional Review Board and participants provided written informed consent. OF specimens were collected from 12 male participants administered increasing daily doses of 40-120 mg oral synthetic THC (dronabinol) every 3.5-6 h for eight days. 499 OF specimens were collected with the Quantisal™ device and blank OF was obtained from healthy volunteers (n=9). All specimens were tested with the Immunalysis Sweat/OF THC Direct ELISA kit according to provided instructions (Rev. 10/2007). Limit of detection (LOD) absorbance values were calculated by subtracting three times the standard deviation of the 9 negative specimens from their mean absorbance. Plate linearity was investigated by calculation of the regression line by the method of least squares and expressed as the coefficient of determination (r^2) for THC concentrations at 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 10, 15, 20, 25, 30, 50, 75, 100, 125, 150, and 200 ng/mL in pooled negative OF. Intra- and inter-day imprecision were determined with in-house controls at 1.0, 3.0, 5.0, and 6.0 ng/mL THC. For intra-day imprecision, 5 replicates at each concentration (n=20) were assayed. For inter-day imprecision, 2 replicates of controls at each concentration were assayed on 10 plates (n=20). Cross-reactivity was characterized by analyzing 11-hydroxy-THC (11-OH-THC), cannabidiol, cannabinol, Δ8-tetrahydrocannabinol (Δ8-THC), 11-nor-9-carboxy-THC (THCCOOH), THCCOOH-glucuronide, and THC-glucuronide at 0.1, 1, 4, and 400 ng/mL, as well as potential interference from 30 other commonly encountered licit and illicit drugs at 1000 ng/mL. Analytical sensitivity and specificity were evaluated at an ELISA screening cutoff of 4 ng/mL and GCMS confirmation at 2 ng/mL with 499 authentic OF specimens. Specimens were quantified by two-dimensional EI-GCMS (THC) and NCI-GCMS (THCCOOH) with limits of quantification (LOQ) of 0.5 ng/mL and 7.5 pg/mL, respectively.

On five different days, immunoassay LOD values of 0.88, 0.77, 0.82, 0.91, and 0.77 ng/mL were obtained. Linearity samples yielded a logarithmic regression line of $y = -0.6176\ln(x) + 0.2228$ (r^2=0.9835), indicating good linearity to 50 and fair linearity to 200 ng/mL THC in OF. Intra-plate imprecision (%CV) ranged from 0.4% at 3 ng/mL to 12.9% at 5 ng/mL. Inter-plate imprecision (%CV) ranged from 2.9% at 1 ng/mL to 7.5% at 5 ng/mL. Immunoassay cross-reactivity at 4 ng/mL was 198% for 11-OH-THC, 121% for THCCOOH, 128% for Δ8-THC, 2.4% for cannabidiol, 87% for cannabinol, 10% for THC-glucuronide, and 11% for THCCOOH-glucuronide. Cross-reactivities for the 30 common drugs or chemicals were less than 0.05% and all samples were qualitatively negative. Of the 499 OF specimens collected after 40-120 mg/day oral Marinol®, 52 confirmed positive (THC 2.0–290 ng/mL), with no false negatives at the proposed SAMHSA 4 ng/mL screening cutoff and 2 ng/mL GCMS cutoff; therefore, sensitivity was 100%. 47 specimens screened positive but were not confirmed, yielding a specificity of 89.5% and efficiency of 90.6%. 32 of the 47 false positive OF specimens were from a single participant and contained THCCOOH >500 pg/mL, potentially contributing to decreased specificity and efficiency.

The Immunalysis Sweat/OF THC Direct ELISA kit is a highly sensitive, simple and rapid screening procedure to detect cannabinoids in OF at the proposed SAMHSA guidelines.

Supported by the National Institute on Drug Abuse, NIH. Immunalysis kindly supplied immunoassay screening kits.

Keywords: **Oral Fluid, THC, ELISA**

P15 Simultaneous Quantification of Nicotine, Cotinine, *trans*-3'-Hydroxycotinine and Norcotinine in Human Oral Fluid by LCMSMS

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Objective: A quantitative LC-TurboIonSpray-MSMS method for rapid and simple assay of nicotine and metabolites in human oral fluid was developed and validated.

Method: Oral fluid specimens were collected with the Quantisal™ device (Immunalysis, Pomona, CA) and analyzed for the presence of nicotine, cotinine, *trans*-3'-hydroxycotinine, and norcotinine. 2 mL Quantisal™ buffer, containing 0.5 mL oral fluid, was added to 2 mL of 2M sodium phosphate buffer pH 6, prior to solid phase extraction (SPE) on CleanScreen SPE columns (United Chemical Technologies, Bristol, PA). Analytes were separated on a Phenomenex Synergi Polar-RP analytical column with a gradient mobile phase of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile with a gradient program of 5% B increasing to 50% over 3 min and hold for 1 min, and 50% to 5% B over 1 min. Including re-equilibration for 2 min, the total run time was 7 min. The flow rate was 250 µL/min. Mass spectrometry data were collected in positive ion mode with selected reaction monitoring. Transitions included *m/z* 163.2 to 132.2, 84.2 for nicotine, *m/z* 167.2 to 136.1 and 121.0 for nicotine-d_4, *m/z* 177.2 to 80.1, 98.1 for cotinine, *m/z* 180.2 to 101.2 and 80.2 for cotinine-d_3, *m/z* 193.2 to 80.2, 134.0 for OH-cotinine, *m/z* 196.2 to 134.1 and 79.9 for OH-cotinine-d_3, *m/z* 163.2 to 80.2, 118.2 for norcotinine, and *m/z* 167.2 to 139.2 and 84.2 for norcotinine-d_4. The following criteria evaluated method performance over four unique assays: selectivity, sensitivity, limits of detection and quantification, linearity, imprecision, analytical recovery, extraction efficiency, matrix effect, process efficiency, carryover, dilution integrity, and stability.

Results: Stability of the LC method was evaluated by calculating retention time variabilities. The percent relative variation for retention times were ≤ 0.18 % for all analytes over 30 consecutive runs. Calibration by linear regression analysis utilized deuterated internal standards and a weighting factor 1/x. Linearity was achieved from 1-2000 µg/L for nicotine and norcotinine; 0.5-2000 µg/L for *trans*-3'-hydroxycotinine; and 0.2-2000 µg/L for cotinine with limits of detection of 0.5, 0.3, and 0.1 µg/L, respectively. There was no endogenous signal for any analyte in ten blank human oral fluid specimens demonstrating good specificity for the method. In addition, no selected over-the-counter or abused drugs at a concentration of 10000 µg/L interfered with the low quality control of the analytes. Suitable analytical recovery >91% (to the nominal concentration values) was achieved with extraction efficiencies (recovery from the matrix) >56% and matrix effects <29% CV.

Conclusion: This assay provides quantitative analysis of nicotine and metabolites in oral fluid to support clinical studies determining the most appropriate nicotine biomarker to differentiate active, passive and environmental nicotine exposure.

Supported by the Intramural Research Program, National Institute on Drug Abuse, NIH.

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

P16 Automated Immunoassay for the Detection of Barbiturates in Oral Fluid on Roche Instrument Platform

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Introduction: A homogeneous immunoassay is being developed for the qualitative and semi-quantitative determination of Barbiturates in oral fluids on Roche automated clinical analyzers[#]. In recent years, interest in oral fluids as an alternate matrix for measuring drugs of abuse has increased. Sample collection is less invasive than with urine, more cost-effective, and adulteration is more difficult. Results are more indicative of recent abuse, and better reflect drug doses at the site of action.

Methods: The assay utilizes KIMS technology (Kinetic Interaction of Microparticles in Solution) with ready-to-use liquid reagents. The two-reagent system consists of a first reagent containing a polyclonal antibody with a broad reactivity profile for barbiturate compounds plus an accelerant, and a second reagent containing a protein-drug conjugate covalently coupled to carboxy-modified polystyrene microparticles.
The Barbiturates oral fluid assay utilizes a cutoff of 20 ng/mL when using the Intercept® Oral Specimen Collection Device from OraSure Technologies, Inc. The test range of the assay is 0 - 160 ng/mL. Multi-calibrators based on a proprietary synthetic matrix are provided by OraSure Technologies, Inc.

Results: When run in a semi-quantitative mode with 6-point calibration on a Roche/ Hitachi 917 analyzer, calibration curves are generated reproducibly with an analytical sensitivity (mean – 3*SD) of ca. 10% of the cut-off concentration (i.e. 1.3-2.5 ng/mL), while maintaining good dynamics over the entire measurement range (span cutoff-to-zero dose ≥40% of total span, i.e. ≥500 mAU). Even at 20% accelerant overdose, no non-specific agglutination is found in the absence of antibody. Individual oral fluid samples spiked at +/-25% of the cut-off concentration are recovered within a range of 100 +/-10%, with no crossovers. Within-run precision studies (n = 21) at these levels yield %CV values of 2.5 – 5.5%. Preliminary studies showed broad cross-reactivity with a variety of barbiturate drugs. Accelerated stability studies with samples spiked ad 15 and 25 ng/ml, respectively, indicate a real-time stability period in excess of 18 months at 4°C.

Conclusion: In summary, the test reagents and assay procedure show performance characteristics suitable for testing Barbiturates in oral fluids.

[#] These assays are currently in development and have not been approved for use in the US by the FDA.

Keywords: **Oral Fluid, Barbiturates, Automation**

P17 Automated SPE-LC-MS-MS Analysis of 6-Acetylmorphine in Human Urine Specimens: Application to a High-Throughput Urinalysis Laboratory

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The views expressed in this article are those of the authors and do not necessarily reflect the official policy of the Department of the Navy or the U.S. Government.

Abstract: An automated solid-phase extraction liquid chromatography tandem mass spectrometry (SPE-LC-MS-MS) method using the Spark Holland Symbiosis Pharma SPE-LC coupled to a Waters Quattro Micro MS-MS was developed for the analysis of 6-acetylmorphine (6-AM) in human urine samples. The method was linear (R^2 = 0.9938) to 200 ng/mL, with no carryover at 200 ng/mL. Limits of quantification and detection were found to be 2 ng/mL. Inter-run precision was evaluated by analyzing five samples at the 10 ng/mL over six batches (n=30). The %CV was found to be 4.8 % over the course of the validation. Other opioids (e.g., codeine, morphine, oxycodone, oxymorphone, hydromorphone, and hydrocodone) did not interfere in the detection and quantification of 6AM or the deuterated internal standard. Forty-eight negative urine specimens spiked with 6-AM both below (5 ng/mL) and 48 above (12.5 ng/mL) the cutoff concentration (10 ng/mL) were analyzed by SPE-LC-MS-MS and a previously-validated gas chromatographic mass spectrometry (GC-MS) method. Additionally, 24 authentic human urine samples previously found to contain 6-AM by the GC-MS procedure were compared to the SPE-LC-MS-MS method. The automated SPE procedure eliminates the human factors of specimen handling, extraction, and derivatization, thereby reducing labor costs and rework resulting from human error or technique issues. Additionally, method runtime is greatly reduced by approximately 50% when compared to the GC/MS analysis.

Keywords: **6-Acetylmorphine, LC/MS/MS, Automated**

P18 Comparison of Fisher Tissuemiser® and Bullet Blender™ in Homogenizing Meconium for Drugs Testing

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Meconium begins accumulating in the digestive tract of a developing fetus from about twelve weeks of gestation until birth. It is currently the specimen of choice for detecting pre-natal drug exposure, as it chronicles exposure over the last 4-5 months of pregnancy. Meconium is a complex matrix and thus necessitates an appropriate homogenizing procedure in order to produce a workable specimen for further solid phase extraction and analysis for detection of drugs. One instrument used in our laboratory is the Fisher Tissuemiser® homogenizer (Thermo Fisher Scientific, Waltham, MA). The Tissuemiser® is placed in a tube containing meconium and extraction solvent and operated for approximately one minute to homogenize the sample. It is then cleaned through a series of washes before use with another sample. This process is time consuming, labor intensive, and presents the opportunity for carryover and repetitive motion injuries. In attempts to overcome these issues, we evaluated the Bullet Blender™ (Next Advance, Averill Park, NY) for use with confirmation assays for seven different drug classes. The bullet blender is a vortexer/homogenizer that can process up to 24 samples simultaneously. Samples are placed into polypropylene tubes to which beads and extraction solvent are added. Sample tubes are then placed in the Bullet Blender™ which agitates the sample thousands of times per minute resulting in sample homogenization.

Twenty-seven patient specimens were homogenized with the Bullet Blender™ in duplicate, and processed for drug testing. Results were compared with those generated with separate aliquots of the same samples that were homogenized using the Fisher Tissuemiser®. Of the drug classes that were represented; there were five positive results each for amphetamine, cocaine, and methadone, and four positive results each for benzodiazepines, PCP, and barbiturates. Opiates were also validated with the Bullet Blender™. They were part of more comprehensive new assay development project, therefore 32 patient specimens (78 total positive results) were evaluated but not in duplicate. Drug concentrations were quantitated by GC-MS or LC-MS/MS.

Analytes from each drug class were tested and a first order linear regression fit of $y = 1.014x - 3.6694$, and an $r^2 = 0.9895$ were obtained for all results ($n = 126$). Good correlation ($y = 1.05x – 6.9788$ and $r^2 = 0.9952$) was also seen when comparing the duplicate specimens homogenized using the Bullet Blender™.

The Tissuemiser® required approximately one minute of a technologist's time to adequately homogenize each sample, however the Bullet Blender™ homogenized up to 24 samples in 5-8 minutes. Only 30 seconds of the Bullet Blender™ homogenization time required technologist's attention, allowing the technologist to accomplish other tasks during the remaining time.

In conclusion, both the Bullet Blender™ and the Fisher Tissuemiser® methods homogenized samples completely for use in drugs of abuse testing. However, the Bullet Blender™ has several advantages: namely, it is less labor intensive, less time consuming (especially with larger runs), and, most importantly, eliminates the possibility of homogenizer carryover and repetitive motion injuries.

Keywords: **Meconium, Sample Preparation, Homogenize**

P19 Two Unusual Carbamate Pesticide Case Reports

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Carbamate pesticides are derived from carbamic acid, kill insects in a similar fashion as organophosphate insecticides. They are widely used in homes for gardening and agriculture.

Case #1: A young woman stole from her family's debit card $17,000 to buy a car. Days later prepares a pork chop dinner for the whole family and even the dog. The meat was seasoned with "Adobo", a local seasoning. The family felt "sick" with symptoms compatible with of a viral syndrome and even the dog was described as behaving "funny". Upon investigation the seasoning was sent for analysis and contained aldicarb (Temik®) that she intentionally added to murder her family. Fortunately the family and the dog recovered from the intoxication episode. She was sent to a mental institution.

Case #2: A 26 year old male farmer, whose family described as "quiet", was found dead on his bed. Upon autopsy, the gastric contents were bight "Hulk-like green" in color with green particulate matter. Green fluid remains were found in a glass at the scene and submitted to the toxicology lab as evidence. The evidence, blood and gastric contents were positive to oxamyl (Vydate L®).

Both cases were screened using LC/MS and confirmed by UPLC/MS/MS

Keywords: **Carbamate Pesticides, Oxamyl, Aldicarb**

P20 Extraction of Cocaine and Metabolites Using Resin-based Mixed-mode Cation Exchange SPE with LC-MS/MS Analysis

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Introduction: Cocaine is one of the most widely abused illicit drugs available and not confined to any particular socio-economic class. Available in various forms it is highly addictive, however, instantaneous euphoric effects has led to huge popularity. This widespread misuse has led to the necessity of rapid and reliable methods for analysis and quantitation from various matrices. EVOLUTE CX is a resin-based mixed-mode strong cation exchange SPE sorbent designed for the extraction of basic drugs. The dual retention mechanism of hydrophobic interaction and strong cation exchange enables a rigorous interference wash regime resulting in cleaner final extracts.

Aims: This poster shows the application of EVOLUTE CX to the extraction of cocaine and its major metabolites from various human biological fluids.

Methods: Initial work investigated blank human plasma, urine and whole blood samples spiked with cocaine and metabolites at 50 ng/mL and 100 µL extracted using EVOLUTE CX in the 25mg 96-Well plate format. The generic method is based on a 50mM ammonium acetate buffer at pH 6, however, to incorporate all metabolites for analysis modification was necessary. Column conditioning takes place with 1 mL of methanol follow by 1 mL of buffer. 100 µL of matrix was pre-treated with buffer and extracted through the SPE procedure. The dual retention enables subsequent washes of buffer, 2% formic acid (aq) and methanol (1 mL). Analyte elution is afforded by 5% ammonium hydroxide in methanol. The SPE extracts were evaporated to dryness and reconstituted in appropriate mobile phase for analysis. All samples were analyzed using a Waters 2795 liquid handling system coupled to a Quattro Ultima Pt triple quadrupole mass spectrometer. Positive ions were acquired using electrospray ionization operated in the MRM mode.

Results: The analyte suite consisted of: cocaine, norcocaine, benzoylecgonine, ecgonine methyl ester, anhydroecgonine methyl ester and cocaethylene. Preliminary results showed excellent extraction efficiencies for all analytes. Recoveries greater than 80% with corresponding RSD's below 10% were observed for all analytes. Full results will be shown in the final poster.

Conclusion: This poster shows the application of EVOLUTE CX to the extraction of cocaine and major metabolites from various matrices. High reproducible recoveries were obtained for all matrices tested.

Keywords: **Cocaine, Mixed-mode SPE, LC-MS/MS**

P21 Detection of Cotinine in Oral Fluid from Three Human Subjects using Liquid Chromatography-Electrospray-Tandem Mass Spectrometry and Enzyme-Linked Immunosorbent Assay

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Objectives: (1) To develop and validate a sensitive and specific liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay for the simultaneous quantification of nicotine (NIC), cotinine (COT), nicotine-N-*β*-glucuronide (NIC GLUC), cotinine-N-*β*-glucuronide (COT GLUC), *trans*-3-hydroxycotinine (3-HC), norcotinine (NCOT), *trans*-nicotine-1'-N-oxide (NNO), cotinine-N-oxide (CNO), nornicotine (NNIC), anatabine (AT) and anabasine (AB) in oral fluid samples collected as part of a clinical study investigating biomarkers of low-level smoking; (2) To obtain preliminary data on the use of a COT microplate enzyme-linked immunosorbent assay (ELISA) as a screening tool in oral fluid from low-level smokers.

Clinical Study: Oral fluid was collected from three human subjects prior to administration of a 7 mg nicotine transdermal patch (Novartis®, Basel, Switzerland) and then at 0.5 h and 0.75 h following patch removal (IRB approval #21414, University of Utah). 1 mL oral fluid was collected using the Quantisal™ collection device (Immunalysis®, CA, U.S.A) at each time point.

LC-MS/MS Method: Deuterated internal standard solution and 1 mL of 10 % aqueous trichloroacetic acid solution were added to 0.5 mL of oral fluid/ buffer (1:3 v/v), followed by vortexing and centrifugation. The oral fluid supernatant was then subjected to solid-phase extraction using a combination of Oasis® HLB and Oasis® MCX mixed mode cartridges. Chromatographic separation was achieved using a Discovery® HS F5 HPLC column (100 mm x 4.6 mm, 3 µm, Supelco®, MO, U.S.A.) with a 10 mM ammonium acetate + 0.001 % formic acid (pH 4.97), and methanol gradient system at a flow rate of 0.6 mL/min. Two MRM transitions were monitored for each analyte (except COT GLUC), which produced only one fragment ion) using a Quattro Premier XE™ triple quadrupole mass spectrometer (Waters Corporation®, MA, U.S.A) with MassLynx™ v 4.1 software. The method was validated to include an evaluation of linearity over the expected range for each analyte, limit of quantification (LOQ), specificity, extraction recovery and intra-(n=5) and inter-assay imprecision (n=20) over 4 batches.

ELISA Method: Buffered oral fluid samples (20 µL) were tested using a commercially available COT micro-plate ELISA (Immunalysis®, CA, U.S.A). The ELISA assay was validated to include dose response curve for COT, an assessment of cross-reactivity for 8 additional NIC metabolites, and intra- (n=8) and inter-assay (n=80) imprecision data for COT in oral fluid over 10 batches.

Method Results: The selected LC-MS/MS calibration range calculated by simple linear regression (all R^2 >0.99) for each analyte in plasma was: NIC GLUC, NCOT, NNIC, NIC (0.5-50 ng/mL); 3-HC, NNO, COT (0.5-100 ng/mL); CNO, AT (0.75-100 ng/mL); and COT GLUC (50-500 ng/mL). No endogenous matrix interferences were observed. Intra- and inter-assay imprecision were < 20 % for all analytes. The mean extraction recovery (n=5) was > 90 % for all analytes. The ELISA dose response range for COT was linear over the range 5-200 ng/mL. Intra- and inter-assay imprecision were < 3%. Nicotine metabolites demonstrated < 1 % cross-reactivity with the ELISA kit, except for COT (100 %) and 3-HC (120 %).

Clinical Data Results: Baseline oral fluid was negative by both methods for all subjects. At 0.5 h and 0.75 h after NIC patch removal, COT concentrations were 5.7-24.5 ng/mL and 7.8-26.3 ng/mL respectively, as measured by LC/MS/MS. Consistent with the 10 ng/mL ELISA cut-off, and as predicted by the LC/MS/MS data, two of the three subjects had positive oral fluid samples at the two collection points after transdermal NIC.

Conclusion: A sensitive and specific LC-MS/MS and ELISA method have been applied for the detection of COT in oral fluid samples which have been collected following administration of a 7 mg nicotine transdermal patch.

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Keywords: **Nicotine and Metabolites, Oral Fluid, LC-MS/MS**

P22 Development of a High Throughput LC/MS/MS Assay for 13 Commonly Prescribed Pain Management Drugs from Urine with Clean Up Using Solid Phase Extraction (SPE)

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Objective: Chronic pain affects some estimated 86 million Americans. Pain management centers have been established all over the country to help treat these chronic conditions. During treatment, routine testing is critical to prevent abuse. This work represents an analysis solution for 13 commonly prescribed compounds for pain management. The methodology utilizes a strong cation-exchange solid phase extraction (SPE) cleanup and concentration followed by fast (< 2 minutes) analysis using new UHPLC column technology coupled to LC/MS/MS.

Methods: Five different lots of urine samples were spiked at therapeutically relevant concentrations of 13 commonly prescribed drugs including Hydrocodone, Nalterxone, Tramadol, and Fentanyl. Urine samples were first hydrolyzed using beta-glucuronidase for 2 hours at 60 °C. Clean up was done using a 30 mg/3 mL polymer based high ion-exchange capacity media to remove endogenous matrix components. Analysis was done using UHPLC coupled to an ABI 4000 QTrap.

Conclusion: The analytical method resulted in an LLOQ of 2.5 ng/mL with accuracy of 83-98%. At 3 times the LLOQ the RSD was well below 15% indicating a wide linear working range for all compounds. The use of a new UHPLC column technology allowed for a significant increase in chromatographic efficiency. Average peak width at base was 1-2 seconds, resulting in a significant increase in sensitivity requiring the dilution of some extracts by up to 20 times in order to fall within the linear range of the system.

Keywords: **Pain Panel, SPE, LC/MS/MS**

P23 High Throughput Extraction of Opiates from Urine and Analysis by GC/MS or LC/MS/MS

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Objective: The prescription of opiate drugs is common for the treatment of chronic pain. Due to the addictive nature of these drugs, opiate abuse has significantly increased in recent years, creating a need for faster, more accurate analysis. Many opiates are regulated under Schedule I and routinely tested as part of pre-employment screening or workplace performance monitoring. The confirmation of these analytes by GC/MS can be complicated by two factors, 1) removal of endogenous compounds from urine that can co-elute with target analytes and 2) the presence of non-target opiates which have similar mass ions. The objective of this work was to develop a high throughput assay for opiates in urine utilizing solid phase extraction (SPE) for sample clean up and analysis by either GC/MS or LC/MS/MS.

Methods: Urine samples were spiked at 2,000 ng/mL with morphine and codeine and other commonly encountered opiate related interferences. Samples were then hydrolyzed using acid and heat to remove the glucuronide. The hydrolyzed samples were then extracted using a 30 mg/3 mL SPE tube containing a polymeric cationic exchange sorbent. Wash conditions were optimized to remove polar cationic contaminants that interfered with the GC/MS analysis. Separation by GC/MS of morphine and codeine from other opiate related inferences was evaluated using two different derivatization procedures. Samples were simultaneously analyzed by LC/MS/MS without the need for derivatization.

Results: The resulting extraction procedure using a 30 mg SPE bed mass allowed for sample load, wash, and elution volumes to be significantly reduced, significantly decreasing blow down time prior to subsequent derivatization steps. Recoveries for morphine and codeine were 97% and 98%, respectively, with %CVs <4% for replicate injections over three different concentrations. The use of an alternative derivatization procedure significantly improved chromatographic resolutions and eliminated the need to perform an oximation step to prevent multiple reaction products. Samples prepared for analysis by LC/MS/MS did not require derivatization. This study will help laboratories meet the increasing demand for high throughput monitoring of opiates.

Conclusion: A new procedure for the extraction and analysis of opiates from urine was developed that dramatically reduced overall sample processing time and improved chromatographic results by either GC/MS or LC/MS/MS.

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

P24 The Simultaneous Solid Phase Extraction and Qualitative Analysis of Diuretics, Beta-Blockers and Other Xenobiotics in Human Urine by HPLC-MS/MS and UPLC-MS/MS

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This poster presents two general screening procedures based on liquid chromatography tandem mass spectrometry (LC-MS/MS), for the simultaneous detection of 48 xenobiotics (21 diuretics, 19 beta-blockers, 6 stimulants and 2 steroids) in human urine. Sample preparation was accomplished through a single solid phase extraction procedure using Varian Nexus™ SPE columns, resulting in analyte recoveries of 30 – 99%. The LC-MS/MS assay of the analyte species was accomplished using both an Agilent 1200 series HPLC coupled to an Applied Biosystems 3200 triple quadrupole mass spectrometer and a Waters Acquity UPLC coupled to a Waters Micromass Quattro Premier XE mass spectrometer. In each case, all analytes were analyzed simultaneously using a single solvent gradient. Mass detection was carried out using electrospray ionization with polarity switching between positive and negative ion modes and with multiple reaction monitoring (MRM) as the data acquisition method. Benzoylecgonine-d3 and furosemide-d5 were uses as internal standards for positive and negative analyte species, respectively. The overall time needed for an LC analysis was 12.5 minutes for the HPLC screen and 6 minutes for the UPLC screen. All compounds showed good reproducibility in terms of both the retention times and the relative peak area responses of the diagnostic transition ions. The limits of detection (LOD) for both the HPLC and UPLC screening methods ranged from 3 to 200 ng/ml, depending on the compound, and were found to easily satisfy the minimum required performance limits (MRPL) set by the World Anti-Doping Agency (WADA). On the whole, LODs were lower for the HPLC/MS/MS method, likely due to the fact that the injection volume was 20 µl compared to 2 µl for the UPLC/MS/MS method. However, these LODs could likely be substantially reduced by either increasing the injection volume, reducing the reconstitution volume, or both. In conclusion, analysis by UPLC/MS/MS reduced the analysis time by 50% while maintaining LODs that readily fulfilled the requirements of WADA.

Keywords: **Anti-Doping, LC/MS/MS, UPLC**

P25 Solid Phase Extraction and Analysis of THC and Carboxy-THC from Whole Blood using a Novel Fluorinated SPE Sorbent and Fast LC-MS/MS

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Abstract: In this presentation, attendees will learn about the solid phase extraction (F-SPE) using a novel fluorinated (Heptadecafluorotetrahydrodecyl (C10H4F17)) phase to efficiently isolate THC and its primary metabolite Carboxy-THC from whole blood samples.

Method:

F-SPE was performed in hydrophobic mode after samples of whole blood (1 mL) were precipitated with acetonitrile (2 mL) and evaporated to 200µL. The samples were applied to the F-SPE in 5 mL of aqueous phosphate buffer (pH 7). The sorbent was washed with deionized water, phosphate buffer (pH 7) (3 mL of each, respectively), and dried under full vacuum. Each F-SPE column was eluted with 3 mL of a solvent consisting of ethyl acetate/ hexane (50:50 3 mL) containing 2% acetic acid. The eluates were collected, evaporated to dryness and dissolved in mobile phase (50 µL) for analysis by fast LC-MS/MS in positive/ negative MRM mode. Data is presented for MRM's of THC, THC-D3, Carboxy-THC, and Carboxy-THC-D3, respectively.

Liquid chromatography was performed in gradient mode employing a 50 x 2.1 mm C18 analytical column and a mobile phase consisting of acetontitrile and 0.1% aqueous formic acid. The gradient was programmed to run from 50% to 90% acetonitrile in 4.5 minutes and equilibrate. The total run time for each analysis was less than 5 minutes. In this presentation, representative chromatograms are shown to illustrate the efficiency of separation of the cannabinoid analytes.

Results: The limits of detection/ quantification for this method were determined to be 0.1 ng/ mL and 0.25 ng/ mL, respectively. The method was found to be linear from 0 ng/ mL to 50 ng/ mL (r2>0.999). Recoveries of the individual cannabinoids were found to be greater than 80%. In this report, results of authentic samples analyzed for THC and Carboxy-THC are presented using this new methodology.

Conclusion: This procedure will be of great use to analysts in the field of drug/driving toxicology as it will serve to lower the time and levels of detection in reporting results in such cases.

Keywords: **THC, LC-MS/MS, F-SPE**

P26 A Sensitive Method for Quantification of Buprenorphine and Norbuprenorphine in Human Whole Blood, Plasma and Serum by UPLC/MS/MS

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A procedure for the rapid extraction and quantification of buprenorphine and its major metabolite, norbuprenorphine, from human whole blood, plasma and serum using liquid-liquid extraction and ultra performance liquid chromatography coupled with tandem mass spectrometry (UPLC/MS/MS) is described. Buprenorphine is a Schedule III semi-synthetic opiate used for the treatment of chronic pain and opiate addiction. It is rarely reported as a sole contributor to cause-of-death, but can be a significant contributor when other psychotropics are present, especially benzodiazepines. Due to poor oral bioavailability combined with its potency, buprenorphine is typically found in low concentrations (0.1-76 ng/mL) in forensic blood samples (therapeutic range for buprenorphine is 2-8 ng/mL). Buprenorphine has been found to cause significant respiratory depression when in combination with benzodiazepines even at therapeutic levels.

Previous methods have required extensive extractions (such as SPE), longer total run-times, derivatization or had reduced selectivity through the use of single-quadrupole MS. The present study introduces a sensitive technique for the quantitation of buprenorphine and norbuprenorphine using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). The use of UPLC-MS/MS adds an additional layer of selectivity that is important in forensic applications where unknown specimens could contain a myriad array of potential interferences. Subsequent separation and quantification using UPLC/MS/MS was achieved in 4.25 minutes total run-time per sample. Linearity over the range 1 ng/mL to 100 ng/mL was established using deuterated analogs as internal standards. The methodology showed excellent intra-run precision in whole blood (QCs spiked at 20 and 80 ng/mL) with %CV values ranging from 1.27-3.81% for buprenorphine, 1.56-3.56% for norbuprenorphine. Inter-run precision experiments produced %CV values ranging from 4.88-5.02% for buprenorphine, 5.61-6.51% for norbuprenorphine. The reported method proved to be a rapid, robust and sensitive assay for the quantitation of buprenorphine and norbuprenorphine in forensic applications and was also found to be relevant in clinical and therapeutic drug monitoring applications such as pain treatment compliance monitoring.

Keywords: **Blood, UPLC/MS/MS, Buprenorphine**

P27 The Extraction and Analysis of Urinary Antitussive Metabolites Using MEPS™ and ESI-LCMS

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Microextraction Packed Sorbent (MEPS™) is an adaptation of Solid-Phase Extraction (SPE) into a miniaturized device with a typical void volume of less than 10 µL. With operating volumes of this scale and its compatibility with autosampler syringes, MEPS™ allows the specificity of the solid-phase process to be harnessed for digital chromatography using discontinuous changes in solvent polarity. The eluant volumes are sufficiently small to be injected directly into a HPLC system and therefore permit the on-line use of solid-phase extraction methodology in real time with the HPLC. In this case, the MEPS™ sorbents are nominally 50 µm silicas modified with C18, C8 or SCX chemistries.

Robitussin DX Dry Cough Forte syrup (equivalent to 30 mg dextromethorphan hydrobromide, Wyeth Consumer Healthcare Pty Ltd, NSW, Australia), Vicks Cough Syrup (equivalent to pentoxyverine citrate 15 mg, Proctor and Gamble Australia Pty Ltd, NSW, Australia) or Mersyndol day Strength Caplets (equivalent to paracetamol 1000mg and codeine phosphate 19.2 mg, Sanofi Aventis, NSW, Australia) were administered orally to a healthy 80 kg human male volunteer who was not receiving any other medication. With single subject administrations and inter-administration washout times of 48 hours, metabolite profiles are intended for demonstration of the extraction technique. Naturally voided urine samples were collected at 0, 2, 3 and 4 hours following administration and were stored frozen at -20 °C until required for analysis.

Typically, urine (3ml) was diluted with 0.2M sodium phosphate buffer (3ml, pH 6.0) and the sample enzyme hydrolysed by incubation with 50IU of glucuronidase enzyme (*E.coli*) at 50 °C for 1 hour. The specimen was centrifuged at 2000g to remove suspended materials and the supernatant used for either conventional SPE or MEPS extraction. A 20 minute automated sample preparation by conventional SPE was performed as described previously [1]. MEPS extraction was performed on C18 MEPS BINS fitted to a 100 µL MEPS syringe. BINS were conditioned sequentially with methanol (50 µL) and water (100µL). The sample (12 x 80 µL) was passed through the sorbent, followed by water (80 µL), saturated sodium tetraborate solution (50 µL), water (80 µL) and air (2 x 80 µL at 50 µL/sec). Retained analytes were eluted directly to an autosampler vial with methanol (2 x 20 µL), diluted with 40 µL of 0.1% aqueous acetic acid for analysis. Extraction time was less than 5 minutes.

Samples were analysed by HPLC on a Shimadzu Prominence LC-20 system (Kyoto, Japan) on a ProteCol-P C18 HQ105 (1500 mm x 4.6 mm i.d., SGE Analytical Science, Melbourne, Australia) at temperature of 40 °C an a flowrate of 0.7mL/min. The mobile phase was varied on a linear gradient from 1 % aqueous acetic acid in 10 % methanol to 1 % aq. acetic acid in 90 % methanol over 10 minutes then held isocratically for a further 10 minutes. Detection was by positive ion ESI-MS using a LCQ Classic MS (Themo, San Jose, USA). Speculative structural elucidation of metabolites was made on the basis of mass fragmentography using MS^3 or MS^4 as required. The effectiveness of MEPS™ for the on-line extraction of biological fluids and subsequent LCMS analysis of urinary antitussive metabolites was evaluated by a comparison with the analytical results obtained when the same specimens were prepared off-line using a conventional SPE cartridge.

Recoveries were qualitatively and quantitatively similar for both techniques. While the sensitivity and precision of MEPS™ was shown to be comparable to conventional SPE methods, MEPS™ has the advantage of much shorter extraction times and the easy adoptability to automated systems. The extraction time using MEPS was less than five minutes per sample while extraction time of the same specimen using conventional SPE was twenty minutes. Experimental parameters of the SPE and the MEPS™ extractions in this paper will highlight the differences between these methods.

[1] SPE extraction was performed on Bond-Elut Certify™ columns using methods described previously. (Wynne PM, Batty DC, Vine JH and Simpson NKJ., Chromatographia, 59 (4/5), S50-S61, (2004).

Keywords: **Urine, MEPS, ESI-LCMS**

P28 Inert Column Hardware for the Separation of Difficult Samples in RP-HPLC

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We will investigate the role of metal surfaces on the broadening and tailing of some peaks in reversed phase chromatography. Analytes containing high amounts of oxygen such as ortho-polyphenols or ß-hydroxy ketones have the potential to form chelates with iron resulting in poor peak shape when analyzed with HPLC. The largest source of these non specific interactions is the stationary phase due to its large surface area but other surfaces such as the frit, the column wall, and the connection capillaries contribute as well.

We will evaluate a range of column hardware with coated surfaces (either glass- or PEEK™-lined stainless steel) and metal-free frits to minimize non-specific binding of analytes. These columns were also packed with highly inert C18 silica to optimize column performance. We will show that eliminating all sources of metal in the flow path significantly improves the peak shape of various active compounds.

Keywords: **HPLC, Inertness, Chelates, Active Compounds**

P29 Analysis of Absinthe by Solid Phase Extraction and LC-MS/MS: A Simple Test for Thujone Concentration

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Introduction: Absinthe, once a beverage subject to a ban in the United States is now finding a new audience. It is euphemistically called the "Green Fairy" after the color of the liquid. The main constituent of the beverge is Anethole but some samples of the drink may contain α/βThujone, which is thought to give rise to its hallucinogenic properties. The drink is also known to contain upto 50 percent acohol by volume. The focus of this presentation is to to demonstrate a simple and efficient procedure for the analysis of Absinthe which will determine the presence (or absence) of α/ β thujone as well as the anethole. This should be of great assistance to those actively testing such drinks and offer an alternative method of analysis

Aims: The focus of this work, was to demonstrate the use of both an alternative solid phase procedure employing a long chain sorbent (C_{30}) and LC-MS/MS in the analysis of an alternative matrix i.e. Absinthe thus offering analysts a different route for thujone testing.

Methods: Calibrators and controls were used for extracting anthethole and α/ β thujone from 50 PC aqueous alcohol samples (0.2 mL). In this method, to the calibrators, controls and genuine samples of absinthe (10) menthol (IS) was added before dilution with DI water (5 mL). and applied to solid phase extraction columns (3 mL containg 25 mg of CEC30 sorbent (UCT Inc.). The columns were conditioned with methanol and DI water (1 mL, respectively). After washing with 0.4 mL of DI water the samples were eluted with 4 x 50 µL of methanol and the eluates transferred directly to auto sample vials for analysis by LC-MS/MS in positive electrospray (MRM) mode. The quantifying transitions were: Menthol (156 .1→83.1), Thujone (153.1→135.1), and Anethole (149.1→121.2), respectively.

From the analysis of the calbrators and controls: r^2 value> 0.995, recoveries > 90%, and a limit of detection of 10 µg/ mL, respectively were acheived. The method was found to be linear upto 1000 µg.
Tandem mass spectrometry was performed on API 2000 MS/MS, whilst chromatographywas carried out with a 50 x 2.1mm (3 µm) Phenyl column (Selectra ™) for separation of the analytes. A mobile phase consisting of acetonitrile (with 0.1% formic acid) and DI water (with 0.1% formic acid) was used in gradient mode in the analysis.

Results: Data is presented in this poster along with LC-MS/MS chromatograms showing those samples of genuine absinthe containing thujone and those containing only anethole. The range of α/ β thujone concentrations was found to be from 0 to over 750 µg/ mL (348 to 769 µg/ mL (n=5)) whilst all of the samples contained anethole at various levels (2480 to 7720 µg/ mL (n=10)) far exceeding that of the α/ β thujone.

Conclusions: This simple amd effiecient procedure for the analysis of Absinthe (especially Thujone) is the first method using both SPE and LC-MS/MS. The use of this procedure should assist those analysts involved testing Absinthe beverages for the presence of thujone. This method should also help analysts testing pre-ban samples of Absinthe for authenticity, as the presence or absence of the thujone should assist in the establishment of legitimacy of the sample.

Keywords: **Absinthe, SPE, LC/MS/MS**

P30 Three Little Steps-Two Cases of Fatal Aldicarb Ingestion

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Aldicarb is a carbamate pesticide approved in United States for professional use, but not for household use. Aldicarb is also the active ingredient of "Tres Pasitos", a rodenticide illegally imported into the United States from Latin American countries.

Case 1: A 44 year old woman was found unresponsive at home with a drinking glass containing a residue indicated by the family to be “Tres Pasitos”. She was transported to the hospital where a CT scan revealed anoxic brain injury. Life support was withdrawn and she expired within 24 hours of admission.

Case 2: A 60 year old man was found unresponsive in bed by his daughter. A cup believed to contain rat poison was found next to the bed along with several empty bottles of vodka. Granules of “Tres Pasitos” were found in a closet.

Both decedents were Hispanic and had a history of depression. In both cases the cause of death was acute aldicarb intoxication and the manner of death was determined to be suicide (intentional consumption of rat poison). Autopsy specimens were submitted for toxicologic analysis Residue in the glass from Case 1 was also available for analysis.

Aldicarb analysis was performed by solid phase extraction at pH 6.0 followed by high-performance liquid chromatography with ultraviolet absorbance at 205 nm measured using a diode array detector.

Toxicology findings were as follows:

ALDICARB (mg/L or mg/kg)

	BLOOD	VITREOUS HUMOR	BILE	GASTRIC CONTENT	URINE
CASE 1	1.1			detected	detected
CASE 2	12.1	10.1	14.6	217 (15.4 mg in 71.2 g)	detected

Aldicarb was identified in the residue from the glass found in Case 1. Lidocaine was the only other finding in that case. Ethanol was detected in the blood, vitreous humor and urine in Case 2 at concentrations of 0.20 g%, 0.25 g% and 0.26 g%, respectively.

Keywords: **Aldicarb, Postmortem, HPLC**

P31 An Investigation of Norpropoxyphene Cyclization in Methanol

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Dextropropoxyphene, a synthetic opioid analgesic, is widely prescribed for the relief of mild to moderate pain. It is primarily metabolized to norpropoxyphene in humans through N-demethylation. In most methods, analysis of norpropoxyphene involves a strong base-catalyzed conversion to norpropoxyphene amide to help improve the chromatography.[1]

In the absence of strongly basic conditions, the solution behavior of norpropoxyphene in a protic solvent (methanol) was examined using HPLC, LCMS, and ^{1}H NMR. The results support the formation of a cyclic intermediate in solution. Literature precedence exists for the involvement of a cyclic intermediate in the base-catalyzed conversion of norpropoxyphene to norpropoxyphene amide[2] as well as in the formation of cyclic dinorpropoxyphene.[3]

Based on these results we propose a mechanism for the formation of this cyclic intermediate and its potential role in the analysis of norpropoxyphene by LCMS. We offer HPLC and ^{1}H NMR evidence which corroborate the possible formation of this cyclic intermediate in methanol. The effects of storage conditions and time on the formation of this cyclic intermediate in solution will also be discussed.

1. Amalfitano, G., Bessard, J., Vincent, H., Eysseric, H., and Bessard, G. Gas Chromatographic Quantitation of Dextropropoxyphene and Norpropoxyphene in Urine after Solid Phase Extraction. (1996) *Journal of Analytical Toxicology* 20, 547-548.
2. Norheim, G. and Bye, E. X-Ray Crystallographic and Gas Chromatographic-Mass Spectrometric Studies on Norpropoxyphene. (1976) *Archives of Toxicology* 36, 98.
3. Nash, J.F., Bennett, I.F., Bopp, R.J., Brunson, M.K., and Sullivan, H.R. Quantitation of Propoxyphene and Its Major Metabolites in Heroin Addict Plasma after Large Dose Administration of Propoxyphene Napsylate. (1975) *Journal of Pharmaceutical Sciences* 64, 431.

Keywords: **Norpropoxyphene, Cyclization, LCMS**

P32 Practical Method Validation for the Quantification of Lacosamide by UPLC-MSMS

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Prior to implementing an analytical procedure it is vital to validate the method to ensure accurate and precise results. At minimum, methods should be evaluated for: within- and between-run accuracy and precision, recovery, limit of detection (LOD), analytical range, specificity, and sample and extract stability. Additional criteria that may be included are: extraction efficiency, dilution integrity, carryover, and matrix effects. The validation of an ultra performance liquid chromatography-positive ion electrospray- tandem mass spectrometry (UPLC-MS/MS) method for the quantification of lacosamide in serum/plasma, whole blood and urine is used to illustrate the validation protocol used at NMS Labs.

Lacosamide is a novel anticonvulsant, the presence or absence of which may provide important information in postmortem and suspected impaired driving cases. It was extracted from biological matrices using liquid-liquid extraction with d_3-lacosamide as an internal standard. Quantification was achieved in a 2.1 minute run. The MS was operated in multiple reaction-monitoring mode and two transitions were monitored for lacosamide and the internal standard. The following parameters were included in the method validation performed over 6 days: linearity, LOD, analytical range, within- and between-run precision, accuracy, specificity, extraction recovery, extract and sample stability, dilution integrity, matrix effect, and carryover.

Lacosamide was quantified employing a 5-point 2^{nd} order calibration curve with 1/x weighting. The coefficient of determination was ≥ 0.999 and calibrators back-calculated within ±4.8% of target values. The method LOD was 0.02 □g/mL and the analytical range was 0.5-60.0 □g/mL. Accuracy and precision was evaluated at the limit of quantification (0.5 □g/mL), 2.0 □g/mL (low control, LC) and 40.0 □g/mL (high control, HC). The maximum within-run imprecision was 3.7%, 3.1% and 3.2% for LOQ, LC and HC, respectively. Between-run imprecision was ≤ 4.5% at all concentrations. The average LOQ, LC and HC accuracy was 100.1%, 99.4%, and 96.4%, respectively. Specificity was evaluated by fortifying blank matrix with 48 potentially interfering substances. No interferences were noted. Extraction efficiency was 63-73% across the analytical range. Stability of lacosamide in serum, whole blood and urine was evaluated at room temperature, ≈3ºC and ≈-10ºC for 1 day, 3 days, 7 days, 15 days and 30 days. Concentrations remained stable in urine and serum for 15 days at all conditions, but was only stable in blood at room temperature for 3 days. The validation also confirmed stability during specimen preparation, three freeze/thaw cycles, and on the instrument auto-sampler. A ten-fold dilution of the high control resulted in calculated concentrations within 11% if target. Matrix matching evaluated the methods' ability to accurately quantify lacosamide in whole blood and urine collected in a variety of tube types using a plasma calibration curve. Blank matrix from different collection devices did not contain any component that would interfere with the quantification of lacosamide. All matrices spiked with low and high concentrations of lacosamide quantitated within 16.4% of target. Injection of a blank following the high calibrator indicates that there is no carryover with the analytical method at the high calibrator concentration.

The validated method has been used to quantify lacosamide in serum/plasma for routine therapeutic drug monitoring. This thorough method validation protocol is used prior to implementation of all new methods at NMS Labs providing accurate and precise methods for clinical and forensic toxicology analyses.

Keywords: **Lacosamide, UPLC-MSMS, Therapeutic Drug Monitoring**

P33 The Detection and Quantification of Brodifacoum in Embalmed Exhumed Liver by High Performance Liquid Chromatography/Tandem Mass Spectrometry (LC-MS/MS) and High Performance Liquid Chromatogrpahy/Tandem Quadrupole-Time of Flight Mass Spectrometry

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The analysis of biological specimens for the presence of anticoagulants is of special interest in both the clinical and forensic laboratory. Anticoagulants such as the frequently prescribed warfarin may be used therapeutically to prevent thrombosis and embolism while others such as brodifacoum and difenacoum are common ingredients of pesticides and rodenticides. Because this group of substances is not always implicated or suspected at the time of a death, the analysis is often challenging since by the time testing does become a necessity the type of specimens and the amount remaining may be limited, and their conditions variable.

In 2007, liver and brain tissue collected from an embalmed exhumed body, buried approximately 7 years prior, were submitted for toxicological testing as part of an ongoing criminal investigation. Based upon direction, testing was performed on the liver specimen for ethylene glycol and the anticoagulant class of drugs. Even though ethylene glycol was also detected, this presentation discusses the analytical strategy that was applied to the anticoagulant portion of this work, and the ultimate detection and quantification of brodifacoum using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) on a tandem quadrupole instrument under multiple reaction monitoring (MRM) conditions, and on a Quadrupole-Time of Flight (Q-TOF) instrument under repetitive product ion scanning conditions at high resolution.

The first test performed was a screen for anticoagulants by LC-MS/MS. The anticoagulants included in the scope of the analysis were warfarin, dicumarol, diphacinone, chlorophacinone, difenacoum, brodifacoum and bromadiolone. This qualitative method is briefly described as follows: After addition of internal standard (Chloro-Warfarin), a protein precipitation is performed followed by solvent extraction with Methyl t-Butyl Ether. The solvent is evaporated to dryness and the residue reconstituted with mobile phase. Subsequent analysis is by MRM in the negative ion mode on a triple-quadrupole mass spectrometer coupled with an electrospray ionization source and an Ultra-Performance Liquid Chromatography (UPLC) system. Two ion transitions are monitored for each analyte as shown in the following table:

Analyte	Quant Ion	Ratio Ion
Warfarin	307.1 > 161.1	307.1 > 117.1
Dicumarol	335.1 > 161.1	335.1 > 117.1
Diphacinone	339.2 > 172.1	339.2 > 167.0
Chlorophacinone	373.1 > 201.1	373.1 > 144.9
Difenacoum	443.1 > 135.1	443.1 > 93.0
Brodifacoum	523.1 > 135.1	523.1 > 80.9
Bromadiolone	527.1 > 250.2	527.1 > 80.9
Chloro-Warfarin (Internal Standard)	341.1 > 161.1	341.1 > 284.2

This method provides a limit of quantitation (LLOQ) of 10 ng/mL for blood or 40 ng/g for tissue. For this particular case, it was from this analysis that the presence of brodifacoum was indicated. From this point forward all additional testing was specifically designed and optimized for the analysis of brodifacoum.

While the second test performed also employed LC-MS/MS, the extraction procedure was adjusted with the intent of creating a cleaner sample extract. Specifically, after addition of the same internal standard described above, samples underwent sequential solvent extraction with Methanol, Chloroform, Acetic Acid and Hexane. The final solvent was evaporated to dryness and the residue reconstituted with mobile phase. In addition, taking into account that the same analytical technique was used to screen the sample, the monitored ion transitions were expanded (m/z 523.1 to 135.1, 93.1 and m/z 521.1 to 135.1, 93.1) to ensure the accuracy of the identification. This quantitative method provides a limit of quantitation (LLOQ) of 5 ng/mL for blood or 20 ng/g for tissue. Testing was performed using two levels of standard addition and based upon standard addition calculations the liver was found to contain brodifacoum at a concentration of 98 ng/g.

Due to the circumstances of this case, the presence of brodifacoum was further substantiated by determining its full product ion spectra in the liver specimen using LC-MS/MS-TOF. For this work, samples were prepared as described above for the second LC-MS/MS test performed, and native and spiked specimens were qualitatively compared.

Taking into account the totality of the analytical findings we concluded and reported that this specimen was positive for brodifacoum. The results, for both the ethylene glycol and brodifacoum, were subsequently presented at trial in early 2009. The overall approach to these analyses, in conjunction with the analytical techniques detailed above, may be applied to a variety of sample types and situations where the possibility of anticoagulant exposure needs to be assessed.

Keywords: **Brodifacoum, Anticoagulants, LC-MS/MS**

P34 A Comprehensive Analysis of 24 Opioid Analgesics Utilizing LC-MS/MS

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An extensive confirmation/quantitation method for 24 opioid analgesics in blood, serum, and tissue has been developed using a solid phase extraction (SPE) followed by LC-MS/MS analysis. The method presented here incorporates a traditional opiate panel analysis for oxycodone, hydrocodone, dihydrocodeine, codeine, morphine, 6-monoacetylmorphine (6-MAM), hydromorphone, and oxymorphone with an extended opioid panel including propoxyphene, norpropoxyphene, tramadol, O-desmethyltramadol (ODT), methadone, EDDP, meperidine, normeperidine, and fentanyl which are all currently assayed by GC-MS in this laboratory. Other significant analytes including naloxone, naltrexone, nalbuphine, pentazocine, buprenorphine, alfentanil, and sufentanil, many of which require low limits of detection, are also included in this extended opioid panel. This method should be considered forensically noteworthy as it precludes the use of a derivitizing agent (notably BSTFA) and acetonitrile, which is currently in short worldwide supply.

Blood, serum, and tissue samples are first spiked with a mix of deuterated internal standards (18 total deuterated analogs of analytes included in the panel) at a concentration of 100 ng/mL (10 ng/mL for d5-fentanyl and d4-buprenorphine) and then extracted using United Chemical Technologies CLEAN SCREEN DAU extraction columns, collecting the basic fraction for analysis. The evaporated basic fraction is transferred to high-recovery sample vials and then reconstituted in 150 mcL of 0.1% formic acid for LC-MS/MS analysis. Analyses were performed on a Varian ProStar® HPLC with a Model 410 Autosampler coupled to a Varian 1200L Quadrupole MS/MS. Injections of 30 mcL were made onto a Varian Pursuit XRs 3.0 micron 2.0 x 150 mm C18 column with a Varian Metaguard 3.0 micron 2.0 mm guard column. The mobile phase was established as A: 0.1% formic acid in diH_2O and B: methanol with a flow of 0.200 mL/min. The initial solvent gradient held at 10% B for 2.0 minutes, ramped to 90% B by 23.0 minutes, with a total run time of 30.0 minutes. This gradient provided complete chromatographic resolution of analytes that share a common precursor ion (m+1), namely morphine and hydromorphone, codeine and hydrocodone, and oxymorphone and dihydrocodeine. Analytes were quantitated using the precursor ions and two transitions as qualifiers per drug, if available, at an optimized collision energy.

By utilizing deuterated internal standards, matrix interferences would be expected to be negligible for each analyte. Still, these effects were studied and it was found that any ion suppression would take place before any of the compounds eluted off the column, which is most likely attributed to the cleanliness of the extracts. Intraday (n=10) and interday (n=10) precisions were generally less than 5% at three different concentrations for each analyte. Appropriate lower limits of quantitation were observed for each compound, and each analyte was linear in the range 10.0 ng/mL to 1000 ng/mL (or 1.0 ng/mL to 50.0 ng/mL) with correlation coefficients (r^2) above 0.99. Suitable extraction efficiencies were also observed for all analytes.

Keywords: **Opioids, LC-MS/MS, Solid Phase Extraction**

P35 A Novel Validated Method for the Simultaneous Analysis of Nicotine, Eight Metabolites and Two Minor Tobacco Alkaloids in Human Plasma and Urine by Solid-Phase Extraction Coupled With Liquid Chromatography-Electrospray Ionization-Tandem Mass Spectrometry

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Objectives: To develop and validate a sensitive and specific liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the simultaneous quantification of nicotine (NIC), cotinine (COT), nicotine-N-*β*-glucuronide (NIC GLUC), cotinine-N-*β*-glucuronide (COT GLUC), *trans*-3-hydroxycotinine (3-HC), norcotinine (NCOT), *trans*-nicotine-1'-N-oxide (NNO), cotinine-N-oxide (CNO), nornicotine (NNIC), anatabine (AT) and anabasine (AB) in human plasma and urine.

Method: Deuterated internal standard solution was added to 1 mL of human plasma or urine (50 ng/ml). For plasma, 1mL of 10 % aqueous trichloroacetic acid was added, followed by vortexing and centrifugation. For urine, 1.5 mL of 5 mM aqueous ammonium formate (pH 2.5) was added, followed by vortexing. The plasma supernatant and the buffered urine were then subjected to solid-phase extraction using a combination of Oasis® HLB and Oasis® MCX mixed mode cartridges (Waters® Corporation, MA, U.S.A). Chromatographic separation was achieved using a Discovery® HS F5 HPLC column (100 mm x 4.6 mm, 3 µm, Supelco®, MO, U.S.A.) with a gradient system consisting of 10 mM ammonium acetate with 0.001 % formic acid (pH 4.97), and methanol at a flow rate of 0.6 mL/min. Two MRM transitions were monitored for each analyte with the exception of COT GLUC (which produced only one fragment ion) using a Quattro Premier XE™ triple quadrupole mass spectrometer (Waters® Corporation, MA, U.S.A) with MassLynx™ v 4.1 software.

Validation: To assess low concentrations of analytes**,** the method was fully validated to include an evaluation of linearity over the expected range for each analyte, limit of quantification (LOQ), specificity, extraction recovery, matrix effects, matrix stability, and intra- (n=5) and inter-assay imprecision (n=20) for 4 batches.

Results: The plasma and urine calibration curves were calculated using simple linear regression (all $R^2 >$ 0.99). The selected range for each analyte in plasma was: NIC GLUC, CO, NNIC, AB (0.75-50 ng/mL); 3-HC, NO, COT, AT (0.75-100 ng/mL); NCOT, NIC (1-50 ng/mL); and COT GLUC (50-500 ng/mL). In urine, the selected range for each analyte was: NIC GLUC and AB (2.5-50 ng/mL); 3-HC, NNO, COT and AT (2.5-100 ng/mL); CNO, NCOT, NIC; AB (1-100 ng/mL); and COT GLUC (50-500 ng/mL). Intra- and inter-assay imprecision were < 20 % for all analytes in both matrices. The mean extraction recovery (n=5) in plasma was: COT GLUC, CNO, NNO, COT, NNIC and NIC > 70 %; NIC GLUC, 3-HC, NCOT and AB > 60 %; and AT 57 %. In urine, the mean extraction recovery (n=5) was > 82 % for all analytes with the exception of COT GLUC which was 57 %. No endogenous matrix interferences were observed for either matrix. Furthermore, matrix effects (n=5) resulting in either ion suppression or enhancement were calculated to be < 20 %. Matrix stability tests (n=5) have shown the analytes to be stable after 3 freeze-thaw cycles.

Conclusion: A novel LC-MS/MS method for the simultaneous extraction and quantification of nicotine, eight metabolites and two minor tobacco alkaloids from human plasma and urine has been successfully developed and validated. The methods have been applied in the analysis of plasma and urine samples collected as part of a clinical study investigating biomarkers of nicotine use in low-level smokers.

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Keywords: **Nicotine Metabolites, Plasma/urine, LC-MS/MS**

P36 Method Development & Measurement of Native Levels of Smoking markers in Candidate Standard Reference Material (SRM 3671), Nicotine Metabolites in Human Urine, using Solid Phase Extraction (SPE) and Liquid Chromatography / Mass Spectrometry (LC/MS)

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The National Institute of Standards and Technology (NIST) is currently developing a new candidate Standard Reference Material, SRM 3671 Nicotine Metabolites in Human Urine, which represents 3 levels of exposure to tobacco smoke. The SRM consists of unspiked fresh frozen urine collected from 3 donor pools: a high level (level 3) from smokers known to smoke at least 1 pack of cigarettes per day, a mid-level (level 2) from non-smokers with passive exposure to cigarette smoke, and a low level (level 1) from non-smokers with no known exposure to cigarette smoke. This candidate SRM is being developed as a replacement for NIST RM 8444 Cotinine in Freeze Dried Human Urine. The previous material was prepared by spiking urine with cotinine, a nicotine metabolite. Cotinine levels in RM 8444 were considerably lower than those expected in smokers.

The candidate SRM will be certified for cotinine and related nicotine metabolites. As part of this effort, analytical methodology is currently being developed for these markers and other native species of interest. Two independent sample preparation schemes, either a solid phase or a liquid-liquid extraction procedure will be followed by quantification analysis using gas chromatography / mass spectrometry (GC/MS) or liquid chromatography/ mass spectrometry (LC/MS). This will be the first NIST reference material with assigned values for native metabolites associated with tobacco smoke. Because this new candidate SRM was prepared as a fresh-frozen material (rather than freeze-dried like RM 8444) potential issues with reconstitution should be minimized and commutability should be enhanced.

We discuss herein the method development for the extraction of nicotine and a suite of its metabolites from urine using an SPE technique followed by detection using an LC/MS.

Keywords: **Nicotine Metabolites, Urine, LC/MS**

P37 Simultaneous Determination of Ethyl Glucuronide and Ethyl Sulfate in Urine by HPLC-MS/MS

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Testing of urine samples for alcohol metabolites such as ethyl glucuronide (EtG) and ethyl sulfate (EtS) has recently grown in popularity due to the fact that these compounds can indicate alcohol ingestion for much longer than conventional tests for ethanol. EtG and EtS are both hydrophilic compounds that are difficult to retain on reversed-phase HPLC columns. Many methods for the analysis of these compounds use long columns with highly aqueous mobile phases. Lack of retention and a highly aqueous mobile phase both play a role in reducing MS sensitivity. The objective of this project was to develop an RP-HPLC method to adequately retain both EtG and EtS on a short column. The use of a shorter column allows for faster run times due to reduced re-equilibration time. Adequate retention reduces the chance for ion suppression from co-eluting matrix components and allows for the use of a mobile phase containing more organic solvent, which improves LC-MS/MS sensitivity.

The method developed to analyze EtG and EtS in urine used dihexylammonium acetate (DHAA), a commercially-available, volatile, LC-MS-compatible ion-pairing reagent and methanolic mobile phase. The use of a similar ion-pairing reagent has been previously documented for environmental LC-MS/MS methods. Mobile phases A and B consisted of 5mM DHAA in water and methanol, respectively. A simple step gradient was used to elute the compounds of interest as well as eliminate buildup of matrix components in the LC column. A 5µm Ultra II Biphenyl 50mm x 2.1mm column was used for this work. Initial exploratory work was performed on a Shimadzu LCMS-2010, while validation work was performed on an API-3200 QTRAP™ LC-MS/MS system. Both instruments were operated in ESI(-) mode. Two transitions were monitored for both EtG (221/75, 221/85) and EtS (125/80, 125/97). Quantitation for both compounds was accomplished using deuterated internal standards. Transitions for creatinine were also monitored. A simple 10:1 dilution of urine was used for sample preparation.

EtG and EtS were well-separated from most matrix components and exhibited good retention with k' values of 2.75 and 4.0, respectively. The total run time for the method, including re-equilibration, was 7.5 min. Validation experiments included LOD/LOQ, ion-suppression, specificity, linearity, precision, and accuracy.

The method presented here allows for fast, quantitative, simultaneous analysis of EtG and EtS in urine samples.

Keywords: **Ethyl Glucuronide, Ethyl Sulfate, LC-MS/MS**

P38 Validation of a Liquid Chromatography Mass Spectroscopy Method for the Determination of Fentanyl, Norfentanyl, Despropionylfentanyl, Hydroxyfentanyl, and Hydroxynorfentanyl in Human Urine

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The objective of this study was to validate a LC/MS/MS method for the determination of fentanyl and its metabolites in human urine. Fentanyl is a synthetic opioid that is approximately 100 times more potent than morphine. Since it is used mostly to treat post operative and chronic pain, it has a very high potential for abuse. The principal metabolite of fentanyl found in urine is norfentanyl along with hydroxyfentanyl, hydroxynorfentanyl, and despropionylfentanyl as minor metabolites.

For the LC/MS/MS method, fentanyl and norfentanyl with their appropriate deuturated analogs (fentanyl D5 and norfentanyl D5) were extracted from human urine with a basic liquid-liquid procedure. The same procedure was also used to determine approximate concentrations of other minor metabolites like, despropionylfentanyl, hydroxyfentanyl, and hydroxynorfentanyl. All extracts were analyzed with a Micromass Quattro Micro triple quad mass spectrometer that was coupled to a Waters 2795 Alliance HPLC system. The instrument was operated in a positive electrospray mode, where analysis was performed by multiple reaction monitoring, where the protonated molecules were used as precursor ions for all analytes. Using a simple gradient method, 20 µl of the supernatant was injected onto a Waters Xterra column (5 µm, 2.1 mm * 100 mm) set to a flow rate of 0.325 ml/min of 90:10 methanol to water both containing 5.0 mM ammonium acetate and 0.1% formic acid.

To test the robustness, sensitivity and specificity of the LC/MS/MS method, the following validation studies were carried out; intra- and inter-day precision and accuracy, percent recovery, linearity, and limit of quantitation for fentanyl and norfentanyl. Since analytical grade standards are not available at this time for the minor metabolites, not all validation studies were carried out. The calibrator range established on the LC/MS/MS demonstrates good linearity from 0.5 ng/mL to 100.0 ng/mL for both analytes. The intra and inter-day precision and accuracy CV's were all less than 15%. The extraction efficiency of fentanyl and norfentanyl at a concentration of 10ng/mL is 87% and 67% respectively. The LOD for fentanyl is 0.050 ng/mL, and for norfentanyl is 0.250 ng/ml.

This LC/MS/MS method provides a reliable, precise, and rapid method that was successfully applied to the analysis of fentanyl and its metabolites in human urine.

Keywords: **Fentanyl, LC/MS/MS, Enzyme Immunoassay**

P39 Comparison of Urinary Clonazepam Detection by Immunoassay and LC/MS/MS in a Pain Management Population

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Pain management patients are often prescribed benzodiazepines as well as opiates as part of their regimen of pain management medication. The patients are tested to ensure compliance with this group of drugs. Failure to observe the presence of the prescribed benzodiazepine can lead to patient dismissal with dire consequences for their pain control. Therefore the analyst is challenged to provide accurate information regarding compliance with benzodiazepine medications.

A retrospective analysis of the Millennium Laboratory test data base over a two month period was conducted to compare the ability of immunoassay and LC/MS/MS to detect the benzodiazepine clonazepam in a pain management population. The test data were filtered to include patients with prescriptions for clonazepam and exclude samples containing oxazepam, nordiazepam, temazepam, alpha-hydroxyalprazolam, and lorazepam allowing for an evaluation of immunoassay responses representative of clonazepam and its metabolites that were not influenced by other benzodiazepines. 180 samples from this cohort were tested using the Microgenics DRI ® benzodiazepine assay with a 200 ng/mL cutoff on Olympus AU640 and AU5400 analyzers. Quantitative analysis of 7-aminoclonazepam (7-AC) was performed on the same samples by LC/MS/MS with a cutoff of 40 ng/mL using the Agilent model 6410 triple quad in MRM mode. Samples were prepared for MS analysis using a simple “dilute and shoot” technique that incorporates glucuronidase hydrolysis. The positivity rates were 21% (38 samples) and 87% (157 samples) for immunoassay and LC/MS/MS respectively. These differences are not attributed only to the lower cutoff of the MS assay; at a cutoff of 200 ng/mL, the MS positivity rate was 70%. This shows that the nominal 200 ng/mL cutoff of this immunoassay does not apply to 7-AC . The low immunoassay positivity rate observed here relative to MS is consistent with the manufacturer’s published cross reactivity data for clonazepam and 7-AC. Concentration distributions plotted using the same data set reveal a significant fraction (7%) in the 40-100 ng/mL range. These data illustrate the limitations of immunoassay when used to monitor clonazepam compliance and suggest that an MS cutoff of 40 ng/mL or less is needed to reliably monitor use of this drug.

Keywords: **Clonazepam, Pain Management, LC/MS/MS**

P40 Extraction of Cocaine and Metabolites using Resin-based Mixed-mode Cation Exchange SPE with LC-MS/MS Analysis

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Introduction: Cocaine is one of the most widely abused illicit drugs available and not confined to any particular socio-economic class. Available in various forms it is highly addictive, however, instantaneous euphoric effects has led to huge popularity. This widespread misuse has led to the necessity of rapid and reliable methods for analysis and quantitation from various matrices. EVOLUTE CX is a resin-based mixed-mode strong cation exchange SPE sorbent designed for the extraction of basic drugs. The dual retention mechanism of hydrophobic interaction and strong cation exchange enables a rigorous interference wash regime resulting in cleaner final extracts.

Aims: This poster shows the application of EVOLUTE CX to the extraction of cocaine and its major metabolites from various human biological fluids.

Methods: Initial work investigated blank human plasma, urine and whole blood samples spiked with cocaine and metabolites at 50 ng/mL and 100 µL extracted using EVOLUTE CX in the 25mg 96-Well plate format. The generic method is based on a 50mM ammonium acetate buffer at pH 6, however, to incorporate all metabolites for analysis modification was necessary. Column conditioning takes place with 1 mL of methanol follow by 1 mL of buffer. 100 µL of matrix was pre-treated with buffer and extracted through the SPE procedure. The dual retention enables subsequent washes of buffer, 2% formic acid (aq) and methanol (1 mL). Analyte elution is afforded by 5% ammonium hydroxide in methanol. The SPE extracts were evaporated to dryness and reconstituted in appropriate mobile phase for analysis. All samples were analyzed using a Waters 2795 liquid handling system coupled to a Quattro Ultima Pt triple quadrupole mass spectrometer. Positive ions were acquired using electrospray ionization operated in the MRM mode.

Results: The analyte suite consisted of: cocaine, norcocaine, benzoylecgonine, ecgonine methyl ester, anhydroecgonine methyl ester and cocaethylene. Preliminary results showed excellent extraction efficiencies for all analytes. Recoveries greater than 80% with corresponding RSD's below 10% were observed for all analytes. Full results will be shown in the final poster.

Conclusion: This poster shows the application of EVOLUTE CX to the extraction of cocaine and major metabolites from various matrices. High reproducible recoveries were obtained for all matrices tested.

Keywords: **Cocaine, Mixed-Mode SPE, LC-MS/MS**

P41 Comparison of Plasma and Whole Blood Extraction Using Resin-based Mixed-mode Cation Exchange SPE with LC-MS/MS Analysis

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Introduction: The extraction of whole blood is becoming increasingly widespread in bio-analytical applications. This approach is gaining popularity due to the requirement to analyse the entire sample in order to avoid overlooking drugs or metabolites not present in the plasma or serum fraction alone. Resin-based mixed-mode cation exchange SPE is widely used for the extraction of basic drugs and provides clean extracts due to the dual retention mechanism afforded by the sorbents. Extract cleanliness is an extremely important consideration when sensitive analytical equipment such as mass spectrometry is being used.

Aims: This poster will assess the performance of resin-based mixed-mode SPE, EVOLUTE CX for the extraction of whole blood compared to plasma.

Methods: Human plasma and whole blood (100 µL) was extracted using generic SPE methods throughout. Overall ion suppression (cleanliness) experiments extracted blank plasma and whole blood through the SPE, reduced to dryness and reconstituted in mobile phase spiked with 1µg/mL caffeine solution. The amount of matrix interference was calculated by comparing peaks area with an authentic caffeine standard. Recovery experiments investigated matrix spiked with a suite of basic analytes at 50 ng/mL (procainamide, salbutamol, atenolol, ranitidine, naltrexone, metoprolol, brompheniramine, mianserin, amitriptyline and fluoxetine) and extracted with the SPE procedure. Extracts were evaporated to dryness and reconstituted in appropriate mobile phase for LC-MS/MS analysis. Chromatographic ion suppression experiments investigated matrix ion suppression exerted on basic analytes by comparing peak area response to an authentic standard. Blank plasma and whole blood were extracted through the SPE and reduced to dryness. Extracts were reconstituted and spiked with various basic analytes. All samples were analyzed using a Waters 2795 liquid handling system coupled to a Quattro Ultima Pt triple quadrupole mass spectrometer.

Results: Overall ion suppression comparing plasma and whole blood extracts was measured by flow-injection analysis (FIA). Extract cleanliness comparing calculated matrix factors shows more ion suppression when extracting whole blood relative to plasma. The recovery experiment showed consistently high recoveries from both whole blood and plasma. The majority of analytes exhibited recoveries greater than 80% with corresponding RSD's less than 10%. Chromatographic ion suppression indicated good consistency comparing extract cleanliness in terms of matrix factors between plasma and whole blood with all analytes showing calculated responses above 0.75. Full results will be shown in the final poster.

Conclusion: Mixed-mode SPE shows high, reproducible basic drug recoveries for plasma and whole blood, however, plasma samples result in slightly cleaner extracts.

Keywords: **Whole Blood, Mixed-mode SPE, LC-MS/MS**

P42 Understanding the Distribution of Buprenorphine and Its Metabolites in Blood

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Recent adaptation of buprenorphine as an agent for "treating" heroin addiction in Taiwan has prompted our interest in pharmacokinetics study related to this compound. This study was designed to understand whether the concentrations of buprenorphine and its metabolites derived from the common practice in the analysis of plasma are representative of these compounds' contents in blood. To the best of our knowledge, no study reported in the literature has addressed this issue.

In addition to parameters associated with GC-MS, we have studied various combinations of approaches adapted in the sample preparation steps. The following protocols were found effective for preparing blood samples for the analysis of free buprenorphine and norbuprenorphine: (a) deproteinization by acetonitrile; (b) liquid-liquid extraction by dichloromethane/isopropanol (9:1) in 0.1 M ammonium carbonate buffer (pH 10); and (c) derivatization by acetic anhydride. For the analysis of glucuronides, an additional step was required. Specifically, the sample was first hydrolyzed by β-glucuronidase, followed by the analysis of total buprenorphine and norbuprenorphine by the same protocols shown above. The concentrations of the glucuronides were calculated based on the differences of the findings resulting from these two analyses.

By analyzing plasma, blood cell, and whole blood separately we have concluded that: (a) the amounts of buprenorphine-related analytes found in the plasma were most likely less than that in the whole blood; and (b) the plasma/whole blood ratios of buprenorphine and its metabolites were significantly different. Data derived from *spiked* samples indicate the amounts of buprenorphine, norbuprenorphine, buprenorphine-3-β-D-glucuronide, and norbuprenorphine-3-β-D-glucuronide found in plasma represent approximately 72%, 55%, 86%, and 100% of their contents in the whole blood. GC-MS methods may not be adequately sensitive to detect and quantitate all analytes in all clinical specimens. Potentially more sensitive LC-MS-MS approaches are currently under study to better analyze clinical specimens.

Keywords: **Buprenorphine, GC-MS, LC-MS-MS**

P43 Disposition of Cannabinoids in Mice Brain Following Marijuana Inhalation Determined by HPLC/MS/MS

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A validated liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) method was developed for the analysis of marijuana cannabinoids in brain tissue using an Applied Bio systems 3200 Q trap with a turbo V source for TurbolonSpray with a Shimadzu SCL HPLC system. The method included cannabichromene (CBC), cannabidiol (CBD), D^9-tetrahydrocannibial (THC), 11-Hydroxy-tetrahydrocannabinol (11-OH-THC) and D^9-tetrahydrocannabinol carboxylic acid (THC-COOH).
Whole brain specimens were weighed and then, diluted with 1.5 mL of DI water. Specimens were homogenized using a glass homogenizer. Internals standards, THC-d3 and THC-OH-d3, were added to each sample. These samples were mixed and allowed to equilibrate overnight. The cannabiniods were then isolated by liquid/liquid extraction using cold acetonitrile. The extracts were then dried in a Savant AES1000 and reconstituted in 100µL of acetonitrile. The cannabiniods were separated using a Zorbaz eclipse XDB-C18, 4.6 x 75 mm, 3.5 micron (Agilent Technologies, USA) analytical column using water/methanol (10:90 v:v) and 0.1mM ammonium formate as the mobile phase with a flu flow rate: 0.5 mL/min. The following transition ions were monitored by multi reaction monitoring (MRM): THC/CBD/CBC 315>193, 315>259 m/z; THC-OH 331>193, 331>105; THC-COOH 345>299, 345>193 and THC-d3 389>346 m/z THC-OH-d3 318>196, and THC-COOH-d3 348>302. Linearity of THC, THC-OH and THC-COOH was 1 – 200 ng/gm; CBC, and CBD was 0.5 – 20 ng/gm. In run and between run precisions for all the analytes yielded coefficients of variation of < 20%. Four C57 mice were sacrificed twenty minutes after exposure to the smoke of 200 mg of marijuana containing: CBC, 0.44 mg; CBD, 0.93 mg and THC, 8.81 mg. The mean brain concentrations in ng/gm were: CBC, 3.9; CBD, 21; THC, 364. 11-THC-OH and THC-COOH were not detected. The relative mean brain cannabinoid concentrations were related to the amounts of the cannabinoid in the inhaled marijuana.

Keywords: **LC/MS/MS, Brain Cannabinoids, Marijuana**

P44 The Prevalence of Impairing Substances in US Aviation Accident Pilot Fatalities Between 1989 and 2008: Part II, Benzodiazepines

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Introduction: Benzodiazepine medications including, diazepam, nordiazepam, alprazolam, alpha-hydroxyalprazolam, temazepam, and midazolam are commonly prescribed and frequently abused in America. The side effects of these medications include drowsiness, dizziness, decreased alertness, and/or memory loss which can lead to impairment and a decreased ability to properly control an aircraft. Determining the presence of these medications in postmortem specimens of aviation accident victims can help determine the cause of the aviation accident and potentially result in serious legal consequences. Our laboratory is in a unique position as a medical history is available to investigators for most certified pilots. With this in mind we compared benzodiazepine compounds found following postmortem analysis with the available medical history for each victim. This evaluation was conducted to determine if their records supported the use of such medications or if the aviator was taking the compound without the approval of their aviation medical examiner and/or possibly abusing the substance.

Method: In fatal aviation accidents, specimens from accident victims are routinely sent to the Federal Aviation Administration's Forensic Toxicology Research Laboratory for toxicological analysis. Toxicological information from these analyses is stored in a database at the Civil Aerospace Medical Institute's (CAMI's) Forensic Toxicology Research Laboratory. Case histories and accident information were obtained from the National Transportation Safety Board (NTSB). Medical histories were obtained from the Document Imaging Workflow System (DIWS) database.

Results and Discussion: Over this 20-year period, there were 6112 fatal aviation accidents, 159 (2.6%) pilots were found positive for a benzodiazepine. Fatal aviation accidents involving pilots who had taken a benzodiazepine compound prior to the flight are an infrequent event. This presentation will correlate the use of benzodiazepine compounds with past medical history; highlight the 20-year trend of benzodiazepine use in the pilot community; and present a variety of demographic information about the pilots involved in such incidents.

Keywords: **Forensic Toxicology, Benzodiazepines, Aviation**

P45 Automated Homogeneous Enzyme Immunoassay for the Detection of Fentanyl and Norfentanyl in Human Urine

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The objective of the study was to develop a homogeneous enzyme immunoassay for the semi-quantitative determination of Fentanyl in human urine. Fentanyl is a synthetic opiate analgesic similar to morphine. Fentanyl is 50-100 times more potent than morphine. It is prescribed mainly for patients with chronic pain and is generally used to manage pain after surgery. Fentanyl is prescribed as intravenous anesthetic (Sublimaze®), transdermal patch (Duragesic®), and transmucosal Lozenge form (Actiq®). The fentanyl dose in the Duragesic ranges from 2.5-10 mg and in Actiq, it ranges from 0.2 mg-1.6 mg. The fentanyl patches have become the potential targets for abuse and diversion. These patches are sold on the street as Apache, China white, jackpot and Tango. Half-life of Fentanyl is 3-12 hours. More than 90% of the dose is eliminated as N-dealkylated and hydroxylated metabolites.

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

The assay consists of two reagents-antibody/substrate reagent and enzyme conjugate reagent. The calibrator levels are 0,10,25,50 and 100 ng/mL. Reagents, calibrators and controls are liquid ready-to-use. Norfentanyl is used as the calibrator drug. The performance of the assay was evaluated on the Hitachi 917 analyzer using the following parameters to generate the calibration curve: sample volume 25 uL, reagents 1 and 2 at 85 uL each, assay method Rate A, measuring cycles 19-22 and calibration type Logit-Log4P. The assay range is 5-100 ng/mL. The precision results for the three controls are: Within-run: 5 ng/mL 2.2%, 15 ng/mL 2.5% and 35 ng/mL 1.7%, Total Precision: 5 ng/mL 2.2% 15 ng/mL 3.4% and 35 ng/mL 2.8%. The limit of detection (LOD) was 0.6 ng/mL. No significant interference was observed from endogenous substances. The assay demonstrated 100% cross-reactivity to norfentanyl and significant cross-reactivity to the hydroxylated metabolites of fentanyl and norfentanyl with minimal cross-reactivity to other opiate compounds. Comparison of the immunoassay results (n=83) with LC-MS/MS showed >95% agreement between the two methods.

DRI Fentanyl Assay is a simple, precise and convenient method for the detection of Fentanyl and its metabolites in urine. The assay can be applied to high throughput clinical chemistry analyzers such as Hitachi and Olympus analyzers.

Keywords: **Fentanyl, Enzyme Immunoassay, G6PDH**

P46 Internal Standard Choices for Fast and Accurate Determination of Blood Alcohols in Post Mortem Samples

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Objective: Last year, nearly 35% of all traffic fatalities were alcohol related, reinforcing the need for accurate methods of determining blood alcohol content (BAC). Gas chromatography has become the standard analytical testing device for BAC in both confirmatory testing as well as post mortem investigations. To improve accuracy of this test, many labs are moving towards dual column approaches with the use of an internal standard. The use of n-propanol as an internal standard is common, but can be problematic when analyzing post mortem samples because it can also be formed in the body's natural decay process. The objective of this work was to provide a method for post mortem samples using a different internal standard while still retaining fast analysis, with good accuracy and precision.

Methods: Samples were run by headspace GC using dual columns connected to a single injection port via a Y-splitter. Sample concentrations ranged from 0.025% to 0.400%. Three possible internal standards were explored including t-butanol, n-propanol, and n-butanol all at 0.100%. Replicate samples were run for 0.025% as well as 0.100% to determine reproducibility.

Results: A headspace and chromatographic method was developed that provides baseline resolution for all compounds including t-butanol which has previously found to be problematic for some methods. Improved resolution was achieved for ethanol compared to other methods. The calibration curve for all compounds was found to be linear with correlation coefficients (R^2) within a range of 0.9980 – 0.9998. Replicate samples showed RSD values that were below 3.0% using any of the three internal standards. Method LOD and LOQ were determined to be less than 0.0003% and 0.001% respectively for all compounds.

Conclusion: The use of t-butanol or n-butanol was found to be viable alternatives to n-propanol using the conditions outlined within this method. This method allows for accurate and fast analysis (under two minutes using t-butanol as internal standard) of blood alcohols with detection limits that are over 80 times lower than the 0.08% cutoff.

Keywords: **BAC, Ethanol, Alcohol**

P47 Microwave Assisted Extraction in the Study of the Effect of Dose-Death Interval on Meperidine Detection in Skeletal Tissue

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The effect of dose-death interval and tissue distribution on the detection of meperidine in selected skeletal tissues was examined using a rapid microwave assisted extraction (MAE) methodology. Rats (n=14) were dosed with 0 (n=2) or 30 mg/kg (n=12) meperidine (i.p.). Drug-positive rats were then sacrificed with CO_2 after 20, 30, 90 and 150 minutes (n=3 per group). Heart blood was collected immediately after death. Tibiae were excised and frozen for further analysis. The remaining carcasses were placed outside in secured cages and allowed to decompose to the point of complete skeletonization in a rural Northern Ontario location during the late summer months. Vertebrae and pelvi were collected for each animal.

Tibiae were cracked open with pliers and bone marrow was removed with a syringe. Bone marrow was then sonicated in 3 mL PB6 (Phosphate Buffered Saline, pH=6) for 30 minutes. Fresh tibiae, and decomposed vertebrae and pelvi were cleaned in PB8.5 (Phosphate Buffered Saline, pH=8.5) and sonicated to remove remaining soft tissue. Samples of dried, ground bone (0.5-1 g) were combined with 2 mL PB6 and then irradiated in a domestic microwave oven (1100 W) at atmospheric pressure for 15 minutes, in cycles of 10 seconds to prevent boiling. The supernatant was then removed for further extraction. Samples of vertebral bone (1 g) were also extracted by passive incubation in methanol (3 mL, 50 ^{o}C, 72 hours). Blood (100 µL) was diluted in PB6 (300 µL). All samples were further extracted by solid-phase extraction and analyzed by GC-MS, using electron impact ionization in the Selected Ion Monitoring (SIM) mode and D4-meperidine as an internal standard. Standard solutions of meperidine (5-500 ng/mL) prepared in drug-free bone extract and subjected to SPE displayed a linear relationship ($R^2 = 0.995$) between response ratio and meperidine concentration.

Mean values for mass-normalized response ratio (RR/m, where RR represents the ratio of peak areas for the m/z 247 and 251 ions) were negatively correlated with dose-death interval (r = -0.44, -0.32, -0.81, -0.87, -0.58, -0.82, -0.84, respectively) for the vertebrae (MAE), pelvi (MAE), vertebrae (passive extraction), tibial epiphyseal bone (MAE), tibial diaphyseal bone (MAE), marrow and blood. One-way ANOVA with post-hoc analysis showed dose-death interval to be a main effect ($p<0.05$) with respect to RR/m for blood, marrow, tibial epiphyses and vertebral bone prepared by passive extraction, but not for tibial diaphyses, pelvi or vertebrae prepared by MAE. These data suggest that MAE is advantageous as a rapid extraction tool for screening purposes in skeletal tissues. However, further work is required to characterize whether the MAE process is contributory to the limited analytical precision, or if drug extraction from skeletal tissue is the dominant factor. Overall, it remains clear that assignment of significance to quantitative expressions of skeletal drug concentrations is complex and should be approached with caution.

Keywords: **Meperidine, Skeletal Tissues, Microwave-Assisted Extraction**

P48 Deadly Ingestion Involving Zolpidem, Clonazepam and Ethanol: A Case Report

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A 34 year old male residing in a hotel was found dead sitting in a chair. He had a history of alcohol abuse, depression and anxiety. An autopsy was performed, and postmortem peripheral blood, vitreous humor, urine, liver tissue and brain tissue were submitted for toxicological analysis. The autopsy disclosed pulmonary edema and congestion, moderate fatty change of the liver, and a white-tan chalky and pasty material in the stomach consistent with pill residue.

Toxicologic testing revealed high concentrations of zolpidem, clonazepam and ethanol and low concentrations of ibuprofen and lamotrigine. Quantitative analysis by gas chromatography using a flame ionization detector determined ethanol concentrations of 319 mg/dL and 337 mg/dL in femoral blood and vitreous humor, respectively. Quantitative analysis by high performance liquid chromatography determined 109 ng/mL of clonazepam and 4.8 mcg/mL of lamotrigine in subclavian blood. Quantitative analysis by gas chromatography using a nitrogen phosphorus detector determined zolpidem at 10 mg/L, 3.9 mg/L and 8.5 mcg/g in femoral blood, subclavian blood and liver tissue, respectively.

The 10 mg/L femoral blood level of zolpidem is the highest reported blood level of this drug to date. The disparity between the 10 mg/L femoral level and the 3.9 mg/L subclavian level remains unclear, but the remarkably high concentrations of zolpidem in this case may have attributed to this disparity.

Keywords: **Zolpidem, Multiple Drug Toxicity, Postmortem**

P49 A Preliminary Investigation into Retrospective Calculation of In-Vivo Drug Concentrations in Dried Crime Scene Blood

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Dried blood left at a crime scene is at times of forensic toxicological interest. Such as in determining if an injured motorist who has left the scene of an accident, and not apprehended until many hours or days later, may have been under the influence of drugs at the time of the accident. Or in determining if a wounded individual who has escaped the scene may have been under the influence of drugs during a violent dispute.

Dried blood spots have been used for many years in clinical laboratory testing. These tests rely on premeasured volumes of blood or standardized blotter material for calculating in-vivo concentration. However, dried blood discovered at a crime scene can rely on no such convention to determine original specimen volume. Furthermore, dried crime scene blood is an unfamiliar matrix, which by its nature is unpreserved and unrefrigerated. These factors raise the questions of drug recovery and stability in this matrix. The objective of this study is to provide forensic toxicologists with a conversion factor to accurately estimate blood volume from the weight of a dried blood specimen as well as to provide data on recovery and stability of drugs in this matrix.

The authors investigate the potential for back calculating in-vivo drug concentration using the weight of dried blood to predict the original volume. Specimens submitted for CBC analyses were selected for this study. The specimens were collected in 5 mL purple top tubes containing EDTA as an anticoagulant, but otherwise unpreserved. One half milliliter of blood was transferred by volumetric pipette to a weighing boat and the total weight was immediately recorded. The specimens were allowed to dry at room temperature for a minimum of 72 hours and then re-weighed. The difference in weight was corrected for the weight of the anticoagulant and the ratio of the non-volatile components of blood to its volume was determined for each specimen.

The authors also investigated the recovery and stability of several drugs of abuse in dried blood. Amphetamine, methamphetamine, MDA, MDMA, codeine, morphine, hydrocodone, hydromorphone, cocaine, ecgonine methyl ester, and benzoylecgonine were spiked into pooled unpreserved drug-free blood. The initial concentration of each analyte was determined and 1 mL aliquots were prepared and dried as previously described. The spiked dried blood specimens were analyzed for the target compounds periodically over the following weeks and months.

While this is a limited study and further investigations are warranted, it was found that the volume from which a specimen of dried blood originated could be calculated within 23% with a confidence level of 95%. Furthermore, with the expected exception of cocaine and ecgonine methyl ester, the drugs investigated were quite stable in a dried blood matrix over a period of months, and recoveries for most drugs investigated were well above 90%. These findings along with future investigations may allow for the calculation of original drug concentration from the analysis of dried crime scene blood and provide an estimate of the uncertainty of this process.

Keywords: **Dried Blood, Drugs**

P50 The Prevalence of Impairing Substances in US Aviation Accident Pilot Fatalities Between 1989 and 2008: Part III, Stimulants

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Introduction: Stimulants, including amphetamine, methamphetamine, MDMA (ecstasy), and cocaine, can severely affect a pilot's ability to safely operate their aircraft. The side effects of these medications including increased heart rate and higher blood pressure can have adverse affects on a pilot during flight. Demonstrating the presence of these drugs in postmortem specimens of aviation accident victims can have serious legal consequences and can help determine the cause of the aviation accident. Our laboratory is in a unique position as a medical history is available to investigators for most certified pilots. Though few of these compounds are prescribed by physicians, we compared the stimulant compounds found following postmortem analysis with the available medical histories for each victim in order to determine if the deceased pilots had an incident in their past requiring such compounds.

Method: In fatal aviation accidents, specimens from accident victims are routinely sent to the Federal Aviation Administration's Forensic Toxicology Research Laboratory for toxicological analysis. Toxicological information from these analyses is stored in a database at the Civil Aerospace Medical Institute's (CAMI's) Forensic Toxicology Research Laboratory. Case histories and accident information were obtained from the National Transportation Safety Board (NTSB). Medical histories were obtained from the Document Imaging Workflow System (DIWS) database.

Results and Discussion: Over this 20-year period, there were 6112 fatal aviation accidents, 87 (1.4%) pilots were found positive for a stimulant. Fatal aviation accidents involving pilots who had taken a stimulant compound prior to the flight are an infrequent event. This presentation will correlate the use of stimulant compounds with past medical history; highlight the 20-year trend of stimulant use in the pilot community; and present a variety of demographic information about the pilots involved in such incidents.

Keywords: **Forensic Toxicology, Stimulants, Aviation**

P51 Tissue Distribution of Newer Anticonvulsant Drugs in Postmortem Cases

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Over the past 20 years, a number of anticonvulsant drugs have been developed to replace or augment the use of classical anticonvulsant drugs such as phenobarbital, phenytoin and carbamazepine. Among the group of newer anticonvulsant drugs are levetiracetam (Keppra®), oxcarbazepine (Trileptal®) and topiramate (Topamax®). Associated with their increased therapeutic use is their increased detection in cases investigated by medical examiners' and coroners' offices, as deaths due to seizure disorder often fall under the jurisdiction of these offices. The distribution of these drugs in blood, liver and kidney were compiled in cases investigated by the Office of the Chief Medical Examiner, State of Maryland. None of these deaths were ruled by the medical examiner to be caused by an intoxication of the anticonvulsant drug identified.

Levetiracetam, hydroxycarbazepine (the primary product of oxcarbazepine use) and topiramate were quantified using the acid/neutral drug screening procedure employed by this laboratory. Briefly, blood or tissue homogenates spiked with an internal standard (cyclopal) were buffered to pH 5 and applied to Chem Elut® columns. The columns were washed with methylene chloride, collected and evaporated to dryness. The residue was reconstituted with 0.033 M TMAH and injected into a gas chromatograph equipped with a DB-5 analytical column (25 m x 0.32 mm id) and a nitrogen phosphorus detector. A calibration curve using 4 calibrators ranging in concentration from 2 to 20 mg/L was used for quantification.

The collected data is compiled in Table 1. Despite the limited number of cases for each drug, there were some trends suggested by the data. None of the drugs displayed significant differences in concentration between the heart blood and peripheral blood specimens. Only the hydroxycarbazepine quantifications in case 5 showed significant differences between the 2 blood sites. This is consistent with other acid/neutral drugs such as acetaminophen and meprobamate. It also appears that the liver and kidney concentrations of the 3 drugs are on the same order of magnitude as the blood concentrations. Only the levetiracetam concentration in case 3 reflected a liver or kidney concentration greater than 5 times the blood concentration. The kidney hydroxycarbazepine concentration in case 6 was less than one-tenth of the blood concentration.

Keywords: **Postmortem, Anticonvulsant, Distribution**

Table 1. Tissue Distribution of Newer Anticonvulsant Drugs in the Investigated Cases.

No.	Drug	(Concentrations (mg/L or mg/kg)			
		Ht. Blood	P. Blood	Liver	Kidney
1	levetiracetam	17	19		
2	levetiracetam	19	18	44	
	hydroxycarbazepine	29	29	38	
3	levetiracetam	1.8	1.4	14	1.8
	hydroxycarbazepine	7.7	7	11	7.6
4	hydroxycarbazepine	5.8	9		5.6
5	hydroxycarbazepine	81	32	118	
6	hydroxycarbazepine	52		46	2.3
	topiramate	20		13	10
7	topiramate	12	13	12	9.5
8	topiramate	10	9.2	8.6	
9	topiramate	7.5	8.6	10	9.1
10	topiramate	10		13.7	8.7

P52 Identification and Validation of Gene Expression Changes Occurring During Acute Ethanol Use

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Introduction and Objective: As part of the aviation safety program to define the adverse effects of ethanol on flying performance, we present results of our DNA microarray analysis of samples from a timecourse study of individuals given ethanol orally, and then evaluated by breathalyzer to monitor blood alcohol content (BAC). At five blood alcohol levels, T1-T5, blood was drawn such that the samples represented 0%, 0.04%, 0.08%, return to 0.04%, and 0.02% BAC. Microarray analysis showed that changes in gene expression could be detected across the timecourse. We verified these expression changes by quantitative polymerase chain reaction (qPCR). Candidate target genes identified from the microarray analysis were clustered by expression change pattern and examined for shared functions and functional network membership. The results demonstrate that it is possible to detect significant changes in gene expression due to the acute presence of imbibed ethanol using total RNA isolated from whole blood.

Results: The qPCR results supported the microarray analysis results for 11 of 12 genes tested. The candidate target genes clustered into five coordinately expressed groups and functional analysis showed shared transcription factor binding sites and functions for members of the clusters. These functions include protein synthesis and modification, expected for changes in gene expression, hematological and immune functions, expected for a blood sample, and pancreatic and hepatic function, expected as response to ethanol.

Conclusion: It appears that the methods used for microarray data analysis are valid and complementary. They identified genes whose expression indicated change with time and alcohol concentration that could be validated via qPCR. Therefore these methods can be utilized as part of a workflow to identify target genes by timecourse changes in gene expression that may affect pilot performance.

Keywords: **Ethanol, mRNA, Expression**

P53 GC-MS Analysis of Carisoprodol and Meprobamate in Blood Using Deuterated Internal Standards

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The analysis of carisoprodol, and its metabolite meprobamate, is common in toxicology laboratories due to carisoprodol's frequent use and potential misuse. With the recent availability of deuterated internal standards for both analytes, a new method for the quantitation of carisoprodol and meprobamate in blood was developed using solid-phase extraction (SPE) coupled with gas chromatography-mass spectrometry (GC-MS).

Carisoprodol, meprobamate, carisoprodol-d_7, and meprobamate-d_7 were purchased from Cerilliant Corp. (Round Rock, TX). A series of extractions, which included 5 calibrators, a negative control and 6 known positive controls prepared in 0.5 mL of drug-free blood, were run on 3 separate occasions in order to determine the assay's accuracy and precision. Calibrators were prepared in a concentration range from 1.0-25 µg/mL. The positive control target concentration was 5 µg/mL for both analytes. The internal standard target concentration was 1.0 µg/mL for carisoprodol-d_7 and meprobamate-d_7.

In preparation for the SPE procedure, 0.1 M phosphate buffer (pH 6) was added to each specimen. After centrifugation, the specimens were poured into SPE biphasic cartridges (United Chemical Technologies, Inc, Bristol, PA) preconditioned with methanol, water, and 0.1 M phosphate buffer (pH 6). The cartridges were washed with water, acetic acid, and hexane before the analytes were eluted with a hexane:ethyl acetate (50:50, *v/v*) solution. The extracts were dried under a gentle stream of nitrogen at 40°C and reconstituted in ethyl acetate.

The extracts were analyzed using an Agilent Technologies (Santa Clara, CA) 6890 Gas Chromatograph with a 5973 Mass Selective Detector and 7683 Automatic Liquid Sampler. The gas chromatograph was equipped with a Varian (Walnut Creek, CA) VF-5MS capillary column (30-m x 250-µm, 0.25-µm film thickness) under a constant flow of helium at 1.0 mL/min. The quantifying ions selected for data collection were *m/z* 158 for carisoprodol, *m/z* 165 for carisoprodol-d_7, *m/z* 144 for meprobamate, and *m/z* 151 for meprobamate-d_7. The qualifying ions selected for data collection were *m/z* 245 and *m/z* 184 for carisoprodol, *m/z* 252 for carisoprodol-d_7, *m/z* 114 and *m/z* 96 for meprobamate, and *m/z* 108 for meprobamate-d_7.

The study demonstrated linearity from 1.0-25 µg/mL, a limit of quantitation of 1.0 µg/mL and a limit of detection of 0.25 µg/mL. All positive control results were within ±20% with inter-assay %CV values for carisoprodol and meprobamate of 3.9% and 3.9%, respectively. In addition, a set of 14 authentic post-mortem blood specimens were analyzed by this method and compared with previously reported results obtained with an established method using a non-deuterated internal standard. The correlation coefficient between the two methods was 0.97.

In conclusion, a validated method has been described for the analysis of carisoprodol and meprobamate from post-mortem blood utilizing SPE extraction followed by GC-MS analysis.

Keywords: **Carisoprodol, Meprobamate, GC-MS Analysis**

P54 Suicide by Azide Ingestion

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A 59 year male laboratory technologist intentionally ingested an unknown amount of sodium azide. He was promptly discovered in his office at work and transported to Hospital Emergency Department. He had convulsed and vomited; he was incoherent and required intubation. His hypotension was treated with fluids and presser agents. His acidosis was treated with bicarbonate. Later on, he was treated by Extracorporeal Membrane Oxygenation and blood exchange transfusions. He arrested and was resuscitated twice. Life support system was withdrawn 24 hours after ingestion following a neurological assessment in which no brainstem activity was detected.

Azide was measured in ante mortem and post mortem blood and plasma samples. The blood azide concentration upon hospital admission, 1.4 hours after ingestion, was 5.6 μg/mL. After peaking at about 4 hours, the blood azide was eliminated at a constant rate of 0.84 μg/mL per hour. No azide was detected in the post mortem blood samples. The plasma to blood azide concentration ratios ranged from 1.05 to 1.18.

Blood and plasma azide concentrations were measured by gas chromatography equipped with a nitrogen-phosphorus detector. Azide was reacted with propionic anhydride to form propionyl azide in a headspace sampling bottle. A portion of the headspace was introduced into the heated injection port of the gas chromatograph, whereupon the propionyl azide was converted to ethyl isocyanate, according to the Curtius Rearrangement. The ethyl isocyanate peak was quantitated using propionitrile as the internal standard.

Keywords: **Azide, Blood, Plasma, Suicide, GC-NPD**

P55 Detection and Quantitative Analyses of Cyanide by Cyantesmo® Paper and Headspace GCMS in Two Cases of Suicide

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We describe results obtained for the qualitative analyses of cyanide by Cyantesmo® paper (Macherey-Nagel, Düren, Germany) and its quantitative analyses in postmortem blood, urine, other fluids and gastric contents by headspace-gas chromatography/mass spectrometry (HS-GCMS). Two suicide cases by ingestion of cyanide are presented. Case 1 is a 34 year old female who was found on the floor in her home with an open bottle of champagne and a vial marked KCN on a kitchen counter top. Case 2 is a 60 year old male found dead in a chair in his residence with a bottle of KCN nearby. The cause of death in both cases is acute cyanide intoxication with the manner of death being suicide. Table 1 summarizes the toxicology findings in both cases.

Cyantesmo® paper provides utility as a rapid presumptive test for cyanide in postmortem specimens. The procedure is simple, inexpensive and uses 3 mL of specimen and exhibits adequate sensitivity (LOD = 0.62 mg/L) for investigating suspected cyanide poisoning cases. The HS-GCMS quantitative analysis entails use of isotopically labeled internal standard (IS), the addition of ascorbic acid and phosphoric acid to sample prior to incubation, and injection of 0.5 mL headspace sample into a GCMS Agilent 5973 equipped with a PLOT-Q (30 m, 0.33 mm i.d., 20 μm film) column. The calibration curve extends over the range 0.025 mgL to 2.5 mg/L (blood controls are 0.30 mg/L and 1.5 mg/L). Operating in SIM mode, the mass spectrometer monitors the following ions: 29 (IS, $K^{13}C^{15}N$) and the native cyanide ions 26 and 27.

Combining rapid presumptive screening with the HS-GCMS instrumental method provides effective analytical schemes for resolving suspected postmortem cyanide intoxication cases.

Table 1. Summary of Toxicological Findings.

	Case 1	**Case 2**
SPECIMEN	Cyanide (mg/L) or (mg)	Cyanide (mg/L) or (mg)
Blood	Cyantesmo® Paper ++++	Cyantesmo® Paper ++
Blood	Heart 42	Heart 6.9
	Inferior Vena Cava 14	Femoral 1.0
Urine	0.52	0.05
Vitreous Humor	1.3	1.4
Bile	10	1.8
Gastric Contents	10 mg in 50 mL	141 mg in 150 mL
	Volatiles (g%) or (g/dL)	Volatiles (g%) or (g/dL)
Heart Blood	Ethanol : 0.06	Isopropanol: 0.01, Acetone: 0.14
Vitreous Humor	Ethanol : 0.06	Isopropanol: 0.01, Acetone: 0.18

Keywords: **Cyanide, Cyantesmo® Paper, Headspace GCMS**

P56 Postmortem Redistribution of Fentanyl in Femoral Blood

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The purpose of this study was to determine whether postmortem redistribution of fentanyl occurs in femoral blood. Seven medical examiner cases had femoral blood (FB) collected from the same vein at two postmortem times, at the time that the body arrived at the morgue (FB1) and at the time of autopsy (FB2). Heart blood (HB), liver tissue, and heart tissue were also collected from the same cases. Fentanyl concentrations in FB1 ranged from undetectable (<2.0 µg/L) to 14.6 µg/L and in FB2 from 2.0 to 52.5 µg/L. Heart blood fentanyl ranged from 5.0 to 58.8 µg/L (n=6), liver tissue fentanyl (n=6) from 45.0 to 161.0 µg/kg and heart tissue fentanyl (n=4) from 24.8 to 179.6 µg/kg. The postmortem interval from death to FB1 ranged from 2.5 to 6.0 hours (mean 4.0 h), while the interval to FB2 ranged from 7.0 to 53 h (mean 21.6 h). Four of the seven cases (57%) had fentanyl concentrations that increased from undetectable at FB1 to detectable at FB2, 1.5 to 50 h later. The ratio of fentanyl HB to FB1 ranged from 1.00 to 17.26 (mean 8.39) and was consistently higher compared to the corresponding HB to FB2 ratio, which ranged from 0.94 to 14.91 (mean 3.48). The change in concentration between FB1 and FB2 compared to the time elapsed between their collections showed a poor correlation ($r = 0.18$). In conclusion, 57% of the cases studied had FB concentrations that increased over the postmortem interval. Caution is urged in interpreting postmortem blood fentanyl concentrations during cause of death assessment, as the postmortem FB concentration may not be equivalent to the FB concentration present at the time of death.

Keywords: **Fentanyl, Postmortem Redistribution, Femoral Blood**

P57 Evaluation and Validation of the BAC Tracker® in Two Drinking Studies

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The Forensic Toxicologist is faced with a number of variables to consider in alcohol related scenarios. Zuba and Piekoszeski (2007) have shown that using traditional equations such as the Widmark alone will result in a variance when other subject data are not included. Especially when volume of distribution (Vd) is concerned, when height, weight and age are included a more precise estimation of alcohol values can be achieved. There are many available computer programs that calculate BrAC and BAC levels when types and amount drinks are known along with other variables such as absorption, elimination and others. One such program is the BAC Tracker®. The aim of this study is to validate the BAC Tracker® by comparing and contrasting results from two drinking studies distinguished by the prandial state of the volunteers, non-fed and fed state, using a variety of absorption rates and Vd formulae. The BAC Tracker® uses Vd formulae published by Posey and Mozayani (2007), Widmark (1932), Watson et al. (1989), Forrest (1986), Seidl et al. (2000) and Ulrich et al. (1987). We used the program to evaluate and validate the program using a fed (n=14 healthy volunteers) and a non-fed study (n=23 healthy volunteers). Before study initiation, standard volunteer participation documents were signed by all. All volunteers were questioned as to drinking and health history by law enforcement personnel. Ages ranged from 24-63 years, weights were 56-102 kg, heights were 137-178 cm. In the fed study, volunteers were given three separate drinks of alcohol for a total mean consumption of 0.61g/kg (range 0.47g/kg to 0.74g/kg) over a course of one hour. Analysis was done using Intoximeters. These instruments were certified and quality control performed according to DOT standards. The two operators who performed the anlysis were DPS certified. Intoximeter readings were taken on three separate occasions in the course of the subsequent hour after each drink. Age range for the non-fed study ranged from 21-35 years, weights were 70-90.9 kg and heights were 167-191 cm. Food intake was restricted for a minimum of three hours prior to the study. The volunteers were given five separate drinks of alcohol for a total mean consumption of 1.0g/kg (range 0.80g/kg to 1.3g/kg) over a course of two hours. Intoxilyzer 5000® readings were taken on three separate occasions—once after the third drink and two others after the fifth drink spaced 1.5 hours Results will be reported as to the performance of the BAC tracker® in both scenarios to validate the software based on the data from the non-fed and fed state studies using a combination of various absorption rates and Vd formulae.

Keywords: **Absorption Rates, Volume of Distribution, Drinking Studies**

P58 Assessment of Buprenorphine Among Cases Submitted to the Miami Valley Regional Crime Laboratory

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The Miami Valley Regional Crime Laboratory is located in Dayton, Ohio and provides toxicological testing for greater than 100 agencies in southwest Ohio. Case submissions include, but are not limited to: driving under the influence of alcohol or drugs (DUI/DUID), child endangering, vehicular homicide, probationary testing, and drug-facilitated sexual assault. In early 2009, local Drug Abuse Monitoring Networks reported an increase in the abuse potential of buprenorphine. Buprenorphine (Suboxone®, Subutex®) is a semi-synthetic thebaine derivative with both analgesic and opioid agonist/antagonist properties. The Food and Drug Administration (FDA) currently approves it for maintenance therapy in opiate addiction, in both a sublingual and parenteral dose delivery.

Beginning in January 2009, all cases submitted to the toxicology section of the Crime Lab were screened for the presence of buprenorphine. Biological matrices submitted for testing included blood and urine. All specimens were screened using an Immunalysis® ELISA Buprenorphine Kit with a Tecan® Automated System. Positive and low positive cutoffs were spiked at 2 ng/mL and 1 ng/mL, respectively. Positive screens were then confirmed by solid phase extraction similar to the laboratory's method for confirmation of opiates. Samples were placed in phosphate buffer (pH 6.0) and passed through a co-polymeric bonded phase column. The samples were eluted with a 4% ammonium hydroxide/ethyl acetate solution, evaporated at room temperature, and derivatized with BSTFA/1% TMS. Samples were injected onto a 6890 gas chromatograph/mass spectrometer (GC/MS) with an inert source. Buprenorphine was measured using buprenorphine-d_4 as the internal standard. The calibration range for buprenorphine was established from 1.0 to 50.0 ng/mL, with a coefficient of determination (r^2) greater than 0.99.

Screening of the cases submitted to the Crime Lab produced very few buprenorphine positives (~2%). Of these cases, approximately half confirmed positive for the presence of buprenorphine. Because norbuprenorphine has relatively strong cross-reactivity with the buprenorphine kit and the analytical method employed had difficulty identifying the metabolite at low concentrations, it is possible that the negative confirmations may have contained only norbuprenorphine. Further testing and studies are planned to optimize a method for both parent and metabolite drug as well as assessing abuse potential of buprenorphine in casework from the Montgomery County Coroner's Office. Though there seems to be potential for use of buprenorphine in the Miami Valley and surrounding area, current toxicological testing shows that usage is minimal in the DUI population.

Keywords: **Buprenorphine, ELISA, GC/MS**

P59 Preparation and Uncertainty of Ethanol Standards

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Ethanol standards are widely used in the forensic/toxicology and clinical industries for determination of blood alcohol content. Accuracy of the reference standards is critical to the quantitative determination of ethanol in samples. The manufacture, certification and uncertainty of Cerilliant Ethanol Standards in accordance with ISO/IEC 17025 and ISO Guide 34 requirements are presented. Factors critical to accurate preparation of an ethanol standard including demonstrating traceability to a NIST SRM reference are outlined along with the uncertainty associated with the certified concentration of the standard.

Standards are prepared gravimetrically and provided as single use standards in flame sealed ampules. Production and testing are controlled to reduce uncertainty. The standards are tested for homogeneity and certified for concentration by a validated GC/FID headspace analysis method.

Combined expanded uncertainty calculations are presented for the gravimetrically prepared concentration along with validation results for the GC/FID headspace method.

Identifying sources of uncertainty and establishing an accurate concentration value are critical in manufacturing ethanol certified reference standards. A thorough analysis of each step of the preparation process and certification was performed and the results of that study are provided in this presentation.

Keywords: **Ethanol, Uncertainty, Reference Standard, Blood Alcohol**

P60 Ultra-Performance Liquid Chromatographic Separation with Enzyme Immunoassay Detection for Rapid, Sensitive and Selective Drug Screening

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Ultra-performance liquid chromatography (UPLC) facilitates rapid, high resolution separations, often completed within 5 minutes or less. Flowrates are on the order of 0.2-0.6 ml/min with observed peak widths often 10 seconds or less at baseline. This allows for direct collection of low fractionated eluent volumes (~20-50 μL) into ELISA microwells for immunoassay, which may resolve cross-reacting compounds. This exploits the extremely high sensitivity of ELISA (~0.1-10 ng/mL) while providing high selectivity achieved through UPLC separation. Consequently, analytes that are below the limit of detection for the UPLC-PDA instrument may still be detected with a concentration-dependent response, with sensitivity rivaling that of LC/MS/MS.

Here, standard solutions of model drugs/metabolites (fentanyl, cocaine, benzoylecgonine, diazepam, nordiazepam and oxazepam) were analyzed at high concentration (400-500 ng/mL) using UPLC-PDA, equipped with a 2.1×100 mm column with C8 stationary phase on 1.7 μm particles, for method optimization and retention time calibration. Mobile phase composition was fixed at 57:43 A:B (A – 0.1% formic acid in 10:90 methanol:water, B - methanol) for fentanyl analysis and 40:60 A:B for analysis of cocaine and benzoylecgonine. Retention times were 1.31 min, 1.44 min and 1.66 min for fentanyl, benzoylecgonine and cocaine, respectively. Mobile phase composition was fixed at 40:60 (A - 10:90 methanol:water, B - methanol) for analysis of the benzodiazepines. Retention times were 1.16 min, 1.37 min and 1.48 min for oxazepam, nordiazepam, and diazepam, respectively. Raw ELISA absorbance data were converted to the decrease in absorbance, expressed as a percentage relative to values corresponding to chromatographic baseline collected in the fractionated eluent (%DA).

As a significant focus in our laboratory is the characterization of drug disposition in skeletal tissues, in which drug concentrations may be very low, analyte and negative control solutions were prepared in 1 mL drug-free bone tissue extract, with concentrations at or below the limit of detection for the UPLC-PDA system. Sample preparation included lipid removal *via* precipitation in cold methanol:acetonitrile (1:1 v/v) and acetone, followed by solid-phase extraction. Extracts were fractionated by UPLC with eluent collected directly into ELISA microwells containing 30 μL phosphate buffer and assayed by standard ELISA protocols. Fentanyl-positive extract (0.5 ng/mL) yielded cumulative (i.e., summed over appropriate fractions) %DA = 14.4%, where $3\times SD_{Blank}$ = 8.2%. Extracts of oxazepam, nordiazepam and diazepam at 5 ng/mL yielded cumulative %DA = 26%, 123.6% and 96.9%, respectively, where $3\times SD_{Blank}$ = 14%. Finally, extracts of cocaine and benzoylecgonine at 20 ng/mL yielded cumulative %DA = 64.9% and 86.9%, respectively, where $3\times SD_{Blank}$ = 4.1%. These results suggest that the rapid, efficient separating power of UPLC may be combined with the high sensitivity of EIA detection to provide highly selective and sensitive drug screening to facilitate substance identification in complex sample types. This technique merits further investigation with samples from postmortem casework.

Keywords: **UPLC, ELISA, Drug Screening**

P61 Assessment of Response of the Intoxilyzer® 8000C to Volatiles of Forensic Relevance *In Vitro* Part II: Toluene, Xylene, Methyl-Ethyl-Ketone and Ethyl Acetate

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In Canada, the Intoxilyzer® 8000C is used for breath alcohol analysis using combined infrared absorbance measurements at both 3.4 and 9.4 μm wavelengths to give a measure of equivalent blood alcohol content (BAC). As with prior instruments, the potential exists for this instrument to report elevated apparent BAC values due to the presence of forensically relevant interferents. This potential was examined using aqueous and ethanol-positive (50 mg/dL) mixtures of toluene (0-21 mg/L), xylene (0-20 mg/L), methyl ethyl ketone (MEK, 0-50 mg/L) and ethyl acetate (EA, 0-20 mg/L), introduced using a wetbath simulator. Solvent mixtures were prepared at forensically-relevant concentrations in both deionized water and diluted standard alcohol solution (50 mg/dL target concentration, expressed as a BAC equivalent using 2100:1 blood-breath ratio). Headspace vapour from each mixture (34.0 ± 0.2 ^{o}C) was analyzed in replicates of 20, and both the apparent BAC (mg/dL) and frequency of the "INTERFERENT DETECT" message (IDM) were noted.

Maximum apparent BAC values of 0-12 mg/dL, 0-10 mg/dL, 0 mg/dL and 0-24 mg/dL were observed in assays of toluene, xylene, MEK and EA, respectively, using the solvent concentration ranges in aqueous solution. The IDM was actuated in a maximum of 75% of trials at a toluene concentration of 14 mg toluene/L, 100% of trials at a xylene concentration of 20 mg xylene/L, 100% of trials at a MEK concentration of 20 mg MEK/L, and 100% of trials at an EA concentration of 20 mg EA/L. The IDM was actuated in 0% of trials at a toluene concentration of 1 mg xylene/L or lower, an MEK concentration of 30 mg MEK/L, and an EA concentration of 10 mg EA/L or lower.

Maximum apparent BAC overestimations were 1-19 mg/dL, 2-5 mg/dL, 2-8 mg/dL and 6-25 mg/dL observed in assays of toluene, xylene, MEK and EA, respectively, using these solvent concentration ranges in aqueous alcohol standard, relative to initial solvent-free trials (n=5 for each solvent system). In cases where the IDM was actuated in less than 100% of trials, maximum apparent BAC overestimations were 1-8 mg/dL, 5 mg/dL, 2 mg/dL and 6-11 mg/dL observed in assays of solutions of toluene, xylene, MEK and EA, respectively.

When superimposed with a positive BAC measurement, the potential for BAC overestimation in the absence of an IDM will be largely eliminated through truncation of evidentially reported breath measurements and instrument-derived underestimation of BACs through use of a blood-breath ratio of 2100:1.

Keywords: **Breath, Intoxilyzer®, Interferents**

P62 Evaluation of the Lin-Zhi International Immunoassay for the Detection of Buprenorphine and Norbuprenorphine in Urine

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We present an evaluation of a new Buprenorphine Enzyme Immunoassay [BUPI] (Lin-Zhi International, Inc., Sunnyvale, CA) for the detection of buprenorphine (BUP) and norbuprenorphine (NBUP) in urine. The Lin-Zhi assay is based on competitive antibody binding between BUP and NBUP in urine and glucose-6-phospahate dehydrogenase labeled NBUP. When BUP or NBUP are present in urine, active unbound enzyme reduces the co-enzyme NAD to NADH that results in an increase of measured absorbance at 340 nm.

The BUPI was evaluated by testing 33 urine specimens collected from criminal justice clients and substance abuse treatment patients. All 333 specimens were tested with the assay in an ADVIA 1200 Chemistry System auto-analyzer (Bayer Health Care, Diagnostics Division, Tarrytown, NY) with calibrators containing 0 and 10 ng (cut-off calibrator) of NBUP. Controls containing 3 ng/mL of NBUP (negative control) and +25% (positive control) of the 13 ng/mL cut-off calibrator (Lin-Zhi International, Inc., Sunnyvale, CA) were analyzed with each batch of samples. All urines were then analyzed by GC/MS for BUP and NBUP at a cut-off concentration of 10 ng/mL.

In the testing of the 333 urine samples, the assay demonstrated good overall agreement with GC/MS results 93.1%. There were 0 false positives and 23 false negatives using the Lin-Zhi assay. Using the 10 ng/mL norbuprenorphine cut-off, the Lin-Zhi assay had a sensitivity of 0.848 and a selectivity of 1.000. The precision of the assay was determined by the absorbance rates of the negative and positive controls. The within-run precision expressed as %CV (n=8) was <3% to <10%; while the between-run precision of the controls was <9% to <13%. Cross-reactivity of various drugs to test the selectivity of the assays was also performed. The Lin-Zhi assay gave a false positive for Codeine at 100,000 ng/ml. The Lin-Zhi BUPI provides a precise, reliable method for the routine detection of phencyclidine in urine specimens.

Keywords: **Enzyme Immunoassay, Buprenorphine, Norbuprenorphine, Drug Testing**

P63 Gene Expression Changes in Response to 36 Hours Sleeplessness

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Objectives: Discover genes that change expression levels over time in response to 36 hours of total sleeplessness by microarray analysis. Genes found to change over time will be used to increase our understanding of the biology of fatigue and develop assays for use in forensics and improved regulations.

Methods: Air battle managers were recruited to participate; total RNA was purified from four blood samples drawn from each participant at approximately 24 hour intervals to diminish circadian effects in our analysis. Samples were drawn beginning the day before the sleepless period began and ended about 22 hours after the sleepless period ended to measure recovery. The purified RNA was amplified and used as target material for Affymetrix hG-133plus2.0 GeneChips(R). A test statistic estimating deviance from static expression over time ranked genes in order of greatest to least probability of differential expression and gene lists were taken from the output for further analysis.

Results: The most stringent list contained genes considered most likely to be useful in development of an assay for fatigue These will be targeted for further investigation by assays such as quantitative polymerase chain reaction in subject samples from this and other studies. This list is enriched for genes that play a role in hematological system development and molecular transport. A role for calcium in the response to fatigue is strongly implied by the presence of genes that bind and/or are regulated by this ion (annexin, PITPNM2, REPS2) and a fourth gene, PTPRJ, that controls intracellular calcium levels.

The biology of fatigue was investigated using a less stringent list. Genes involved in signaling through multiple pathways are represented in this gene list. Pathways represented include: B-cell, insulin, toll-like, LXR/RXR, and glucocorticoid receptors; GM-CSF, VEGF, and IL-12 extracellular effectors; ERK/MAPK as a mediator; and the NFk-B transcription complex as an intracellular effector.

Conclusions: From a research perspective, fatigue can be divided into two categories, chronic and acute. Chronic fatigue is defined as resulting from repeated cycles of reduced sleep where sleep debt accumulates over a period of time measured in weeks or months. Conversely, acute fatigue defines the sleep debt that arises due to total sleeplessness over some number of days. The results of this study increase our understanding of fatigue at the molecular level and are the basis of a test for fatigue that adds to the aerospace medicine tool bag. These tests will be useful for causal determination in accident investigation and result in a real-time assay allowing for an on-the-spot determination of fitness-to-perform leading to decreased accident and incursion rates across the aviation and transportation industries.

Keywords: **Fatigue, Gene Expression Markers, Whole Blood**

P64 Analysis of Federal and Federally Regulated Urine Drug and Specimen Validity Test (SVT) Results Reported by SAMHSA Certified Laboratories: CY 2008

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Background: Urine specimens are collected as part of a comprehensive Drug Free Workplace Program conducted under separate legal authority for Federal employees and US Department of Transportation federally regulated industry employees. These specimens are drug tested for cannabinoids (delta-9-THCA), cocaine metabolite (benzoylecgonine, BZE), phencyclidine (PCP), opiates (morphine, codeine, 6-acetylmorphine), methamphetamine and amphetamine. Specimen validity tests are also conducted to determine if each specimen is consistent with the chemical characteristics of normal human urine. The SAMHSA inspection process includes data audit as an essential element focusing on drug positive, adulterated, invalid and substituted specimen results. Laboratories submit reported test results to the National Laboratory Certification Program (NLCP). The NLCP databases this information in a "Non-Negative Specimen List", from which specific specimens are chosen for complete data audit.

Objective: To analyze Federal and federally regulated urine drug and SVT results reported by SAMHSA Certified Laboratories during calendar year 2008.

Methods: A database was designed and implemented for federally regulated results reported from the SAMHSA Certified Laboratories. In February 2009 this database was completed and NNSLs were imported in an Excel format. The database now contains information from 118,971 non-negative specimens reported in 2008.

Results: In calendar year 2008, 6,260,730 specimens were received at the laboratory, 6,250,111 were tested, and 3,851 were retested. The 1.7% non-negative specimens (NNSs) which numbered 108,352 are characterized in Table 1. The justifications for the 10,619 specimens that were rejected for testing are summarized in Table 2.

Table 1 Analysis of NNSs	**# of Specimens**	**% Positive Specimens**
Amphetamine pos	14,714	13.6
BZE positive	20,208	18.7
Opiate positive	11,106	10.2
PCP positive	1,428	1.3
THCA positive	51,183	47.2
Adulterated	891	0.8
Invalid	6,439	5.9
Substituted	2,383	2.2
Total	108,352	100.0

Table 2 Rejected Specimens	**# of Specimens**	**%**
Insufficient volume	5,787	54.5
Broken seals	1,805	17.0
No collector name or signature on CCF	1,136	10.7
Mismatched info on CCF or no CCF	1,338	12.6
"Other" reasons	553	5.2
Total	10,619	100.0

In addition, 99.6% of the 3,851 retested specimens reconfirmed the positive, substituted or adulterated result reported by the primary laboratory.

Conclusions: The analysis of nearly 6.2 million specimens reported in 2008 indicated an overall non-negative rate of 1.7%, a reconfirmation rate of 99.6% and a 0.2% rate for specimens reported as Rejected for Testing. These rates are similar to overall rates noted in 2006 and 2007.

Keywords: **SAMHSA Certified Laboratories, Specimen Results, Calendar Year 2008**

P65 Two Case Reports Involving 1-Benzylpiperazine and 1-(3-Trifluoromethyl)phenylpiperazine in Urine Specimens Admitted to ED

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A2, Bliss, Charge, Herbal Ecstasy, Benny Bear, Benny, Frenzy, Nemesis, Legal E and Legal X are slang words used to describe the designer/rave drug 1-benzylpiperazine (BZP). 1-(3-trifluoromethyl)phenylpiperazine (TFMPP) is a designer drug commonly sold with BZP. The combination of BZP and TFMPP is reported to produce an effect similar to that achieved by Ecstasy but with a hallucinogenic "kick."

BZP is a federal schedule 1 drug and its sale is illegal in the United States. However, it is legally available for purchase in other countries, including Canada. TFMPP was a U.S. federal schedule 1 controlled drug from 2002-2004 under a temporary schedule. In 2004 TFMPP was not renewed as a federally scheduled drug and remains a federal noncontrolled substance. Some state statutes, such as Hawaii's Uniform Controlled Substances Act, ban the substance.

We report two unrelated cases in which subjects who presented to our emergency department (ED) tested positive for BZP and TFMPP. The first subject presented to the ED with symptoms of intoxication and complained of head and stomach pain. The second subject was believed to have ingested Ecstasy. In both cases, urine was collected and submitted to the toxicology laboratory for a comprehensive drug screen. The comprehensive drug screen included a volatile quantitation (ethanol, isopropanol, acetone and methanol) by GC-FID, a nine panel drug of abuse screen by enzyme immunoassay and a liquid alkaline extraction for GC-MS and TLC analyses. The volatile quantitation and amphetamine screen were negative in both cases.

TFMPP showed no cross-reactivity with our EMIT® d.a.u.® amphetamine reagent and BZP only cross-reacted at a very high concentration (400 ug/mL). A drug screen absent GC-MS analysis would likely have not detected either drug. Literature research indicates that BZP and TFMPP are often sold as "MDMA" or "Ecstasy" at rave parties. Therefore, we believe it is important to routinely check for these two drugs in any suggested MDMA or club drug intoxication.

Keywords: **1-Benzylpiperazine (BZP), 1-(3-Trifluoromethyl)phenylpiperazine (TFMPP), Ecstasy**

P66 Utilization of Two Dimensional Gas Chromatography Time-of-Flight Mass Spectrometry (GCxGC-TOFMS) Analysis for Enhanced Detection and Separation in Anti-doping Control Screening

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The illegal use of anabolic steroids to enhance athletic performance has reached disturbing levels worldwide. Anabolic steroid screening analysis in urine is complex and labor intensive requiring sensitive instrumentation and optimized chromatographic separations. This research investigates the use and practical application of comprehensive multidimensional gas chromatography time-of-flight mass spectrometry (GCxGC-TOFMS) for the analysis of androgenic anabolic steroids in urine. Conventional methods for steroid analysis in urine rely heavily on one dimensional gas chromatographic separations and selected ion monitoring (SIM) mass spectrometry methods. This study utilizes GCxGC to increase peak capacity and resolution in combination with time-of-flight mass spectrometry (TOF-MS) detection followed by data processing with deconvolution software algorithms for the identification and quantitation of anabolic steroids in urine. This study focuses on the identification of 3-hydroxystanazolol at the 2ppb level, which is known to be particularly difficult to detect and separate chromatographically.

A steroid mixture containing stanazolol, 4-hydroxystanazolol, boledenone, norandrosterone, and 3-hydroxystanazolol was prepared from standards obtained commercially. Methyl-testosterone was used as an internal standard (ISTD). Sample preparation followed a well established extraction and derivatization procedure used for steroid profiling in doping control. Two mL aliquots of urine spiked with the steroid standard mixture was used for the analysis. Hydrolosis was performed for 1 hour at 50°C after addition of 0.8M sodium phosphate buffer pH 7.0, ß-glucoronidase, and methyl-testosterone (ISTD). The solution was then alkanalized with potassium carbonate solution to pH 9.0 prior to liquid-liquid extraction with tert-butylmethylether (MTBE). The extractions were dried under nitrogen and then the residues were derivatized with 100 uLs of MSTFA-NH4I-ethanethiol (1000:2:6, v/m/v) for 1 hour at 60°C. The derivatized samples were subsequently analyzed by GCxGC-TOFMS. GCxGC analysis was conducted using an orthogonal column set of distinct column phases and dimensions equipped with a dual-stage quad jet thermal modulator between the first and second dimensions. Separated components were detected by TOFMS which provides continuous full range nonskewed mass spectral information along with the fast acquisition rates required for optimal detection of the peak data density generated by comprehensive GCxGC analysis.

Results from this study show significant improvements in chromatographic resolution and peak capacity, as well as the enhanced detectability that GCxGC-TOFMS provides for this steroid urine screening analysis. Successful trace level identifications of the five steroid standard mixture will be shown at the 2ng/mL (2ppb) level. Utilizing the increased resolving power and peak capacity of GCxGC coupled with the advantage of simultaneous full mass range data acquisition, TOFMS provides optimal peak identification and mass spectral deconvolution. This exploratory research investigation demonstrates favorable and practical applicability of GCxGC-TOFMS for the positive identification of anabolic steroids at the lowest allowable concentration limits meeting the strict guidelines set by the World Anti-doping Agency (WADA).

Keywords: **GCxGC-TOFMS, Deconvolution, Anabolic Steroids**

P67 Prevalence Patterns of Prescription Opiates and Metabolites in a Chronic Pain Population

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Interpretation of urine tests for morphine-related "opiate" drugs is often complex because they are frequently metabolized to other commercially available drugs. For example, morphine (MOR), hydromorphone (HM), hydrocodone (HC), dihydrocodeine (DHC), and oxymorphone (OM) have been identified as metabolites of other opiates. Consequently, it may not be readily apparent from urine tests what drugs were administered. Inclusion of unique nor-metabolites (NMs) of opiates such as norcodeine (NCOD), norhydrocodone (NHC), and noroxycodone (NOC) in a test panel could assist interpretation of drug source(s). We evaluated the prevalence NCOD, NHC and NOC, together with prescription opiates in a database of 13,126 positive confirmed urine results from pain patients undergoing treatment for chronic pain. The study was approved by an Institutional Review Board. Specimens were enzymatically hydrolyzed and tested by liquid chromatography (LC) tandem mass spectrometry (MS) for ten analytes as follows: codeine (COD); NCOD; MOR; HC; DHC; NHC; HM; oxycodone (OC); NOC; and OM. Cutoff concentrations and limits of detection were 100 ng/mL and 50 ng/mL, respectively for the analytes. Table I lists the number and % positives for each analyte (≥LOD). The percentage of positive specimens (≥LOD) that contained only parent drug (D), D in combination with NM, and only NM is shown in Table II. The number of analytes measured in each specimen varied from

Table I

Analyte	# Positives	% Positive
COD	267	2.0
NCOD	50	0.4
MOR	2704	20.6
HC	5595	42.6
NHC	5372	40.9
DHC	3698	28.2
HM	4282	32.6
OC	5046	38.4
NOC	4757	36.2
OM	4544	34.6

Table II

D/NM	#Positives (D + NM)	D, # (%)	D + NM, # (%)	NM, # (%)
COC/NCOD	275	225 (81.8)	42 (15.3)	8 (2.9)
HC/NHC	6538	1166 (17.8)	4429 (67.7)	943 (14.4)
OC/NOC	5748	991 (17.2)	4055 (70.5)	702 (12.2)

1-8 with 3 (34.8%) being most prevalent. In many cases D was absent and NM was present, often in combination with other metabolites of D. The presence of NMs clearly indicated that D had been ingested. The authors conclude that inclusion of NMs in pain monitoring panels provides important interpretative information about drug sources.

Keywords: **Opioids, Nor-metabolites, Urine Tests**

P68 Performance Characteristics of Methadone Immunoassays and Correlation of Analyte Concentrations as Determined by Immunoassays and GC-MS

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As a potent analgesic and sedative, methadone has long been used as a substitution drug for treating heroin addiction. The main metabolic pathway is *N*-demethylation with spontaneously cyclization to form two major metabolites, 2-ethylidene-1,5-dimethyl-3,3-diphenyl-α-pyrrolidine (EDDP) and 2-ethyl-5-methyl-3,3-diphenylpyrroline (EMDP).

Performance characteristics of four methadone enzyme immunoassay (EIA) kits (Roche, CEDIA, EMIT, DRI) currently available from commercial sources were examined to better understand their analytical parameters, including calibration, cross-reacting, and other performance characteristics. None of these reagents cross-reacts significantly to related compounds they are not targeting. Roche and EMIT methadone kits appear to exhibit narrower linear ranges. Various sample preparation procedures were explored for GC-MS analysis of methadone and its metabolites in urine specimens. Liquid-liquid extraction by dichloromethane in 1.5 M carbonate buffer (pH 10.2) was found effective to achieve high recoveries of the analytes of interest: 84.9%, 78.3%, and 92.1% for methadone, EDDP, and EMDP, respectively. Ions (m/z) suitable for monitoring these analytes are 223, 294, 295; 262, 276, 277; and 115, 130, 208 for methadone, EDDP, and EMDP, respectively.

Information derived from the EIA and GC-MS studies were applied to the analysis of clinical urine specimens. Resulting EIA apparent analyte concentrations were correlated against the concentrations of methadone as determined by GC-MS to better understand the effects of these EIAs' specificity characteristics. With the specific sets of clinical specimen studied, the correlations between the apparent methadone concentrations derived from each of these reagent kits and specific GC-MS concentration are similar. If 200 ng/mL of methadone is selected as the GC-MS cutoff, the corresponding cutoffs would be approximately 295–310 ng/mL for the DRI, CEDIA and Roche kits, and slightly higher (320 ng/mL) for the EMIT kit.

Keywords: **Methadone, EIA, GC-MS**

P69 Preliminary Results of a Homogeneous Enzyme Immunoassay (HEIA) for the Detection of Zolpidem in Urine

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The increasing popularity of non-benzodiazepine drugs such as zolpidem (Ambien®) to alleviate sleep disorders have increased incidences of driving while impaired by such drugs. We describe initial evaluation of a highly sensitive and rapid homogeneous enzyme immunoassay (HEIA) that detects zolpidem in urine. The antibody is highly specific for zolpidem, and it does not cross react with other sleep aids such as zopiclone and zaleplon. The assay has a detection limit of 5 ng/mL.

A time dependent dosage study was performed using two volunteers* to determine the efficacy of the homogenous enzyme immunoassay. Prior to the ingestion of a 10 mg dose of zolpidem (Ambien®) the subjects provided initial pre-dose urine samples. Sample collections post-dose were taken hourly at 1, 4, 8, and 16 hour time points. All urine specimens were tested directly in the HEIA with no pre-treatment or dilution. The enzyme HEIA assay was able to detect zolpidem in urine at least 8 hours after ingestion of a 10 mg dose as shown in the table.

Time (hrs)	Subject #1 (infrequent user)		Subject #2 (chronic user)	
	HEIA (ng/mL)	LC-MS/MS (ng/mL)	HEIA (ng/mL)	LC-MS/MS (ng/mL)
Pre Dose	0	0	<LOD	1
1	91	5	114	283
4	36	5	102	96
8	16	4	117	85
16	< LOD	1	98	73

Using the technique, the presence of zolpidem in urine was detected up to 8 hours following a single oral dose.

*Both subjects had prescriptions for zolpidem (Ambien®) and gave informed consent.

Keywords: **Zolpidem, Enzyme Immunoassay, Urine**

P70 Direct Analysis of Common and Abused Drugs in Urine via Solid-Phase Microextraction (SPME) Coupled with Desorption Electrospray Ionization (DESI) / Tandem Mass Spectrometry

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Objective: Urine testing for the presence of drugs of abuse requires methodology sensitive enough to monitor well below therapeutic levels and selective enough to identify specific drugs without false positives. Urine analysis is typically by using an auto analyzer or ELISA reagent kit while confirmation testing is performed by HPLC-MS/MS or GC-MS. Recent advances in direct desorption ionization techniques and tandem mass spectrometry make this combination of technologies applicable for direct analysis in matrix such as urine without the time-consuming chromatography step. Cocaine, benzoylecgonine, coca-ethylene, norfentanyl, methadone, EDDP, and meprobamate were selected as representative drugs or metabolites in urine.

Relevance: We describe a simple and rapid method for determination of seven common and abused drugs in urine using SPME extraction and direct analysis of the fibers with DESI-MS/MS.

Methodology: A new line of SPME C18 coatings suitable for liquid desorption has been developed to address limitations of current commercially available coatings. Stable isotope labeled internal standards for each analyte were prepared and added to urine samples. In this study, a 15 minute exposure time was used to extract the analytes from urine. However, shorter exposure times were also investigated. Each fiber was rinsed with water, secured in a prototype device for positioning the SPME fiber in the DESI spray, and analyzed by scanning with a 1- D Automated DESI source (Prosolia, Inc) coupled to a Thermo TSQ Quantum Discovery Max triple quadrupole mass spectrometer. A 7 mm length of the SPME fiber was scanned at a surface scan rate of 100 μm/sec. The DESI-MS/MS analysis was completed in approximately 1 minute.

Results and Conclusion: The method was investigated over a concentration range of 20 to 1000 ng/mL for cocaine, coca-ethylene, benzoylecgonine, methadone, norfentanyl, and EDDP. Meprobamate was examined over a concentration range of 0.20 to 10 μg/mL Results were calculated from calibration curves generated from the ratio of analyte to internal standard area versus analyte concentration. QC samples were run at different concentration levels within the calibration range to validate the method. Data will be presented comparing DESI-MS/MS results to those obtained by ELISA and HPLC-MS/MS techniques. The results from SPME-DESI-MS/MS indicate that this methodology is suitable for rapid analysis of urine samples with minimal preparation.

Keywords: **SPME, DESI, Urine**

P71 A Retrospective Examination of Cocaine with Levamisole Cases in the State of New Mexico

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Ergamisol (levamisole HCl) is used as an antihelminthic to treat worm infestations in animals and in aquariums for the control of internal parasites. Originally discovered by Janssen Pharmaceutical in 1966, levamisole was approved by the FDA in 1990 as an adjuvant therapy for the treatment of colorectal cancer; however, the drug is no longer available for human use in North America. Currently, levamisole is a known cocaine adulterant and in October 2008, the United States Department of Justice, Drug Enforcement Agency estimated that 30% of the illicit cocaine supply arriving in the US contained levamisole. Most recent DEA statistics report 57% of cocaine coming into the United States contains levamisole.

The Drug Confirmation Section of the Toxicology Bureau performs drug analysis by state statute for the Office of the Medical Investigator and the Implied Consent Drugs and Driving program in New Mexico. In 2008, we noticed an increase in levamisole-cocaine positive cases. In late 2008, concern was raised in Canada due to an increase in agranulocytosis cases associated with levamisole-tainted cocaine. We were interested in the recent increased identification of cocaine-levamisole related cases and decided to do a retrospective analysis of cases involving cocaine from 2003-2008.

Implied Consent cases with a BAC less than 0.08% and all OMI cases underwent ELISA screening. Presumptive positive cocaine cases were confirmed using solid phase extraction and GC/MS by SIM method in EI mode. Levamisole was identified using a basic, acidic and neutral extraction by SPE, GC/MS full scan in EI mode.

Our laboratory first detected levamisole with cocaine in 2005. In 2007, we found three cases of cocaine and levamisole. Nineteen cocaine-levamisole related cases were identified in 2008, and 2009 data supports this increasing trend. The majority of cases involved male subjects in the range of 46-50 years of age. The highest occurrence was from July through September of 2008, and half of all cases were within Bernalillo County, the largest major metropolitan area in New Mexico. To date, one death investigation has been attributed to the use of cocaine with levamisole by the Office of the Medical Investigator.

In 2008 the number of cocaine cases that also contained levamisole rose significantly in New Mexico. This trend coincides with DEA reports documenting the increasing prevalence of levamisole-adulterated cocaine seizures in the United States. This study will present toxicological results as well as demographic data and medical autopsy findings in identified cases. In addition, limitations of our data will also be presented and discussed.

Keywords: **Levamisole, Cocaine, Agranulocytosis**

P72 Development of a High Sensitivity Antibody for the Detection of Salvinorin A

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Salvinorin A is a potent and selective kappa-opiod receptor agonist. Its CNS effects and hallucinogenic properties have been compared to LSD, leading to its illegal status in many countries. Its use as a recreational drug is increasing due to its ready availability and potent activity. The use of immunoassays enabling specific and sensitive detection of this compound are useful for toxicological, therapeutic and clinical applications.

We report for the first time in our knowledge, the development of a high sensitivity polyclonal antibody for the specific detection of salvinorin A, which is of value for the development of effective immunoassays for its determination and application to toxicology and clinical settings.

The immunogen comprising salvinorin coupled to bovine thyroglobulin (BTG) as carrier was administered to adult sheep on a monthly basis to provide target-specific polyclonal antiserum. IgG was extracted from the antiserum and evaluated via competitive ELISA. Competition between free analyte and horseradish peroxidase labelled conjugate for antibody binding sites after 1 hour incubation at 25^0C was measured by reading the absorbance at 450nm. The absorbance was inversely proportional to the concentration of the analyte.

Initial evaluation of the antibody shows specificity for salvinorin A with a % cross-reactivity of 100 and cross-reactivity of 12% for salvinorin B. For the calibration range of 0-10 ng/ml, the sensitivity value expressed as IC50 is 0.3 ng/ml. The intra-assay precision (n=3), expressed as %CV, is typically $\leq$10 for eight different concentration levels.

This data show that the polyclonal antibody exhibits high sensitivity in the specific detection of salvinorin A. This is valuable for the generation of effective immunoassays to be used in toxicological and clinical settings.

Keywords: **Salvinorin A, Antibody, Immunoassays**

P73 High Throughput, Rapid Multiplexed Testing for Toxicology and Forensic Applications-Technology Advance

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To accommodate the demand for tests and the appearance of new drug classes, new analytical methods are needed for clinical, regulatory, toxicological and forensic applications. The use of screening methods enabling the rapid and simultaneous detection of drugs of abuse facilitates the application to regulatory control, as only positive results need confirmation. Evidence biochip array technology provides a platform for the simultaneous determination of multiple analytes from a single sample. This leads to an increase in the results output and a reduction in the consumption of the sample/reagent.

The core of this technology is the biochip (9mm x 9mm), which represents the chemically activated solid phase where the ligands are immobilised and stabilised defining microarrays of test sites and also the vessel where the reactions take place. For drug testing, competitive chemiluminescent simultaneous immunoassays are applied. Different sample matrices e.g. urine, blood, oral fluids can be tested with dedicated reagents and the cut-off levels can be individually selected avoiding re-calibration and the use of different calibrators. Furthermore, the dedicated analysers: the high throughput fully automated Evidence^{R} (FDA cleared) and the semi-automated bench top Evidence Investigator^{TM} for lower throughput, incorporate the software to process, report and archive the data generated.

The drugs of abuse biochip array I allows the simultaneous detection of the following drug classes: amphetamine, methamphetamine, cocaine(benzoylecgonine), barbiturates, cannabinoids, opiates, methadone, benzodiazepines, phencyclidine. In urine, the % cross-reactivity of the amphetamine assay for a cut-off of 1000ng/ml presented values ranging from 32 (phentermine) to 544 (MDA) and <0.2 for methamphetamine (relative to d-amphetamine, %cross-reactivity 100). With the same cut-off, the methamphetamine assay showed % cross-reactivity 36 for MDMA, 89 for MBDB and 0.7 for d-amphetamine (relative to (+)-methamphetamine, %cross-reactivity 100). Two separate test sites for benzodiazepines on the biochip allowed detection of oxazepam and lorazepam respectively (%cross-reactivity 100 at a cut-off concentration of 200ng/ml) with different cross-reactivities for other benzodiazepines ranging from 23.9 (lorazepam glucuronide) to 1818.2 (alprazolam). The qualitative detection of drugs of abuse in urine samples (n=2408) presented and average agreement of 90.3% with GC/MS on Evidence.^{R} In oral fluid, for an incubation time of 30 minutes, the system allowed the detection of these drug classes and also metabolites e.g. cannabinoids, a cut-off of 4 ng/ml was applied and the immunoassay showed specificity for the parent compound Δ^{9}-THC (%cross-reactivity 100) and for 11-nor-Δ^{9}-THC-9-carboxylic acid (%cross-reactivity 324), 11-hydroxy-Δ^{9}-THC (%cross-reactivity 114), cannabinol (%cross-reactivity 41). Simultaneous semi-quantitative determination of the analytes was obtained in urine and whole blood samples on both immunoanalysers, with sensitivity in urine ranging from 0.02ng/ml (methadone) to 125ng/ml (methamphetamine) and in whole blood from 0.1ng/ml (benzoylecgonine and phencyclidine) to 6.2ng/ml (methamphetamine) for neat sample on Evidence.^{R}

The target drugs of abuse classes for simultaneous detection with biochip array II are buprenorphine, ketamine, LSD, MDMA, methaqualone, fentanyl, oxycodone, generic opioids and propoxyphene. In urine, the %cross-reactivity values of the ketamine assay at a cut-off of 300ng/ml were 100 (norketamine) and 56 (ketamine), the MDMA assay at a cut-off of 500ng/ml presented %cross-reactivities of 62.8 (MBDB) and 328.2 (MDEA) relative to MDMA (%cross-reactivity 100), the methaqualone assay at a cut-off 300ng/ml presented %cross-reactivities 45.3 (6-hydroxymethaqualone), 408.9 (methaqualone) relative to 2'OH methaqualone (%cross-reactivity 100), different test sites for oxycodone compounds allowed detection at a cut-off of 100ng/ml of oxycodone (%cross-reactivity 100) with different cross-reactivities for other opioids ranging from 20.1 (levorphanol) to 2224.9 (hydrocodone) and the propoxyphene assay presented at a cut-off of 300ng/ml a %cross-reactivity value of 1921 (propoxyphene) relative to norpropoxyphene (%cros-reactivity 100). Assessment of urine samples on Evidence Investigator showed % agreement with confirmatory methods ranging from 89.6 (buprenorphine: cut-off 2ng/ml, n=48) to 100% (LSD: cut-off 500pg/ml, n=80, MDMA: cut-off 500ng/ml, n=60, methaqualone: cut-off 300 ng/ml, n=10, oxycodone 2: cut-off 100ng/ml, n=36). Initial results in oral fluid for opioids showed the following %cross-reactivity values for a cut-off of 30ng/ml: 100 for oxycodone,62.6 for thebaine, 48.3 for morphine and 30.9 for hydrocodone.

Biochip array technology detects multiple drugs of abuse classes at a single point in time from a single sample. It represents a flexible multi-analyte approach as test sites for new drugs can be incorporated to the arrays. This methodology is a very applicable tool for the rapid screening of large numbers of batches of samples, as only positive sample determinations require further confirmatory analysis.

Keywords: **Drugs of Abuse, Biochip Arrays, Multiplex**

P74 Forensic Toxicology Screening for 725 Compounds Using a GC/MS/NPD System and Automated Spectral Deconvolution Software

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Objective: The purpose of this work is to show the benefits achievable by combining several recent GC/MS techniques into the toxicology screening process. The goals are to: 1) decrease the GC/MS run time 2) decrease the data review time and 3) decrease the number of false positives and false negative identifications in the screening process.

Introduction: With recent advances in GC/MS technology, there are several opportunities to substantially increase the number of targets screened for and simultaneously reduce the time required per sample. With the system described here, samples are screened for 725 compounds using automated spectral deconvolution and reporting software. Data review time is substantially reduced compared to conventional techniques. Post run bakeout of heavy matrix compounds is replaced with column backflushing, which is faster and reduces system maintenance. Run time is reduced by using a fast GC run (9.75 min injection to injection) and simultaneously collecting scan, SIM, and NPD data. The scan data is deconvoluted and used to identify any of the 725 target compounds. SIM data is used to look for select low level compounds not detectable in scan mode. The nitrogen response of the NPD is used to highlight non-target nitrogen compounds, identity confirmation, and can be used for quantitation if needed. Using extracts of whole blood samples, the system finds all the compounds detected by the conventional method in significantly less time.

Methods: Whole blood extracts prepared for GC/MS analysis were supplied by NMS Labs (Willow Grove, PA). The whole blood was prepared with a single step liquid/liquid extraction into a solvent, evaporated to dryness, and reconstituted in toluene at 1/10th volume. Samples were run on a quadrupole GC/MS system with electron impact ionization. The GC column effluent was divided between an NPD detector and the MS with a splitter device. The splitter also provides for solvent venting and post-run backflushing of the column. The system simultaneously collects the full scan, SIM, and NPD data. The scan data is deconvoluted and screens for the presence of 725 compounds. The SIM data is used to analyze for 25 compounds at lower levels, and the NPD is used for identity confirmation and highlighting non-target nitrogen containing compounds.

Results: Significant time savings were obtained by collecting all three data signals simultaneously in a shortened run. Backflushing further reduced analysis time by eliminating the need for long post run column baking. The most significant time savings came in data review, where the deconvolution software identified the compounds present in ~10 minutes. The conventional review process required at least ~1 hour and was more prone to false negatives, especially where matrix interferences were present. The SIM data was useful for low level compounds like fentanyl, and the NPD prevented missing non-target nitrogen containing compounds.

Conclusion: The combination of techniques used here demonstrates that GC/MS toxicology screening can be significantly improved in both productivity and accuracy. Of the techniques evaluated, automated spectral deconvolution software makes the largest improvement in the screening process.

Keywords: **GC/MS/NPD, Deconvolution, Backflushing**

P75 Evaluation of Immunalysis Immunoassays for Detection of Buprenorphine, Oxycodone, Meperidine, and Tramadol in Urine by EMIT

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Use and abuse of narcotics is a significant social concern. Patients of pain management and rehabilitation/abstinence programs often must submit to urine drug testing to verify compliance with prescribed therapy. Testing to detect drugs beyond the traditional SAMHSA panel is necessary due to the common use and potential for abuse of many synthetic or semi-synthetic opioid analgesics.

Here Enzyme Multiplied Immunoassay Technique (EMIT) kits (Immunalysis Corporation, Pomona, CA) designed to detect buprenorphine (5 ng/mL), oxycodone (100 ng/mL), tramadol (200 ng/mL), and meperidine (200 ng/mL) were evaluated using the cutoff concentrations indicated in parenthesis and an Olympus AU400e (Olympus America, Center Valley, PA) analyzer. Calibrators and quality control materials were prepared in-house and target concentrations verified by LC-MS/MS. De-identified urine samples that previously confirmed positive by LC-MS/MS for oxycodone (n=29, at concentrations from 40 ng/mL to > 5000 ng/mL) or buprenorphine (n=21, at concentrations from 9.8 ng/mL to > 2000 ng/mL) as well as prepared urine samples containing meperidine (n=25) and tramadol (n=25) at concentrations ranging from 50% below the cutoff to ten times above the cutoff were analyzed.

All samples with target drug concentrations above their respective cutoffs screened positive (n=99). Five of the 29 oxycodone specimens with concentrations below the EMIT cutoff ranging from 40 ng/mL to 169 ng/mL screened positive. One sample with an oxymorphone concentration of 27 ng/mL and no detectable oxycodone also screened positive. Oxymorphone cross-reactivity is stated to be 100% in the kit literature. Seven of the buprenorphine specimens screened positive for oxycodone in addition to buprenorphine; these seven specimens were then analyzed by LC-MS/MS but failed to confirm for oxycodone or oxymorphone. Four specimens confirmed positive for other opioids (morphine, 6-acetylmorphine, codeine, and hydromorphone) and three failed to confirm for any opioids. The kit insert states that there should be no cross-reactivity with drugs other than buprenorphine and norbuprenorphine. The five meperidine and tramadol samples fortified at the cutoff (200 ng/mL) screened negative, but those fortified at 250 ng/mL all screened positive.

The Immunalysis EMIT kits successfully detected buprenorphine, oxycodone, meperidine, and tramadol in fortified samples and patient specimens. Further studies are required to better understand specificity of the buprenorphine reagents and define precision at the cutoff concentrations for all assays.

Keywords: **Pain Management, EMIT, Urine**

P76 Single Nucleotide Polymorphisms on the CYP3A4 Gene May Contribute to Unexpected Methadone Death

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West Virginia and Kentucky rank among the top ten states for increase in fatal methadone overdoses from 1999 to 2004 according to the United States Department of Health and Human Services. Methadone is a difficult medication to prescribe due to extreme variability in interindividual pharmacokinetics, increasing the risk of an unexpected overdose. The rate of conversion of methadone to 2-ethyl-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) can be expressed as [methadone]/[EDDP] ratio. In a retrospective study from the West Virginia Office of the Chief Medical Examiner from 2003 to 2008, the methadone to EDDP ratio was found to be 18.3 for "methadone-only" deaths compared to a ratio of 5.3 for individuals successfully undergoing methadone maintenance treatment (MMT) in another study by Burmejo *et al* [1]. The higher ratio may be associated with one or more single nucleotide polymorphisms (SNPs) on the CYP3A4 gene that affect the function of the P450 product. CYP3A4 is a key cytochrome P450 (CYP450) involved in the metabolism of methadone as well as numerous other drugs. The hypothesis of this study is that one or more genetic polymorphisms within the CYP3A4 gene could lead to decreased methadone metabolism, allowing an individual to achieve a fatal concentration of methadone at normal dosing levels.

In addition to the 76 methadone-only deaths from West Virginia were combined 49 "methadone-only" deaths from the state of Kentucky were included. Although, methadone/EDDP ratios were not available for the Kentucky cases, SNP genotyping in this group could help determine the role of CYP3A4 variants in methadone overdose. The average methadone concentration for the deceased individuals (from combined WV OCME and Kentucky data) was 0.601 mg/L. We determined genotypes for seven SNPs within the CYP3A4 gene. Genomic DNA was isolated from blood spots prepared from autopsy samples. SNP genotypes were determined by Taqman Allelic Discrimination Analysis. All loci studied were within Hardy-Weinberg Equilibrium with the exception of the rs2740574 SNP, which was most likely due to the low minor allele frequency. The observed genotypic frequencies were not significantly different from those expected based on population statistics for four of the SNP loci. Genotypic frequencies were significantly different from the frequencies for the general population ($p<0.01$) for the other three SNPs (rs2246709, rs2242480, and rs2740574). Although, none of these SNPs were found to be significantly associated with an increase in methadone/EDDP ratio, there is an apparent enrichment of rare homozygotes in the methadone fatality group. These initial findings indicate that a genetic polymorphism on the CYP3A4 gene may contribute to unexpected methadone death. Determining the correlation of CYP3A4 with methadone toxicity and genotyping the CYP3A4 gene could aid in identifying current and future methadone users who may be at risk for overdose when given methadone at a normal dosing level.

1. Burmejo AM, Lucas AC, Tabernero MJ. 2000. Saliva/plasma ratio of methadone and EDDP. J of Anal Toxicol. 24(1): 70-2.

Keywords: **Methadone, CYP3A4, Polymorphism**

P77 Development and Validation of a Novel Homogeneous Immunoassay for Detection of Fentanyl in Urine

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Fentanyl is an extremely potent synthetic opioid that is widely used for chronic pain treatment. This drug is highly addictive and prone to abuse. The objective of this project was to develop a high throughput homogeneous enzyme immunoassay (HEIA) for rapid detection of fentanyl in human urine. An in-house antibody was selected capable of measuring fentanyl at low concentrations. Ahighly sensitive HEIA for fentanyl in urine has been confirmed with authentic human urine samples that have been confirmed positive or negative for fentanyl/norfentanyl by LC-MS/MS.

This qualitative assay has a detection limit that was determined to be 0.5 ng/mL. A cut-off concentration of 2 ng/mL for urine. The intra-day (n = 15) and inter-day (n =60) rate precision of the assays were less than 10% CV. No interferences from structurally unrelated and commonly ingested drugs were observed at a concentration of 10,000 ng/mL.

A total of 93 LC-MS/MS verified urine specimens (61 positive and 32 negative samples) were analyzed by HEIA. Sixty-one tested positive and twenty-nine tested negative by HEIA as listed in the following table. Three samples that tested borderline positive by the HEIA were negative by LC-MS/MS at a 1 ng/ml cut-off. The sensitivity, specificity, positive predictive value and negative predictive value are 100%, 91%, 95% and 100% respectively as shown in the following table.

		LC-MS/MS	
		Positive	Negative
HEIA	Negative	0	29
	Positive	61	3

In summary, a high throughput, highly sensitive HEIA has been developed for the detection of fentanyl in human urine which correlates well with LC-MS/MS. This is the first report of a homogeneous immunoassay for fentanyl.

Keywords: **Fentanyl, Enzyme Immunoassay, Validation**

P78 Postmortem Identification of Intoxication Tendencies of the Last Decade in Puerto Rico

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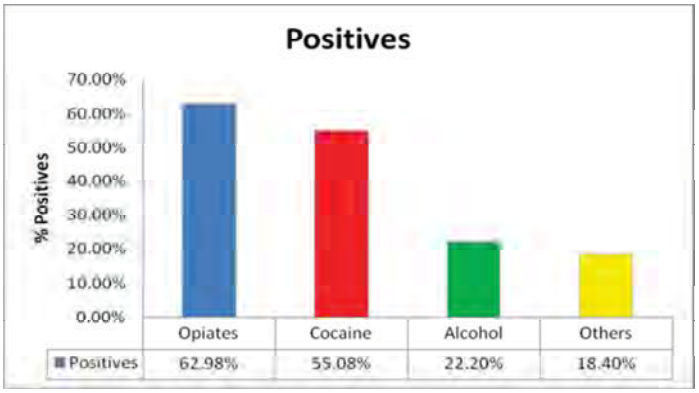
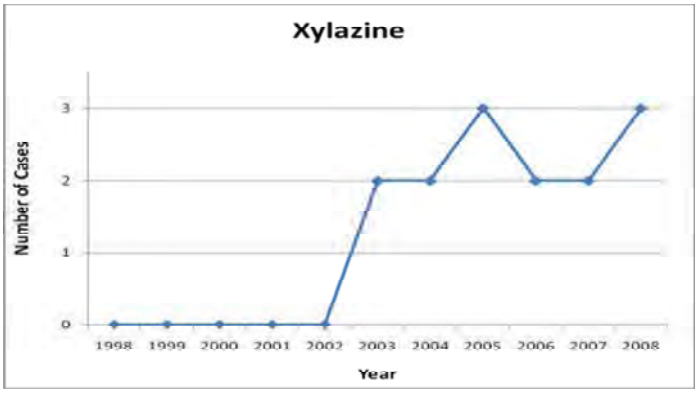
This retrospective study analyzes the intoxication pattern present during the last 11 years (1998 - 2008) in Puerto Rico. A total of 3522 intoxication cases were classified as accidental, suicidal, homicidal or natural. The main group of overdose deaths were associated with drugs use, primarily opiates (62.98%), followed by cocaine (55.08%) and alcohol (22.20%). Xylazine (a veterinarian anesthetic) was present in 14 cases in the last 6 years, in combination with heroin, alcohol or cocaine. Only the 18.4% of the cases were positive to pesticides, therapeutic drugs, carbon monoxide or other substances. The therapeutic drugs cases were divided by year to observe the decrease or increase on a particular usage. Tendencies among gender were also evaluated, in suicide cases, as well as accidental cases. The predominant gender are men with few women (11.44%).





Keywords: **Intoxication, Postmortem, Tendencies, Opiates, and Xylazine**

P79 Deaths Caused by Difluoroethane

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Difluoroethane is an odorless, colorless, and highly flammable gas. It is used as a refrigerant blend component and aerosol propellant such as the computer cleaning spray Dust-Off. It is also a choice of abuse as an inhalant, especially among teenagers. Inhalant abuse or huffing is the intentional inhalation of a chemical vapor to achieve euphoric effects. Intentional direct exposure to difluoroethane can cause freezing of airway soft tissue, oxygen displacement and sensitization myocardium. Overexposure can result in fatality possibly due to displacement of oxygen.

We are reporting three recent deaths in Broward County caused by difluoroethane. The ages of the decedents are 18, 20 and 42. In all three cases, aerosol cans were found at the scenes. Difluoroethane was identified by GCMS and quantitated by GC headspace. The blood concentrations of difluoroethane are 116mg/L, 141mg/L and 199mg/L. In all three cases, the cause of deaths were ruled as difluoroethane inhalation.

Keywords: **Difluoroethane, GC Headspace, Inhalant**

P80 Intra-Individual Variability in Linear Elimination Rates from Breath Alcohol Profiles Following Social Drinking

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An examination of the variability in linear ethanol elimination rate within individuals following social drinking conditions conducted over different sessions is reported. In each drinking session, healthy volunteers ($n = 10$, $n_{male} = 5$) consumed ethanol at their own pace and in the beverage form of their choice, at a dose of 0.6-0.7 g/kg (2-4 standard drinks) over the course of roughly one hour. Each subject participated in at least two drinking sessions, separated by at least 3 weeks; one subject participated in 3 sessions, while another subject participated in 4 sessions. Breath alcohol concentration was measured using an Intoxilyzer 8000C equipped with a PS-590 portable wetbath simulator, beginning approximately 5 min after end of drinking and continuing until each subject displayed a BrAC (expressed as BAC equivalent, using a blood-breath ratio of 2100:1) of 10-20 mg/dL. Breath samples were thus collected every 5-15 min over roughly 4-7 hours. In general, subjects were not regulated with respect to food intake prior to each session or the rate of ethanol ingestion. Each subject consumed the same dose of ethanol in the same beverage form for each session attended.

Linear ethanol elimination rates were determined by linear regression analysis. Linear elimination rates in any given session ranged from 10.5-22.3 mg/dL/hr. The unsigned intra-individual change in linear elimination rate between sessions ranged from 0.2 to 4.8 mg/dL/hr. In one subject, the linear elimination rate varied between 13.0-17.8 mg/dL/hr over four different sessions conducted over 12 weeks. This has substantial implications for litigation in which a subject-specific elimination rate derived from a single breath alcohol profile generated at some time after an incident is used in pharmacokinetic calculations.

Keywords: **Ethanol, Elimination Rate, Variability**

P81 RNA Degradation in Victims of Aviation Accidents: Implications for Post-Mortem Marker Analysis

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Objective: Due to post-mortem degradation, RNA is poorly characterized as a source of material for marker analysis in forensic samples and has generally been assumed to be of little use. Gene expression-based marker characterization of factors important in aerospace medicine such as fatigue and hypoxia is performed in whole blood from living subjects. However, for these assays to have efficacy in forensic specimens, the post-mortem stability of whole blood RNA must be assessed. In this study, we assess RNA quality on whole blood samples at time points up to 48-hours post collection and from a set of samples collected at autopsy after aviation accidents.

Methods: Blood samples were collected from aviation accident victims in PaxGENE Blood RNA tubes containing a RNA stabilizing solution at autopsy. The post-mortem interval (PMI) was noted in most cases. In a controlled experiment, blood samples were collected in serum tubes and aliquots added to PaxGENE tubes at time points from 0 to 48 hours after collection. RNA was purified from both sets of samples (accident victims and serum tubes). Quality was assessed on an Agilent BioAnalyzer 2100 with the RNA-nano and small RNA chips.

Results: In the accident victim samples, the RNA Integrity Number (RIN) calculated from the RNA-nano chips decreased as the PMI increased ($r = -0.545$). RINs ranged from 7.5 at a PMI of 5 hours to 2.4 in samples that had PMIs of 3 days. Furthermore, with decreasing RINs, there was an apparent enrichment for small-RNAs. However, visual inspection of the electropherograms from the small-RNA chips showed the apparent enrichment was amorphous, lacking the characteristic peaks of true small RNAs. We conclude that the apparent increase in small RNA enrichment with longer PMI is actually degraded messenger and ribosomal RNA.

Samples collected at specific time points in serum tubes likewise degraded over time ($r = -0.78$) although the degree of degradation was not as obvious as the range of RINs was from 8.75 at 0.5 hours to 7.8 at 36 hours. Here, it is apparent that sample collection in a sterile environment does not adequately mimic the real-world vagaries of the aviation accident site.

Conclusions: Predictably, RNA integrity decreases as a function of increasing time between death and autopsy. While samples collected under sterile conditions do degrade over time, time from collection does not correlate well to accident sites. However, keying to RINs may adequately predict sample efficacy for gene expression marker analysis.

Keywords: **RNA Integrity, Blood, Gene Expression Marker Analysis**

P82 Correlation of Ethanol Concentrations in Urine by Enzymatic Assay and Headspace Gas Chromatography

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Background: Blood Alcohol Concentration (BAC) and Urine Alcohol Concentration (UAC) are both reflections of the amount of alcohol present in an individual at the time of collection. The recommended conversion factor between UAC and BAC is approximately 1.33, although it has been reported that in the early absorption phase the UAC/BAC ratio may be less than 1.0; in the late absorption/distribution period the ratio varies between 1.0-1.2 and on reaching the post-absorptive phase, the UAC/BAC ratios averages 1.3-1.4[1]. This conversion factor needs to be taken into account when determining the alcohol concentration of the urine sample in question.

The purpose of this study was to evaluate the linearity of an enzymatic assay (EA) for detecting ethanol in urine, and determine the quantitative correlation that exists between those urine samples analyzed using the enzymatic assay and a standard headspace gas chromatography (GC) method.

Methods: Standards were purchased from Cerilliant (Round Rock, TX) at concentrations of 20, 100, 200, and 400mg/dL. Negative synthetic urine, drug standards and authentic samples were diluted 1:10 with phosphate buffered saline (PBS). A 10 µL sample volume was added to the microplate EA assay (Immunalysis Corporation, CA). 100 µL of 0.6M Tris buffer with 0.1% Sodium (RA) was added to each well, followed by 100 µL of alcohol dehydrogenase + nicotinamide adenine dinucleotide (NAD) in Tris buffer (RE); the microplate was covered and incubated at room temperature for 30 minutes. The absorbance was measured at 340 nm.

Results: The linearity of the standard curve over the range of 20 mg/dL to 400 mg/dL was $r^2 = 0.999$. The intraday precision testing (n=8) resulted in coefficient of variations (CVs) all below 4%, and the interday precision testing (n=5) resulted in CVs all below 10%. Authentic samples were analyzed using the EA method described above and by headspace GC, producing a correlation coefficient of $r^2 = 0.989$ between the two assays.

Summary: The enzymatic assay consistently produced results with good linearity, excellent precision and a high quantitative correlation with a headspace gas chromatography method. Based on the results of this study, this enzymatic assay would be a highly suitable choice for accurately measuring the concentration of ethanol in urine samples.

[1]. Jones AW. Urine as a biological specimen for forensic analysis of alcohol and variability in the urine-to-blood relationship. Toxicol Rev. 2006;25(1):15-35

Keywords: **Ethanol, Enzymatic Assay, Headspace GC**

P83 An Abuse Death Involving the Insufflation of Extended-Release Oxymorphone Tablets**

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Oxymorphone is a semi-synthetic opiate 6-8 times more potent than morphine. Recently, an extended release form of the drug (Opana® ER) has been approved for the treatment of chronic pain. Two cases are reported, including one fatality, of abuse of oxymorphone in combination with alprazolam (Xanax®), one of the most potent benzodiazepines. Two juvenile females were discovered unresponsive and hypoxic by a male acquaintance. Early in the evening the trio had reportedly crushed and snorted Opana® ER tablets and each taken a Xanax® for recreational purposes. Emergency personnel were called to the scene where cardiopulmonary resuscitation was performed on both individuals. One female was stabilized and transported to the Trauma center where she was treated for a drug overdose. The second female was pronounced dead at the scene. Blood and urine samples from the surviving female were collected at the Trauma Center between 48-96 hours post incident after she was removed from intensive care. Toxicology results showed the presence of oxymorphone, doxylamine, dextromethorphan, alprazolam, α-hydroxyalprazolam, oxazepam, and temazepam in her urine only. No drugs were detected in her blood. Toxicology on the deceased female revealed the presence of 0.13 mg/L oxymorphone and 0.04 mg/L alprazolam in her blood. Gastric content contained 0.25 mg/L and 0.93 mg/L of oxymorphone and alprazolam, respectively. Oxymorphone, alprazolam, and α-hydroxyalprazolam were present in her urine. Quantitative results were achieved by gas chromatography mass spectrometry (GC/MS) monitoring selective ions for the oxymorphone-oxime-trimethyl-silyl derivative, alprazolam and the α-hydroxyalprazolam tert-butyl-dimethyl-silyl derivative. The methods had limits of quantitation (LOQ) of 0.050 and 0.025 mg/L for the opiate and benzodiazepines, respectively. The level of oxymorphone in the decedent's blood was high enough to be the single cause of death. However, the presence of alprazolam in her blood at therapeutic levels and the reported adverse interactions with opiates could not be minimized. The cause of death was reported as multiple drug toxicity and the manner of death was accidental.

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

P84 A Comparison of Confirmation Techniques for the Detection of Δ9-THC in Oral Fluid

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Oral fluid is becoming an increasingly popular biological fluid as an alternative to blood and urine to analyze for drugs of abuse. Since Δ9-THC (THC) is one of the most common illegally used drugs, its analysis in oral fluid has become very important. This analysis can be extremely challenging due to the low concentrations of THC typically found in oral fluid, and the low volumes of oral fluid which are generally available for analysis. Also, like many biological fluids, chemical noise from the matrix can be problematic, especially at these low levels.

Several gas chromatographic strategies have been employed to overcome these difficulties. These include combining the high sensitivity of selected ion monitoring (SIM) on a single quadrupole with the use of a GC with a programmable temperature vaporization (PTV) injector programmed to discriminate between analyte and matrix on the basis of boiling point temperature. Also, multidimensional chromatographic techniques have been used with single quadrupole instruments to remove matrix interference through "heart-cutting" the THC peak from the oral fluid matrix background. A third technique is to use the high selectivity and sensitivity of the selected reaction monitoring (SRM) mode on a GC-triple quadrupole to remove matrix interference at low sample concentration. The use of a GC-triple quadrupole in SRM mode was selected to validate this assay because it proved better at eliminating matrix background than using the PTV with a GC-single quadrupole, and it is less complicated to set up and maintain than the multidimensional GC approach, making it more flexible for development of additional assays.

For validation of this assay, negative oral fluid calibrating solution was first spiked with THC-D3 and the appropriate amount of THC. The samples were then extracted using solid phase extraction, followed by derivatization with BSTFA. The derivatized samples were then transferred to a TSQ Quantum GC triple stage quadrupole for analysis with an electron ionization source. Chromatographic separation was accomplished on a 15 m x 0.25 mm x 0.25 µm TR5 MS column with an immediate temperature ramp from 60 ºC to 320 ºC at 35 ºC/min. SRM transitions from m/z 386 to 303 and 371 to 289 were monitored for THC, and m/z 389 to 306 and 374 to 292 were monitored for THC-D3.

Results include excellent linearity from 0.2 ng/mL to 20 ng/mL, with the 0.2 ng/mL level quantitating within 5% of the spiked amount for four consecutive injections. Carryover was not seen even after injection of a known negative sample after four 40 ng/mL sample injections. Finally, precision was demonstrated for inter- and intra-day batch analysis of 6% CV and less at levels of 0.8 and 2.5 ng/mL.

Keywords: **Δ9-THC, Oral Fluid, GC Triple Quadrupole**