## S01 An Automated SPE/LC/MS Method for the Analysis of THC and Metabolites in Biological Fluids

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This method utilizes an online solid-phase extraction (SPE) coupled to a liquid chromatography-tandem mass spectrometry (SPE-LC/MS/MS) for the analysis of $\Delta^9$- tetrahydrocannabinol (THC), 11-hydroxy- $\Delta^9$-tetrahydrocannabinol (THC-OH) and 11-nor-9-carboxy-$\Delta^9$-tetrahydrocannabinol (THC-COOH), Cannabinol (CBN) and Cannabidiol (CBD, an isobar of THC) in urine and whole blood.

This is a unique procedure that interfaces the Spark Holland Symbiosis™ to the Applied BioSystems 4000 QTrap™ so the two instruments function as a single system. The Symbiosis™ system is an online SPE system that consists of a storage compartment, autosampler, two pumps and an extraction unit. Positive ion electrospray mode was used for THC, THC-OH, THC-COOH, CBN, and CBD in both urine and whole blood. The deuterated analogues (d3-THC, d3-THC-OH and d3-THC-COOH) of THC, THC-OH and THC-COOH were used as the internal standards.

In developing this method, eight solid phase cartridges were evaluated to determine the optimal SPE material for the extraction of all analytes from urine and blood. The results demonstrated that the C8-EC cartridge was successful in retaining all five of the analytes.

The analytical HPLC column (Xterra C18, 3mm x 50mm i.d., 5µ) was used with the optimal gradient from 95% of 0.1% formic acid in water to 95% of 0.1% formic acid in acetonitrile. The total runtime was 10 minutes, including solid phase extraction and chromatographic separation. A total of thirty-two MRM channels were monitored with four transitions for each analyte.

The method was validated over a range from 0.5 – 500 ng/mL in urine and 0.5 – 200 ng/mL in whole blood, with an average correlation coefficient > 0.99 for all analytes. The standard curves were plots of the ratios of analytes/internal standard responses (peak area) as a function of the analyte concentration. The data were fit to a linear least-squares regression curve with a weighting index of 1/ι.

The limits of detection for THC, THC-OH, THC-COOH, CBN, and CBD in urine were 0.4 ng/mL, 0.4 ng/mL, 0.7 ng/mL, 0.3 ng/mL and 0.2 ng/mL, respectively. The limits of detection for THC, THC-OH, THC-COOH, CBN, and CBD in blood were 0.6 ng/mL, 1.0 ng/mL, 0.9 ng/mL, 2.5 ng/mL and 1ng/mL, respectively. Likewise, the limits of quantitation for THC, THC-OH, THC-COOH, CBN, and CBD in urine were determined to be 1.0ng/mL, 1.5 ng/mL, 2.0 ng/mL 1.0 ng/mL and 0.5 ng/mL, respectively. The limits of quantitation for THC, THC-OH, THC-COOH, CBN, and CBD in blood were determined to be 2.0 ng/mL, 3.5 ng/mL, 3.0 ng/mL 7.5 ng/mL and 3.0 ng/mL, respectively.

The bias and precision were determined using a simple analysis of variance (ANOVA: single factor). The data for the bias and precision were run at four levels (10, 50, 100 and 200 ng/mL) in triplicate over five days for urine, and at three levels (10, 50, 100 ng/mL) in triplicate over five days for whole blood. The results demonstrated that the bias and precision was < 11 % for THC, THC-OH, THC-COOH, CBN, and CBD in urine at each level. The bias and precision in blood was < 5% for all analytes at each QC level.

Keywords: **SPE, THC, Metabolites, LC/MS/MS**

## S02 Completely Automated GC/MS Analysis of Drugs and Metabolites Using Disposable Pipette Extraction with Cooled Injection System

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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 of the GC (or HPLC) and the samples can be extracted, derivatized and analyzed in one sequence 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".

For concentration, a GERSTEL CIS (cooled injection system) is used to vent solvent and permit the injection of 50 µL or more of eluent and derivatizing reagent.

Benzodiazepines in urine were analyzed by simply transferring 0.5 mL of hydrolyzed and centrifuged urine samples into sample tubes. The GERSTEL MPS processed the samples with DPX-RP (reversed phase) and DPX-CX (cation exchange) tips, and the eluents were directly injected (30 µL) with derivatizing reagent (20 µL 50% MTBSTFA in acetonitrile). The benzodiazepines tested included diazepam, alprazolam, nordiazepam, oxazepam, temazepam, α-hydroxy alprazolam, clonazepam, 7-aminoflunitrazepam, flunitrazepam, and lorazepam. All analytes were readily detected at less than 50 ppb with most having less than 10% C.V.

Several drugs were analyzed by the automated DPX that required no chemical derivatization. Using just 0.25 mL of whole blood, which was protein precipitated with 0.5 mL of acetonitrile (with 0.1 mL of 0.1 M HCl subsequently added), less than 100 ppb of cocaine, amitriptyline, imipramine, methadone, codeine, PCP, meperidine, methaqualone, and methamphetamine could be detected by automated DPX using DPX-CX. The extracts were very clean, and hence no additional deterioration of the injection port was noted.

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

## S03 Trace Analysis of Non-Derivatized Drugs in Urine by Two Dimensional Gas Chromatography Time-of-Flight Mass Spectrometry (GCxGC-TOFMS)

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This research investigates the potential use and practical application of comprehensive multidimensional gas chromatography time-of-flight mass spectrometry (GCxGC-TOFMS) in forensic toxicology analysis for a diverse range of illicit drugs in urine without sample derivatization. Conventional methods for the analysis of drugs in urine use time consuming sample derivatization for GC/MS analysis to enhance chromatographic performance. This study utilizes GCxGC to increase peak capacity and resolution in combination with TOFMS detection followed by data processing with deconvolution software algorithms for the identification and quantitation of non-derivatized drugs in urine.

A multiple drug standard mixture was prepared from standards obtained from Sigma-Aldrich. The stock standard mixture was spiked in 5mL aliquots of urine at various concentrations from 10 to 1500ng/mL. An internal standard, hexachlorobenzene (HCB), was added to each sample at a concentration of 500ng/mL. Solid phase microextraction was performed by submersion of the SPME fiber (50/30µm DVB / Carboxen™/PDMS Stable Flex, Supelco, Bellefonte, PA) for 30 minutes at room temperature while stirring at 1000 rpm. Sample preparation was followed by comprehensive two dimensional gas chromatography and TOFMS detection. 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. Separation in the first dimension was conducted on a 30m x 0.25mm x 0.25µm film thickness, Rxi-5ms, (Restek Corp., Bellefonte, PA) followed by sample cryofocusing and thermal modulation unto the second dimension column, a 1.5m x 0.18mm x 0.20µm film thickness, Rtx-200, (Restek Corp., Bellefonte, PA). 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.

Trace level non-derivatized sample analysis of the illegal drugs methamphetamine, cocaine, codeine, and diacetylmorphine will be shown. Solid phase microextraction (SPME) followed by GCxGC-TOFMS analysis was utilized for this research with a minimum LOD of 10 ng/mL (ppb) for these target analytes. SPME was performed on non-derivatized urine samples spiked with a multiple drug standard at different concentrations prior to sample injection and subsequent analysis by GCxGC-TOFMS. Calibration curves were developed from prepared standards in the concentration range of 10 to 1500 ng/mL by a splitless SPME GC sample injection. Results from this study demonstrated favorable linearity values for the low level concentration range from 10 to 1500 ng/mL. Correlation coefficient values of 90 percent or greater were achieved for codeine, diacetylmorphine, and cocaine. While methamphetamine exhibited poor chromatographic behavior it still achieved correlation coefficient values of approximately 85 percent and was detectable at 10ng/mL. Successful identification was observed in this study for other non-derivatized drug standards. Positive identifications were made for the drugs oxycodone, ecstasy, acetylcodeine, monoacetylmorphine, and LSD. The identifications were confirmed by mass spectral library search matches as compared to the NIST 05 spectral library.

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 shows favorable promise towards successful identification and quantitation of illicit drugs in urine without complex sample derivatization.

Keywords: **GCxGC-TOFMS, Drugs of Abuse, Solid Phase Microextraction**

## S04 Multi Target Screening (MTS) For More Than 400 Drugs by QTRAP LC-MS/MS and Library Searching – On Its Way To Routine Use

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**Objectives:** Development and application of a drug screening procedure with LC-MS/MS for general unknown screening.

**Methods:** A library with ESI MS/MS spectra of 1250 compounds has been developed using a QTrap 3200 tandem-mass spectrometer (Applied Biosystems) with a turbo ionspray source. After standardization of a chromatographic system using a 50 mm x 2.1 mm Allure PFP column (Restek), the library has been used for the identification of drugs and metabolites in urine and serum/blood samples using a "multi target" general-unknown screening approach. Retention times of 600 compounds have been determined and transitions for each compound were selected by a "scheduled" survey-MRM scan, followed by an information dependent acquisition (IDA) using the sensitive enhanced product ion scan of the Qtrap hybrid instrument. A library search was performed for compound identification. Due to the selection of MRM transitions, the method is called Multi Target Screening, covering more than 400 compounds in a single LC-run (drugs of abuse, psychoactive drugs and many others). Automation of data exploration has been performed.

**Results:** Standardization of the procedure has been performed for its applicability in different laboratories, using a reference standard test mixture ("Suitability Test Mix"), and also internal deuterated standards for semiquantitative analysis for several drugs. First applications of this procedure have been developed for the detection and identification of drugs of abuse and drugs for substitution (opiates, amphetamines, cocaine, LSD, cannabinoids, buprenorphine, and methadone), psychopharmaceuticals (benzodiazepines, hypnotics, antidepressants, neuroleptics) and pain relief drugs. Urine samples of drug abusers, from clinical and forensic cases (material from autopsy) have been investigated, with the aim of testing the reproducibility and robustness of the system, especially in terms of comparison of different sample preparation procedures (dilution 1:10 and 1:3 [v/v], or extraction with chlorobutane at pH 9) and matrix effects. With the use of the internal standards, the system could be used for drug identification as it has been demonstrated by GC/MS and HPLC-DAD analysis performed in parallel. The optimized method allows the detection and identification of a great variety of compounds within one analytical run of 15 min using a gradient elution with steadily increasing flow rate. Limits of detection were in many cases lower than those of classical immunoassays (amphetamines, opiates, benzodiazepines, cocaine-metabolite).

**Conclusions**: The application of this screening method is in the fields of clinical toxicology (for target analysis in intoxication cases), psychiatry (antidepressants and other psychoactive drugs), and forensic toxicology (drugs and driving, workplace drug testing, oral fluid analysis, drug facilitated sexual assault) – whenever different classes of drugs are relevant. Further challenges are the automation by on-line solid-phase extraction for plasma or oral fluid samples.

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

## S05 Comparison of MRM vs. Full Scan MS/MS for LC/MS/MS Drug Confirmation

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**Objective:** To determine the advantage and limitations of using MRM ratio versus full scan MS/MS spectra for compound confirmation when performing LC/MS/MS analyses.

**Methods:** Standards of various drug compounds were spiked into drug free urine at various concentrations and diluted 10x with mobile phase. Analysis was performed on an LC interfaced to a hybrid triple quadrupole/linear ion trap (LIT) mass spectrometer. All compounds were analyzed using positive mode electrospray ionization.

For the MRM only method, two MRM transitions per analyte were monitored with the second transition functioning as a qualifier ion. The ratio of the peak areas of the target MRM to the qualifier MRM was calculated. Three different MRM ratio criteria were compared.

When full scan MS/MS spectra were used for confirmation, an MRM survey scan was used to detect the presence of an analyte. If an analyte was detected, the system automatically acquired a full scan MS/MS spectrum of the compound using Q3 operating in LIT mode. The resulting spectrum could be searched against a library for identification and confirmation. A purity match of 60% or higher was required for confirmation.

**Results:** Both MRM and LIT full scan MS/MS were sufficient for confirmation at mid-level concentrations. Differences were observed at the extremes – very low levels (10x below cutoff levels) or very high levels (>50x cutoff levels). Cutoff levels for most analytes were approximately 100 ng/mL with the exception of fentanyl, which was 1 ng/mL. MRM confirmation worked slightly better at very low levels as long as the qualifier ion had sufficient intensity. If the qualifier ion had low intensity, as was the case with certain amphetamines, LIT MS/MS yielded better confirmatory results. At very high concentrations, LIT confirmation showed significantly better results.

**Conclusion:** Initial findings indicate that both the full scan method using a LIT and the MRM with two transitions method are robust and precise in performing confirmations. The ability to successfully confirm drug identifications depended on many factors, including qualifier ion intensity and analyte concentration

Keywords: **LC/MS/MS, Drug Screening, Drug Confirmation**

## S06 A Novel Validated Method for Quantification of Methadone, Cocaine, Opiates and Metabolites in Human Placenta by LCMSMS

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**Objectives:** To develop and validate a sensitive and specific assay for the simultaneous quantification of methadone, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), cocaine, benzoylecgonine (BE), morphine, codeine and 6–acetylmorphine (6AM), and for determining disposition of these analytes in placenta of opioid-dependent women receiving methadone pharmacotherapy.

**Method*:*** Five mL of 0.1% perchloric acid (v/v) were added to 1 g of human placenta and homogenized in a blender until complete homogenization. Deuterated internal standard solution was added to the homogenate, followed by centrifugation. The supernatant was subjected to solid phase extraction with mixed mode-reversed phase cartridges. Chromatographic separation was performed on a Synergi Polar RP column (75 mm x 2 mm, 4 μm, Phenomenex®, Torrance, CA) with a gradient of (A) 0.1% formic acid and (B) acetonitrile. Transitions monitored were: *m/z* 310.1 to 265.0, 247.1, 219.2 for methadone, *m/z* 278.2 to 249.1, 234.2 for EDDP, *m/z* 304.1 to 182.1, 150.1, 82.1 for cocaine, *m/z* 290.1 to 168.1, 150.0, 82.1 for BE, *m/z* 286.2 to 201.1, 229.0, 268.1 for morphine, *m/z* 300.2 to 215.1, 243.0, 282.2 for codeine and *m/z* 328.2 to 211.1, 268.1, 193.1 for 6AM.

**Validation:** The method was validated including linearity, limit of quantification (LOQ), carryover, intra- (n=5) and inter-assay imprecision (n=20) and analytical recovery (n=20) over four different assays at 3 quality control (QC) concentrations (7.5, 75 and 375 ng/g). Endogenous and exogenous interferences were evaluated. Extraction efficiency (n=5) and matrix effect (n=10) also were studied.

**Results:** All the analytes eluted within 10 min, for a total chromatographic run of 15 min. Calibration curves were linear between 10-2000 ng/g for methadone, and 2.5-500 ng/g for the rest of the analytes. Linearity was verified using 1/x weighted linear regression, with coefficients of determination > 0.99. LOQ for methadone was 10 ng/g and 2.5 ng/g for rest of the analytes. Carryover was observed for methadone after injection of blank sample fortified at 4000 ng/g, although the signal was 6 times less than that of the LOQ. Intra and inter-assay imprecision were <15%, and intra- and inter-assay analytical recovery were >85% for all the analytes. No endogenous or exogenous interferences were observed at low QC sample. Extraction efficiency was >46%, with significant matrix effect, although it was compensated by using the deuterated internal standard. Placentas obtained from a controlled administration study of methadone and buprenorphine in opioid-dependent pregnant women will be analyzed by the validated assay.

**Conclusion:** A method for quantification of methadone, EDDP, cocaine, BE, morphine, codeine and 6AM was developed and fully validated, satisfying acceptance criteria. This method will contribute to the knowledge of *in utero* drug exposure, focusing on the disposition and effects of methadone prenatal exposure.

Supported by the National Institutes of Health, Intramural Research Program, National Institute on Drug Abuse, and the Galician Government, Galicia, Spain.

Keywords: **Methadone, *In Utero* Drug Exposure, Placenta, EDDP**

## S07 Comparison of Oral Fluid Collection Devices for the Detection of Opiates and Benzodiazepines

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Urine analysis can often be used to assess the compliance of individuals in the rehabilitation setting. Obtaining a urine sample involves witnessing the passing of urine to lessen the possibly of adulteration. This process can be distressing for both the patient and the collector. Oral fluid testing can be used to analyse drugs present in the body at the time of collection. Collecting oral fluid can be witnessed easily and is available for sampling at any time.

An assessment of oral fluid testing in the rehabilitation setting was carried out by the simultaneous collection of oral fluid, using two different devices, urine and blood. Samples were collected from 20 patients entering a residential rehabilitation centre who self-reported heroin use within the last 24 hours; samples were taken for five consecutive days. The samples were analysed for opiates and benzodiazepines using LC-MS-MS.

The results showed that on day 1 after admission to the centre, 32 % of Intercept® collected oral fluid samples, 50 % of the modified Omni-Sal® device collected oral fluid samples, 56 % of blood samples and 100 % of urine samples were positive for morphine. The median concentrations of morphine positives found in each matrix throughout the five days were 22 ng/mL (range 2-312 ng/mL) using the Intercept® device, 32 ng/mL (range 3 – 437 ng/mL) using the modified Omni-Sal® device, 7 ng/mL (range <3 – 28 ng/mL) for blood and 197 ng/mL (range <3 - >>200 ng/mL) for urine. This study clearly shows there are marked differences between oral fluid and urine testing. Concentrations of analytes were much lower in oral fluid than in urine and consequently were present for a shorter period of time in oral fluid than in urine.

Keywords: **Oral Fluid, Rehabilitation, Opiates**

## S08 Potential Biomarkers of Fentanyl Utilizing Pyrolysis-GC/MS

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Fentanyl is a potent opioid analgesic that is increasingly becoming a choice drug of abuse. Fentanyl transdermal patches are easily obtained and are consumed by smoking the gel and/or the whole patch due to the increased bioavailability when the drug is inhaled. Recent work in our laboratory has demonstrated the utility of analytical pyrolysis for studying smoked drugs of abuse. These methods were used here to identify possible biomarkers associated with smoked fentanyl and fentanyl patches.

Pyrolysis was carried out under reductive and oxidative conditions, using helium and air, respectively. The pyroprobe was interfaced to a GC/MS using a heated transfer line. Pyrolytic temperature profiles were optimized with a final temperature of 750°C. A RTX-5ms column (30 m, 0.25 mmID, 0.50 μm) was used. Analysis of fentanyl was reproducibly done in three ways: without a trap, with a trap, and with a trap plus air as a reactant gas. Sample was introduced into the pyroprobe via methanolic solutions and as a solid.

Pyrolysis of the drug under both reductive and oxidative conditions consistently yielded propionanilide as the major product, along with pyridine and the reported metabolites norfentanyl and despropionyl fentanyl. However, the pyridine was poorly retained and identification was problematic without the trapping conditions. Chlorine-containing compounds, presumably formed from the HCl salt of fentanyl, were also tentatively identified. When oxidative trapping was employed, a more complex chromatogram resulted, but most identifications were tentative due to the lack of reliable commercial standards for comparisons. These conditions greatly enhanced the recovery of pyridine and afforded a positive identification.

Pyrolysis of fentanyl patches, new and recovered from cases at autopsy, were conducted under the same reductive, oxidative, and trapped conditions. Not surprisingly, significant polymeric and hydrocarbon compounds were seen as well as those likely derived from adhesives. While trapping improved chromatography, no significant differences were seen beyond this observation. Fentanyl on the patches was in the citrate salt form; not surprisingly, the chlorine-containing pyrolytic products obtained with the neat drug were not observed.

Pyrolysis of fentanyl and fentanyl patches identified several potential biomarkers distinctive to fentanyl and not seen as typical metabolites. One of these, pyridine, could be amenable to simple headspace assay. When patches are smoked directly, a large suite of potential biomarkers were identified. In this application, it may also be possible to identify what salt form of the drug was smoked based on pyrolytic products.

Keyowrds: **Fentanyl, Pyrolysis, Biomarker**

## S09 Mass Spectral Evaluation for Compound Identification in Forensic Toxicology

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One of the most critical aspects in reporting analytical toxicology results is the criteria to be used for establishing the positive identification of a given compound in a submitted specimen. For most modern analytical toxicology, mass spectral data is a key element in establishing these identifications, and the use of clear and unambiguous procedures for evaluation of this data is essential for ensuring consistent and defensible reporting of results. Unfortunately, no universally accepted standard for evaluation of mass spectra in compound identification exists. While several accrediting and oversight bodies, including NCCLS and WADA, do provide guidelines, these are not entirely consistent between different accrediting bodies, often do not provide clear information on practical implementation, and sometimes even exhibit clear deficiencies in methodology.

We present the guidelines used in our laboratory for the evaluation of mass spectra, which draw and expand upon some of the best aspects of other existing guidelines, focusing on electron-impact and tandem electrospray analyses, along with the reasoning behind the chosen criteria. The value of direct comparison to contemporaneously analyzed standards, as opposed to library matching, will be emphasized. Reasons for using integrated peak areas, as opposed to mass spectral "stick height" lists, for calculation of ion ratios will be explained. The rationale for using sliding scales for ion ratio matching tolerances, and for using different tolerances in EI and ESI data will be addressed. Finally, the importance of information content in evaluation of mass spectra will be considered, particularly as it applies to the limitations of SIM and SRM analyses when compared to full scan experiments. Application of these guidelines will be demonstrated with examples drawn from validation studies and actual casework in our laboratory.

Keywords: **Mass Spectrometry, Tandem MS, Drug Standards**

## S10 Plasma Cannabinoid Concentrations in Six Chronic Cannabis Users After Frequent Oral THC Administration

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Individuals requiring appetite stimulation, relief of pain or nausea have benefited from cannabinoid pharmaceuticals such as synthetic $\Delta^9$-tetrahydrocannabinol (THC) (dronabinol, Marinol®) and Sativex®. Knowledge of cannabinoid pharmacokinetics after multiple administered doses would be useful for therapeutic drug monitoring and for interpretation in forensic investigations. This research provides data on plasma cannabinoid concentrations during and after frequent oral dosing with synthetic THC.

Six adult African-American males self-reporting daily cannabis smoking provided written informed consent and resided for 10 d on a closed clinical research unit while participating in this IRB-approved protocol. Participants received 20 mg oral THC twice on Day 1 (day after admission), 5 times on Days 2 – 4, 6 times on Days 5 – 7 and twice on Day 8. Plasma was collected on admission, 12 times on Day 1, 3 times on Days 2 – 7, 12 times on Day 8 and once prior to discharge on Day 9. Free THC, 11-hydroxy-THC (11-OH-THC) and 11-nor-$\Delta^9$-9-carboxy-THC (THCCOOH) were extracted from plasma by solid phase extraction and quantified with 2-dimensional chromatography with cryofocusing. Limits of quantification were 0.25 ng/mL (THC and THCCOOH) and 0.5 ng/mL (11-OH-THC).

Plasma specimens were refrigerated after collection (never frozen) and usually analyzed within 14 days from discharge; a few specimens required multiple analyses, extending testing time. Mean [SD] plasma concentrations on admission (Day 0) were 9.6 ± 11.7, 3.6 ± 4.1 and 59.2 ± 41.0 ng/mL for THC, 11-OH-THC and THCCOOH, respectively, representing previously self-administered cannabis. 20 h later (Day 1) prior to the first THC dose, concentrations decreased to 4.3 ± 2.7, 1.3 ± 1.2 and 34.0 ± 20.5 ng/mL, respectively. After the 1st THC dose, mean Cmax and Tmax were 16.5 ± 8.3 ng/mL at 2.8 ± 0.8 h for THC; 8.2 ± 4.9 ng/mL at 2.5 ± 0.4 h for 11-OH-THC, and 75.5 ± 23.7 ng/mL at 3.1 ± 1.2 h for THCCOOH. During multiple daily doses, mean THC Cmax was 47.7 ± 19.8 ng/mL occurring 99.1 ± 23.7 h after 1st dose, 23.9 ± 7.6 ng/mL for 11-OH-THC at 141.1 ± 30.0 h and 327.2 ± 130.0 ng/mL for THCCOOH at 153.1 ± 24.3 h. On Day 9, 22.5 h after the last THC dose, mean plasma concentrations were 3.7 ± 1.2, 3.0 ± 1.8 and 195.7 ± 100.0 ng/mL for THC, 11-OH-THC and THCCOOH, respectively.

THC concentrations were >1.9 ng/mL 20 h after admission in chronic cannabis users. Oral THC bioavailability is low and variable due to acidic degradation and erratic absorption in the gastrointestinal tract, and enterohepatic recirculation. These data provide the first reference for interpretation of THC, 11-OH-THC and THCCOOH plasma concentrations during frequent high-dose oral cannabinoid administration.

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Keywords: **THC, Cannabinoids, Plasma, Oral**

## S11 Whole Blood Cannabinoids in Daily Cannabis Users During Eight Days of Controlled Multiple Oral THC Doses

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Cannabis is the illicit substance most commonly detected in blood of driving under the influence of drugs (DUID) cases and in hospitalized or fatally injured drivers. Whole blood is often the only specimen available in such cases, yet controlled cannabinoid administration studies have primarily examined plasma pharmacokinetics of Δ9-tetrahydrocannabinol (THC) and metabolites 11-hydroxy-THC (11-OH-THC) and 11-nor-9-carboxy-THC (THCCOOH). Additionally, while frequent or daily cannabis use is common, few pharmacokinetic data for whole blood are available from specimens collected during repeated controlled cannabinoid administration.

Six male African Americans (aged 18-32) provided written informed consent for this IRB-approved protocol. Subjects reported average daily smoking of 1 to >5 cannabis cigarettes, blunts or bong inhalations over the 3 months prior to study admission to a closed research unit. Participants received 20 mg oral synthetic THC (dronabinol) twice on Day 1 (1 day after admission), 5 times on Days 2 – 4, 6 times on Days 5 – 7 and twice on Day 8. Whole blood was collected upon admission, 8 times on Day 1, once on Days 2 – 7, 4 times on Day 8 and once on Day 9 prior to discharge. Free THC, 11-OH-THC and THCCOOH were extracted from whole blood by solid phase extraction and quantified by GCMS (electron impact/selected ion monitoring) using two-dimensional chromatography with cryofocusing. Limits of quantification (LOQ) were 0.25 ng/mL for THC and THCCOOH and 0.5 ng/mL for 11-OH-THC. Whole blood specimens were refrigerated (never frozen) after collection and generally analyzed within 14 days of discharge; some specimens required re-analysis extending storage to no longer than 22 days after discharge.

On admission, mean [SD] whole blood THC, 11-OH-THC, and THCCOOH concentrations were 5.9 ± 7.1, 2.3 ± 2.5 and 35.4 ± 27.0 ng/mL, respectively. 20 h later (Day 1), prior to dosing, mean concentrations decreased to 2.7 ± 1.7, 1.0 ± 0.9 (n = 4) and 20.2 ± 13.9 ng/mL, respectively. $T_{max}$ was 2.8 ± 1.0 h for THC ($C_{max}$ 9.7 ± 5.5 ng/mL), 2.6 ± 0.5 h for 11-OH-THC ($C_{max}$ 4.5 ± 2.5 ng/mL) and 3.0 ± 0.6 h for THCCOOH ($C_{max}$ 37.8 ± 10.7 ng/mL). Concentrations returned to near pre-dose levels (3.9 ± 0.8 ng/mL for THC, 2.3 ± 0.7 ng/mL for 11-OH-THC, and 31.7 ± 8.3 ng/mL for THCCOOH) within 5 h. During chronic dosing, THC and metabolite concentrations increased until Day 5. Thereafter, THC and 11-OH-THC decreased while THCCOOH plateaued at approximately 140 ng/mL. 22.5 h after the final dose (Day 9), concentrations of THC, 11-OH-THC, and THCCOOH were 2.8 ± 1.4 ng/mL, 2.2 ± 1.2 ng/mL, and 108.0 ± 46.5 ng/mL, respectively.

Irregular absorption, individual differences in first-pass metabolism and enterohepatic re-circulation may have resulted in the highly variable cannabinoid concentrations during frequent oral THC dosing. Whole blood THC was >1.1 ng/mL in all participants 20 h after admission and 22.5 h after the last oral THC dose. To our knowledge, these are the first data on whole blood THC concentrations during and after frequent oral THC dosing, providing a scientific basis for interpretation of cannabinoid concentrations in DUID and other forensic cases.

Supported by the National Institute on Drug Abuse, NIH and Sanofi-Aventis.

Keywords: **Whole Blood, Cannabinoid Pharmacokinetics, Oral THC Administration**

## S12 Methylecgonidine and Ecgonidine in Urine Following Four Different Routes of Cocaine Administration

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**Background:** Methylecgonidine (MED), also called anhydroecgonine methyl ester, and its metabolite ecgonidine (ED) have been proposed as urinary markers of cocaine smoking. The objective of this study was to identify and quantify MED and ED in urine following smoked (SM), intravenous (IV), intranasal (IN) and oral routes of cocaine administration to test the effectiveness of this proposal.

**Methods:** Participants provided written informed consent to participate in this NIDA Institutional Review Board-approved research. 6 male subjects were administered cocaine by the following routes and doses: SM 42 mg, IV 25 mg and IN 32 mg. Six different males received SM 0, 10, 20, 40 mg; IV 0, 11.2, 22.4, 44.8 mg and Oral 22.4 mg. Urine specimens (n= 632) were collected prior to and up to 166 h after dosing and stored at -20°C until analysis for MED and ED by a validated GCMS method with LOQs of 10 and 25 ng/mL, respectively (Paul et al. *Biomed Chromatogr* 19:677-88, 2005). Urine cocaine and 6 other metabolites were determined by GCMS and published previously (Huestis et al. *J Anal Toxicol* 31:462-8, 2007). Blind QC urine samples (n= 51) stored at -20°C were analyzed simultaneously with subject specimens.

**Results and Discussion:** Following SM cocaine, MED (11-166 ng/mL) was found in 20 of 235 urine specimens, ED (25-144 ng/mL) was measurable in 59, and 13 specimens contained both analytes. MED and ED were detectable for up to 8 h and 51 h, respectively. Subjects with the highest peak urine cocaine concentrations had the highest MED concentrations. After oral ingestion, no urine specimens contained either analyte. One subject in the IN group had 5 positive specimens: 3 specimens with 14-19 ng MED/mL and 3 with 30-31 ng ED/mL. The same subject had two positive specimens following IV administration: one with 19 ng MED/mL and the other 31 ng ED/mL. A different IV subject produced urine with 25 ng ED/mL. There were 2 of 12 subjects and 8 of 322 specimens with benzoylecgonine or ecgonine methyl ester >LOQ that also contained MED and/or ED following nonsmoking routes of administration. Benzoylecgonine or ecgonine methyl ester concentration in these 8 specimens was >1000 ng/mL and ecgonine >98 ng/mL. Mean %CV for MED and ED in frozen stored blind QCs were 8.8 and 3.1%, respectively. Possible explanations for MED and ED in urine following nonsmoking routes of administration include unusual metabolism and/or excretion, or degradation during storage. Contaminants in administered cocaine and analytical artifact production are unlikely; only a few of the subjects administered the same lots of cocaine were MED or ED positive, and no artifact controls included in each assay were positive.

**Conclusion**: Based on these results, MED and ED may be present in stored frozen urine specimens following nonsmoking routes of cocaine administration. Further investigations are needed to determine the genesis of these substances.

Supported by the American Registry of Pathology, Armed Forces Institute of Pathology, and Intramural Research Program, NIH, National Institute on Drug Abuse. Opinions are those of the authors and not necessarily those of the Department of Defense or NIDA/NIH.

Key words: **Methylecgonidine, Ecgonidine, Smoked Cocaine**

## S13 Urinary Excretion Profiles of Flunitrazepam Metabolites

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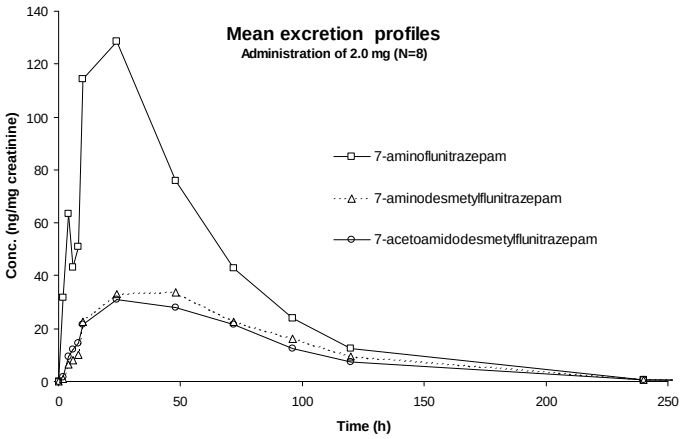
The objective of this paper was to investigate the excretion of five metabolites of flunitrazepam after a single dose of either 0.5 mg or 2.0 mg. Flunitrazepam is a low dose sedative known to be used in connection with sexual assaults or robbery to incapacitate the victim. Blood and urine samples in connection with sexual assaults are commonly obtained for toxicological analysis. However, samples are often collected a considerable time after the assault and present with negative results. We developed a sensitive method for flunitrazepam metabolites in urine to study excretion patterns and detection times in a controlled dosing study. Sixteen subjects were included. Urine samples were obtained prior to dosing and then after 2, 4, 6, 8, 12, 24, 48, 72, 96, 120, 240, and 360 hours. Samples were screened for benzodiazepines using CEDIA reagent on an ADVIA 1650 using the 300 ng/ml cut-off. All samples were also analyzed by LC-MS-MS after enzymatic hydrolysis and solid phase extraction. The confirmation cut-off was 0.5 ng/ml for flunitrazepam, desmethylflunitrazepam (dmFlu), 7-aminoflunitrazepam (7AFlu), 7-aminodesmethylflunitrazepam (7AdmFlu), 7-acetamidoflunitrazepam (7AAFlu), and 7-acetamidodesmethylflunitrazepam (7AAdmFlu).

A total of 204 urine samples were collected. None of the samples from subjects given 0.5 mg flunitrazepam presented with a positive screening result. Twenty-three of those from subjects receiving 2.0 mg were screened positive. These samples were obtained between 4 and 72 hours post dose. The predominant metabolite was 7AFlu followed by 7AdmFlu and 7AAdmFlu that both had similar excretion profiles then followed by 7AAFlu. The parent compound and dmFlu were only found in very low concentrations. Peak concentrations ranged between 11-100 ng/ml for 7AFlu, between 9-22 ng/ml for 7AdmFlu, and between 4-41 ng/ml for 7AAdmFlu after the low dose and 122-412 ng/ml, 28-95 ng/ml, and 10-199 ng/ml, respectively for the high dose. After the low dose the three predominant metabolites were detected together up to 5 days after dose, however in four subjects either 7AFlu or 7AdmFlu were found above the 0.5 ng/ml cut-off also after ten days. After the high dose six of the subjects showed positive samples for all three predominant metabolites up to ten days after dose. One subject was positive for 7AdmFlu and 7AAdmFlu even after 15 days. The mean excretion profiles corrected for creatinine are depicted in the figure.




We conclude that using a sensitive analytical method even a low dose of flunitrazepam can be traced in the urine up to ten days after the administration. The use of routine immunochemical screening methods may leave an intake undiscovered. Therefore, we emphasize that the analysis of cases involving involuntary ingestion of drugs should be carefully investigated using target method rather than screening procedures.

Keywords: **Flunitrazepam, Excretion, LC-MS**

## S14 Evaluation of the Response of Novel Buprenorphine Metabolites, M1 and M3, in Urine to Anti-Retroviral Treatments That Induce and Inhibit Buprenorphine Metabolism

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The traditional metabolism of buprenorphine is N-dealkylation to norbuprenorphine and then glucuronidation of both. Recently a number of hydroxylated metabolites of buprenorphine and norbuprenorphine have been identified. Two have been identified in urine of subjects taking therapeutic doses of buprenorphine. These two have a hydroxyl group introduced onto the alkyl-hydroxy side chain of buprenorphine (M1) and norbuprenorphine (M3). Both are also glucuronidated, but M1 much more so than M3 (Chang et al. *Drug Metab Dispos* **34**: 440-448, 2006). The purpose of this study was to evaluate 24-hour urine samples collected from subjects on maintenance doses of buprenorphine before and after co-treatment with the antiretroviral agents efavirenz, delavirdine, nelfinavir, ritonavir, and lopinavir/ritonavir. The results for buprenorphine and traditional metabolites in plasma were previously published (McCance-Katz, et al. *Clin Infect Dis* **43**: S224-S234 and S235-S246, 2007).

After subjects (10 per treatment group) had been on stable maintenance doses of buprenorphine/naloxone 16/4 mg sublingual for at least 2 weeks, and then again after treatment with efavirenz (600 mg daily for 15 days), delavirdine (600 mg twice daily for 7 days), nelfinavir (1250 mg twice daily for 5 days), ritonavir (100 mg twice daily for 7 days), and lopinavir/ritonavir (400/100 mg twice daily for 7 days), 24-hour pharmacokinetic sessions were performed that included collection of plasma samples and pooled urine samples. Pooled urine samples had volume measured and then aliquots, as well as the plasma specimens, were stored frozen until time of analysis. Analysis for buprenorphine and traditional metabolites in plasma and urine was completed using solid phase extraction and liquid chromatoagraphy-electrospray ionization-tandem mass spectrometry. The same methodology with liquid/liquid extraction and different transitions was used to obtain semiquantitative values (peak area ratios compared with d4-buprenorphine and d3-norbuprenorphine as internal standards) for M1 and M3.

There was no significant change in urine volume excreted between buprenorphine only and antiretroviral co-treated groups. The changes in plasma area under the curve and amount excreted in urine of buprenorphine and traditional metabolites were similar for all treatments except for lopinavir/ritonavir. Increased renal clearance was found for norbuprenorphine and its glucuronide after co-treatment with these lopinavir/ritonavir. These findings suggested that urine amounts of M1 and M3 would adequately reflect systemic changes for all treatments except lopinavir/ ritonavir. Efavirenz decreased M1 and decreased M3 consistent with its ability to induce cytochrome P450s, particularly P450 3A. Delavirdine increased M1 and decreased M3 consistent with its ability to inhibit P450 3A. Both nelfinavir and ritonavir decreased both M1 and M3 consistent with their ability to inhibit both P450 3A and 2C8. These results provide further information on the in vivo response of novel secondary metabolites of buprenorphine to inhibitors and inducers of buprenorphine metabolism. (Supported in part by NIDA grants R01 DA 10100, R01 DA 13004, K02 DA 00478, and K24 DA 023359)

Keywords: **Buprenorphine Metabolites, Antiretroviral Medications, Altered Metabolism**

## S15 A Novel Method for Simultaneous Quantification of Buprenorphine, Norbuprenorphine, Buprenorphine Glucuronide and Norbuprenorphine Glucuronide in Human Placenta by Liquid Chromatography Tandem Mass Spectrometry

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**Objective:** A method for the first simultaneous determination of buprenorphine and metabolites, norbuprenorphine, buprenorphine glucuronide and norbuprenorphine glucuronide, in human placenta by liquid chromatography tandem mass spectrometry (ion trap) was developed and fully validated.

**Method**: Two g placenta was homogenized in 6 mL of 0.1% perchloric acid in water in the presence of deuterated buprenorphine and norbuprenorphine. After 15 min centrifugation at 4000 rpm, the liquid supernatant was subjected to solid-phase extraction with preconditioned Strata-XC cartridges. Reverse-phase separation was achieved with a Synergi Polar column (2.1x75mm, 4µm) in 20 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 selected ion monitoring of precursor ions *m/z* 468.4 for buprenorphine; 414.3 for norbuprenorphine; 644.3 for buprenorphine glucuronide and 590 for norbuprenorphine glucuronide. Buprenorphine and norbuprenorphine were identified by $MS^2$, with monitored fragments of 396.3, 414 and 426.2 for buprenorphine, and 340.2, 364.2 and 382.2 for norbuprenorphine. Glucuronide conjugates were identified by $MS^3$ by *m/z* 396 and 414 for buprenorphine glucuronide and *m/z* 340.1 and 382 for norbuprenorphine glucuronide. Validation parameters included linearity, limits of detection (LOD) and quantification (LOQ) determined empirically over four separate assays. Intra-day (n=5) and interday (n=20) imprecision and bias, extraction efficiency (n=5), process efficiency (n=5), and matrix effect (n=10), were evaluated at 3 concentrations (1.75, 15 and 40 ng/g) across the linear dynamic range of the assay. Matrix interference was assessed with 10 unique placentas to evaluate potential endogenous interferences and selectivity tested with 33 licit and illicit drugs. The potential for carryover also was tested.

**Results:** The assay was linear from 1 to 50ng/g. Intra- and inter-assay imprecision were <14.7% and bias was <13.1%. Extraction efficiencies ranged from 40.7-66.5%, and process efficiency from 38.8-70.5%. No significant matrix effect was detected for any compound. The method was specific (no endogenous or exogenous interferences) and sensitive (LOD 0.8 ng/g). No carryover was detected. In order to prove method applicability, an authentic placenta from an opioid-dependent pregnant woman under buprenorphine treatment was analyzed. Interestingly, buprenorphine was not detected above the LOQ. Concentrations of the metabolites were norbuprenorphine 15.7 ng/g, buprenorphine glucuronide 3.2 ng/g, and norbuprenorphine glucuronide 46.6 ng/g.

**Conclusion:** This novel method permits simultaneous determination of buprenorphine and its main metabolites in human placenta, with good selectivity and sensitivity in this complex matrix. This method will be employed to analyze placentas collected in a clinical study investigating buprenorphine as substitution therapy for opioid treatment during pregnancy.

Supported by the Intramural Research Program, NIH, National Institute on Drug Abuse, and the Ministerio de Educación y Ciencia of Spain funding for Marta Concheiro.

Keywords: **Buprenorphine, Placenta, LC/MS/MS (Liquid Chromatography-Tandem Mass Spectrometry**)

## S16 Urinary Excretion of Buprenorphine, Norbuprenorphine, Buprenorphine-Glucuronide and Norbuprenorphine-Glucuronide in Pregnant Opioid-Dependent Women Receiving Buprenorphine Pharmacotherapy

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Buprenorphine (BUP) is under investigation as pharmacotherapy for opioid-dependent pregnant women. BUP and metabolite concentrations in urine from women maintained on BUP during the 2nd and 3rd trimesters of pregnancy and postpartum were determined with a new liquid chromatography-tandem mass spectrometry (LCMSMS) method to determine drug disposition.

BUP, norbuprenorphine (NBUP), buprenorphine glucuronide (BUP-Gluc) and norbuprenorphine glucuronide (NBUP-Gluc) concentrations were determined in 515 urine specimens collected thrice weekly during pregnancy and post-partum. An additional 152 specimens were collected during 24 h in-patient dosing sessions designed to examine changes in BUP excretion during pregnancy. Specimens were analyzed by a fully validated LCMSMS method with limits of quantification of 5 ng/mL for BUP and BUP-Gluc and 25 ng/mL for NBUP and its conjugated metabolite. Ratios of metabolites were examined across trimesters and postpartum to identify possible changes in metabolism during pregnancy.

Overall, 1.2, 63.9, 89.9, and 96.5% of urine specimens contained BUP, NBUP, BUP-Gluc and NBUP-Gluc, respectively. NBUP-Gluc was the primary metabolite identified in urine and exceeded BUP-Gluc concentrations in 99% of specimens. While BUP-Gluc was identified in more specimens than NBUP, NBUP exceeded BUP-Gluc concentrations in 77.9% of specimens that contained both analytes. In the thrice-weekly urine specimens, mean creatinine corrected BUP, NBUP, BUP-Gluc, and NBUP-Gluc were 32.0 ± 37.1, 169.3 ± 280.2, 137.5 ± 474.9, and 1055.5 ± 1832.2 ng/mL, respectively. Among all participants, mean BUP-Gluc to NBUP-Gluc ratio was significantly higher in the second trimester as compared to the third and there were significant intra-subject differences between trimesters in 71% of participants. Percent of dose excreted in 24 h during pregnancy was higher than post-pregnancy results, consistent with other data indicating increase in renal clearance of drugs during pregnancy.

These are the first data evaluating urinary disposition of BUP and metabolites in a cohort of pregnant women. Variable BUP excretion during pregnancy may indicate metabolic changes requiring dose adjustment during later stages of gestation.

This research was funded by the National Institutes of Health, National Institute on Drug Abuse, Intramural Research Program.

Keywords: **Buprenorphine, Urine, Disposition**

# ~ 2008 ERA Award Recipient ~

## S17 Evaluation of Buprenorphine Immunoassays for Compliance Monitoring in Heroin Substitution Therapy Program

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"Substitution therapy" and the use of buprenorphine (B) as an agent for treating heroin addiction continue to gain acceptance and was recently implemented in Taiwan. Low cost and sensitive immunoassay (IA) approaches greatly facilitate the implementation of analytical tasks associated with compliance monitoring and pharmacokinetic/pharmacodynamic studies. Following a study on the cross-reacting and calibration characteristics of commercially available IAs, those found favorable were applied to the analysis of urine specimens collected from a B substitution therapy program. Resulting analytical findings were compared against data derived from a gas chromatography-mass spectrometry (GC-MS) method.

All IAs studied exhibit low cross-reactivity toward morphine, codeine, hydrocodone, hydromorphone, and oxycodone, but dissimilar cross-reactivities toward different B metabolites, resulting in better correlation to the concentrations of different analytes as determined by GC-MS. Responses derived from three microplate-well and one analyzer-based IAs were found to correlate to the total concentration of B (B plus B glucuronide) with correlation coefficients ranging from 0.4 to > 0.9 (Figure 1). Respective correlation equations indicate corresponding apparent B concentrations derived from the responses of these four IAs range from 5 to 10 ng/mL for a urine specimen that was found to contain 10 ng/mL of total B as determined by GC-MS. While the microplate-well IAs exhibit low detection limits, the analyzer-based IA exhibits a wider calibration range and higher sensitivity. This latter product was further investigated to determine whether it could generate test results that are consistent with the GC-MS method. For this purpose, test data resulting from a group of clinical specimens (Table 1) were evaluated. As a result, when 25 and 35 ng/mL (apparent B concentration) are adopted, respectively, as the cutoffs for the GC-MS and CEDIA test methodologies, the IA method would result in 0 "false positive" and 1 "false negative". The corresponding data would be 1 and 2 if 50 and 60 ng/mL concentrations are adopted as the cutoffs. Since the analyte concentrations in all specimens fall in the vicinity of the critical cutoff concentrations, the number of specimens included in this study is significant and the evaluation result informative. In conclusion, the analyzer-based IA can be highly effective under high-volume testing settings.

***Disclosure Information:*** *This study was partially supported by a grant from the (Taiwanese) Bureau of Controlled Drugs (DOH96-NNB-1006). Immunoassay reagents were provided free of charge (or with special discount) from the following sources: Diagnostix Corp. (Mississauga, ON, Canada), Immunalysis Corp. (San Dimas, CA, USA), International Diagnostic Systems Corp. (St. Joseph, MI, USA), Neogen Corp. (Lexington, KY, USA), Microgenics (Fremont, CA, USA). The authors are also grateful to the technical assistance provided by Cheng-Chuan Chiang of Beam International Inc. (Taipei, Taiwan) and Mr. Ching-Chiang Lin of the Department of Laboratory Medicine, Fooyin University Hospital (Pingtong, Taiwan).*

Keywords: **Buprenorphine, Immunoassay, Glucuronide**

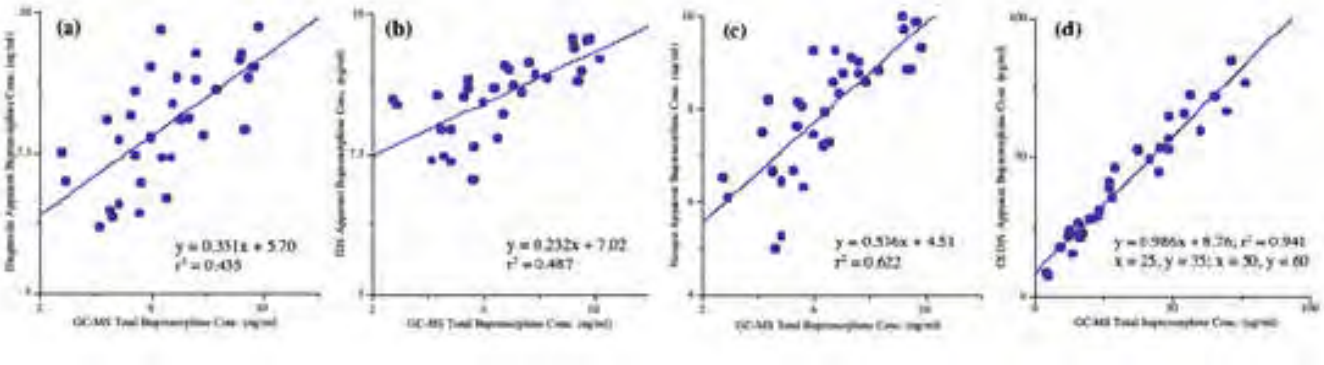


**Figure 1** Correlation of "IA apparent B" vs. "GC-MS B" concentrations with reagent from Diagnostix (a), International Diagnostic Systems (b), Neogen (c) and Microgenic (d).

**Table 1.** Comparison of test results generated by CEDIA immunoassay and GC-MS

| | Total B conc. & P/N by GC-MS | | | Apparent B conc. & P/N by CEDIA | | |
|---|---|---|---|---|---|---|
| Specimen | ng/mL | $(P/N)_{25}$ [a] | $(P/N)_{50}$ [b] | ng/mL | $(P/N)_{35}$ [a] | $(P/N)_{60}$ [b] |
| 1 | 75.9 | P | P | 77.0 | P | P |
| 2 | 55.8 | P | P | 72.6 | P | P |
| 3 | 69.3 | P | P | 67.0 | P | P |
| 4 | 12.0 | N | N | 22.1 | N | N |
| 5 | 41.5 | P | N | 49.9 | P | N |
| 6 | 50.2 | P | P | 58.7 | P | N (false N) |
| 7 | 23.4 | N | N | 31.3 | N | N |
| 9 | 37.0 | P | N | 46.0 | P | N |
| 10 | 71.7 | P | P | 13.3 | N (false N) | N (false N) |
| 11 | 15.2 | N | N | 27.1 | N | N |
| 13 | 4.35 | N | N | 8.90 | N | N |
| 15 | 36.8 | P | N | 52.9 | P | N |
| 16 | 9.19 | N | N | 18.0 | N | N |
| 17 | 20.3 | N | N | 34.0 | N | N |
| 18 | 16.0 | N | N | 21.2 | N | N |
| 19 | 23.0 | N | N | 29.1 | N | N |
| 20 | 23.7 | N | N | 31.6 | N | N |
| 22 | 20.8 | N | N | 28.5 | N | N |
| 25 | 15.9 | N | N | 0.00 | N | N |
| 26 | 22.1 | N | N | 32.0 | N | N |
| 29 | 33.0 | P | N | 41.3 | P | N |
| 30 | 44.8 | P | N | 45.1 | P | N |
| 31 | 48.3 | P | N | 65.2 | P | P (false P) |
| 33 | 59.8 | P | P | 60.0 | P | P |
| 34 | 27.0 | P | N | 39.2 | P | N |
| 35 | 48.5 | P | N | 57.1 | P | N |
| 36 | 12.6 | N | N | 24.2 | N | N |
| 38 | 48.3 | P | N | 53.5 | P | N |
| 39 | 65.1 | P | P | 72.1 | P | P |
| 40 | 70.7 | P | P | 85.0 | P | P |
| 42 | 15.7 | N | N | 22.9 | N | N |
| 44 | 27.2 | P | N | 42.0 | P | N |
| 45 | 19.6 | N | N | 28.4 | N | N |
| 48 | 45.5 | P | N | 53.8 | P | N |
| 49 | 28.0 | P | N | 35.8 | P | N |
| 50 | 29.0 | P | N | 46.7 | P | N |
| 51 | 54.3 | P | P | 66.4 | P | P |
| 53 | 16.9 | N | N | 23.3 | N | N |
| 57 | 13.4 | N | N | 15.6 | N | N |
| 58 | 5.26 | N | N | 7.90 | N | N |

[a] Cutoffs for GC-MS and CEDIA IA were set at 25 and 35 ng/mL, respectively.
[b] Cutoffs for GC-MS and CEDIA IA were set at 50 and 60 ng/mL, respectively.

# ~2008 YSMA Award Recipient~

## S18 Oxymorphone Concentrations in Postmortem Blood

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**Objective**: The purpose of this investigation is to report oxymorphone concentrations in postmortem blood and to highlight the recent trend of increased incidences of oxymorphone in this population.

**Method**: During routine toxicological analyses at The Office of the Chief Medical Examiner, Chapel Hill, postmortem blood is screened for a broad spectrum of drugs utilizing a combination of ELISA (cocaine metabolite, fentanyl, benzodiazepines, and opiates) and GC-NPD methodologies. Presumptive positive results are confirmed with mass spectrometry in a second aliquot of blood. Commencing January 2008, an oxycodone/oxymorphone-specific immunoassay screen was implemented in addition to the existing opiate ELISA test. Specimens that screen positive for opiates, either by immunoassay or GC-NPD, are confirmed by a previously published GC/MS method, which allows for the simultaneous detection of codeine, morphine, 6-acetylmorphine, hydrocodone, hydromorphone, oxycodone, and oxymorphone.

A retrospective study of cases involving oxymorphone in the absence of oxycodone was conducted from 1995 to March, 2008.

**Results**: Oxymorphone, unless specifically targeted, evaded detection during routine screening tests. Due to the recent increase in number of relatively young people who were dying but in whom no anatomical or toxicological cause of death could be found, an improved screening regimen for oxymorphone was implemented with the addition of an oxycodone/oxymorphone-specific immunoassay kit (cutoff 10 ng/mL). Prior to the immunoassay implementation, oxymorphone was only detected by a directed GC/MS method if the history suggested its use.

Looking at cases from 1995 to date, 14 cases were reported in which oxymorphone was involved. Of these cases, twelve were deaths that occurred since May 2007. Greater than 80% of the cases were ruled accidental oxymorphone toxicity deaths. The median concentration of oxymorphone in postmortem heart blood was 0.14 mg/L (range: < 0.05–0.31). The median age of the decedents was 37.5 (range 15-54). Eleven of the cases involved white males while the remaining three were white females. Eight of the subjects had a known history of drug abuse.

**Conclusion**: Since the advent, in June 2006, of a new formulation of oxymorphone (Opana®; Endo Pharmaceuticals, Inc., PA), there has been a significant increase in oxymorphone-related deaths. To date, there is no published data for oxymorphone concentrations in postmortem blood. Since the implementation of the oxycodone/oxymorphone immunoassay screen, two cases have been confirmed to contain oxymorphone, which would otherwise have gone undetected. The number of oxymorphone-related deaths prior to May 2007, when awareness of oxymorphone prevalence began, will remain an unknown but going forward these deaths will be detected. Additionally, concentration reference ranges in postmortem fluids and tissues will be published.

Keywords: **Oxymorphone, Postmortem, Blood**

# ~2008 ERA Award Recipient~

## S19 Correlations Between Maternal Buprenorphine Dose, Meconium Buprenorphine and Metabolite Concentrations and Neonatal Outcomes

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This study investigates the relationship between maternal buprenorphine (BUP) dose, meconium BUP and metabolite concentrations and neonatal outcomes in 10 infants born to nine women treated with cumulative BUP doses of 1330 – 2196 mg for the last 12 – 22 weeks of pregnancy. After delivery, infants were hospitalized for at least four days per standard hospital medical practice and evaluated for neonatal abstinence syndrome (NAS) six to eight times a day using a 19-item modified Finnegan Scale and given a score from 0-42. Birth weight (g), head circumference (cm), length (cm) and length of hospital stay were obtained from medical records. Time to NAS onset (h), peak NAS score, NAS duration (h), frequency of NAS (% NAS scores greater than 4) were compared to cumulative and third trimester BUP dose and meconium BUP and metabolite concentrations.

The first sensitive and specific liquid chromatography-tandem mass spectrometry method for the quantification of BUP and norbuprenorphine (NBUP) was developed and validated. Limits of quantification were 20 ng/g for BUP and NBUP with a linear dynamic range of 20 – 2000 ng/g. Free and total BUP and norbuprenorphine were determined by analyzing meconium specimens with and without enzymatic hydrolysis to allow estimation of the extent of conjugation. Total BUP concentrations ranged from 24-297 ng/g with a mean (±SE) of 131 ± 27 ng/g and a median concentration of 110 ng/g. Free BUP ranged from 24-240 ng/g (mean = 93 ± 23; median = 60 ng/g). Specimens contained higher concentrations of total and free norbuprenorphine, 324-1880 (mean = 754 ± 136 ng/g; median = 660 ng/g) and 331-1229 ng/g (mean = 610 ± 88 ng/g; median = 501 ng/g), respectively.

No relationship was identified between maternal buprenorphine dose and meconium concentrations or neonatal outcomes. A statistically significant negative correlation was found for free BUP and head circumference. Since there was a significant difference between free BUP and total BUP concentrations, the % free BUP was calculated (Free BUP/Total BUP X 100) and this also negatively correlated with head circumference. Additionally, there was a negative correlation between the ratio of free BUP to free NBUP and time to NAS onset and between %NBUP and time to peak NAS score. Total BUP, free BUP to free NBUP ratio and total BUP to total NBUP ratio were all significantly correlated to percent NAS scores greater than 4.

Due to the small specimen size (N = 10), trends in positive and negative correlations also are reported. Positive trends included free BUP concentrations and duration of NAS ($p=0.06$) and total NBUP concentrations and time to peak NAS score ($p=0.05$). Also, Free BUP concentrations tended to predict ($p=0.07$) percent NAS scores greater than 4. A negative trend between the ratio of total BUP to total NBUP and NAS onset also was observed ($p=0.05$). Free BUP concentrations tended to predict smaller head circumference, with and without the inclusion of twin data. Finally, when twins were excluded, there was a negative trend between free NBUP concentrations and head circumference ($p=0.08$) and the concentration of free BUP in meconium and infant length ($p=0.06$).

These data suggest the safety of BUP pharmacotherapy during pregnancy and give preliminary information on the effectiveness of buprenorphine and metabolite concentrations in meconium to accurately predict onset and frequency of NAS signs and symptoms.

Keywords: **Buprenorphine, Meconium, Neonatal Abstinence Syndrome**

## S20 Case Report: 'Knocked Out by Toxicology': The Use of Enflurane in a Domestic Homicide

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This case report details the murder of a 49 year old female by her husband, a bio-medical scientist employed at Doncaster Royal Infirmary, England, UK. The suspect knocked-out his wife using an anaesthetic-soaked cloth and then suffocated her with cushions, whilst their two children slept upstairs.

In the early hours of the 12th July 2007 the emergency services received a phone call from the husband stating that his wife, a heavy drinker, was unconscious on the sofa; she was not breathing, her lips were blue and her legs appeared mottled. Life was pronounced extinct less than 30 minutes later. A police investigation ensued following concerns being raised by friends of the deceased. There were allegations made which implicated the husband; it was alleged that he had spiked his wife's drinks and injected her, whilst she was sleeping, with pure alcohol.

Enflurane (ethrane) is a non flammable halogenated methyl ethyl ether that was introduced in the 1970's as an alternative to other halogenated anaesthetics such as halothane and methoxyflurane, whose biotransformation products have been implicated in hepatic and renal damage in man. Enflurane is a liquid at room temperature with a boiling point of 56.5°C and saturated vapour pressure is 23kPa at 20°C. It is no longer used as an anaesthetic, however, when it was used medicinally the drug was administered from an accurately calibrated vaporiser. The current threshold limit value for occupational exposure is 75ppm in the workplace air.

At lower concentrations the effects of enflurane are similar to those of alcohol; loss of inhibition, euphoria, drowsiness, dizziness, amnesia, nausea and vomiting. In high concentrations, blood pressure decreases and breathing is depressed. Several cases of severe acute hepatoxicity after enflurane use have been reported, including 2 fatalities.

Toxicology analysis of blood and urine samples was undertaken at the Forensic Science Service Laboratory in Chorley, Lancashire using enzyme immunoassay (EIA), high pressure liquid chromatography (HPLC) and gas-chromatography headspace and mass spectrometry (GCHS, GCMS). Hair samples collected from both the suspect and the deceased were analysed at ChemTox, France using liquid chromatography tandem mass spectrometry (LCMS/MS). Analysis confirmed the presence of enflurane in the victim's blood and urine, together with the hypnotic drug zopiclone (Zimovane®) in the suspect's hair.

Further investigations followed whereby the defendant's version of accounts was challenged scientifically by investigating the volatility of the anaesthetic at room temperature and pressure.

After hearing the forensic evidence, the jury returned a guilty verdict (11-1 majority) to the charge of murder. The husband was sentenced to life imprisonment, with a recommendation to serve a minimum of 15 years before being granted parole.

Keywords: **Anaesthetic, Homicide, Volatile**

## S21 Two Fatal Case Reports of Oxymorphone Occurring in Massachusetts and Virginia

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In June 2006 the Food and Drug Administration approved the new prescription drug Opana® for the treatment of moderate to severe pain. Two forms of the drug, Opana®ER and Opana®, contain oxymorphone hydrochloride and are formulated as extended-release and immediate-release versions, respectively. The objective of this presentation is to describe the toxicological findings for two fatal cases which included the detection and quantitative analyses of oxymorphone in Massachusetts (Case 1) and Virginia (Case 2).

In Case 1, a 28 year-old male, attending a bachelor party, was last seen alive by friends during the late pre-midnight hours and then early hours of the next morning. Friends stated the decedent had been drinking the entire night. Case 2 is a 21 year-old male, with a past history of OxyContin® abuse, who attended a party but was too intoxicated to drive home. At 7 am he was heard to be snoring loudly, but at 2 pm he was discovered dead. The toxicological findings for both cases are summarized below.

Toxicology Results

| Case 1 | Heart Blood | Vitreous Humor | Urine | Bile |
|---|---|---|---|---|
| Ethanol (% w/v) | 0.11 | 0.14 | 0.25 | 0.11 |
| Oxymorphone (mg/L) | 0.09 | NP | 0.21 | NP |
| **Case 2** | **Femoral Blood** | **Vitreous Humor** | **Urine** | |
| Oxymorphone (mg/L) | 0.08 | 0.05 | 1.9 | |
| Hydrocodone (mg/L) | ND | NP | 0.54 | |

NP - Not Performed ND - None Detected

Conclusion: Detection of oxymorphone in postmortem blood and other body fluids, resulting from intoxication by Opana®ER or Opana®, poses analytical challenges to laboratories relying on ELISA (cross reactivity with oxycodone) and conventional GCMS full scan front line testing modalities. Case 1, temporarily classified "pending" by the medical examiner, with an ELISA oxycodone OD just above the cutoff for a positive result was resolved by directed analysis for oxymorphone when additional police report information (e.g., confirmed finding of Opana®ER, 40 mg at the death scene) became available. The oxycodone ELISA result for Case 2 fell just below the positive cutoff concentration with other unremarkable toxicological findings. Case circumstances, the decedent's age, past history of OxyContin® abuse, and borderline oxycodone ELISA result collectively prompted the laboratory to conduct direct synthetic opioid analysis.

Keywords: **Opana®, Oxymorphone, Fatalities**

## S22 Ethylene Glycol Poisoning: Validation and Forensic Application of a GC-MS Method for Ethylene Glycol and Glycolic Acid in Postmortem Blood

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Ethylene glycol (EG), a relatively infrequent cause of fatal intoxication, presents an analytical challenge for forensic confirmation in the postmortem toxicology setting. We report the modification and validation of a previously reported serum GC-MS method for use in the confirmation and quantitation of ethylene glycol and its major acid metabolite, glycolic acid (GA), in postmortem blood following identification of EG by GC-FID screening. Postmortem blood, along with matrix matched calibrators and controls, was prepared for analysis by dilution, sonication, protein precipitation with acetonitrile containing internal standard (1,3-propanediol) and centrifugation. A water-scavenger assisted reduction in supernatant volume was performed with *N*,*N*-dimethylformamide and 2,2-dimethoxypropane treatment and partial dry down, followed by derivatization with *N*-methyl-*N*-(tetra-butyldimethylsilyl) trifluoroacetamide. Chromatographic separation was performed with a Varian 3900 GC using a 5% diphenyl / 95% dimethyl polysiloxane liquid phase capillary column (Restek Rxi-5ms). Detection was by mass spectral analysis using a Varian Saturn 2100T ion trap operated in full scan mode with selective quantification of EG (m/z 147 and 233) and GA (m/z 147 and 247) ions. EG and GA measurement was linear over a range of 50 to 2000 mg/L, with a positive threshold of 50 mg/L. Between assay precision (% CV) for EG (2.1-8.6%) and GA (4.3-6.0%) was determined along with accuracy of 91-102% (EG) and 94-102% (GA). EG and GA levels were below the positive threshold in postmortem blood from a series of cases (n=20) without evidence of EG exposure. The method was applied in twelve medical examiner cases where EG poisonings was suspected. EG concentration range in nine fatalities was 1010-8180 mg/L with a glycolic acid concentration range of 880-2000 mg/L. In two cases the EG concentrations was near threshold (64 and 95 mg/L) with corresponding GA concentrations of 1200 and 980 mg/L, and in one case the EG was negative with a GA of 100 mg/L. The EG concentration in postmortem blood by GC-MS showed close correlation ($R^2$=0.97) with EG quantitation performed by the GC-FID screening method but showed inconsistent correlation with GA concentration ($R^2$=0.22) by GC-MS. Mass fragmentation pathway was determined for EG and GA by GC-MS-MS analysis under resonant conditions as an additional molecular confirmation by transition ion identity. In conclusion, a sensitive method for confirmation and quantitation of EG and GA has been validated and postmortem blood studies show variable EG concentration but consistent elevation in GA concentration in EG associated fatalities.

Keywords: **Ethylene Glycol Poisoning, Glycolic Acid, Postmortem Blood**

## S23 Quantitation of 1,1-Difluoroethane from Post Mortem Tissues Using a Gas in Equilibrium Method of Calibration and Headspace Gas Chromatography.

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The volatile compound 1,1 difluoroethane (DFE), or Freon 152a, is a propellant and refrigerant used in a variety of commercially available aerosol products used for cleaning electronic equipment. When inhaled or "huffed", this halogenated hydrocarbon produces a sudden onset of intoxication, loss of muscular control and may precipitate sometimes-fatal cardiac arrhythmia. This presentation describes the method of analysis and the toxicological findings of a case in which impairment resulting from the abuse of DFE is thought have been a contributing factor in a fatal motor vehicle accident. The deceased, a 30 year old male, was observed by another motorist to drive erratically into the path of an oncoming passenger van. He was pronounced dead at the scene. Investigators found a canister of Dust Off® in the decedent's vehicle.

DFE was quantitated utilizing a recently developed gas in equilibrium method of calibration and headspace gas chromatography. The validated method uses two calibration curves based on 0.5 mL whole blood calibrators. Combined, these curves range from 0.225-180.0 mg/L. Linearity was 0.9992 and 0.9995 respectively, limit of detection (LOD) was 0.018 mg/L, limit of quantitation (LOQ) equaled 0.099 mg/L (recovery 111.9%, C.V 9.92 %), and upper limit of linearity (ULOL) was 27,000.0 mg/L. Combined recovery results of a 98.0 mg/L DFE control prepared using an alternate technique was 102.2% with C.V of 3.09%. Matrix interference was not observed in DFE enriched blood, urine or brain specimens. Using analysis of variance (ANOVA) no significant differences were detected ($\alpha$=0.01) in the area under the curve (AUC) of blood, urine or brain specimens at three identical DFE concentrations.

Application of this method to tissues collected at autopsy detected DFE at the following concentrations; femoral blood 11.5 mg/L, pulmonary artery blood 67.7 mg/L, aortic blood 55.8 mg/L, brain 55.2 mg/kg, vitreous 14.3 mg/L, liver 42.2 mg/kg, lung 47.1 mg/kg, adipose 5.1 mg/kg, bile 43.5 mg/L, and urine 27.1 mg/L. The concentrations of DFE were compared to the findings in four other fatal DFE related cases. Based upon these comparisons the author's conclude that the levels detected are consistent with abuse of and intoxication with DFE, which is likely to have contributed to the accident.

Keywords: **Difluoroethane, Huffing, Inhalant Abuse**

## S24 Homicidal Insulin Overdose or Hirata's Syndrome?

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**Objective:** When it is alleged that insulin has been used for homicidal purposes the defense may raise issues of causation. In two recent English cases it was suggested that fatal hypoglycemia may have been caused by Hirata's Syndrome rather than insulin overdose. This presentation describes this defense and the responses that may be made to it.

The use of insulin for homicidal purposes by health care staff is not uncommon in relative terms but rare in absolute terms. The prior probability than any episode of hypoglycemia is due to a deliberately administered insulin overdose is thus low.

The primary diagnostic investigation in hypoglycemia is to obtain a blood sample during the period of hypoglycemia. This should promptly analyzed for glucose, insulin, C-peptide, cortisol and β-hydroxybutyrate, with the remaining sample being stored frozen. Modern insulin immunoassays use high affinity antibodies that effectively measure the total rather than the free insulin in serum. Normally the pancreas secretes one molecule of C-peptide with each molecule of insulin. When hypoglycemia is present, the insulin concentration is high and the C-peptide concentration is low (undetectable with most assays), then the presumptive diagnosis is insulin overdose.

Hirata's syndrome, also known as Insulin Autoimmune Syndrome (IAS), is associated with circulating insulin antibodies. When the pancreas secretes insulin in response to a carbohydrate load, where insulin antibodies are present, these may bind insulin, releasing it later at a time when the blood glucose is normal and the pancreas is no longer secreting insulin or C-peptide. Such patients may become hypoglycemic with high insulin concentration and low C-peptide concentrations. Differentiation of insulin overdose from IAS depends on both clinical findings and on meticulous laboratory investigation, ideally at an early stage in any criminal investigation

**Conclusion:** Toxicologists must be prepared for challenges to the paradigm that the triad of hypoglycemia, high insulin and low C-peptide concentrations in plasma always means insulin overdose.

Keywords: **Homicide, Insulin, Antibodies**

## S25 Oxycodone Related Fatalities in the West of Scotland

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**Objectives:** In the United Kingdom, oxycodone is available on prescription in two forms, OxyContin (slow-release tablets) and OxyNorm (liquid/capsules). In Scotland, the number of prescriptions for oxycodone has risen by 430% since prescribing began in 2002 due to a shortage of diamorphine. Oxycodone misuse and related fatalities have been widely reported in the USA but it is hoped that stricter regulations will prevent a similar trend in the UK. The focus of this study is to review fatalities involving oxycodone in the West of Scotland using an LC-ESI-MS-MS method developed for the determination of oxycodone and its metabolites in post-mortem specimens.

**Methods and Materials**: Four cases were identified where oxycodone was implicated in the cause of death from a review of over 3000 cases. Deuterated internal standards were added to blood samples collected post-mortem and analytes extracted using Bond Elut C18® cartridges. Separation was achieved using a Synergy Polar RP column (150 x 2.0 mm, 4 μm) and gradient elution (mobile phase with (A) 10 mM ammonium formate, pH 3 and (B) acetonitrile) at a flow rate 0.3 ml/min using a Thermo-Finnigan LCQ Deca Plus instrument in the ESI SRM mode.

**Results**: The developed method for oxycodone and its metabolites, noroxycodone and oxymorphone was linear over the concentration ranges 5 - 250 ng/mL and 50 - 5000 ng/ml with correlation coefficients ($R^2$) greater than 0.999. Limits of detection and lower limits of quantification were 0.2 - 0.4 ng/mL and 1.0 - 1.2 ng/mL, respectively. Oxycodone, noroxycodone and oxymorphone were detected in three cases of oxycodone intoxication. In one case of poly-drug intoxication (tramadol, m,orphine, codeine, temazepam, oxycodone and amitriptyline), oxycodone and noroxycodone were detected at low levels but oxymorphone was not present.

**Conclusion:** A sensitive, selective and robust method for the determination of oxycodone, noroxycodone and oxymorphone was validated and applied to oxycodone intoxication cases. High levels of oxycodone in combination with low levels of noroxycodone and oxymorphone were identified in three cases of suicide involving the deliberate ingestion of multiple tablets of OxyContin. In all three cases a number of undigested OxyContin tablets were identified in the stomach contents. Oxycodone prescriptions have risen sharply in Scotland in recent years and the identification of four oxycodone-related death in the past 10 months in the Strathclyde region of Scotland alone highlights the importance of including this drug in routine laboratory screening and confirmation procedures.

Keywords: **Oxycodone, OxyContin, LC-ESI-MS-MS**

## S26 Free Oxymorphone Distribution in Biological Matrices: A Collection of Case Studies

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Oxymorphone is a schedule II, semi-synthetic opioid analgesic that, until recently, was administered predominantly in hospitals via an injection or suppository for pain management. It exists as both a parent drug and a metabolite of oxycodone. In 2006, an oral dosage was approved by the Federal Drug Administration for chronic pain in standard and extended release form. Beginning in 2007, the Montgomery County Coroner's Office/Miami Valley Regional Crime Laboratory began seeing postmortem and antemortem casework where oxymorphone was present without, or in a greater concentration than, oxycodone.

Cases positive for oxymorphone were initially detected by an opiate test kit for ELISA immunoassay. Due to the typically poor sensitivity of these kits for this drug, an oxycodone test kit with better cross-reactivity for oxymorphone was also used for identification. All positives were then confirmed by solid phase extraction on a co-polymeric bonded phase column. The specimens were eluted with a 4% ammonium hydroxide/ethyl acetate solution and derivatized with 1:1 BSTFA/1% TMS and ethyl acetate. The specimens were injected on one of two instruments based on availability: a 6890 and 7890 Gas Chromatograph/Mass Spectrometer (GC/MS), both with an inert source. Oxymorphone was measured using both oxycodone-$d_3$ and oxymorphone-$d_3$ as internal standards. At this time, both internal standards are under review to determine which is more suitable for this method. Quantitative testing was performed on whole blood, vitreous fluid, and urine based on specimen availability. If oxymorphone was confirmed, other available matrices including liver, brain, cerebrospinal fluid, and hair, were tested. All cases within this study contained multiple drugs in addition to oxymorphone.

The results showed that oxymorphone was distributed into all of the matrices tested. However, determining distribution ratios was not possible due to so few cases being available at this time. Oxymorphone is shown to have abuse potential and should be considered when opiates are a suspected avenue of toxicity. Its low therapeutic values and minimal cross-reactivity with most ELISA screening kits make it a drug that can be easily overlooked.

Keywords: **Oxymorphone, Matrix Distribution, Opioid Analgesic**

## S27 A Review of Cases Analyzed for Zopiclone/Eszopiclone

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Eszopiclone (Lunesta®), the s-isomer of zopiclone, is a relatively new drug that was approved for use by the U.S. FDA in 2004 for the treatment of insomnia. Pharmacokinetic data shows that a single 3 mg dose produces peak serum concentrations of 20 – 30 ng/mL within 2 hours. Over the course of 16 months NMS Labs, a national reference laboratory, has analyzed a variety of specimen types, including bodily fluids (e.g., blood, serum/plasma, urine) and liver tissue for the presence of zopiclone/eszopiclone by LC-MS/MS.

The analytical method utilized is briefly described as follows: After addition of internal standard (zopiclone-d4), specimens are prepared for analysis using a liquid-liquid extraction procedure with mixed solvent (63% Pentane/32% DCM/5% Isopropanol) at basic pH. The extract is evaporated to dryness under nitrogen, reconstituted and analyzed. Quantification is achieved by using HPLC separation with positive ion electrospray tandem mass spectrometry for detection. The method provides a limit of quantitation (LLOQ) of 2 ng/mL.

Even though the case histories for each specimen submitted were not readily accessible, the specimens were separated into three main groups for assessment purposes based upon the source of the specimen and the identity of the investigating agency. It should be noted that directed analysis for zopiclone/eszopiclone was performed because it was either specifically requested by the submitting agency or because its presence was indicated by comprehensive GC-NPD/GC/MS screening. The first group (Group I) represents postmortem specimens and consists of 94 blood specimens, 3 liver specimens and 1 urine specimen. The second group (Group II) contains 5 blood samples that were submitted by police departments and the last group (Group III) contains the remainder of the specimens, 27 blood specimens, 7 serum/plasma specimens and 23 urine specimens. Group III includes samples submitted from different types of facilities including hospitals, forensic science centers and reference laboratories.

The following table shows the data for the positive blood samples for zopiclone/eszopiclone by group:

| Group ID | Mean | Median | Range |
|---|---|---|---|
| I (n = 78) | 138 ng/mL | 32 ng/mL | 2.7 – 1800 ng/mL |
| II (n = 3) | 18 ng/mL | 16 ng/mL | 3.5 – 37 ng/mL |
| III (n = 18) | 127 ng/mL | 30 ng/mL | 5.4 – 570 ng/mL |

For the cases submitted by police agencies (Group II) it is of importance that all three of the positive blood specimens contained other substances that may potentially contribute toward impairment. The complete findings, by case, are as follows:

| Case # | Zopiclone/Eszopiclone Concentration | Other Drug Findings |
|---|---|---|
| 1 | 3.5 ng/mL | Norfluoxetine: 25 ng/mL, Zolpidem: 140 ng/mL, Tacrolimus: 7.7 ng/mL, Venlafaxine: 290 ng/mL and o-Desmethylvenlafaxine: 280 ng/mL |
| 2 | 16 ng/mL | Ethanol: 184 mg/dL and Gabapentin: 3.1 mcg/mL |
| 3 | 37 ng/mL | Diazepam: < 50 ng/mL, Nordiazepam: < 50 ng/mL and BZE: < 50 ng/mL |

Additional findings for Group I show that one liver specimen was positive with a concentration of 110 ng/g and that the urine specimen contained 23 ng/mL. Of note, for the blood specimens that had higher concentrations (> 100 ng/mL); many contained other drugs (e.g., oxycodone, hydrocodone, alprazolam, etc.) at toxicologically relevant concentrations. For Group III, a bimodal distribution was seen with 12 of the blood specimens having a concentration range of 5.4 – 40 ng/mL and 6 of the blood specimens having a concentration range of 260 – 570 ng/mL. Two serum samples were positive with concentrations of 110

ng/mL and 170 ng/mL, and four urine specimens were positive with a concentration range of 54 – 860 ng/mL.

Finally, while the analytical method described above does not differentiate eszopiclone from its optical isomer zopiclone, analytical methods that allow for this distinction may be employed if warranted.

Keywords: **Eszopiclone, Blood Concentrations, LC-MS/MS**

## S28 Preliminary Studies: Development of Drugs of Abuse in Liver Reference Materials

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**Introduction:** Matrix reference materials are an important component in the development, validation and ongoing QC/QA programs of analytical laboratories. This is challenging for forensic toxicology laboratories particularly for postmortem toxicology as matrix matched reference materials for tissue analysis are essentially nonexistent. RTI International has begun a preliminary study to develop liver reference materials containing drugs of abuse. The long-range goal of the study is to develop a product suitable for use as certified reference materials, quality control samples and proficiency test samples in forensic toxicology and other fields that involve testing of tissue samples.

**Objectives:** 1) To prepare pilot test materials of bovine liver material fortified with methadone and bovine liver material fortified with cocaine. 2) To achieve vial to vial variability of less than 10% in the pilot test materials. 3) Evaluate the handling of the material in practicing ME laboratories.

**Methods:** In RTI's fortification scheme, liver tissue was placed in a solution containing cocaine or methadone. After fortification, the test materials were freeze-dried, ground to a powder and packaged in serum vials at 4 grams each (equivalent to 14.4 grams of wet tissue). Ten vials of each test material (methadone-fortified liver and cocaine-fortified liver) were submitted to a reference laboratory for homogeneity testing. The methadone-fortified test material was analyzed for methadone and 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP). The cocaine-fortified test material was analyzed for cocaine and benzoylecgonine. Additional testing is currently being conducted in 4 postmortem laboratories.

**Results:** The theoretical concentration range for each of the test materials was 6,700-10,000 ng/g (freeze-dried powder). The methadone-fortified test material gave results of 32,614 ± 2,083 ng/g (freeze-dried powder) (RSD=6.39%). EDDP was detected in one of the test vials, but not detected in the remaining nine vials. The cocaine-fortified test material gave results of 11,503 ± 348 ng/g (freeze-dried powder) (RSD=3.03%). Benzoylecgonine was not detected in any of the test vials.

Homogeneity Testing of Drug-Fortified Liver

| | ***Methadone (ng/g)*** | ***Cocaine (ng/g)*** |
|---|---|---|
| ***Mean*** | ***32614*** | ***11503*** |
| ***SD*** | ***2083*** | ***348*** |
| ***%RSD*** | ***6.39*** | ***3.03*** |

Keywords: **Matrix Reference Materials, Postmortem Toxicology, Quality Control**

## S29 The Temporal Fate of Drugs in Decomposing Porcine Tissue[α, ß]

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**Introduction*:*** Determining the presence of drugs and interpreting the meaning of their concentration in decomposed tissues remains is difficult (1) because there is currently little, or no information, available to help guide the toxicologist, and subsequently the pathologist, in evaluating whether a drug(s) played any significant role in causing the death. This study monitored concentrations of drugs in multiple tissues and organs during whole body decomposition. Specific questions addressed were: 1) how does the concentration of drugs change during decomposition and 2) which collection sites are most useful for detecting drug exposure and interpreting results?

**Materials and Methods:** The concentration of 16 drugs in tissue from decomposing pigs (*Sus domestica),* 120-150 lbs, was monitored in blood, brain, liver, kidney, muscle, maggots and soil during one week. Pigs were divided into groups (n=5) with each group receiving 4 drugs. Drug cocktails were prepared from pills (capsules or tablets) obtained as pharmaceutical formulations. Sacrifice of pigs (intracardiac pentobarbital) was 4 hours after dosing (gavage), with tissue collection at 4, 24, 48, 96, and 168 hrs post-dosing. Samples were frozen until assay. Detection and quantitation of drugs was through SPE followed by GC/MS analysis. Morphine was derivatized with heptafluorbutyric anhydride and assayed by SIM.

**Results:** This study was performed during summer months resulting in rapid loss of tissues. Brain and kidneys were not available after 48 hrs. Muscle and liver persisted for 1 wk. Results for liver are shown below. Analysis of muscle showed average levels of drugs increasing but amitriptyline was the only drug where the trend of increase achieved statistical significance.

Table 1: Average Concentration of Drugs (µg/g) in Decomposing Pig Liver Over Time

| | ----------Time---------- | | | | | Relative Increase | |
|---|---|---|---|---|---|---|---|
| Drugs | 4 hr | 24hr | 48 hr | 96hr | 168 hr | 4 to 96 or168 hrs | p value[τ] |
| Morphine | 0.23 | 0.44 | 1.22 | 4.2 | | 18.6 | 0.119 |
| Amitriptyline | 14.6 | 35.4 | 92.9 | 169.2 | 248.3 | 17.7 | 0.014 |
| Citalopram | 5.0 | 17.0 | 38.8 | 75.4 | 49.2 | 9.8 | 0.304 |
| Diazepam | 4.7 | 12.2 | 16.8 | 42.7 | 51.5 | 10.9 | 0.010 |
| Methadone | 2.5 | 10.8 | 7.8 | 23.8 | 25.3 | 10.1 | 0.081 |
| Fluoxetine | 16.1 | 38.0 | 27.7 | 55.2 | 83.6 | 5.2 | 0.060 |
| Doxepin | 66 | 177 | 146 | 339 | 398 | 6.0 | 0.011 |
| Acetaminophen | 5 | 20 | 66 | 74 | 63 | 12.6 | 0.002 |
| Oxycodone | 1.1 | 2.7 | 3.0 | 3.9 | 3.6 | 3.3 | 0.270 |
| Diphenhydramine | 33 | 72 | 116 | 113 | 151 | 4.6 | 0.129 |
| Venlafaxine | 67.2 | 135.7 | 273 | 304 | 405 | 6.0 | 0.012 |
| Carisoprodal | 21.3 | 38.8 | 139 | 216 | 300 | 14 | 0.001 |
| Verapamil | 6.9 | 9.6 | 10.1 | 12.4 | 20.4 | 3.0 | 0.145 |
| Zolpidem | 3.2 | 3.4 | 6.6 | 6.9 | 13.1 | 4.0 | <0.0001 |
| Propoxyphene | 13.4 | 44 | 62 | 78 | 86 | 6.6 | 0.005 |

[τ]Significance of the F statistic on the trend by one-way analysis of variance (ANOVA)

**Conclusion:** Liver and muscle persisted longer than other tissues and the concentration of the drugs analyzed increased with time during decomposition. Attempting to interpret drug levels in decomposed bodies may lead to incorrect conclusions about cause and manner of death.

[α]*This research was performed in accordance with the National Research Council's "Guide for the Care and Use of Laboratory Animals", and was approved by the ILACUC, The Ohio State University, Columbus, OH.*

[ß]*This research was funded by a grant from Midwest Forensics Resource Center, Ames, IA.*

Keywords**: Drugs, Decomposing, Tissue**

## S30 Direct Determination of Ethyl Glucuronide and Ethyl Sulphate in Post-Mortem Specimens using Hydrophilic Interaction Liquid Chromatography–ESI-MS

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**Background**: The interpretation of alcohol results in post-mortem specimens can be difficult due to the possibility of post-mortem production of alcohol, which may take place in the body or in the autopsy samples. Ethyl glucuronide (ETG) and ethyl sulphate (ETS) are primary ethanol metabolites and have been shown to be useful markers of alcohol consumption for several hours after death or when ethanol itself has been completely eliminated from the body. ETG and ETS are very polar metabolites requiring very low percentages of organic modifiers (less than 5%) for elution from a conventional reversed phase column, which results in poor retention, large matrix effects and low sensitivity in LC-MS. Post-column addition of organic solvent can enhance ESI-MS-MS response while preserving good chromatographic peak shapes. Recently, hydrophilic interaction chromatography (HILIC) has been introduced as an alternative to reverse phase LC separation of polar compounds. HILIC is suitable for ESI-MS as a high percentage of organic modifiers can be used, up to 90%, without reducing analyte retention.

**Aims**: This work was aimed at developing and validating a HILIC-ESI-ion trap-MS-MS method for identification and quantification of ETG and ETS as ethanol biomarkers and at employing this method for routine analysis of post-mortem samples.

**Methods**: Following addition of pentadeuterated internal standards for ETG and ETS, 200 µl of acetonitrile was added to 0.1 ml of urine and centrifuged at 10000 rpm. The supernatant was then evaporated before reconstituting with 100 µl of initial mobile phase. Analytes of interest were separated on a ZIC-HILIC column (150 x 2.1 mm, 3.5 µm) (SeQuant, Umea, Sweden) connected to a Thermo-Finnigan LCQ Deca Plus LC-MS-MS instrument operated in the ESI-SRM mode. Gradient elution used a mobile phase with (A) 5 mM ammonium acetate and (B) acetonitrile. Analytes were identified on the basis of their retention times and the relative intensities of their pseudo-molecular ions and two product ions.

**Case samples**: 90 urine case samples were divided into three groups depending on the ethanol concentration found in blood and analysed by the developed method: group A with post-mortem blood ethanol higher than 0.2 g/100 mL; group B with ethanol concentration in the range 0.08 to 0.2 g/100 mL and group C with ethanol concentration less than 0.08 g/100 mL.

**Results:** ETG and ETS had high recoveries of 98-99 % and the HILIC column produced fine, sharp peak shapes and achieved baseline separation in less than 7 min. The calibration model was linear over a concentration range of 0.05-10 mg/L and correlation coefficients ($R^2$) were better than 0.999. In addition, a lower limit of quantification of 0.001 mg/L was obtained and no matrix effects were observed. The method has been used for analysis of ETG and ETS in routine case samples and has been shown to be a useful tool to indicate recent ethanol consumption. Both ethanol markers were detected in all groups with overall median concentrations of 113.6 mg/L and 23.2 mg/L for ETG and ETS, respectively.

**Discussion and Conclusions:** It can be concluded that the risk of false positive ethanol results increased in the low ethanol concentration group as several cases tested negative for both biomarkers in group C. ETG was detected at low concentrations in some cases for which ETS tested negative, suggesting that ETG may have a longer half life in urine or else ETS is unstable. Our data were compared with previous studies and confirm that both ethanol biomarkers should be determined in heavily putrefied cases and when the ethanol level in post-mortem blood is low, which suggest the production of ethanol after death. To the authors' knowledge, this is the first report of the determination of ETS using an LC-ESI-ion trap-MS-MS method, and of a HILIC-ESI-ion trap-MS-MS method for the simultaneous determination of ETG and ETS in post-mortem urine samples.

Keyword: **Ethyl Glucuronide, Ethyl Sulphate, HILIC-ESI-MS-MS**

## S31 Simultaneous Quantification of Nicotine, Opioids, Cocaine and Metabolites in Human Postmortem Brain by Liquid Chromatography Tandem Mass Spectrometry

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**Objective:** A quantitative LC-TurboIonSpray-MSMS method for the simultaneous, rapid and simple determination of nicotine, opioids, cocaine and metabolites in small specimens of human postmortem brain was developed and validated.

**Method:** Preparation of calibration curves and quality control samples employed ultrasonic homogenization of 100 mg drug-free brain tissue in pH 6 sodium phosphate buffer and solid phase extraction. Analytes of interest included nicotine, cotinine, *trans*-3'-hydroxycotinine (OH-cotinine), cocaine, benzoylecgonine (BE), ecgonine methyl ester (EME), morphine, codeine, and 6-acetylmorphine (6AM). Separation and quantification were accomplished on a Phenomenex Synergi Hydro-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 4 min. The flow rate was 200 µL/min, with re-equilibration for 3 min. Mass spectrometry data were collected in positive ion mode and analyte identification and quantification were based on selected reaction monitoring. The following transitions were monitored: *m/z* 163 to 132, 84 for nicotine, *m/z* 177 to 80, 98 for cotinine, *m/z* 193 to 80, 134 for OH-cotinine, *m/z* 304 to 182, 82 for cocaine, *m/z* 290 to 168, 105 for BE, *m/z* 200 to 182, 82 for EME, *m/z* 286 to 152, 165 for morphine, *m/z* 300 to 152, 165 for codeine and *m/z* 328.3 to 165, 152 for 6AM.

**Validation:** Specificity, sensitivity, limits of detection and quantification, linearity, precision, accuracy, extraction efficiency, carryover effect, matrix effect, and stability were evaluated. Method validation was accomplished in five days in five unique assays.

**Results:** Total chromatographic run time was 10 min. Calibration was by linear regression analysis with deuterated internal standards and a weighting factor of 1/x. Linearity was achieved from 5-5000 pg/mg for BE and cocaine; 25-5000 pg/mg for cotinine, EME and 6AM; 50-5000 pg/mg for OH-cotinine, and codeine; 250-5000 pg/mg for nicotine with limits of detection of 2.5, 15, 30 and 200 pg/mg, respectively. There was no endogenous interference for any analyte in ten blank human brain specimens, demonstrating good specificity. In addition, 29 common over-the-counter or abused drugs at 50,000 pg/mg did not interfere with any analyte in low quality control samples. Intra- and inter-assay recoveries were ≥ 92% and intra- and inter-assay imprecision was ≤ 14.6% RSD for all analytes. Parameters were tested at three concentrations across the linear dynamic range of the assay for each analyte. Extraction efficiency from brain tissue was ≥ 67.2% for all analytes.

**Conclusion**: The assay provides simultaneous quantitative analysis of nicotine, opioids, cocaine and metabolites in human brain with sufficient sensitivity to allow quantification of drugs in small amounts of tissue. This procedure will be employed to monitor drugs in fetal brain tissue.

This research was funded by the National Institutes of Health, National Institute on Drug Abuse, Intramural Research Program.

Keywords: **Nicotine, Brain, LCMSMS**

## S32 Cocaine Analytes in Human Hair I: Evaluation of Concentration Ratios in Drug User Populations

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**Introduction:** The disposition of controlled substances in hair and effects of environmental contamination on hair drug-testing results is not yet understood. Current interpretation practices employ cocaine analyte cut-off concentrations and a benzoylecgonine-to-cocaine (BE/COC) ratio ≥0.05 to distinguish COC use from external contamination; however, ratios of other analytes, such as cocaethylene (CE) and norcocaine (NCOC), have yet to be fully investigated. The primary research goal was to evaluate COC concentrations and analyte-to-parent drug concentration ratios in human hair of two drug use populations (e.g. street use with unknown dose and controlled drug administration with known dose).

**Methods:** 38 head hair specimens were collected from drug users in a street environment (e.g., STREET). Another 20 head hair specimens were collected during inpatient clinical studies at NIDA Intramural Research Program following controlled drug administration (e.g., CLINICAL) and written informed consent. Hair specimens were collected at peak drug concentrations after subcutaneous low (75 mg/kg) and high (150 mg/kg) COC HCl doses. Hair specimens were analyzed by liquid chromatography-tandem mass spectrometry with limits of detection, quantification and linearity for cocaine analytes of 25, 50 and 10,000 pg/mg, respectively. Deuterated internal standards, a quantitative ion and 2 confirming ions were employed for all analyte concentration determinations. COC analyte concentration ratios were statistically evaluated (ANOVA) to determine significant differences**.**

**Results:** Cocaine analyte concentrations in hair specimens were determined and 5 different criteria for positive results were evaluated. The first 3 criteria were originally proposed by Substance Abuse and Mental Health Services Administration (SAMHSA) Mandatory Guidelines (Federal Register 69: 19673-10732, 2004) and additional criteria were evaluated for CE/COC and NCOC/COC ratios (See Table). While the COC and BE concentrations were significantly greater in STREET drug users than CLINICAL specimens, the CE and NCOC concentrations were not ($p > 0.05$). Similarly, the BE/COC and NCOC/COC ratios were significantly different in STREET drug users than CLINICAL specimens ($p < 0.0001$). STREET drug users' specimens were never positive by CE or NCOC criteria without also meeting BE criteria. For CLINICAL drug users' hair, there were 3 instances in which a positive test met CE and NCOC, but not BE criteria.

| **STREET Drug User Specimens** | | | | | **CLINICAL Drug User Specimens** | | | |
|---|---|---|---|---|---|---|---|---|
| | **COC*** | **BE*** | **CE*** | **NCOC*** | **COC** | **BE** | **CE** | **NCOC** |
| N | 38 | 38 | 20 | 32 | 20 | 17 | 7 | 14 |
| Range (pg/mg) | 159 – 218,276 | 65 – 67,253 | ND* – 5,003 | ND – 1,810 | 725 – 32,786 | ND – 1,501 | ND - 397 | ND – 2,075 |
| Mean (pg/mg) | 27,889† | 8,132† | 901 | 345 | 6,171† | 424† | 123 | 290 |
| Median (pg/mg) | 12,470 | 1,746 | 436 | 253 | 4,141 | 336 | 75 | 142 |
| **Criteria for Positive Result (pg/mg)** | | **# Positive Tests** | | **%** | **Criteria for Positive Test** | | **# Positive Tests** | **%** |
| COC ≥ 500 & BE ≥ 50 & BE/COC ≥ 0.05 | | 36 | | 95 | COC ≥ 500 & BE ≥ 50 & BE/COC ≥ 0.05 | | 14 | 70 |
| COC ≥ 500 & CE ≥ 50 | | 19 | | 50 | COC ≥ 500 & CE ≥ 50 | | 6 | 30 |
| COC ≥ 500 & NCOC ≥ 50 | | 33 | | 87 | COC ≥ 500 & NCOC ≥ 50 | | 14 | 70 |
| COC ≥ 500 & NCOC ≥ 50 & NCOC/COC ≥ 0.01 | | 24 | | 63 | COC ≥ 500 & NCOC ≥ 50 & NCOC/COC ≥ 0.01 | | 14 | 70 |
| COC ≥ 500 & CE ≥ 50 & CE/COC ≥ 0.002 | | 17 | | 45 | COC ≥ 500 & CE ≥ 50 & CE/COC ≥ 0.002 | | 6 | 30 |

* Cocaine, benzoylecgonine, cocaethylene, norcocaine, none-detected; † Indicates values significantly different at $p<0.05$

**Conclusions:** Results were consistent with lower dosages administered in clinical inpatient studies as compared to self-reported drug use from street environments. Furthermore, criteria for distinguishing COC

positive tests (e.g., decision points) indicated that proposed NCOC and CE cut-off concentrations of ≥50 pg/mg and NCOC/COC ratio ≥0.01and CE/COC ratio ≥0.002 did not identify as many subjects as proposed BE criteria. Generally, COC analyte concentrations in drug user populations suggest that NCOC and CE concentrations and ratios to parent COC may not identify drug use more effectively than COC and BE criteria for hair drug testing. However, following low cocaine exposure, additional cocaine analytes may be valuable to identify cocaine use.

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Key Words: **Cocaine Analytes, Hair, Concentration Ratio**

## S33 Cocaine Analytes in Human Hair II: Evaluation of Concentration Ratios in Different Cocaine Sources and Surface-Contaminated Specimens

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**Introduction:** This study's primary goal was to evaluate COC analyte concentrations and analyte-to-parent drug concentration ratios in human hair to include COC source (e.g., pharmaceutical, street drug) and COC purity. This research evaluated the effect of COC composition and external contamination on COC analytes and analyte-to-parent ratios found in hair.

**Methods:** Hair specimens were contaminated by a previously published contamination model (Stout et al., *J Anal Toxicol*, 2006) using confiscated illicit COC hydrochloride (COC-HCl; Source-*DEA and NIDA*) and commercially available COC-HCl (Source- *U.S. Pharmacopeia*). Drug free hair was contaminated with powdered cocaine of varying purity (see Table). The contaminated hair specimens were subjected to daily hygienic treatments and collected weekly for 10 weeks following a decontamination procedure. All specimens were blinded prior to shipment to the testing laboratory, and drug-free blind control specimens were included with the study specimens. The contaminated hair and the COC-HCl samples used to contaminate the hair were analyzed using liquid chromatography-tandem mass spectrometry. COC analyte concentrations and ratios were statistically evaluated to determine significant differences.

| Sample ID of Contaminating Cocaine | Cocaine | BE | CE | NCOC |
|---|---|---|---|---|
| **COC-HCl_27 (DEA)** | **82.20%** | **10.10%** | **1.43%** | **0.80%** |
| **COC-HCl_20 (NIDA)** | **88.5%** | **0.16%** | **<0.01%** | **8.70%** |
| **COC-HCl_28 (USP)** | **98.9%** | **<0.01%** | **1.10%** | **Not reported** |

**Results:** All three COC sources resulted in significant quantities of COC remaining on the hair over the course of 10 weeks. As previously observed, there was a significant decline in the COC analyte content of contaminated hair over the course of the study. Hair contaminated with COC containing higher CE, BE, or NCOC concentrations resulted in significantly higher concentrations ($p=0.0001$) of each of these drug compounds in the respective hair specimens. At some time point in every hair contamination study, the analyte-to-parent concentration ratios were determined to be above 0.05. BE/COC was consistently greater in the contamination studies using DEA and USP cocaine sources while the NCOC/COC concentration was greater for the contamination study using the NIDA cocaine source. Furthermore, evaluation criteria using CE/COC and NCOC/COC drug concentration ratios did not provide a means to distinguish COC use from possible environmental exposure.

**Conclusions:** These results indicate that the concentration of a COC analyte found in hair can be affected by its relative amount in the cocaine specimen used for the contamination process, although the relationship may not be linear. BE/COC ratio increased significantly over time and could not be used reliably to identify COC-contaminated hair. The results also indicate that the COC cut-off concentrations and ratios currently used by many forensic drug-testing laboratories may not effectively discriminate between drug use and environmental exposure. Further research is needed to determine if additional decision criteria including unique COC metabolites, wash criteria or mathematical criteria that compare the presence of a drug in wash solutions to concentrations in hair will adequately and reliably identify external COC contamination.

Key Words: **Cocaine, Hair Analysis, Contamination**

## S34 Urine Ethanol, Ethyl Glucuronide (EtG) and Ethyl Sulfate (EtS) – What do the Numbers Show?

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**Objectives:** The purpose of this study was to evaluate trends in urine ethanol and urine EtG/EtS concentrations obtained from thousands of paired ethanol and EtG/EtS specimens. EtG and EtS concentrations in urine specimens were also correlated. The data is presented to the scientific community in an attempt to help establish guidelines for EtG and EtS testing in urine and to aid in understanding and interpreting urine ethanol and EtG/EtS results.

**Methods**: Specimens submitted to the laboratory for urine ethanol and EtG/EtS were first screened for ethanol using an Enzyme Assay method with a 0.04gm/dL screening cutoff. The screened positives were subjected to GC confirmation. The limit of quantitation was 0.02 gm/dL. Since the specimens were unpreserved, a microscopic examination for the presence of yeast (Candida sp.) was performed on all ethanol positives to rule out fermentation as a consideration. All specimens were also screened for EtG using a fully validated LC/MS/MS method with a screening cut off of 100ng/mL. A separate aliquot of the screened EtG positives was extracted and subjected to separate LC/MS/MS analysis for confirmation. Both EtG and EtS were monitored in the confirmatory method to increase analytical specificity. A cutoff of 100 ng/mL for EtG and 25 ng/mL for EtS was used. Both EtG and EtS were required to be present in the specimen above the laboratory cutoff levels in order to call a specimen positive for EtG/EtS.

**Results**: 59438 specimens were received in a six month period for ethanol and EtG/EtS testing. 7488 (12.6%) were positive for ethanol, EtG/EtS or both. These specimens were divided into three categories:

**1** – Ethanol and EtG/EtS positives. 547 specimens out of 560 were positive for ethanol, EtG and EtS with 83.5% specimens having EtG concentrations above 10000ng/mL, 11.9% had EtG concentrations between 1000-10000ng/ mL and 4.4 % had EtG concentrations below 1000ng/mL. EtS was above 1000ng/mL in 93% of these specimens. 13 specimens (2.3%) were negative for EtS but positive for ethanol and EtG. 10% (56) of the total specimens in this category (**1**) contained yeast.

**2** – Ethanol positives, EtG negatives. 147 specimens tested positive for ethanol and negative for EtG. Yeast was detected in 69% of these specimens indicating that ethanol may have been present due to fermentation. 4% of the specimens were positive for EtS only with concentrations in excess of 5000 ng/mL indicating EtG may have been subjected to bacterial decomposition.

**3** – Ethanol negatives, EtG/EtS positives. 6781 specimens were negative for ethanol but positive for EtG and EtS (95.9%) or EtG only (4.1%). This is over 90% of the total positives in all three categories indicating the importance of EtG/EtS testing in ethanol abstinence programs. 62.9% of the specimens had EtG concentrations in excess of 1000 ng/mL, and 25.6% had EtG concentrations above 10,000 ng/mL in this category.

**Conclusions**: 1) The majority of ethanol positive urine specimens were positive for both EtG and EtS and had very high concentrations for both; EtG>10,000 ng/mL and EtS> 1000 ng/mL. 2) EtS concentration in more than 90% of the specimens, in general, is lower than EtG; with the EtS concentration being 33% of EtG on an average. This should be useful when establishing cutoff values for EtG/EtS in urine. 3) The presence of yeast in more than 69% of the specimens testing positive for ethanol and negative for EtG indicated the probability of fermentation in these cases. The absence of EtG can also be attributed to decomposition by bacteria in some cases and hence, EtG/EtS combined test should be used since EtS is not known to be susceptible to bacterial decomposition. Urine preservative use should be recommended as well. 4) Majority of the EtG and EtS positives (90%) are negative for alcohol indicating the importance of EtG/EtS testing in abstinence programs.

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

## S35 Preservatives for Stabilizing Ethyl Glucuronide and Ethyl Sulphate in Urine

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**Objectives:** Investigation of the influence of different preservatives on the stability of ethyl glucuronide (EtG) and ethyl sulphate (EtS) in urine during storage at different conditions, simulating non-cooled transportation of samples and storage under laboratory conditions.

**Methods:** Urine sterilized by filtration was spiked with EtG and EtS. Glucuronidase-positive *Escherichia coli* and different preservatives were added and positive and negative control samples were combined. The preservatives used were thymol, chlorohexidine, boric acid and the combination of chlorohexidine, ethyl parabene and sodium propionate. The urine samples were stored at varying temperatures, 4-8°C, at 18±1°C and at 36±1°C, for a period of 9 days, sampling daily. The EtG- and EtS-analysis was performed by LC-ESI-MS/MS. The number of bacteria was determined by counting the colony forming units on Columbia blood agar plates. In addition, urine samples of 10 healthy, social drinkers were stored in commercially available sample tubes with boric acid and in tubes without preservative for 5 days at 36°C to simulate their use in the daily routine.

**Results:** Irrespective of the storage conditions no significant decrease or increase of EtG and EtS and no growth of *E. coli* was seen in the control samples without bacteria. In urine samples with *E. coli* but without preservatives only refrigerated storage inhibited the growth of bacteria and the decomposition of EtG and EtS. After 4 days at 36°C and after 8 days at room temperature the concentration of EtG was largely reduced (by over 90% on average). The concentration of EtS remained stable. The addition of chlorohexidine alone as well as in the aforementioned combination had a bacteriocidal effect and no significant degradation of EtG and EtS was observed. Boric acid also exhibited a bacteriostatic effect and stable concentrations of EtG and EtS were detected over the time period investigated. The addition of thymol in a concentration of 100 mg/L did not inhibit the bacterial growth and EtG, but not EtS, was largely degraded. The practical test with commercial tubes showed, that boric acid does not interfere with the analytes during LC-MS analysis and can therefore be recommended for routine analysis because of its bacteriocidal effect.

**Conclusions:** Chlorohexidine on its own as well as in combination and boric acid proved to be useful preservatives, while EtG was degraded in samples doped with thymol.

Keywords: **Ethyl Glucuronide, Preservatives, Bacterial Decomposition**

## S36 Correcting Urine Drug Concentrations by Normalization to Specific Gravity and Creatinine

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Drug metabolism and urinary excretion rates are variable from individual to individual. In addition, the amount of fluid intake by individuals on a day-to-day basis can create large variations in urine drug concentrations. Methods for correction ("normalization") of drug/metabolite concentrations in urine have been utilized by anti-doping laboratories, pain monitoring programs and in environmental monitoring, but such procedures are rarely used in workplace, legal and treatment settings. We evaluated two specific correction procedures based on specific gravity and creatinine urinary determinations. The specific gravity correction of drug/metabolite concentrations in urine was based on the Levine-Fahy equation (*J. Indust. Hygiene Toxicol.* 27**:** 217-223, 1945) as follows:

$$Concentration_{SG\ corrected} = Concentration_{specimen} \times (SG_{reference} - 1) / (SG_{specimen} - 1)$$

where drug/metabolite concentration is expressed in ng/mL units, $SG_{reference}$ is a population reference value for specific gravity representing "normal" or "nondiluted urine" and $SG_{specimen}$ is the specific gravity of the test specimen. The $SG_{reference}$ utilized in all corrections was 1.0200. The procedure developed in this study for creatinine correction of drug/metabolite concentrations in urine was based on the following equation:

$$Concentration_{CR\ corrected} = Concentration_{specimen} \times (CR_{reference}) / (CR_{specimen})$$

where drug/metabolite concentration is expressed in ng/mL units, $CR_{reference}$ is a population reference value for creatinine representing "nondiluted urine" and $CR_{specimen}$ is the creatinine concentration of the test specimen. The $CR_{reference}$ utilized in all corrections in this study was 100 mg/dL. It should be noted that the procedure developed in this study for creatinine correction differs from other procedures, often referred to as "creatinine normalization", which involves calculation of the ratio of drug concentration to creatinine concentration.

Drug/metabolite corrections were performed with specimens from three distinct groups of drug users (pain patients, heroin users, and marijuana/cocaine users). Each group was unique in characteristics, study design and dosing conditions. Comparison of concentrations by specific gravity and creatinine corrections were highly correlated (mean correlation coefficient = 0.94; range, 0.78-0.99). Increases in percent positive specimens by specific gravity and creatinine correction procedures were small (0.3% and -1.0%) when applied to specimens from the heroin users (normally hydrated subjects), modest (4.2-9.8%) for pain patients (unknown hydration state), and substantial (1.4% positivity for uncorrected marijuana versus 49.3% and 53.5% for corrected specimens, and 25.3% positivity for uncorrected cocaine versus 86.5% and 84.0% for corrected specimens) for the marijuana/cocaine users (excessively hydrated subjects). Some limitations on use of these correction procedures that are worthy to note include disease processes that increase or decrease specific gravity, under-estimation of high creatinine concentration by some assays, and influences on urinary creatinine from exercise, diet, age, weight, and gender. Despite such limitations, these correction procedures provide alternative means of dealing with highly dilute, dilute and concentrated specimens. Drug/metabolite concentration normalization by these procedures in association with normalized cutoff concentrations is worthy of consideration in drug testing and monitoring programs.

Keywords: **Specific Gravity, Creatinine, Normalization**

## S37 Determination of Drugs of Abuse in Oral Fluid by Liquid Chromatography and Tandem Mass Spectrometry

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There is increasing interest in the use of oral fluid for drugs of abuse testing for employees at workplaces, as oral fluid is less invasive, easy to collect and relatively less susceptible to adulteration compared to urine. Hence an analytical method was developed for the simultaneous determination of multiple drugs in oral fluid using solid phase extraction (SPE), followed by liquid chromatography-tandem mass spectrometry (LC-MS-MS). Multiple drugs including amphetamine, methamphetamine, 3,4-methylenedioxymethamphetamine, 3,4-methylenedioxyethylamphetamine, codeine, morphine, 6-monoacetylmorphine (6-mam), cocaine, benzoylecgonine, ecgonine methyl ester and Δ9-tetrahydrocannabinol (THC) were extracted with ethyl acetate/hexane and dichloromethane/isopropanol using UCT Clean Screen SPE columns. The extract was evaporated and reconstituted into 50% methanol. All analytes were separated by LC Gemini C18 (50 x 2.0 mm, 3um) column using a gradient elution at a flow rate of 0.3 ml/min with 95% and 5% acetonitrile in 10 mM ammonium acetate as a mobile phase. Mass detection was performed by an electrospray ionization in the multiple reaction monitoring (MRM) mode on an Applied Biosystems 3200 Q Trap LC-MS-MS. Analytes were identified by two MRM transitions respectively and quantified by using deuterated analytes as internal standards.

All analytes were detected within 7 min. The standard curve was linear between 2 and 250ng/ml with curve correlation coefficients exceeding 0.99. The coefficients of variation ranged from 2% to12% for intra-assay and from 10% to 17% for inter-assay for all analytes. Limits of detection and quantitation were at 2 ng/ml for THC and 6-MAM, and at 5 ng/ml for the others. Recovery of all analytes from solid phase extraction ranged between 73% and 99%. This LC-MS-MS method provides a rapid, simple, and specific determination of the most commonly abused drugs in oral fluid.

Keywords: **Oral Fluid, LC/MS/MS (Liquid Chromatography –Tandem Mass Spectrometry), Solid Phase Extraction**

## S38 Multiple Drug Use by Ecstasy Abusers in the United States

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The abuse of designer amphetamines such as 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy) and 3,4-methylenedioxyamphetamine (MDA) is generally associated with young adults attending "Rave" parties. Frequently, other "club" drugs also are available and multi-drug use is commonly reported for this population. Little toxicological information has been reported, however, regarding ecstasy usage by individuals undergoing monitoring in prison/probation/parole (Legal) and other settings in the United States. The goal of this study was to determine if multi-drug use also was common to these groups. Our survey of laboratory data over the years 2005-2007 revealed that approximately 284,000 urine specimens had been screened for ecstasy-type drugs. From these, a total of 198 (0.07%) urine specimens were confirmed positive (cutoff concentration = 100 ng/mL) for MDMA and/or MDA from the following types of donors (# positive specimens): Legal (159); Sports (19); Workplace (9); Pain patients (8); and Specialty (3). Of these, 122 (61.6%) were positive for MDMA and MDA, 70 (35.4%) were positive for MDMA, and 6 (3.0%) were positive for MDA. The median concentration ratio of MDA/MDMA (range) was 0.11 (0.01-0.60), suggesting that MDMA was the primary drug of abuse and that MDA was generally present as a metabolite or a minor contaminant of MDMA. A majority (84.3%) of the specimens contained multiple drugs and/or metabolites in addition to MDMA and MDA. The median number of drugs/metabolites reported for the 198 specimens (range) was 5 (1-9). The percentage of specimens positive for one or more analytes were as follows: one, 8.1%; two, 26.3%; three, 23.7%; four, 17.2%; five, 13.1%; six, 5.6%; seven, 2.5%; eight, 2.5%; and nine, 1.0%. Taking into consideration that some drugs are metabolized to other commercially available forms of drugs, the median number of drugs used (range) was 3 (1-6). In addition to MDMA/MDA, the most commonly identified drug groups (%) were as follows: cannabis (THCCOOH) (61.6%); amphetamine/methamphetamine (38.4%); cocaine (BZE) (30.8%); diazepam-related (9.6%); opiates (7.1%); alprazolam (5.6%); and others (5.6%). Of the 76 specimens positive for amphetamines, 75 were positive for methamphetamine and 27 were positive for amphetamine. Only one specimen was positive for amphetamine (absence of methamphetamine) and 49 were positive only for methamphetamine. Twenty-six specimens contained both amphetamine and methamphetamine. The median concentration ratio of amphetamine/methamphetamine (range) was 0.26 (0.04-1.18), suggesting that amphetamine was present primarily as a metabolite of methamphetamine. However, as both methamphetamine and amphetamine have been identified as "contaminants" of illicit ecstasy tablets, differentiation of intentional use from unknowing use of these two drugs was not possible in this study. In summary, this study revealed that the majority of ecstasy users in these monitoring programs (primarily Legal settings) self-administered multiple drugs in combination with MDMA. Almost two-thirds of the ecstasy users had positive urine tests for cannabis and one-third was positive for methamphetamine or cocaine. Numerous examples were documented of individuals who had combined use of ecstasy with cannabis, cocaine and methamphetamine.

Keywords: **Ecstasy, MDMA, Drugs of Abuse**

## S39 An Overview of Arizona's Model Statewide DUI Enforcement Program: Innovation in Enforcement, Education and Partnerships

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This is an overview of the Arizona statewide DUI enforcement program Areas covered will include innovations in enforcement, education, reporting and partnerships. Arizona has long been recognized for its successful Drug Recognition Expert (DRE) program, DUID prosecution and integration of resources to combat drug impaired driving. DUID case facts, including the successful use of DRE, toxicology and prosecution in Arizona will be discussed.

**Enforcement:** Arizona law enforcement agencies statewide, join together regularly for DUI enforcement efforts. The various law enforcement agencies routinely conduct saturation patrols and sobriety checkpoints for alcohol and drug impaired drivers. Working together and sharing resources assists law enforcement efforts and the high visibility enforcement also serves as a substantial DUI deterrent.

Arizona's Law Enforcement Phlebotomy Program is the only one of its kind, providing training and qualifying officers to draw blood on impaired driving cases. Training is provided by three Arizona colleges and is fully funded by the Arizona Governor's Office of Highway Safety (GOHS). Currently, an excess of 180 officers per year are trained, with more than 700 officers trained since the program's inception. The ability to provide expedient blood collection in DUI cases has been highly beneficial to the state's DUI enforcement effort.

**Education:** Arizona is very proactive in regard to public, law enforcement and prosecutor education and training in the area of impaired driving. Several high profile, impaired driving media campaigns are conducted year round. Law enforcement and prosecutors are afforded training opportunities year round throughout the state. These classes include: SFST (Standardized Field Sobriety Testing), ARIDE (Advanced Roadside Impaired Driving Enforcement), DEC (Drug Evaluation and Classification), Law Enforcement Phlebotomy, and Prosecuting the Alcohol and Drug Impaired Driver. Arizona forensic toxicologists are considered a primary resource for DUI training and are regularly involved in law enforcement and prosecutor education, including: DRE Schools, DRE In-Services, statewide impaired driving conferences and prosecutorial trainings and conferences.

**Reporting:** DUI task force agencies are required to input their activity into a database that provides statewide DUI statistics to the media, by the day following enforcement efforts. This assists in keeping the public informed of law enforcement efforts against impaired driving and assists in making Arizona roadways safer.

Arizona is currently implementing LEADRS (Law Enforcement Advanced DUI/DWI Reporting System), an on-line centralized DUI database. This system will help address issues prolonging DUI arrest times by standardizing and simplifying DUI reporting. Officers, prosecutors and toxicologists have assisted in the design of this program for Arizona.

**Partnerships:** The Arizona GOHS has formed partnerships with law enforcement, prosecutors and toxicologists to promote communication and create consistent and innovative training and program management in the statewide DUI effort. The Arizona DRE Steering Committee and DUI Task Force meet regularly to discuss impaired driving issues, with representatives from all areas of the program represented. Because potential obstacles to successful DUI prosecution are often discussed, these partnerships enables possible solutions to be identified before a problem becomes wide-spread. Personnel from each of the areas of expertise work together regularly, in support of statewide traffic safety efforts.

Keywords: **Drug Impaired Driving, DRE**

## S40 Building on 2005 National Roadside Survey Pilot Study: Expansion of Drug Test Profiles

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**Background:** In 2005, the National Roadside survey was carried out with three main goals: To develop crash risk estimates associated with alcohol and drugs; to measure national progress in reducing prevalence of alcohol and drug-impaired driving; and to determine feasibility of oral fluid and blood collection from night-time drivers.

**Methods:** The specimens were collected from a nighttime driving population at six different locations in the USA: Los Angeles County, CA; Gilpin County, Jefferson County, CO; Douglas County, NE; Bibb County, Tuscaloosa County, AL; Wake County, NC; and Ocean County, NJ. Overall, 639 oral fluid and 394 blood samples were collected and shipped to the laboratory for analysis. Samples were tested for a wide range of drugs: cocaine, amphetamines, opiates (including oxycodone), phencyclidine, cannabinoids, benzodiazepines, tricyclic antidepressants, methadone, methylphenidate, sertraline, fluoxetine, barbiturates, tramadol, zolpidem and carisoprodol. All subjects providing a blood sample also provided an oral fluid; all subjects providing oral fluid did not necessarily consent to blood collection.

**Results and Discussion:** Thirty-three (33) pairs of samples were positive for at least one drug and the results correlated very well. Sixty-seven oral fluid samples (10.4%) were positive. The main discrepancy was benzodiazepines, which have low saliva: plasma ratio. Three blood samples were positive; corresponding oral fluid samples were not.

| ***Drug Class*** | ***Oral fluid positive*** | ***Blood positive*** |
|---|---|---|
| Tricyclic antidepressants | 1 | 1 |
| Amphetamines | 5 | 5 |
| Carisoprodol | 1 | 1 |
| Cocaine and metabolites | 4 | 4 |
| Fluoxetine | 4 | 4 |
| Hydrocodone | 2 | 2 |
| Oxycodone | 1 | 0 |
| Sertraline | 5 | 5 |
| Tramadol | 2 | 2 |
| ***Benzodiazepines*** | ***0*** | ***3*** |
| ***Tetrahydrocannabinol (THC)*** | ***15*** | ***11*** |
| ***THCA and /or 11-OH THC*** | ***Not tested*** | ***+ 3 = 14*** |

For cannabinoids, oral fluid detected four more cases than blood for the active component, THC. When the metabolites THCA and 11-OH-THC were included in the confirmation profile for blood, three of those four were identified.

**Future direction:** Several improvements were made for the 2007 study. In order to improve benzodiazepine oral fluid positivity rates, the screening antibody was re-formulated to target low level drugs such as alprazolam, clonazepam and lorazepam; new confirmatory methods using LC/MS/MS were developed; meperidine, ketamine, dextromethorphan and propoxyphene were added to the test profile.

Keywords: **Oral Fluid, DUID (Driving Under the Influence of Drugs)**

## S41 LAPD: Prevalence of Drugs in Addition to Alcohol at BAC Levels Above the Legal Limit

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California Vehicle Code (V.C) 23152(a) states, "It is unlawful for any person who is under the influence of any alcoholic beverage or drug, or under the combined influence of any alcoholic beverage and drug, to drive a vehicle." At the Los Angeles Police Department Scientific Investigation Division, blood samples from individuals arrested in violation of a variety of laws, including V.C. 23152, and measuring at or below 0.08% BAC are subjected to a routine ELISA blood screen using Immunalysis kits for PCP, cocaine, opiates, amphetamine, methamphetamine, THC, barbiturates and benzodiazepines. Recently, we conducted a study to expand the window to include 0.15% BAC and below in order to ascertain what drugs, if any, were found in addition to higher alcohol levels.

The data collected included 431 cases over a 7-month period from October 2007 to April 2008. This information comprised a majority of all samples analyzed in the Blood Alcohol Section of the Toxicology Unit that met the above criteria. Of the 431 samples, 40% of cases with an alcohol level of 0.09-0.15% BAC screened positive for at least one of the eight drugs included in the screen. The most prevalent drugs detected at these levels were THC (with a cutoff of 25 ng/mL), cocaine (50ng/mL) and benzodiazepines (100ng/mL) with percentages of 62%, 24.5% and 17.5% respectively, in relation to positive values. Quantitative results for cocaine-positive samples were also tabulated in relation to alcohol levels since all cocaine-positives are confirmed by GC/MS, owing to degradation issues of cocaine in blood.

Information regarding drug-positive cases can be useful not only in a court of law as an addition to driving under the influence of alcohol, but also as an explanation of symptoms inconsistent with ethanol impairment at the time of arrest. From these results, the number of drivers under the influence of drugs is significantly underestimated in the city of Los Angeles due to the policy of drug testing only those samples that lie at or below the statutory alcohol limit. These data are important as law-makers consider the necessity of expanding legislation with respect to the drug impaired driver. The data presented here may also be an underestimation of drug impairment because of the limitations of the ELISA panel. Toxicology laboratories commonly restrict the cases that undergo drug analysis because of limited resources; however, in this era of increased prescription drug abuse this topic deserves greater attention.

Keywords: **DUID, Blood Drug Screening, Blood Alcohol**

## S42 Crawford Motions & Lab Management OR Which Toxicologist(s) Testifies?

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*Crawford v. Washington*, 541 U.S. 26 (2004) stands for the proposition that the Defendant has the right to confront witnesses against him. In the realm of scientific evidence, who is the witness the Defendant gets to confront? Many jurisdictions allow the introduction of toxicology results with just a sworn certification from the analyst or laboratory director. Other jurisdictions call as a witness in trial the individual that performed the analysis and obtained the results. Finally, jurisdictions have allowed the use of toxicology experts to admit the results of the tests. Currently there is a new case (*Melendez-Diaz v. Massachusetts*, 07-591) under review at the United States Supreme Court. The question presented is:

"Whether a state forensic analyst's laboratory report prepared for use in a criminal prosecution is "testimonial" evidence subject to the demands of the Confrontation Clause as set forth in *Crawford v. Washington*, 541 U.S. 36 (2004)."

If the answer to the question is "Yes" then all laboratory personnel who performed analytical testing will have to be called to the stand to introduce the results in every trial.

A recent vehicular homicide case in Pima County, Arizona, involved the use of prescription drugs and alcohol. The Defendant's blood contained the following drugs: Lamictal, Cymbalta, Nordiazapam, Diazempam, Oxycodone, Morphine, Lamotrigine and alcohol. Two different labs analyzed the multiple blood samples, a local crime laboratory for alcohol analysis and a large out-of-state private laboratory for the drug toxicology. The private laboratory that confirmed the drug samples used different analysts at each stage of the process, resulting in potentially more than 10 witnesses for the drug quantifications alone. Using those witnesses in trial and allocating travel time, would have resulted in serious operational consequences for the laboratory and great expense for the prosecutor's office.

Laboratory directors and/or managers have traditionally used many different personnel to prepare the sample, screen the sample, confirm some drugs in the sample, and confirm other drugs in the sample. There may be at least four potential witnesses (or more) for a trial who will be sitting in the hallway waiting to testify at the courthouse rather than performing toxicology analyses in the lab. This may raise significant issues related to productivity and costs. For laboratories which cover larger geographical areas, it may result in the laboratory being very selective in the drugs which are tested, resulting in less testing, fewer positives and symptoms which may be in conflict with the drug(s) identified. All of these factors may hinder a successful prosecution.

Keywords: **Trial, Toxicology, Crawford**

## S43 The Propensity Towards Phencyclidine, Paraplegia and the Playoffs in Driving Impairment in Lake County, Ohio.

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The authors present the case history and toxicological findings of four impaired driver cases in a one-year period (June 2007 – June 2008) that were positive for phencyclidine. A review of the four case histories will reveal some unusual and interesting scenarios.

Phencyclidine (PCP) is a dissociative drug with hallucinogenic and neurotoxic properties. Initially introduced as a surgical anesthetic post WWII, it was subsequently discontinued due to the preponderance of adverse psychoactive effects. PCP works as an N-methyl-D-Aspartate (NMDA) receptor antagonist; blocks dopamine reuptake and elevates synaptic dopamine levels. PCP has been shown to produce hallucinations, disorientation, image distortion, mood disorders, memory loss and paranoia.

As a recreational drug, PCP is commonly ingested by smoking plant material (marijuana, herbs) dipped in or sprayed with the liquid form. Other routes of administration include insufflation, intravenous injection, oral ingestion and potential transdermal absorption. Toxicology testing was performed on all cases, using ELISA for initial drug screening and GC-NPD and GC-MS for confirmation and quantitation. PCP blood concentrations ranged from 40 to 90 ng/ml. NHTSA reports blood PCP concentrations ranging from 10 to 180 ng/mL (mean 73 ng/mL) in 50 subjects arrested for driving under the influence of PCP.

Case 1 involved a male motorcycle driver traveling at approx. 100 mph in a posted 25 mph zone. Driver ran stop signs and was weaving in and out of traffic during pursuit before losing control and crashing. Driver advised he had been smoking PCP about an hour before the accident. Toxicology revealed PCP only in blood (50 ng/ml). (was PCP only finding?) at what concentration?

Case 2 involved a male driver traveling on the gravel berm for a distance of approx. 200 feet and then crossing the center white line. Driver informed the responding officer that he did 1 – 2 grams of cocaine, smoked a couple of marijuana joints, smoked some PCP and had approx. 5 drinks earlier that day. Toxicology of blood revealed ethanol (16 mg/dl), marijuana metabolite (qns for quant), cocaine metabolites (benzoylecgonine 415 ng/ml, ecgonine ME pos) and PCP in blood (qns for quant) (include quants if you have them); and ethanol, marijuana metabolite, cocaine and metabolites, amphetamine, methamphetamine, MDA, MDMA and PCP in urine

Case 3 involved a paraplegic driver who was observed driving recklessly at a high rate of speed prior to involvement in a serios injury crash. The driver was extricated from the vehicle and transported by life-flight to a trauma center. Toxicology revealed marijuana metabolite (qns for quant) and PCP in blood (90 ng/ml). (Quants)

Case 4 involved a driver weaving in his lane of travel. The driver admitted to smoking marijuana laced with PCP approximately 3 hours prior to stop while watching the NBA playoffs. Toxicology revealed marijuana metabolite (52 ng.ml) and PCP in blood (40 ng/ml). (Quants)

Prior to 2007 there were only two impaired driver cases in a five-year period (2002 – 2006) that were reported as positive for PCP in Lake County.

Keywords: **Phencyclidine, Hallucinogen, Impairment, PCP**

## S44 Carisoprodol and Drug Impaired Driving in Arizona

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The Arizona Department of Public Safety (AZ DPS) Toxicology Section has seen an increase in the number of driving under the influence of drugs (DUID) cases involving carisoprodol, a drug commonly prescribed for musculoskeletal pain and severe muscle spasms. The drug is a potent central nervous system (CNS) depressant, and has a high dependency potential. Carisoprodol is rapidly metabolized into meprobamate, a pharmacologically active metabolite that acts as a CNS depressant with sedative hypnotic properties. Pronounced impairment results from the combined CNS effects of these drugs. A summary of Arizona DUID data involving carisoprodol and a comparison of these findings to previously published DUID data will be provided. The presentation will conclude with a case study that exemplifies the typical impairment exhibited by Arizona drivers under the influence of carisoprodol.

The AZ DPS Laboratory Information Management System (LIMS) database was searched for DUID cases where the presence of carisoprodol and meprobamate were qualitatively confirmed by GC-MS (reporting cut off = 1000 ng/ mL for both drugs). The search was limited to blood samples submitted between January 1, 2006 and June 30, 2008 and to cases where Drug Recognition Expert (DRE) evaluations were performed. From this data, the case frequency of DUID/ DRE cases involving carisoprodol was determined, and drug classes commonly combined with carisoprodol were identified. Police reports and DRE evaluations were located for cases where carisoprodol was the only impairing drug confirmed.

Between January 1, 2006 and June 30, 2008 AZDPS confirmed 138 carisoprodol DUID/DRE cases. In 18 of these cases, carisoprodol was the only impairing drug confirmed. In 84 of the 138 cases, individuals were under the influence of one or more additional CNS depressants (excluding alcohol). Narcotic analgesics and cannabis were also commonly detected in the presence of carisoprodol and often in combination with other CNS depressants (excluding alcohol). The individuals in the 18 DUID/ DRE cases where only carisoprodol was detected displayed the following general signs and symptoms consistent with driving impairment: disorientation, lack of responsiveness, poor balance, poor coordination, and horizontal gaze nystagmus. Of particular interest was the degree to which these drivers were disoriented at the time of police contact. Many drivers believed they had fallen asleep at the wheel, or were completely unaware they had caused a collision. Drivers made statements such as “Where am I? What is going on?” and were unable to respond to simple questions regarding personal information. These findings are consistent with previously published DUID data involving carisoprodol (Logan *et al*, 2000). The related CNS-depression, tendency towards polypharmacy, and observed driving impairment underscores the danger of driving under the influence of carisoprodol.

Keywords: **Carisoprodol, DUID (Drug Impaired Driving), DRE**

## S45 Zolpidem Blood Concentrations in DUI Related and Postmortem Cases in Georgia

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In recent years, zolpidem and its effects on driving have become a widespread topic of national discussion among law enforcement community, compelling the toxicologist to provide expert opinion more frequently on its role in traffic-related cases. In metropolitan Atlanta, the number of traffic related cases with a positive finding of zolpidem has tripled since 2005. This may be due in part to an increased use of the sleep aid as well as expanded screening of traffic related (DUID) cases utilizing LC/MS/MS by the Georgia Bureau of Investigation Headquarters Laboratory.

A total of 199 DUID (2005-2008) and postmortem cases (2007-2008) with a quantitation of zolpidem in a blood specimen were examined. For the majority of cases zolpidem quantitation was preformed using LC/MS/MS with a limit of report of 0.025 mg/L. The DUID cases (n =125) examined had levels ranging from 0.025 mg/L to 2.4 mg/L with mean and median concentrations of 0.36 mg/L and 0.22 mg/L, respectively. Zolpidem was the only drug detected in 14% of those cases. Postmortem cases (n=74) covered a similar range, 0.025 mg/L to 5.3 mg/L with the mean and median concentrations of 0.51 mg/L and 0.15 mg/L, respectively. Zolpidem was the only analyte in just 3% of those cases. A finding of three or more drugs in addition to zolpidem, accounted for 36% of DUID cases and 78% of postmortem cases. Predictably, with the regularity of poly drug use, the combined case percentage for zolpidem in combination with other compounds such as benzodiazepines, opioids, stimulants, SSRI's, and other CNS-acting compounds was prevailing.

Because of the large increase of zolpidem findings and since 66% of all zolpidem DUID cases in the study period were submitted following a traffic accident of some kind, the incident reports for cases containing zolpidem only and cases containing zolpidem and an SSRI were collected and evaluated for trends. Manifestations and behavior resulting from CNS depression having a negative impact on driving performance was recorded in all cases, but could not be correlated to blood levels. Effects exhibited by drivers at "therapeutic" levels were similar to those observed in drivers at the highest blood concentrations of zolpidem.

Keywords: **Zolpidem, Driving Performance, Drug Concentrations**

## S46 Trends in Driving Under the Influence of Drugs: A Register-Based Study of 30,000 DUID Suspects During 1977–2007 in Finland

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Driving under the influence of drugs (DUID) is a significant public health and traffic safety related problem. Illicit drugs and some psychoactive medicines have been shown to impair driving skills and increase the risk of traffic accident. The aims of this study were to describe the incidence and trends in suspected DUID cases over the last three decades, and to look at the demographics and drug findings in these cases.

The study was a longitudinal, retrospective, register-based study of all apprehended DUID cases in Finland during 1977–2007. There were a total of 32259 suspected DUID cases with a positive finding of illicit/licit drug impairing driving performance. Toxicological results were analyzed from blood and/or urine specimens in one central laboratory.

From 1977 – 2007, the incidence of DUID suspects increased 18-fold during. Most of the suspects were male (89.7%) but the male-female ratio decreased from 13.9 to 7.3 thorughout the study period. The mean age of DUID suspects remained static at about 36.2 years except for 2001 when there was a drop in the mean age to 29.1 The most frequently found substances were benzodiazepines (75.7%), amphetamines (46.0%), cannabinoids (27.9%) and opioids (13.8%). The most frequent illicit drugs were amphetamines and cannabinoids, which started to appear at the end of 1980s. There was a sharp increase in suspected DUID cases after the introduction of a zero tolerance law, enacted in YEAR, especially with regards to amphetamines. In 60.8% of the cases, two or more drugs were found in blood and/or urine.

The incidence of DUID in Finland seems to be increasing, partly because legal acts and police activity make it more visible. Driving under the influence of drugs is a problem that needs serious attention.

Keywords: **Drugs, Driving, Road Safety**

## S47 Alcohol and Drug Use Among Motorcycle Driver Fatalities in Washington State From 2005-2007

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Nationally, the number of motorcycle fatalities has been rising every year, with a trend towards increased deaths in the over-40 age group, and for riders riding larger engine motorcycles. Statistics collected by NHTSA have documented the role of alcohol in motorcycle fatalities, with declining rates of alcohol use from 43% of fatalities in 1996, to 34% in 2006[1]. Much more limited data are available regarding rates of drug use by the fatally injured motorcycle population. A study of 106 injured motorcycle operators seen in a trauma center found 31% positive for marijuana use, 47% for ethanol use, and 8% for cocaine use[2]. We report the results of testing performed in the State of Washington of fatally injured motorcycle operators from 2005 to 2007. In each case, blood or urine was screened for alcohol by headspace gas chromatography and for drugs by EMIT ® immunoassay, testing at a minimum for THC metabolites, amphetamines, cocaine metabolite, opiates, methadone, and benzodiazepines. Additional drug screening for other drugs was performed in select cases.

A total of 213 cases were reported in the three year period. Six percent were female and 94% were male. The average age was 39 (range 18-61). Samples from 210 of the 213 reported cases were screened, and the results were as follows: Alcohol (>0.02g/100mL), 36.7%; Marijuana metabolites, 21.9%; Amphetamines, 9.5%; Opioids, 9.0%; Benzodiazepines, 9.5%; and Cocaine metabolite, 5.2%. Overall, 59.5% of cases tested positive in blood or urine for some psychoactive drug. Rates of drug use appear to have remained unchanged over the three year period.

There were marked age dependant differences in the drugs present. In the 18-20 age group (n=14), only 35.7% were positive for psychoactive drugs, with the most common substances being marijuana (28.6%), and alcohol (14.3%). Marijuana positivity demonstrated a decline with age in the over 30 age groups, with positive rates of 33.3%, 21.6%, 21.2%, and 8% in the 21-30, 31-40, 41-50, and >50 age groups respectively. Opiates were most common in the >50 age group (16.0%), cocaine in the 31-40 age group (13.5%), and amphetamines in the 41-50 age group (17.3%).

Rates of psychoactive drug use by this population are extraordinarily high. A study of drug use by all fatally injured drivers in Washington State[3] showed 39% of all drivers testing positive for psychoactive drugs, compared to 59.5% of motorcycle operators.

This survey is preliminary in nature, as not all cases received comprehensive screening beyond the immunoassay. Rates of psychoactive drug use in this population could be higher than these results suggest. Additionally, it is important to note that the presence of drugs and metabolites in blood or urine indicates drug use, not impairment. Further work should be done employing comprehensive drug and alcohol testing of blood from fatally injured motorcycle operators, and these findings considered in the context of operator behavior, and crash causation.

Keywords: **Drugs, Motorcycle, Fatality**

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## S48 Smoked Cannabis and Doping Control: Are We Looking for the Wrong Target Analyte?

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Since 2004, Cannabis is prohibited by the World Anti-Doping Agency (WADA) for all sports at competition. In the years since then, about half of all positive doping cases in Switzerland have been related to Cannabis consumption. In most cases, the athletes plausibly claim to have consumed Cannabis several days or even weeks before competition and only for recreational purposes not related to competition. In doping analysis, the target analyte is 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH), the reporting threshold 15 ng/mL. However, the wide detection window of this long-term THC metabolite does not allow a conclusion concerning the time of consumption or the impact on the physical performance. Therefore, the evaluation of other target analytes with shorter elimination half-life is needed and the aim of the present pharmacokinetic study.

n a 1-session clinical trial (approved by IRB, Swissmedic, and Federal Office of Public Health), 12 healthy, male volunteers (age 26 ± 3 yrs, BMI 24 ± 2 kg/m$^2$) with Cannabis experience (≤ once/month) smoked a Cannabis cigarette standardized to 70 mg THC/cigarette (Bedrobinol® 7%, Dutch Office for Medicinal Cannabis) following a paced-puffing procedure. Plasma and urine was collected up to 8 h and 11 days, respectively. THC, 11-hydroxy-THC (THC-OH), and THC-COOH concentrations were determined by SPE followed by GC/MS-SIM. The limit of quantitation (LOQ) for all analytes was 0.1 ng/mLVisual Analog Scales (VAS) and vital functions were used for monitoring psychological and somatic effects at every timepoint of specimen collection (up to 480 min).

Eight puffs delivered a mean THC dose of 45 mg. Mean plasma levels of THC, THC-OH and THC-COOH were measured in the range of 0.1-20.9, 0.1-1.8, and 1.8-7.5 ng/mL, respectively. Peak concentrations were observed at 5, 10, and 90 min. Mean urine levels were measured in the range of 0.1-0.7, 0.10-6.7, and 0.1-13.4 ng/mL, respectively. The detection windows were 2-8, 2-96, and 2-120 h. No or only mild effects were observed (dry mouth, sedation, tachycardia).

Instead of THC-COOH, the pharmacologically active THC and THC-OH should be the target analytes for doping urine analysis. This would allow the detection of recent Cannabis consumption probably influencing performance during competition

Keywords: **Cannabis, Doping, Clinical Trial, Plasma and Urine Levels**

## P01 The Comparison of Orasure ELISA Reagents with Randox Biochip Reagents in Screening Barbiturates, Benzodiazepines, Opiates and Cocaine

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Due to the nature of postmortem samples some ELISA results can be unreliable. This study will determine if the Randox Investigator can offer more reliable results with postmortem blood and liver samples than Orasure ELISA reagents. To test the analyzer, several decomposed livers and bloods were selected for this study, as well as fresh postmortem samples.

For this study, data was obtained from a Randox Investigator, an ELISA system which utilizes a unique ceramic antibody coated wafer in place of the more commonly used antibody coated well kits. The antibody coated wafer, referred to as a biochip by Randox comes with ten different antibody binding sites for detection of multiple analytes from a single sample. The data obtained from eighty-five post mortem samples were compared to data obtained from a BioChem Immuno Systems Personal Lab. The Personal Lab is a two plate automated ELISA instrument using Orasure reagents. While the Evidence Investigator tests for ten assays, four were utilized in this study: Barbiturates, Opiates, Benzodiazepines and Cocaine. These four analytes are the ones routinely tested in our laboratory by ELISA. All positive results were then tested further to confirm and identify the analytes present.

The following table shows the positive data from both systems tested:

| | Barbiturates | Benzodiazepines | Opiates | Cocaine |
|---|---|---|---|---|
| Orasure Reagents | 7 | 28 | 15 | 20 |
| Randox Reagents | 0 | 26 | 13 | 17 |
| Confirmed Positives | 0 | 27 | 15 | 18 |

The most dramatic difference between the instruments was results obtained with the Barbiturate assay. The 7 Positive Orasure cases were confirmed to be false positives by GC (FID). The Orasure reagents had 2 false positive Cocaine results or 10 % as compared to Randox which had 1 false positive or 5% as confirmed by GCMS. The Opiate results show 2 false negatives or 13% for the Randox and 0% for the Orasure reagents as confirmed by GCMS. Finally the Benzodiazepine assay gave 1 false positive or 3% with the Orasure reagent and 1 false negative or 3% result with Randox reagents as confirmed by GC (ECD) and LCMS.

The Randox Investigator performance with decomposed bloods and liver homogenates clearly showed an improvement in the elimination of many of the false positives compared to our current ELISA technique, particularly the Barbiturate assay. This study has shown that the Randox Investigator may be a promising and useful tool in the Forensic Toxicology Laboratory.

Keywords: **ELISA, Randox Investigator, Postmortem**

## P02 Application of a Biochip Microarray ELISA-based Assay for Drugs of Abuse Screening to Meconium

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According to the Substance Abuse and Mental Health Services Administration (SAMHSA) National Survey on Drug Use and Health Report from June, 2005, 4.3% of all pregnant women surveyed aged 15-44 (n = 1,100) admitted to using illicit drugs. Eight percent of pregnant women aged 15-25 reported illicit drug use. Newborns that have been exposed to drugs of abuse *in utero* may experience withdrawal symptoms consequential to prenatal drug dependence, and suffer from a number of short and long term health problems. Early detection of exposure is critical to guide necessary treatment and improve outcomes. Drug exposure testing results are also used in child-custody proceedings if removal from the home is in the best interest of the child. Meconium is currently the preferred specimen over fetal urine or blood to detect prenatal drug exposure because specimen collection is non-invasive and meconium provides a longer detection window. Drugs and their metabolites collect in meconium beginning at about 5 months gestation. Thus, meconium can identify exposure to drugs during the last 4 months of a full-term pregnancy.

Heterogeneous competitive ELISA assays are commonly used for the screening of urine and blood specimens for drugs of abuse. Reagents developed for detecting drugs in urine and blood have been successfully applied to meconium specimens. Here drug detection performance in meconium was compared between a commercial ELISA (Immunalysis, Pomona, CA) developed for urine and a biochip immunoassay (Randox, Crumlin, UK) developed for blood specimens. For both assays, the meconium was extracted with buffer, centrifuged and the supernatant was analyzed. The biochip is a $9mm^2$ solid substrate with multiple specific antibodies attached at pre-defined sites on the surface. A single biochip can accommodate up to 23 test regions, allowing for performance of multiple assays per sample with a single biochip. The ELISA is performed with separate 96-well plates coated with antibody, each designed to detect a different drug or drug class. Nine drug classes were evaluated in this study.

Seventy-five residual meconium specimens were extracted and analyzed by both methods. The number of confirmed positive results for each drug class were amphetamine = 19, methamphetamine = 19, barbiturates = 6, benzodiazepines = 13, opiates = 32, cannabinoids = 26, cocaine = 26, methadone = 16, and PCP = 2 (n=159, most specimens were positive for more than one drug class). Drug-free meconium spiked at 0-400% of the established ELISA cutoff concentrations was also analyzed and compared between the assays. Agreement was better than 90%. The false positive rate was the same for both techniques for most drug classes, however the biochip array showed better specificity for cocaine metabolites, and slightly better sensitivity for amphetamine. The ELISA assay had better sensitivity for THC and methamphetamine.

Keywords: **Meconium, Drugs of Abuse, ELISA, Biochip Array**

## P03 Evaluation of a UPLC®-TOF Drug Screening Method to Replace the Bio-Rad REMEDi HS Drug Profiling System: Results of a Parallel Run Study Using 1000 Clinical Urine Samples From 4 Hospitals

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**Introduction:** The decision of Bio-Rad to discontinue world-wide support of the REMEDi Drug Profiling System (DPS) has necessitated its replacement in 4 Hong Kong hospitals. In collaboration with laboratories in Denmark and the UK, we have developed a method for comprehensive urine drug screening using UPLC-TOF mass spectrometry. We present our parallel run study using 1000 routine patient samples.

**Method:** Drugs were separated on a UPLC system (Waters) using an ACQUITY HSS T3 column (2.1x100 mm, 1.8 □m) and analyzed using a LCT Premier XE (Waters). To evaluate the transferability of the method between the 4 systems in different hospitals, a standard solution containing 30 drugs (ranging in molecular weight and chromatographic properties) was used. ChromaLynx™ software was used to identify the drugs based on retention time (RT), mass accuracy, isotope distribution and the mass spectral pattern of both the protonated species and the fragments. A total of 1000 clinical urine samples *i.e.* 250 samples from each hospital, were analysed in parallel, by both the REMEDi and the UPLC-LCT method.

To compare the number of drugs and metabolites being detected by both systems, a 'R' score of 1 was assigned to a sample when the REMEDi system detected a drug and/or its metabolite(s) in that patient sample; similarly a 'L' score of 1 was assigned when the UPLC-LCT method detected a drug and/or metabolite(s). To compare the overall efficiency of the 2 systems, an 'I' score (for improvement) of 1 was assigned when the UPLC-LCT method detected one drug and/or its metabolite(s) that were not detected by REMEDi; a 'D' score (for deficiency) of 1 was assigned when the UPLC-LCT method missed a drug and/or metabolites that was detected by the REMEDi DPS.

**Results:** The transferability of the UPLC-LCT method was assessed. RT were demonstrated to be highly reproducible between 4 laboratories, with an average deviation of 1.3% from the mean RT for each of the 30 drugs. ChromaLynx™ calculated the spectral match factors (MF) for both protonated ions and their fragmentation in comparison to both the in-house library and the libraries prepared using the collaborators' instrumentation. The deviation of the MF against the average was acceptable at <9%, indicating the spectra were reproducible between instruments.

For the parallel run study, a preliminary data analysis based on 400 patient samples (100 samples from each hospital) showed a total 'L' score of 1052 and total 'R' score of 655, showing that the UPLC-LCT method detected 1.6 times more drugs and/or metabolites. The total 'I' and 'D' scores were 491 and 96 respectively, showing that the UPLC-LCT method had a significant improvement over the REMEDi system. In all hospitals, both 'L' and 'I' scores were significantly higher than the 'R' and 'D' scores. Data analyses of the 1000 samples will be completed in one month's time and be available for presentation at the meeting.

**Conclusion:** We have developed a screening method based on UPLC-TOF technology. Identification is achieved by comparison of spectral data and RT to a prepared library. Accurate mass measurement and isotope distribution pattern allow the prediction of probable elemental composition. Preliminary data from the parallel run showed that the UPLC-TOF method was able to detect more drugs and metabolites in routine patient urine samples and demonstrated significant improvement over the REMEDi DPS system.

Keywords: **Screening, TOF, REMEDi**

## P04 Confusing Caffeine: Weak Acid Neutral or Base? Analysis of Total Caffeine and Other Xanthines in Specialty Coffees Using Mixed Mode SPE

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**Aim:** Although caffeine is planet earth's most widely consumed drug, and has its main source in the coffee bean, it can be routinely detected in blood and urine. Measurement of caffeine and other xanthines such as theobromine and theophylline can be useful to forensic toxicologists offering interpretation to coroners and medical examiners as to cause and manner of death in forensic investigations. Previous methods of analysis of coffees[1,2] have employed liquid- liquid extractions under basic conditions to isolate caffeine. At this presentation an alternative solution is offered in which caffeine, theobromine, and theophylline can be extracted and analyzed.

**Methods:** In this presentation attendees will learn about solid phase extraction performed in mixed mode operation and the chemistry involved in the isolation of caffeine and other xanthines including theobromine, theophylline from samples of specialty coffees The use of pKa data to determine optimized extraction conditions will also be discussed. Attendees will also learn about how the solid phase extraction was performed in both hydrophobic mode and ion exchange modes using ethyl acetate/ methanol , and ethyl acetate/ acetonitrile/ ammonium hydroxide, respectively as elution solvents. The separation of the xanthines used in this procedure i.e with liquid chromatography in isocratic mode employing a $C_{18}$ column (150 x 2.1 mm ( 3μm)), mobile phase consisting of acetonitrile:formic acid (0.1 % aqueous) (10: 90)) at a flowrate of 0.1 mL/ minute, and detection of the compounds using photodiode array in scanning mode will also be presented. Details of analytical performance i.e recovery and linearity will also be discussed.

**Results:** In this presentation, the results of the analysis of over 30 caffeinated and 10 decaffeinated coffees along with chromatograms of both types of sample will be presented. Analysis of the coffees showed that theophylline was not found in any of the sample. Only 1 coffee contained theobromine (a double chocolate coffee: 2.82 mg/ g). The levels of caffeine found in the caffeinated samples was found to range from 5.18 mg/ g to 12.21 mg/g of powdered material. The decaffeinated coffees were found to contain less than 0.5 mg/g (0.23 – 0.49 mg/ g). This data will be presented

**Conclusions:** By converting grams to ounces, the caffeinated coffees were found to contain between 146 and 346 mg of caffeine/ ounce which can be made available for human consumption. The decaffeinated varieties still contain over 13 mg/ ounce even after the decaffeination process which is also available for ingestion. The use of SPE and liquid chromatography for the analysis of xanthine alkaloids in coffee related materails will greatly help the forensic toxicology community efficiently analyze and better understand the chemistry of these compounds.

References :
1 R.R McCusker *et al.*, J.Anal.Toxicol. **27**, 520, 2003
2 R.R McCusker *et al.*, J.Anal.Toxicol. **30**, 611, 2006

Keywords: **Coffee, SPE, Chromatography**

## P05 Analysis of Total Xanthine Content of Specialty Coffees Using Solid Phase Extraction After Microwave Digestion

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**Aims:** The aim of this poster presentation is to inform analysts and forensic toxicologists involved in both postmortem and human performance fields about the total xanthine levels (especially caffeine, and also theobromine and theophylline) that occur in specialty coffees (both caffeinated and decaffeinated varieties).The data presented may assist toxicologists in casework.

**Methods:** Microwave digestion of over 40 specialty coffees originating in both the United States and Australia was performed using a domestic appliance and fountain drinking water (1g and 2 x 100 mL, respectively). After cooling the digest, an aliquot of 1 mL of the liquid was added to 1 mL of 0.1 M acetic acid containing 25 µg of 8-chlorotheophylline (used as internal standard). To each sample was added a further 1 mL of 0.1 M acetic acid. Each sample was decanted onto a preconditioned mixed mode solid phase extraction (SPE) column (200 mg, 6 mL capacity). The SPE columns were conditioned with methanol, distilled water, and 0.1 M acetic acid, respectively. After loading, the SPE columns were washed with distilled water and 0.1 M acetic acid (1 x 3 mL, sequentially). Each sample was eluted with ethyl acetate:methanol (90:10, 2 mL). The columns were washed with methanol (1 x3mL) and dried before being eluted with ethyl acetate: acetonitrile: ammonia (78:20 2, 3 mL). The combined eluates were evapoated to dryness before being dissolved in 1 mL of 0.1% aqueous formic acid for analysis by liquid chromatography using photo diode array detection. Chromatographic separation was performed using a $C_{18}$ column (150 x 2.1 mm) and a mobile phase consisting of acetonitrile: 0.1% formic acid (10:90) at a flow rate of 0.1 mL/ minute.

**Results:** In this presentation, the results of the analysis of over 30 caffeinated and 10 decaffeinated coffees are displayed along with chromatograms of both types of samples. It was found that none of the coffee samples contained theophylline, and only one sample contained theobromine (a double chocolate coffee: 2.82 mg/g). The levels of caffeine found in the caffeinated samples was found to range from 5.18 mg/ g to 12.21 mg/g of powdered material. The decaffeinated coffees were found to contain less than 0.5 mg/g (0.23 – 0.49 mg/g).The analytical procedure was found to be linear from 1 µg/ mL to 200 µg/mL ($r^2$>0.999) for caffeine, theobromine, and theophylline. Recoveries of the individual xanthines was found to be greater than 95%.

**Conclusions:** By employing a factor of 28.34 to convert grams to ounces, the caffeinated coffees were found to contain between 146 and 346 mg of caffeine/ounce which can be made available for human consumption. The decaffeinated varieties still contain over 13 mg/ounce even after the decaffeination process which is also available for ingestion. This use of SPE and liquid chromatography for the analysis of xanthine alkaloids in coffee related materials will greatly help the forensic toxicology community efficiently analyze and better understand the chemistry of these compounds.

Keywords: **Coffee, SPE, Chromatography**

## P06 Advanced in Analytical Pyrolysis for Forensic Toxicology

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Drugs of abuse ingested by smoking include heroin, cocaine, methamphetamine, and recently, fentanyl. Smoking generates compounds that are ingested via inhalation. These compounds and their metabolic products can be used as characteristic biomarkers. Such information could be of investigative use as well as for monitoring and for identification of toxic effects separate from the parent drug. The objective of this paper is to demonstrate how analytic pyrolysis can be used as a research and investigative tool in forensic toxicology.

Pyrolysis was undertaken using a commercial pyroprobe connected to a gas chromatograph equipped with a mass selective detector. The column used was a thin-film RDX-5MS phase, 30 meters long with a film thickness of 0.25µm. The pyroprobe was connected to the GCMS through a heated transfer line and needle assembly inserted into the injection port. The pyroprobe was operated in two modes: reductive conditions (helium) and oxidative conditions (air). Drugs studied were methamphetamine, cocaine, and fentanyl supplied as hydrochloride salts. Heating profiles varied by drug, but generally included temperatures up to 750°C. The pyrolytic interface was configured with an adsorbent trap containing Tenax. This trapping mode was used to pre-concentrate gaseous produced during the pyrolysis. Flash heating desorbed the trapped materials into the injector port and onto the column

Our laboratory has previously published work related to pyrolytic products of methamphetamine produced using reductive conditions. This and other earlier work has shown that optimized pyrolysis conditions produce metabolites as well as pyrolytic products and potential biomarkers. In the case of methamphetamine, pyrolysis yielded amphetamine, ephedrine, ethyl benzene and *trans*-phenylpropene. A headspace method was developed for detection of the phenylpropene and its presence confirmed in case samples. Specifics of this method have been previously published (Shakleya et. al., *Journal of Analytical Toxicology* 30(8), **2008**, p. 559-562)

With cocaine, reductive conditions yielded the metabolites norcocaine, cocaethylene, benzoylecgonine, and ecgonine methyl ester. Pyrolytic products of AEME and benzoic acid were also produced. When oxidative conditions were applied with trapping, benzene acetic methyl ester and n-methylbenzamide were tentatively identified but not confirmed due to lack of reliable standards. With fentanyl, oxidative trapping was required to isolate pyridine as a significant and reproducible pyrolytic product. This is of importance given the known toxicity and animal carcinogenicity of this compound.

Two advances in instrumentation have improved its utility in forensic toxicology and applications to smoked drugs of abuse. First, the ability to introduce air under high temperature conditions more accurately mimics the smoking event and allows for identification of more target compounds for subsequent investigation. Secondly, trapping capability pre-concentrates pyrolytic products and allows for detection of less abundant but potentially useful products. Trapping improves chromatography by delivering analytes in a focused band onto the column. Finally, trapping facilitates detection of volatile pyrolytic products that might otherwise be missed. The identification of volatile biomarkers is of interest since the more volatile and less soluble the analyte is, the more amenable it is to simple headspace detection schemes.

Keywords: **Smoking, Pyrolysis, Biomarkers**

## P07 Analysis of Selected Drugs of Abuse and Their Pyrolytic Products Using a Linear Ion Trap Mass Spectrometer

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Recent work involving pyrolysis of common drugs of abuse has shown evidence of distinct products. Presence of these pyrolytic products in biological matrices may be an indication of smoking abuse; however, common assays do not include pyrolytic products or their metabolites. Cocaine and fentanyl, two forensically important drugs, produce distinctive pyrolytic products, anyhdroecgonine (AECG) and propionanilide respectively. AECG has been previously reported as unusable as a biomarker due to its production from the hydrolysis of anyhdroecgonine methyl ester (AEME) in GC injector ports and upon prolonged storage of plasma or high pH conditions. However, with careful storage and use of ambient temperature analysis techniques, this analyte could be a useful biomarker for smoked cocaine. Propionanilide has been seen in recent work carried out in this laboratory as a stable pyrolytic product and shows potential for use as a biomarker for smoked fentanyl. Under pyrolitic conditions the two reported metabolites of fentanyl, norfentanyl and despropionylfentanyl, are also observed with propionanilide being the major product. While it would be possible to develop a gas chromatography mass spectrometry assay for detection of these biomarkers, due to the limitations mentioned above, liquid chromatography tandem mass spectrometry (LC/MS/MS) is a more attractive technique offering lower limits of detection. The current study involves the validation of a LC/MS/MS method for analysis of cocaine, fentanyl and their major pyrolytic products and relevant metabolites.

Separation was performed using gradient reverse-phase liquid chromatography using a standard $C_{18}$ column (3 µm particle size, 50 x 2.1 mm). The mass spectrometer was operated in positive mode with electrospray ionization (ESI). A multiple reaction monitoring (MRM) survey scan and enhanced product ion (EPI) dependent scan with information-dependent acquisition (IDA) were performed. Dynamic exclusion of triggered MRM transitions was used to detect coeluting compounds. A minimum of two characteristic MRM transitions for each compound were selected with the most intense being: cocaine 304/182 *m/z*, norcocaine 290/136 *m/z*, ecgonine 186/168 *m/z*, AEME 182/118 *m/z*, AECG 168/122 *m/z*, fentanyl 337/188 *m/z*, despropionylfentanyl 281/188 *m/z*, norfentanyl 233/150 *m/z* and propionanilide 150/106 *m/z*. As an example of method performance norcocaine was observed at 0.1 µg/L.

This study brings to light the potential use of AECG and propionanilide as biomarkers for smoked cocaine and fentanyl respectively. A highly sensitive LC/MS/MS method for the identification of pyrolytic products and metabolites of two commonly abused substances is provided. With the flexibility and ruggedness of this technique the method could be readily adapted for analysis of other pyrolytic products.

Key Words: **Pyrolysis, Biomarkers, LC/MS/MS**

## P08 Determination of Human Biomarkers of RDX Via LC/MS/MS

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The threat of improvised explosive devices is both a forensic and homeland security threat. As such, forensic toxicologists need to develop a new suite of tools to provide probative information to investigators and the judicial system. The ability to detect biomarkers of energetic materials provides toxicologists a new technique for the identification of those who have been exposed to explosives. This study provides information pertaining to human metabolic products of the widely used explosive, hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). RDX is a non-aromatic cyclic nitramine representative of a large class of explosive compounds that has been investigated in the realm of environmental toxicology. However, human metabolism of RDX is poorly understood.

For the present work, an in-vitro metabolism using human liver microsomes and NADPH was employed and the separation and identification of RDX and metabolites were determined using LC/MS/MS by a linear ion trap. The results comparing a control (containing no NADPH) and metabolized product demonstrated that the parent compound remains unchanged after metabolism, illustrating that RDX does not readily metabolize by human liver microsomes containing cytochrome p450 enzymes. This suggests that the detectable biomarker for exposure to RDX in humans is the parent compound. Reported animal metabolites and biodegradation products of RDX such as 1-nitroso-3,5-dinitro-1,3,5-triazacyclohexane (MNX), 1-nitro-3,5-dinitroso-1,3,5-triazacyclohexane (DNX), 1,3,5-trinitroso-1,3,5-triazacyclohexane (TNX), 4-nitro-2,4-diazabutanal and 4-nitro-2,4-diaza-butanamide were not detected. To test the experimental conditions, 2,4,6-trinitrotoluene (TNT) was metabolized in conjunction with RDX under the same conditions. TNT was shown to metabolize to 4-amino-2,6-dinitrotoluene (4-ADNT) and 1,3,5-trinitrobenzene (TNB) which are known TNT human metabolites/biomarkers. Other unexplored metabolic products of explosives used by terrorists are currently being investigated.

Keywords: **Biomarkers, RDX, Metabolites**

## P09 Progress Toward Chromatography-Free Vapor-Phase Screening for GHB

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One of the difficulties presented by GHB and related compounds is the lack of a rapid presumptive testing method analogous to immunoassay-type screening protocols. Methods for headspace sampling coupled to gas chromatography with flame ionization detection have been validated; however, laboratories could benefit from having a simple, rapid, and reliable screening method available. The objective of this paper is to report on progress toward developing and validating such an assay.

Ion mobility spectrometry (IMS) has been used to detect GHB and related compounds and is an accepted approach to presumptive testing for drugs and explosives. In negative ion mode, the instrument operates much as an electron capture detector, with added selectivity imparted by spectra consisting of detector signal versus drift times of ion/molecule clusters. While mobility spectra alone are not adequate for positive identification, the combination of vapor sampling with IMS provides reasonable selectivity and sensitivity for screening purposes. From sample preparation through reporting, the data analysis cycle is measured in minutes.

Previous work in this and other laboratories has demonstrated the utility of IMS for analysis of GHB and related compounds. The emphasis here was on how best to adapt sampling methods for use as a rapid assay. Procedures studied were direct injection of static headspace vapors; deposition of aqueous samples on Teflon substrates and thermal desorption; and deposition of aqueous samples on absorbent substrates (glass fiber filters and filter paper) followed by thermal desorption at 280°C. In direct deposition, 25.0µL of the sample was placed on the substrate. For all studies, aqueous samples of GHB, GBL, 1,4-butanediol, and GVL (a five-carbon analog of GBL) were prepared at concentrations of 10,000 to 1 ppm (w/v). Solution quality was verified using published validated GCFID headspace techniques.

In general, reduced mobility peaks were reproducible at %RSD values of 2% or less (intraday) regardless of sample introduction method. Day-to-day variations are expected and accounted for using an internal calibrant. With direct injection of headspace vapors (25.0µL), peak areas showed excessive variation (>50% RSD) and detection limits were poor, in the low parts-per-thousand range. Substrates performed much better with all three methods resulting in detectable peaks at concentrations of 1.0 ppm (w/v) for all analytes with the exception of GVL. All substrates had detectable background peaks with the glass fiber filters generally displaying the most complex mobility spectra. The filter paper substrate also had notable background peaks but merits further study given its affordability and availability.

Vapor-phase sampling by IMS has potential as a rapid screening method for GHB and related compounds. Sample can be delivered directly to a simple substrate and thermally desorbed to drive off water prior to detection of the analytes of interest. Sample preparation is minimal and analytical cycle times are less than one minute. Detection limits are adequate for screening forensic samples. Further work is needed to optimize substrate preparation and thermal desorption parameters before the method will be viable for deployment.

Keywords: **GHB, Ion Mobility Spectrometry, Screening**

## P10 Validation of Immunalysis® Microplate ELISA Kits for Drug Screening in Post-Mortem Blood in Accordance with IEC/ISO 17025:2005 Laboratory Accreditation

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**Objectives:** Forensic Toxicology laboratories worldwide are recognizing the importance of implementing a quality management system (QMS) to demonstrate management and technical competence for the services they provide. One aspect of the process is the validation of both screening and confirmation methods utilized in the testing laboratory. The focus of this paper will be to summarise the steps completed for the validation of Immunalysis® Microplate ELISA kits for drug screening in postmortem blood in accordance with the international standard IEC/ISO 17025:2005.

**Materials and Methods:** Immunalysis® Direct ELISA kits (supplied by AgriYork 400 Ltd, UK) for amphetamines, benzodiazepines, cocaine, LSD, methadone, methamphetamine and opiates were validated using both positive and negative calibrators and controls. Calibrators and controls were pipetted both manually and automatically (Tecan MiniPrep) on a number of different days by a number of analysts using at least three separate kit Lot numbers to introduce as much potential variability into the validation process. The performance of each kit was assessed by calculating the percentage binding of the calibrator at the cut-off and of the positive controls spiked at ± 50% of the cut-off and plotted on a Shewhart chart with ± 2% and ± 3% warning and action limits.

**Results:** Each assay demonstrated excellent reproducibility for a non-analytical technique with %CV's ranging from 5.3 to 19.7%. The uncertainty of measurement was calculated at twice the relative standard deviation expressed as a percentage (95% confidence level). The ongoing performance of the method is monitored by the inclusion of quality control samples in all batches and participation in quality assurance schemes.

**Conclusions:** The validation data demonstrate that the method used for screening for the presence of amphetamines, benzodiazepines, cocaine, LSD, methadone, methamphetamine and opiates in post-mortem blood is sufficiently reproducible, robust and sensitive to assign presumptive positive results.

Keywords: **IEC/ISO 17025:2005, ELISA, Drug Screening**

## P11 Achieving Lower Limits of Detection Using Pre-Existing Immunoassay Based Screening Techniques

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AIT Laboratories has reviewed the need for more sensitive screening assays capable of achieving lower cutoff levels for several commonly encountered drugs using immunoassay based techniques. Immunoassay based screening techniques have traditionally been performed using unmodified, commercially available kits. These kits often lack the sensitivity required to detect low levels of highly potent drugs and toxins in biological specimens. AIT has developed an approach for improving cutoff levels to allow for detection of low, but forensically and clinically relevant, drug levels. While the ultimate goal of the project is to allow for screening of multiple specimen types, the initial feasibility study presented here has focused on urine specimens.

Serially diluted calibrators of oxycodone, methadone, and benzodiazepines (nitrazepam), along with appropriate blanks and controls purchased from Microgenics (Freemont, CA) were analyzed using an Abbott Aeroset. The analytical suitability of lower screening cutoffs was evaluated through the replicate analysis (n=20) of calibrators prepared at +/- 25% of the investigated cutoff level. Multiple cutoffs were investigated for each drug class and selected cutoff levels showed excellent precision and accuracy indicating that lower cutoff levels can be achieved. Replicate analysis of oxycodone calibrators at concentrations of 37.5 and 62.5 ng/mL produced %CV's in the range of 4.0-4.7, while analysis of methadone calibrators at concentrations of 75 and 125 ng/mL produced %CV's in the range of 4.0-7.7. Replicate analysis of benzodiazepine (nitrazepam) calibrators at concentrations of 56.25 and 93.75 ng/mL produced %CV's in the range of 4.1-6.2.

As a result of these studies, AIT Laboratories now employs an oxycodone screen cutoff of 50 ng/mL (vendor recommends 300 ng/mL), a methadone screen cutoff of 100 ng/mL (vendor recommends 300 ng/mL) and a benzodiazepine screen cutoff of 75 ng/mL (vendor recommends 200 ng/mL). Implementation of lower screen cutoffs will prove beneficial for the detection of highly potent drugs and toxins in biological specimens.

Keywords: **Cutoff, Immunoassay, Screen**

## P12 Immunoassay Detection of Oxycodone in Postmortem Oral Swabs

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Postmortem toxicological analysis is often difficult due to limited sample size and lengthy sample preparation. Studies have demonstrated that analysis of oral fluid and swabs of the oral cavity are useful in therapeutic drug monitoring and establishing illicit drug use. The purpose of this study was to evaluate oxycodone EMIT® screening of oral swabs collected at autopsy in cases of suspected drug overdose to determine whether the identification of oxycodone in postmortem oral swabs is indicative of the presence of oxycodone in other postmortem specimens. If so, immunoassay screening of a postmortem oral swab could be used in place of screening other specimens, preserving them for further analysis and confirmation.

Analysis of postmortem oral swabs was carried out using an elution procedure wherein 1 mL methanol was added to each swab in the 15 mL centrifuge tube in which it was stored after collection. Samples were vortexed for 1 minute and swabs were removed and wiped against the sides of the tubes to maximize methanol collection. Tubes were centrifuged at 3000 rpm for 10 minutes and methanol was decanted and dried under nitrogen in a water bath at 45°C. Residues were reconstituted in 0.5 mL 0.1M phosphate buffer, pH 6.5, before analysis on a MGC 240 analyzer using a DRI Oxycodone Assay (Microgenics, Fremont, CA). Serum calibrators purchased from the manufacturer with concentrations of 100, 300, 500, and 1000 ng/mL were used with the assay to provide semi-quantitative results. According to the manufacturer, the DRI Oxycodone assay demonstrates 103% cross reactivity to oxymorphone; therefore, GC/MS confirmations of blood oxymorphone concentrations are also considered in our final analysis. The linear range of the assay was determined using oxycodone-spiked saliva on swabs and was found to be 20 ng to 500 ng of oxycodone "on-swab." Spiked swabs were stable over a period of 10 days when stored at 4°C, with recoveries ranging from 73.8% to 89.6%. A study of several elution solvents was also performed and it was determined that methanol was the solvent that gave the greatest oxycodone recovery from postmortem oral swabs for our screening purposes.

Swabs from 33 cases were analyzed, 14 of which had blood precipitate immunoassays with no oxycodone detected. The other 19 swabs were taken from cases in which blood screened and confirmed positive for oxycodone or both oxycodone and oxymorphone. No false positive or false negative results were obtained on swabs which were run as controls. The immunoassay response for oral swabs from cases in which blood confirmed positive by GC/MS ranged from 4 to 971 with an average response of 367 and immunoassay responses for blood precipitates from the same cases ranged from 28 to 679 with an average response of 364. In cases where oxycodone was detected by immunoassay in blood precipitates, GC/MS confirmations resulted in blood oxycodone concentrations ranging from 0.06 mg/L to 1.54 mg/L and oxymorphone concentrations (when detected) ranging from less than 0.01 mg/L to 0.22 mg/L.

It should be noted that the oral swabs collected for this study, unlike those collected from living subjects, often contained more material than just oral fluid. The presence of red or brown residue on many of the swabs suggests that materials such as blood or gastric contents were present in the mouth when swabs were collected and the presence of these substances on the swabs could effectively increase positive oxycodone immunoassay responses. Also, it appears that the presence of other drugs on the swabs (determined by positive immunoassay), even other opioids, seems to have no effect on the oxycodone immunoassay responses. In conclusion, the results of this study suggest that swabs of the oral cavity obtained just prior to autopsy provide a useful specimen for oxycodone immunoassay screening, conserving other specimens such as blood and urine for later analysis. Future research involving postmortem oral swabs will determine their usefulness in screening for other common drugs of abuse.

Keywords: **Oral Swabs, Postmortem, Oxycodone, Enzyme Immunoassay**

## P13 Immunoassay for the Detection of PCP** in Oral Fluid on Roche Automated Instruments

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[1]Roche Diagnostics, Indianapolis, IN, USA[2]OraSure Technologies, Inc., Bethlehem, PA, USA

A homogeneous immunoassay is in development for the semi-quantitative and qualitative determination of PCP 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 other bodily fluids, and adulteration is more difficult. The assay utilizes KIMS technology (Kinetic Interaction of microparticles in Solution) with liquid reagents that do not require reconstitution. The two-reagent system consists of a reagent containing a PCP 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 of 1 ng/mL when using the Intercept® Oral Specimen Collection Device from OraSure Technologies, Inc. (OTI). The test range of the assay is 0 - 8 ng/mL.

When run in a semi-quantitative mode with a 6-point calibration on a Roche/Hitachi 917 analyzer, control samples at concentrations of 0.75 and 1.25 ng/mL show mean recoveries of 0.77 ng/mL (103%) and 1.28 ng/mL (102%), respectively. Within run precision studies (n=21) show %CV values of 8.2% and 6.1 %, respectively, for these control levels. The measured lower detection limit (LDL) of the assay is 0.3 ng/mL (mean + 2 standard deviations). The assay is specific to PCP with cross reactivity of less than 0.1% to dextromethorphan. A set of 823 clinical samples from a drug prevalent population were tested and found to have 100% sensitivity and 97.9% specificity when compared to LC/MS/MS.

**Conclusion:** In summary, the assay produces accurate and reliable results and is well suited for routine screening of PCP in oral fluids.

**This assay is currently in development and has not been approved for use in the US by the FDA.



Keywords: **Oral Fluids, PCP, Immunoassay**

## P14 Immunoassay for the Detection of Tetrahydrocannabinol** in Oral Fluid on Roche Automated Instruments

Shaker Rashid[1], Yuhong Zhao[1], **Davina Ananias***[1], Joseph Pasarelli[1], Kristin Blum[2], Dean Fritch[2], Keith Kardos[2], Stephen Lee[2].
[1]Roche Diagnostics, Indianapolis, IN, USA[2]OraSure Technologies, Inc., Bethlehem, PA, USA

A homogeneous immunoassay is in development for the semi-quantitative and qualitative determination of tetrahydrocannabinol (□[9] THC) 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 other bodily fluids, and adulteration is more difficult. The assay utilizes KIMS technology (Kinetic Interaction of microparticles in Solution) with liquid reagents that do not require reconstitution. The two-reagent system consists of a reagent containing a THC monoclonal antibody that is covalently linked to carboxy-modified polystyrene microparticles, with a drug-polymer conjugate in solution as the second reagent. The THC oral fluid assay utilizes a cutoff of 1 ng/mL when using the Intercept® Oral Specimen Collection Device from OraSure Technologies, Inc. (OTI). The test range of the assay is 0 - 8 ng/mL.

The assay was calibrated with □[9] THC in a synthetic oral fluid matrix and run in a semi-quantitative mode with a 6-point calibration on a Roche/Hitachi 917 analyzer. Within run precision studies (n=10) on control samples at a concentration of 0.75 ng/mL showed a mean recovery of 0.80 ng/mL with a CV% value of 9%. Within run precision studies of controls at a concentration of 1.25 ng/mL showed a mean recovery of 1.16 ng/mL with a CV% value of 5%. No cross-overs of the cutoff were observed in the precision studies. Clinical sample collection is ongoing.

**Conclusion:** In summary, the assay produces accurate and precise results for the detection of THC in oral fluids at a concentration of 1 ng/mL.

**This assay is currently in development and has not been approved for use in the US by the FDA.



Keywords: **Oral Fluids, THC, Immunoassay**

## P15 Comparison of Screening Paired Urine and Oral Fluid Specimens for Naltrexone by ELISA and Gas Chromatography-Mass Spectrometry

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Detection of naltrexone, an opiate antagonist, in urine and oral fluid by gas chromatography-mass spectrometry (GC-MS) has been demonstrated. Naltrexone can be detected in urine by the Immunalysis Naltrexone Direct ELISA Kit. Application of the Immunalysis assay to screen paired urine and oral fluid specimens and comparison of the results with a GC-MS method is described.

Paired human urine and oral fluid samples were collected from 30 subjects. The subjects were the first part of a randomized double blind placebo controlled trial. The subjects received either naltrexone or placebo orally once per day for one week. The naltrexone dosing schedule included: 12.5 mg on day one; 25 mg on days two and three; and 50 mg on days four through seven. All urine specimens were screened by the Naltrexone Direct Elisa Kit according to the Immunalysis package insert. Oral fluid was tested in the same way as urine with one exception. Due to the anticipated lower naltrexone levels in oral fluid, the specimens were not diluted prior to testing. For GC-MS testing urine samples were hydrolyzed with glucuronidase. Oral fluid samples were collected with Salivette devices from Sarstedt. All specimens were adjusted to an alkaline pH. Specimen preparation for GC-MS included SPE with BondElut Certify columns. Derivatization with 2% N–trimethylsilyl-imidazole (TSIM) in N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) occurred in capped conical glass tubes at 100 °C for 30 minutes. GC-MS analysis was performed with an Agilent Technologies 5973 GC-MSD System with a Varian Factor Four VF-5ms 30 m x 0.25 mm column. A temperature programmed run from 100 °C to 310 °C at 40 °C/minute was used. The selected ions monitored were naltrexone: 542, 557, 484; d3-naltrexone: 545, 560, 487; and 6-□-naltrexol: 544, 559, 372.

All 21 paired urine and oral fluid samples from the naltrexone dosed subjects tested positive for naltrexone by the Immunalysis Direct ELISA Kit and GC-MS. Whereas, the nine paired samples from the subjects who received placebo, tested negative for naltrexone by the ELISA kit and GC-MS. GC-MS analysis detected both naltrexone and 6-□-naltrexol in all positive urine samples. GC-MS analysis of oral fluid demonstrated naltrexone and 6-□-naltrexol in all positive samples. The cross-reactivity of 6-□-naltrexol with the ELISA assay is reported by Immunalysis to be only 5 %. The presence of naltrexone in the oral fluid samples was significant for detection by the ELISA method.

The Immunalysis Naltrexone Direct ELISA Kit compared satisfactorily to GC-MS in the detection of naltrexone in urine and oral fluid samples.

Keywords: **Naltrexone, ELISA, Oral Fluid**

## P16 Monitoring Naltrexone Compliance in Urine by Enzyme Immunoassay and GC-MS

**Warren C. Rodrigues*,** Guohong Wang, Christine Moore, Alpana Agrawal, Rekha Barhate, Michael Vincent, James Soares. Immunalysis Corporation, Pomona, CA, USA

**Background***:* Naltrexone is a narcotic antagonist, belonging to the opioid class of drugs. Due to its competition for the same brain receptors that bind both heroin and alcohol, it is administered in drug and alcohol rehabilitation programs for treatment of heroin as well as alcohol addiction. A highly sensitive enzyme immunoassay to screen urine specimens has been developed, followed by GC-MS confirmation, which detects the presence of the parent drug naltrexone, as well as the major metabolite 6β-naltrexol.

**Validation***:* The ELISA and GC-MS results obtained for authentic urine specimens were well- correlated, at a screening cut-off of 10 ng/mL. The ELISA dose response curves are shown in Figure 1. Intra and inter-assay precision were <5% using manual pipetting technique. The limit of detection was 1 ng/mL. For GC-MS, the LOD was 0.1 ng/mL and LOQ was 1 ng/mL. Intra and inter-day precision were <5% for GC-MS.

| ELISA | GC-MS | |
|---|---|---|
| | + | - |
| + | 19 | 0 |
| - | 0 | 16 |

Table 1. GC-MS Correlation data

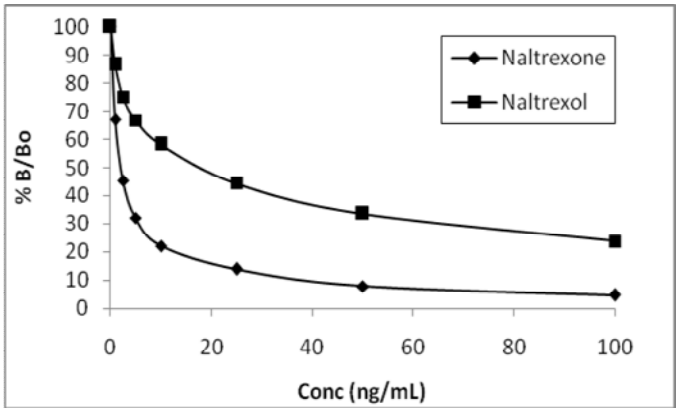



Figure 1. ELISA dose response curves

**Sensitivity***:* 19/(19)=100%; *Specificity:* 16/(16)=100%; *Accuracy* = 100%

**Cross-reactivity***:* The advantage of the ELISA method is that it is possible to detect low levels of the parent drug naltrexone, without picking up high levels of oxycodone, which may be a problem in the toxicology field. The major metabolite, 6β-naltrexol showed 10% cross-reactivity with the antibody, while naloxone, another narcotic antagonist, which exhibits bio-activity similar to naltrexone, showed 15% cross-reactivity in the ELISA. Further, the GC-MS method developed, detected the presence of both naltrexone and morphine

**Summar***y:* Highly sensitive and specific ELISA and GC-MS methods have been developed for the detection of naltrexone and 6β-naltrexol in urine, for compliance within a heroin or alcohol treatment program. The assay can detect low levels of naltrexone, without interference from high oxycodone concentrations, which is a common problem in the toxicology field.

Keywords: **Naltrexone, 6β-Naltrexol, ELISA, GC-MS**

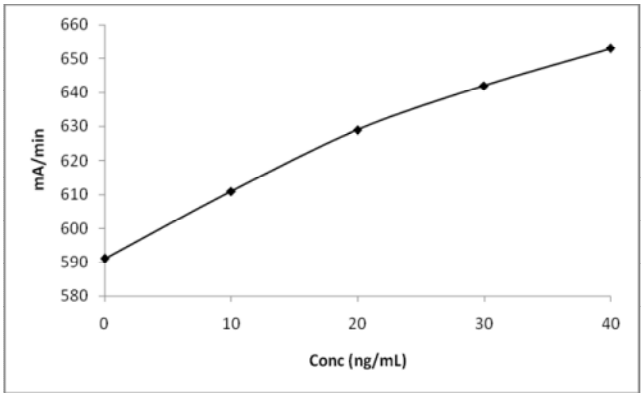
## P17 Detection of Benzodiazepines in Oral Fluid by a Homogeneous Immunoassay Technique

**Warren C. Rodrigues*,** Guohong Wang, Rekha Barhate, Alpana Agrawal, Michael Vincent, James Soares, Christine Moore. Immunalysis Corporation, Pomona, CA, USA

**Background***:* Oral fluid has become a useful biological testing matrix to detect recent drug use, mainly due to observed collection and difficulty of adulteration of specimens. Benzodiazepines, (after marijuana) are the most widely detected compounds in cases of driving under the influence of drugs (DUID). As roadside screening in some jurisdictions is moving to allow oral fluid, new assays are necessary for high volume screening. Sample volumes for oral fluid are usually limited and typically contain low concentrations of drugs. Also, the low saliva:plasma ratio (<1) for benzodiazepines, requires that new oral fluid assays be sensitive and cross-reactive to the lower dosage drugs (alprazolam, lorazepam, clonazepam, etc.).

Oral fluid samples were collected with the Quantisal™ collection device, equipped with a volume adequacy indicator. The device collects 1 mL of neat oral fluid, diluted with 3 mL of extraction buffer. This homogeneous assay uses a 20 µL sample volume, allowing sufficient specimen to remain for GC-MS confirmation as well as other drug screens.

**Validation***:* The limit of detection of the assay is 1 ng/mL. The intra-assay and inter-assay precision at 10, 20, 30 and 40 ng/mL of oxazepam were determined to be < 2%. The percentage recovery from the oral fluid Quantisal™ collection device was found to be 71.3% for oxazepam, 92.1% for diazepam; 86.7% for alprazolam; 83.4% for lorazepam; 88.1% for clonazepam and 84.8% for temazepam. The assay was further validated with oral fluid specimens previously confirmed by GC-MS, at a screening cut-off of 20 ng/mL.




**Cross-reactivity***:* The assay showed >90% cross-reactivity for oxazepam, alprazolam, diazepam, flurazepam, temazepam and lorazepam and 70% for clonazepam. Other common drugs of abuse were spiked in negative oral fluid and showed no interference or cross-reactivity in the assay.

**Summar***y:* The described homogeneous immunoassay method is sensitive, specific and precise, for the detection of a range of benzodiazepines in oral fluid, which are of significance in the toxicology field. The assay format is compatible with most commercially available chemistry analyzers.

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

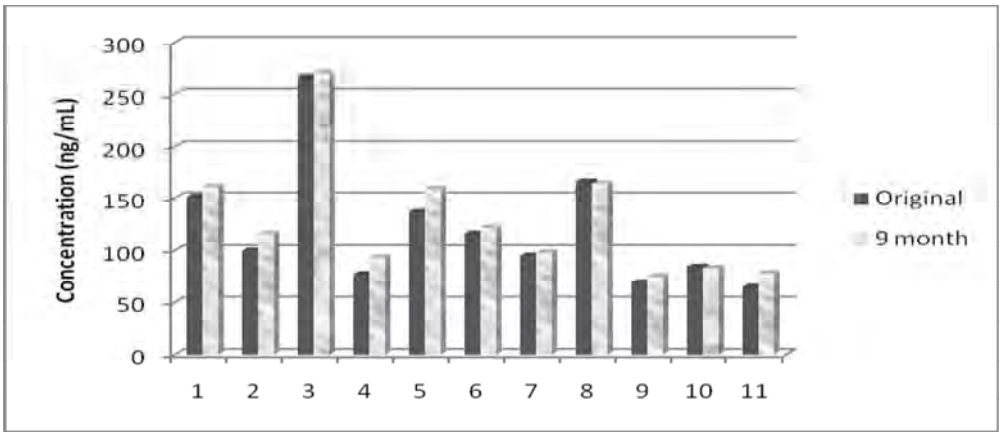
## P18 Amphetamine Stability in Oral Fluid Stored in a Quantisal™ Device Over a Period of Nine Months

**Cynthia Coulter*** [1] Elizabeth Miller[1], Christine Moore[1], Michele L. Merves[2], Bruce A. Goldberger [2], Dennis L. Thombs[2], Robert M. Weiler[2]
[1]Immunalysis Corporation, Pomona, CA; [2]University of Florida, Gainesville, FL, USA

**Background:** Oral fluid is a useful biological specimen to detect recent use of certain drugs and metabolites, and has specific advantages over urinalysis. However, the stability of drugs of abuse in the oral fluid/buffer matrix has not been widely studied.

**Specimen collection***:* In order to assess the epidemiology of alcohol and drug use in a college bar district located in the state of Florida, self-report and biological data were collected from randomly selected and self-selected patrons exiting bars to examine associations between alcohol intoxication level, concomitant drug use, intent to drive a motor vehicle within an hour of study participation, and related behaviors. The protocol was anonymous and approved by a University of Florida Institutional Review Board. Participants provided verbal informed consent. Data were collected from 10:00 p.m. to 2:30 a.m. on four nights in July/August 2007. The participants in the study were mostly men (64.7%) and Caucasian (78.4%) with a reported mean age of 21.4y. To examine drug use, oral fluid specimens were taken using the Quantisal™ collection device, which provides 1mL of neat saliva diluted with transportation buffer (3mL). Specimens were shipped overnight in coolers with ice packs, to the laboratory. Following ELISA screening for amphetamines and 6 other drug classes, confirmation for amphetamine was carried out using an Agilent 6410 LC/MS/MS instrument operating in positive electrospray mode. The specimens were stored at -20$^{o}$C and re-tested after nine months.

**Results and Discussion:** Of 456 specimens, twelve (2.6%) were found to be positive for amphetamine. No specimens indicated the presence of methamphetamine, MDA, MDMA or MDEA. There was sufficient volume remaining to re-test 11 of the specimens. Amphetamine was stable over the 9 month storage period, with all 11 analyses effectively indicating no change from the original concentration. Specimens which appeared to increase may be accounted for by minor variation in analytical approach, but all concentrations were within 20% of the original result.




**Summary:** Amphetamine in the Quantisal™ oral fluid collection device is highly stable over 9 months when stored at -20$^{o}$C.

Keysords: **Oral Fluid, Amphetamine, Drug Stability**

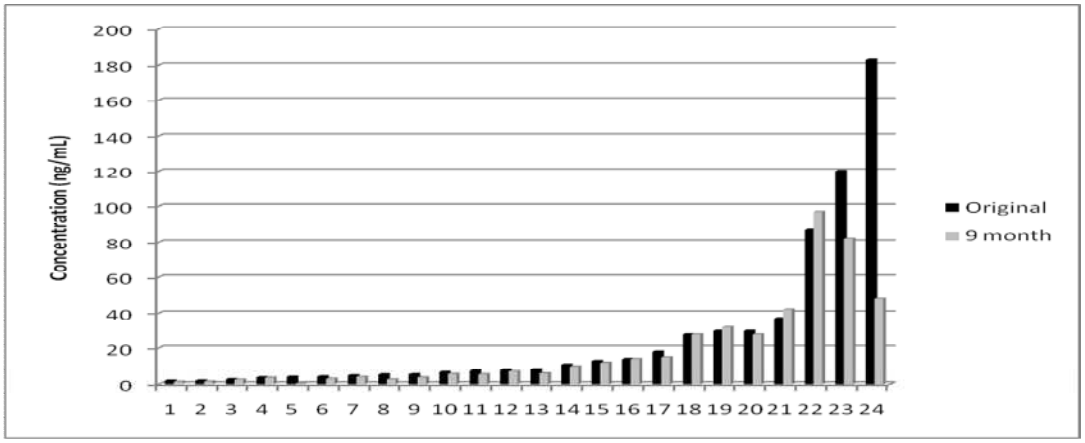
## P19 Stability of Tetrahydrocannabinol (THC) in Oral Fluid Over a Nine Month Period

**Cynthia Coulter*** [1] Elizabeth Miller[1], Christine M. Moore[1], Michele L. Merves[2], Bruce A. Goldberger[2], Dennis L. Thombs[2], Robert M. Weiler[2]. [1]Immunalysis Corporation, Pomona, CA, USA; [2]University of Florida, Gainesville, FL, USA

**Background:** Oral fluid is a useful biological specimen to detect recent use of drugs and has specific advantages over urinalysis. However, the stability of drugs of abuse in the oral fluid/buffer matrix has not been widely studied.

**Specimen collection:** In order to assess the epidemiology of alcohol and drug use in a college bar district located in the state of Florida, self-report and biological data were collected from randomly selected and self-selected patrons exiting bars to examine associations between alcohol intoxication level, concomitant drug use, intent to drive a motor vehicle within an hour of study participation, and related behaviors. The protocol was completely anonymous and approved by a University of Florida IRB. Participants provided verbal informed consent. Data were collected from 10:00 p.m. to 2:30 a.m. on four nights in July/August 2007. The participants were mostly men (64.7%) and Caucasian (78.4%) with a reported mean age of 21.9y. To examine drug use, oral fluid specimens were collected using the Quantisal™ collection device, which provides 1mL of neat saliva diluted with transportation buffer (3mL). Specimens were shipped overnight to Immunalysis Corporation for analysis. Following ELISA screening and confirmation of positives with GC/MS, the specimens were stored at -20$^{o}$C. Nine months later the specimens were re-tested by the same methodology.

**Results and Discussion***:* Of 456 specimens, thirty-two (7%) were found to be positive for THC; of these, 24 had adequate volume remaining for re-testing. Overall, drug loss for THC varied. Three specimens showed significant loss, decreasing from 183 to 48ng/mL in one case; 4.2ng/mL to less than 1ng/mL in the second and 5.5 to 2.7ng/mL in the third. All other concentrations were within 30% of the original result. Assessing those as outliers, the mean loss of THC over 9 months was 14.4%. Specimens which appeared to increase in concentration may be accounted for by analytical variability.




**Summary:** THC in the Quantisal™ oral fluid collection device showed an average decline of 14.4% over the storage period, with some specimens dropping significantly, and others showing no loss at all.

Keywords: **Oral Fluid, THC, Drug Stability**

## P20 Simultaneous Identification and Quantification of *Cannabis Sativa* Cannabinoids and Metabolites in Oral Fluid.

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**Background:** A method for simultaneous identification and quantification of $\Delta^9$-tetrahydrocannabinol (THC), cannabinol (CBN), cannabidiol (CBD), and metabolites 11-hydroxy-THC (11-OH-THC) and 11-nor-9-carboxy-THC (THCCOOH) in oral fluid is presented. A simultaneous assay of these analytes in oral fluid is problematic because of low concentrations, differing concentration ranges, and limited specimen volume. Neutral analytes, such as THC and CBD, are in relatively high (ng/mL) concentrations in oral fluid and acidic THCCOOH is in the low picogram/mL range.

**Objective:** To develop and validate a method for recovery and quantification of THC, CBN, CBD, 11-OH-THC and THCCOOH from a single extraction of oral fluid.

**Methods:** Oral fluid samples were collected with the Quantisal™ device (Immunalysis Inc., Pomona, CA). One mL of oral fluid/buffer was added to Cerex® Polycrom™ (SPEware, Baldwin Park, CA) THC (35 mg) SPE columns and washed with 2.5 mL water/acetonitrile/$NH_4OH$ (84:15:1 v/v). THC, CBD, CBN, and 11-OH-THC were eluted with 2.5 mL of hexane/acetone/ethyl acetate (3:1.5:1 v/v), derivatized with *N*,*O*-bis-(trimethylsilyl)trifluoroacetamide (BSTFA) and quantified by two-dimensional gas chromatography/electron impact mass spectrometry (2D-GC/EI-MS) with cryotrapping. THCCOOH was separately eluted with hexane/ethyl acetate/acetic acid (30:10:1 v/v), derivatized with BSTFA and quantified by 2D-GC/EI-MS. Splitting the eluate enables separate 2D-GCMS chromatographic separation and quantification of THCCOOH after derivatization

**Results:** THC and CBD were simultaneously quantified with dynamic ranges of 0.125 - 10 ng/mL. The dynamic range for CBN and 11-OH-THC was 0.25 - 10 ng/mL and THCCOOH was quantified at 0.25 – 10 ng/mL. Intra and inter-assay imprecision as percent RSD ranged from 2.6%-13.2%. Recovery was within 16.2% of target concentrations. THCCOOH concentrations in oral fluid are in the low pg/mL range (Moore, *et al* 2007). Further development efforts with electronegative derivatives and negative chemical ionization (NCI) MS are underway for THCCOOH, and most likely will be necessary to reach the desired limit of quantification.

**Conclusion:** This new solid phase extraction procedure recovers THC, CBD, CBN, 11-OH-THC and THCCOOH from oral fluid, enabling separation of two analyte groups according to required dynamic ranges. Enhanced analytical sensitivity with improved S/N and detection limits were achieved using 2D-GCMS with cryotrapping for THC, CBD, CBN and 11-OH-THC. Detection of THCCOOH or 11-OH-THC in oral fluid could verify cannabis ingestion and negate passive exposure as a source of positive tests. This method will be applied to quantification of cannabis constituents and metabolites in oral fluid specimens collected from individuals participating in controlled cannabis administration studies and during cannabis withdrawal.

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

Keywords: **Tetrahydrocannabinol, Cannabinoids, Oral Fluid, 11-nor-9-carboxy-Δ9-tetrahydrocannabinol**

## P21 Comparison of Two Enzyme Immunoassays for the Detection of the Cocaine Metabolite, Benzoylecgonine, in Urine

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[1]Kroll Laboratory Specialists, Richmond, VA, USA; [2]Department of Forensic Science,
[3]Department of Pathology, Virginia Commonwealth University, Richmond, VA, USA

We present an evaluation of two enzyme immunoassays for the detection of benzoylecgonine in urine: the DRI® Cocaine Metabolite Assay [DRI] from Microgenics (Fremont, CA); and the Cocaine Metabolite Enzyme Immunoassay from Lin-Zhi International, Inc. [LZ] (Sunnyvale, CA). These assays are based on competitive antibody binding between benzoylecgonine in urine and glucose-6-phosphate dehydrogenase labeled benzoylecgonine. When benzoylecgonine is present in urine, active unbound enzyme reduces the co-enzyme NAD to NADH resulting in an increase of measured absorbance at 340 nm. These assays are calibrated with benzoylecgonine.

The DRI and LZ assays were evaluated by testing 1398 urine specimens from criminal justice and pain management programs. All 1398 specimens were tested with both assays in an ADVIA 1200 Chemistry System auto-analyzer (Siemens Healthcare Diagnostics, Tarrytown, NY). GC/MS data were obtained for all urines that screened positive.

Approximately 46% (644) of the 1398 specimens yielded positive results by the DRI assay, and 47% (664) were positive by the LZ assay. Of these specimens, one was found to have a non-detectable level of benzoylecgonine by GC/MS, indicating one false positive result for each assay. However, 21 specimens yielding negative DRI assay results were found to contain benzoylecgonine above 300 ng/mL, and 29 specimens yielded false negatives with the LZ assay. Therefore, the overall agreement between both the DRI and LZ assays and GC/MS results was 98%. Assay sensitivity was 0.968 (DRI) and 0.958 (LZ); and the selectivity for both assays was 0.999. Urines containing cocaine, additional cocaine metabolites, and other drugs were also tested using both assays. Neither assay demonstrated cross reactivity with these additional compounds.

The within-run precision of the assays, as determined by the absorbance rates of the negative and positive controls, was CV=<3% (n=6); while the between-run precision of the controls was CV=<4% (DRI, n=13) and CV=<7% (LZ, n=9).

The DRI and LZ cocaine metabolite enzyme immunoassays provide a precise, reliable method for the routine detection of benzoylecgonine in urine specimens.

Keywords: **Cocaine, Immunoassay, Urine Drug Screening**

## P22 An ONLINE DAT® Immunoassay for the Detection of Benzodiazepines and Glucuronidated Analogues in Urine

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Roche Diagnostics Operations Inc., Indianapolis, IN, USA

Introduction: A new assay is in development for the detection of benzodiazepines and their glucuronidated metabolites in urine on automated clinical analyzers*.

Method: The liquid, two reagent, ready to use homogeneous assay utilizes the KIMS technology (Kinetic Interaction of Microparticles in Solution), where drug conjugates are covalently linked to carboxy-modified polystyrene microparticles, and purified polyclonal antibody and a β-glucuronidase enzyme are in solution. The assay utilizes cutoff concentrations of 100, 200 and 300 ng/mL for both semi-quantitative and qualitative methods, and features an extended dynamic range compared to currently available assays.

Results: Studies summarized herein evaluated 1) the accuracy and precision, 2) cross-reactivity, and 3) a method comparison of the 100 ng/mL cutoff (comparable results obtained for the 200 and 300 ng/mL cutoff). When run in semi-quantitative mode on Roche/Hitachi 917 analyzers, control recovery at 50, 80, 100, 120 and 600 ng/mL, with n=21, showed a recovery of 50.8 (101.5%), 79.8 ng/mL (99.7%), 101.7 ng/mL (101.7%), 117.3 ng/mL (97.8%) and 610.8 ng/mL(101.8%) with intra-assay %CV precision values of 3.4%, 1.7%, 1.6%, 1.5% and 0.5%, respectively. The inter-assay precision of the same levels ranged from 2.2 to 7.4%. Lorazepam glucuronide, oxazepam glucuronide, and temazepam glucuronide show cross-reactivities of 38.5%, 42.67%, and 40.33% with the enzyme compared to cross-reactivities of 1.34%, 0.87%, and 1.09% without the enzyme, respectively. An internal method comparison of 50 suspected positive clinical samples analyzed against the CEDIA assay showed 11 samples that gave <100 ng/mL results with CEDIA, but positive results with the ONLINE DAT assay. These 11 samples all showed the presence of benzodiazepines upon GC/MS confirmation. The remainder of the samples all showed agreement between the methods. GC/MS confirmed negative clinical samples give an average reading of 1.4 ng/mL.

Conclusion: The above studies demonstrate that this assay provides an accurate and precise method for screening urine for the detection of benzodiazepines and shows evidence of higher cross-reactivity to glucuronidated species compared to assays without the enzyme.

*This assay is currently in development and has not been cleared or approved for use in the US by the FDA.



Keywords: **Benzodiazepine, Immunoassay, β-glucuronidase**

## P23 Qualitative Screening of Cocaine in Hair Using a Homogenous Immunoassay Procedure

**Guohong Wang*,** Michael Vincent, Alpana Agrawal, Catherine Castro, Cecelia Henry, Christine Moore. Immunalysis Corporation, Pomona, CA, USA

**Background:** Hair is a useful specimen to detect long-term use of drugs. Hair generally contains low drug concentrations and sample volume is often limited. Following cocaine use, the main drug detected in hair is the parent drug itself, so a screening immunoassay should be targeted to cocaine.

**Methods***:* Hair (10 mg) from cocaine users (n = 19) as well as hair from drug free volunteers (n = 20) was cut; phosphate buffer was added (pH 2.7; 0.5 mL), and the hair was incubated (3 hrs/75$^{o}$C). The supernatant was analyzed using enzyme linked immunosorbent assay (ELISA) and by homogeneous immunoassay (HEIA) on an Olympus 400 platform. For ELISA, the supernatant was diluted 1:5 with PBS before plating; for homogenous EIA, 20 □L was used directly, making it conducive to commercial chemistry analyzers.

**Results:** An HEIA targeted at cocaine using a screening cutoff of 500 pg/mg has been developed. The intra-assay precision at 250, 500, 1000 and 2500 pg/mg of cocaine was determined to be 7.1%, 12%, 9.3% and 3.8% respectively. All the negative specimens screened negatively using ELISA and EIA. The results of the positive specimens are shown.

| **Sample** | **ELISA** | **HEIA** | **GC/MS results (pg/mg)** | | | |
|---|---|---|---|---|---|---|
| **Cut-off** | *500 pg/mg cocaine* | *500 pg/mg cocaine* | ***BZE*** | ***Cocaine*** | ***NC*** | ***CE*** |
| 1 | P | P | 3492 | >10,000 | 2746 | ND |
| 2 | P | P | 9531 | >10,000 | 2419 | ND |
| 3 | P | P | 375 | 4501 | 62 | 389 |
| 4 | P | P | 9614 | >10,000 | 2847 | 9521 |
| 5 | P | P | >10,000 | >10,000 | 4797 | >10,000 |
| 6 | P | P | 5779 | >10,000 | 662 | ND |
| 7 | P | P | 3978 | >10,000 | ND | 8282 |
| 8 | P | P | 2492 | >10,000 | 644 | 1589 |
| 9 | P | P | 6564 | >10,000 | 722 | 840 |
| 10 | P | P | >10,000 | >10,000 | 3556 | 9868 |
| 11 | P | P | 7672 | >10,000 | 2347 | ND |
| 12 | P | P | >10,000 | >10,000 | 2287 | 756 |
| 13 | P | P | >10,000 | >10,000 | 7927 | 214 |
| 14 | P | P | 132 | 1181 | ND | ND |
| 15 | **N** | P | 231 | 728 | ND | ND |
| 16 | P | P | >10,000 | >10,000 | ND | 7113 |
| 17 | P | P | >10,000 | >10,000 | ND | 833 |
| 18 | P | P | >10,000 | >10,000 | ND | ND |
| 19 | **N** | P | 107 | 573 | ND | ND |

**Cross-reactivity***:* The cocaine antibody cross-reacts 100% with CE, but has lower cross-reactivity to BZE. The assay is precise, specific and sensitive, and is suitable for the rapid screening of hair specimens at a cut-off concentration of 500 pg/mg of cocaine.

Keywords: **Homogeneous Immunoassay, Cocaine, Hair**

## P24 Screening of Amphetamines in Hair Using a Homogenous Immunoassay Procedure

**Guohong Wang*,** Michael Vincent, Alpana Agrawal, Catherine Castro, Cecelia Henry, Christine Moore. Immunalysis Corporation, Pomona, CA, USA

**Background:** Hair is a useful specimen to detect long-term use of drugs. Drug concentrations are usually low and sample volume is often limited, so sensitive screening methods are necessary. Following methamphetamine (METH) use, METH itself is found in hair, but amphetamine (AMP) may be present as a metabolite, or as an independent drug.

**Methods:** Hair (10 mg) from METH users (n = 20) as well as hair from drug free volunteers (n = 20) was cut; phosphate buffer was added (pH 2.7; 0.5 mL) and incubated (3 hrs/70$^{o}$C). The supernatant was analyzed using two enzyme linked immunosorbent assays (ELISA) and two homogeneous immunoassays (HEIA) on an Olympus 400 platform, one for METH, one for AMP. For ELISA, the supernatant was diluted 1:5 with PBS before plating; for HEIA, 10 □L was used, making the process compatible with most commercial chemistry analyzers.

**Results***:* A cutoff of 500 pg/mg was used. The intra-assay precision at 250, 500, 1000 and 2500 pg/mg was determined to be 9.9%, 8.2%, 6.6% and 3.7% for AMP; 11.6%, 8.3%, 7.1% and 2.7% for METH respectively. All the negative specimens screened negatively using both ELISA and EIA. The results of the positive specimens are shown below.

| ***Sample*** | ***ELISA*** | | ***HEIA Cutoff 500pg/mg*** | | ***GC/MS (pg/mg)*** | |
|---|---|---|---|---|---|---|
| | *AMP* | *METH* | *AMP* | *METH* | *AMP* | *METH* |
| 1 | P | P | P | P | 930 | >10,000 |
| 2 | P | P | P | P | 103 | 6121 |
| 3 | P | P | P | P | 283 | 2360 |
| 4 | P | P | **N** | P | 257 | 8671 |
| 5 | **N** | P | **N** | P | 233 | 9273 |
| 6 | N | P | **N** | P | 105 | 2046 |
| 7 | P | P | **N** | P | 2883 | >10,000 |
| 8 | P | P | P | P | 311 | 7953 |
| 9 | P | P | **N** | P | 219 | 2707 |
| 10 | P | P | P | P | 108 | 6665 |
| 11 | P | P | P | P | 2390 | >10,000 |
| 12 | P | P | P | P | 5944 | >10,000 |
| 13 | P | P | P | P | 1089 | 9850 |
| 14 | P | P | P | P | 1825 | >10,000 |
| 15 | P | P | P | P | 3689 | >10,000 |
| 16 | P | P | P | P | 1130 | >10,000 |
| 17 | P | P | P | P | 1135 | >10,000 |
| 18 | P | P | P | P | 1115 | 9512 |
| 19 | P | P | P | P | 775 | 7088 |
| 20 | P | P | P | P | 645 | 5560 |

**Cross-reactivity***:* The METH antibody cross-reacts with MDMA (65%); the AMP antibody cross-reacts with MDA (45%). The assay is precise, sensitive and conducive to rapid hair screening using commercial chemistry analyzers.

Keywords: **Homogeneous immunoassay, Amphetamines, Hair**

## P25 Development and Validation of a Highly Sensitive Homogenous Immunoassay for the Detection of $\Delta^9$-THC in Oral Fluid Collected with the Quantisal™ Device

**Guohong Wang*,** Kim Huynh, Warren Rodrigues, Michael Vincent, James Soares, Christine Moore. Immunalysis Corporation, Pomona, CA, USA

**Background:** Oral fluid is a useful biological specimen to detect recent usage of drugs, and is included as a specimen type in the proposed Federal guidelines for workplace drug testing. While it has many advantages over urinalysis, such as observed collection and difficulty of adulteration, oral fluid also contains lower concentrations of drugs and sample volume is often limited. More importantly, existing urine THC immunoassays are designed specifically for THC metabolites, 11-nor -9-carboxy-THC (THCA) and not sensitive enough for the detection of the parent THC ($\Delta^9$-THC) that is the major component present in oral fluid.

**Sample collection:** Using the Quantisal™ oral fluid collection device, which incorporates a volume adequacy indicator, 1 mL of neat oral fluid is collected (N= 50, x = 0.993 mL, SD =0.029). The pad is then immersed in a transportation tube containing 3 mL of extraction buffer, capped and sent to a laboratory facility.

**Validation:** Taking into account the dilution factor associated with the collector, a highly sensitive homogenous enzyme immunoassay (HEIA) for $\Delta^9$-THC has been developed using a screening cutoff at 8ng/ml (= 2ng/ml of neat oral fluid). This assay uses only 20Δl of the diluted oral fluid specimen ( = 5Δl neat oral fluid). The intra assay precision at concentrations of 4, 8, and 16 ng/mL of THC was determined to be less than 10%. The extraction efficiency from the collection pad was demonstrated to be > 75% for parent drug THC. The buffer also provided stabilization of the extracted drug resulting in negligible losses during transportation to the laboratory. The assay was further challenged with oral fluid specimens previously confirmed by GC-MS. The results indicated that the newly developed THC HEIA assay well-correlated with both GC/MS and ELISA results as showed in the following table.

| | **GC/MS** | | **ELISA** | |
|---|---|---|---|---|
| **HEIA** | **+** | **-** | **+** | **-** |
| **+** | **34** | **4** | **33** | **2** |
| **-** | **3** | **36** | **4** | **38** |

Agreement with GCMS: Sensitivity: 34/(34+3) = 92%; Specificity: 36/(36+4) = 90%.

**Cross-reactivity:** The antibody cross-reacts with THCA at 200%. Potential adulterants including toothpaste, cranberry juice, baking soda, cola, mouthwash and cough syrup were fortified with drug at 50% and 150% of the cutoff. No interferences were noted.

**Summary:** The described assay is precise, specific and sensitive, and is suitable for the screening of oral fluid specimens collected with the Quantisal™ device at a cut-off concentration of 8ng/ml of $\Delta^9$-THC. This assay is compatible with most commercial chemistry analyzers.

Key Words: **Homogeneous Immunoassay, THC, Oral Fluid**

## P26 Qualitative Screening of Opiates in Hair Using a Homogenous Immunoassay Procedure

**Alpana Agrawal*,** Michael Vincent, Guohong Wang, Catherine Castro, Michelle Nguyen, Cecelia Henry, Christine Moore. Immunalysis Corporation, Pomona, CA, USA

**Background:** Hair is a useful specimen to detect long-term use of drugs. Hair generally contains low drug concentrations and sample volume is often limited. Following heroin use, 6-acetylmorphine (6-AM) is detected in hair in addition to morphine, so a screening immunoassay should be targeted to cross react with both analytes. Hydrocodone is also widely prescribed and a broad spectrum opiate assay should cross react with hydrocodone.

**Methods:** Hair (10 mg) from self reported opiate users (n = 20) as well as hair from drug free volunteers (n = 20) was cut; phosphate buffer was added (pH 2.7; 0.5 mL), and the hair was incubated (2 hrs/75$^{o}$C). The supernatant was analyzed using enzyme linked immunosorbent assay (ELISA) and by homogeneous immunoassay (HEIA) on an Olympus 400 platform. For ELISA, the supernatant was diluted 1:5 with PBS before plating; for homogenous EIA, 20 µL was used directly, making it conducive to most chemistry analyzers.

**Results:** An HEIA targeted at morphine using a screening cutoff of 200 pg/mg has been developed. The intra-assay precision at 100, 200, 1000 and 2500 pg/mg of morphine was determined to be 7%, 8.6%, 5.8 % and 10.1% respectively. All the negative specimens screened negatively using ELISA and EIA. The results of the positive specimens are shown.

| **Sample** | **ELISA** | **HEIA** | **GC/MS results (pg/mg)** | | | |
|---|---|---|---|---|---|---|
| **Cut-off** | *200 pg/mg Morphine* | *200 pg/mg Morphine* | **Morphine** | **Codeine** | **6-AM** | **Hydrocodone** |
| 1 | P | P | 1092 | 608 | 4365 | N.D. |
| 2 | P | P | 193 | 191 | 714 | N.D. |
| 3 | P | P | 641 | 393 | 2904 | N.D. |
| 4 | P | P | N.D. | N.D. | N.D. | 371 |
| 5 | P | P | 304 | 265 | 528 | N.D. |
| 6 | P | P | 866 | 428 | 5832 | N.D. |
| 7 | P | P | 552 | 565 | 2031 | N.D. |
| 8 | P | P | N.D. | N.D. | N.D. | 565 |
| 9 | P | P | 734 | 453 | 1430 | N.D. |
| 10 | P | P | 759 | 479 | 4792 | N.D. |
| 11 | P | P | 900 | 637 | 1436 | N.D. |
| 12 | **N** | **N** | N.D. | N.D. | N.D. | N.D. |
| 13 | P | P | N.D. | N.D. | N.D. | 604 |
| 14 | **N** | **N** | N.D. | N.D. | N.D. | N.D. |
| 15 | P | P | 495 | 284 | 1136 | N.D. |
| 16 | P | P | 427 | 215 | 1675 | N.D. |
| 17 | P | P | 647 | 335 | 2785 | N.D. |
| 18 | P | P | 335 | 195 | 1050 | N.D. |
| 19 | P | P | 688 | 431 | 3949 | N.D. |

**Cross-reactivity:** The opiate antibody cross-reacts 125% with codeine, 90% with 6-AM, with lower cross reactivity to hydrocodone. The assay is precise, specific, sensitive, and is suitable for the rapid screening of hair specimens at a cut-off concentration of 200 pg/mg of morphine.

Keywords: **Homogeneous Immunoassay, Opiates, Hair**

## P27 Qualitative Screening of Phencyclidine in Hair Using a Homogenous Immunoassay Procedure

**Alpana Agrawal***, Michael Vincent, Guohong Wang, Catherine Castro, Michelle Nguyen, Cecelia Henry, Christine Moore. Immunalysis Corporation, Pomona, CA, USA

**Background:** Hair is a useful specimen to detect long-term use of drugs. Hair generally contains low drug concentrations and sample volume is often limited.

**Methods:** Hair (10 mg) from proficiency samples and users (n = 10) as well as hair from drug free volunteers (n = 20) was cut; phosphate buffer was added (pH 2.7; 0.5 mL), and the hair was incubated (2 hrs/75$^{o}$C). The supernatant was analyzed using enzyme linked immunosorbent assay (ELISA) and by homogeneous immunoassay (HEIA) on an Olympus 400 platform. For ELISA, the supernatant was diluted 1:5 with PBS before plating; for homogenous EIA, 10 µL was used directly, making it conducive to most commercial chemistry analyzers.

**Results:** An HEIA targeted at PCP using a screening cutoff of 300 pg/mg has been developed. The intra-assay precision at 150, 300, 1000 and 2500 pg/mg of PCP was determined to be 3.7%, 4.5%, 1.3 % and 3.6% respectively. All the negative specimens screened negatively using ELISA and EIA. The results of the positive specimens are shown.

| ***Cut-off*** | ***ELISA 300 pg/mg*** | ***HEIA 300 pg/mg*** | ***GC/MS results (pg/mg) PCP*** |
|---|---|---|---|
| Sample 1 | P | P | 1790 |
| 2 | P | P | 5477 |
| 3 | P | P | 6396 |
| 4 | P | P | 25569 |
| 5 | P | P | 1571 |
| 6 | P | P | 4431 |
| 7 | P | P | 987 |
| 8 | P | P | 2875 |
| 9 | P | P | 5559 |
| 10 | P | P | 8085 |

**Cross-reactivity:** The PCP antibody has <0.4% cross-reactivity with dextromethorphan and < 0.02% cross reactivity with doxylamine. The assay is precise, specific and sensitive, and is suitable for the rapid screening of hair specimens at a cut-off concentration of 300 pg/mg of PCP.

Keywords: **Homogeneous Immunoassay, PCP, Hair**

## P28 Semi-Quantitative Procedure for the Screening of Acetaminophen and Salicylates in Whole Blood Using Enzyme-Linked ImmunoSorbent Assay (ELISA)

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**Background:** Acetaminophen and Salicylic acid derivatives are two of the most commonly used analgesics and antipyretics available on today's market; consequently, due to their ready availability, these two drugs can be taken excessively or in combination with other drugs, or alcohol, leading to potentially fatal results. Having the ability to obtain semi-quantitative results from these screening procedures would enable labs to more rapidly determine overdose cases, aid in assessing dilution factors necessary to accurately complete the confirmation procedures, and potentially cut out costly quantitative procedures currently being used.

The purpose of this study was to determine the ability to use the Immunalysis Direct ELISA Kit in conjunction with the Magellan data-reduction software to produce semi-quantitative results in Acetaminophen and Salicylate screening.

**Methods:** Standards were prepared in synthetic blood at the levels of 10µg/mL, 25µg/mL, 50µg/mL, and 100µg/mL for Acetaminophen and 20µg/mL, 50µg/mL, 125µg/mL, and 250µg/mL for Salicylates. A 1:20 dilution with PBS (Phosphate Buffer Saline) was carried out on all the samples and controls and a volume of 10µL was added to each sample well. Sample addition was followed by the addition of enzyme conjugate, incubation, a wash step, substrate reagent, a second incubation and the addition of a stop reagent, as outlined in the Immunalysis ELISA protocol. The plate was read using the Magellan software at 450nm with a reference wavelength of 620nm.

**Results:** The graphs generated by the software reduction package produced a linearity of $r^2$=0.994 for Salicylates and $r^2$=0.997 for Acetaminophen. The average of each calibrator was calculated from 4 runs completed over 5 days (n=20). Each average fell within a +/-20% range of the target value. Eight external controls were also analyzed over five days, all fell within the +/-20% range. The method was validated using ten blood samples of unknown concentration received from the Los Angeles County Department of Coroner-Toxicology Laboratory, five for each drug. One sample in each drug category fell outside of the curve, so an additional dilution of 1:5 in PBS was carried out, and the samples were re-screened.

**Summary:** Based on the results from this study, it was concluded that this semi-quantitative method is a precise and robust method, ready to be implemented in laboratories interested in using ELISA to screen for Salicylates and Acetaminophen, and procure semi-quantitative results from those screening procedures.

Keywords: **Acetaminophen, Salicylates, ELISA**

## P29 A New Faster One-Step On-Site Oral Fluid Drug Screen System

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Oratect®III, an on-site oral fluid test device, was introduced 3 years ago utilizing a one-step collection and testing procedure to provide results for 7 abused drugs within 7 minutes utilizing 0.5 ml. of oral fluid. This presentation shows the efforts to improve the Oratect®III device (OratectXP™) and the development of a portable reader for the results (OraVue™).

We first redesigned the Oratect®III device housing to accommodate a single test strip for the testing of 4 drugs: Opiates (Opi), Methamphetamine (Met), THC and Cocaine (Coc) using half the amount of oral fluid (0.25 ml). New raw materials for the test strip were evaluated and incorporated to enhance liquid flow. Each drug test was then reformulated to produce cut-off concentration that is in conformance with the SAMHSA proposed guidelines for workplace drug testing (Opi at 10 ng/ml; Met at 50 ng/ml; Coc at 20 ng/ml) except for THC which is at 20 ng/ml. Studies were made on oral fluid collection time required to initiate the test, the time needed to obtain a stable result after the test had been initiated and the correlation of the test results with LC/MS results.

Furthermore, we have studied the use of a handheld reader that can provide a fast display of the test results. Studies were undertaken to evaluate the correlation between results read by eye and those obtained by the reader.

Reproducible cut-off concentrations were obtained for Opi, Coc, Met and Δ9-THC. Collection study on 200 donors showed a mean collection time of 68 sec. (range: 21 sec. to 151 sec.). This compares to the mean collection time of 150 sec. for Oratect®III. Test line intensities reached optimal levels by 3 min for all tests in this new device and were stable for 7 min. Compared to LC/MS, initial studies on a limited number (n=10) of subjects has shown the following:

| | THC | Met | Opi | Coc |
|---|---|---|---|---|
| Sensitivity | 83.3% | 100% | 100% | 100% |
| Specificity | 100% | 100% | 88% | 100% |

Results obtained by the OraVue™ reader and by eye were shown to be 95% in correlation. Further efforts are being undertaken to improve the results. OratectXP™ is demonstrated to be a fast oral fluid drug screen enabling both collection and testing in 5 min. and the OraVue™ is shown to be a useful reader to gather the test results.

Keywords: **Oral Fluid, Drug Screen, Oratect**

## P30 Evaluation of the Lin-Zhi International Immunoassay for the Detection of Methadone in Urine

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We present a comparative evaluation of the Lin-Zhi International, Inc. (Sunnyvale, CA) immunoassay for the detection of methadone in urine to that of the DRI methadone immunoassay from Microgenics (Fremont, CA). These assays are homogenous assays based on competitive antibody binding between the methadone in urine and the enzyme glucose-6-phosphate dehydrogenase labeled methadone. When methadone is present in urine, active unbound enzyme reduces the co-enzyme NAD to NADH that results in an increase of measured absorbance at 340 nm. The assay is calibrated with known methadone concentrations.

The assays were evaluated by testing 764 urine specimens collected from criminal justice clients and pain management patients. All 764 specimens were simultaneously tested with both the DRI and Lin-Zhi assays at a cut-off calibration of 300 ng/mL methadone. The DRI and Lin-Zhi immunoassays were performed in an ADVIA 1200 Chemistry System auto-analyzer (Bayer Health Care, Diagnostics Division, Tarrytown, NY). Controls containing methadone at 0 ng/mL and –25% (negative control) and +25% (positive control) of the 300 ng/mL cut-off calibrator (Bio-Rad Laboratories, Irvine, CA) were analyzed with each batch of samples for both assays. All positive urines were then analyzed on an Agilent 6890 GC and 5973 MSD for methadone at the cut-off concentration of 300 ng/mL.

In the testing of the 764 urine samples, the two assays demonstrated good overall agreement with GC/MS results: DRI-93.5% and Lin-Zhi-93.6%. There were 30 false positives for the DRI assay and 34 false positives for the Lin-Zhi assay. False negatives also appeared under both the DRI and Lin-Zhi assays at 20 and 15, respectively. Using the 300 ng/mL methadone cut-off, both the DRI and Lin-Zhi assays displayed similar efficiencies, but the Lin-Zhi assay was slightly more sensitive (0.946 to 0.931) while the DRI assay was slightly more selective (0.937 to 0.930). The precision of the two assays was determined by the absorbance rates of the negative and positive controls. The within-run precision expressed as %CV (n=8) for both assays was <1% to <13%; while the between-run precision of the controls was <2% to <3%. Cross-reactivity of each assay was also studied. The DRI proved to be very selective for methadone, while the Lin-Zhi assay gave false positives for Brompheniramine, Chlorpheniramine, Diphenhydramine, Doxylamine, and the abundant metabolite of methadone, EDDP. Therefore, the DRI assay at the 300 ng/mL cut-off is more analytically efficient and reliable than the Lin-Zhi assay for routine methadone screening.

Keywords: **Methadone, Immunoassay, Urine Drug Testing**

## P31 Screening DUID Cases for Buprenorphine Using ELISA

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In October 2002, the Food and Drug Administration approved buprenorphine for treatment of opioid dependency. Two forms of the drug, Subetex® and Suboxone®, are available as sublingual buprenorphine tablets alone or in combination with naloxone (4:1 ratio), respectively. The Virginia Department of Health Professionals Prescription Monitoring Records show increasing numbers of buprenorphine tablets dispensed in 2006 (15,927), 2007 (45,844) and 2008 (62,169).

The goal of this study was to develop a screening method for buprenorphine and to assess the prevalence of buprenorphine in suspects arrested for driving under the influence of drugs (DUID) in Virginia. Buprenorphine was added to blood samples in 0.1, 0.2, 0.5, 1, 2, 5, and 10 ng/mL concentrations, diluted in a 1:10 ratio with water and analyzed using Immunalysis® Buprenorphine ELISA Direct Kits. The limit of detection (LOD) for buprenorphine was determined to be 0.5 ng/mL. Controls were established using the LOD, with a positive control (PC) of 1 ng/mL, and a high positive control (HPC) of 2 ng/mL. Three hundred previously analyzed DUID samples dating from the end of 2007 were evaluated using the buprenorphine ELISA kit with no positive results. Due to this discovery, 45 more recent samples from 2008 were evaluated with a result of one positive sample. This sample was confirmed for the presence of buprenorphine using an LC-MS method. Validation of the Buprenorphine ELISA kit by Immunalysis® was found to be successful in blood at and above 0.5 ng/mL concentrations. Furthermore, the findings indicate that at this point in time, buprenorphine does not yet appear to contribute significantly to DUID in the Commonwealth of Virginia. However, Virginia will continue to monitor the impact of buprenorphine on driving using targeted buprenorphine analysis on select DUID cases based on history.

Keywords: **ELISA, Buprenorphine, DUID**

## P32 General Unknown Screening by Ion Trap LC/MS. An Evaluation of ThermoFisher's Instrumentation and ToxID® Software

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The Federal Aviation Administration's Forensic Toxicology and Accident Research Laboratory performs a toxicological evaluation of biological specimens received from the pilot involved in each civil aviation accident that occurs in the US where specimens are available for analysis. Our laboratory has recently acquired a 2D linear ion trap mass spectrometer, the LTQ XL®, and ToxID® software from ThermoFisher Scientific. Since acquiring this system we have screened approximately 100 cases in duplicate for comparative purposes; once with our current procedure that includes liquid chromatography with both UV and fluorescence detectors and GC/MS and then with the new ion trap LC/MS system. Our current extraction procedure is as follows. Three mL of urine that is hydrolyzed, buffered to pH 6.0 and extracted with a Varian BondElut Certify SPE column. The columns are washed with 1 mL of 1 M acetic acid, dried, and then washed with 6 mL of methanol. The methanol wash contains any acids or neutrals present. It is saved for a secondary acid extraction. Basic compounds are eluted with ethyl acetate containing 2% $NH_4OH$. The saved methanol wash is evaporated, buffered to pH 6.0 and extracted with a UCT Styre Screen polymeric SPE column. The columns are then washed with 0.10 M phosphate buffer, pH 6.0, washed with 1 mL of 1 M acetic acid, dried, and then washed with 2 mL of hexanes. Acidic compounds are then eluted with 3 mL of methylene chloride. The LC is run in gradient mode with a total run time of 30 minutes. The mobile phase consists of acetonitrile containing 0.1% formic acid and 10 mM ammonium formate containing 0.1% formic acid. The mass spectrometer collects full scan mass spectra in both positive and negative ionization modes using an ESI source. $MS^2$ spectra are then collected in data dependent scan events throughout the run when a compound in the library is detected. ToxID® includes a library that currently contains 364 structurally diverse compounds with a wide molecular weight range (100-825 amu). Compound identification is based on both $MS^2$ spectra using fit values including both search index and reverse search index and retention time. Numerous compounds have been detected by LC/MS that were not seen by LC/UV, a few examples are: diphenhydramine, clopidogrel, clonazepam, carvedilol, chloroiquine, terazosin, doxazosin, fexofenadine, omeprazole, oxazepam, bisoprolol, amlodipine, THCA, cocaethylene, cocaine, and quinine.
The goal of our laboratory is two-fold; first, the eventual replacement of the LC/UV portion of our screening regimen due to the inherent lack of specificity with that technique, and second, simplification of the current extraction procedure.

Keywords: **General Unknown Screening, LC/MS, Forensic Toxicology**

## P33 Confirmation of Exposure to Gentian Violet in an 11-Week Old Infant with Detection and Quantitation of Leucocrystal Violet in Blood

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This is a report of a case of an 11-week old baby boy who was brought to the hospital by his mother. The infant was dead on arrival, in full rigor, and his mouth and tongue were stained purple/ violet in color. The parents reported that the child was being treated for thrush with gentian violet (GV).

GV is a triphenylmethane dye used as a local anti-infective agent, as an antihelminthic agent by oral administration, and as a blood additive to prevent transmission of Chagas' disease. It is also added to poultry feed to prevent the growth of *Candida*. GV is converted to leucocrystal violet (LV) in the gastrointestinal track by intestinal microflora. Ingestion may cause gastrointestinal irritation with nausea, vomiting, abdominal pain and diarrhea, while intravenous injection has been reported to cause depression in the white blood cell count. Poisonings have not been reported in man and no blood values could be found in the literature.

A detailed review of the full-scan GC/MS data from the blood base extraction showed a positive finding for LV. GV and LV were evaluated analytically and an analytical method was developed for LV. LV was quantitated in the blood using a two-step solvent extraction with no derivatization. Analysis was by gas chromatography with a dual-column, dual-detector (NPD and MS) instrument where quantitation was performed on the NPD and the full scan MS data was used to verify identity of peak and exclude interferences. The data showed acceptable linearity from 10 to 250-ng LV/mL blood, and two levels of standard addition to the blood sample showed acceptable matrix matching. LV was reported at 57-ng/mL blood. There were no other toxicologically significant findings.

Autopsy proceedings revealed that the baby was very small for age with slender limbs (< $5^{th}$ percentile) but the heart was enlarged, greater than two standard deviations above average weight, and the right ventricle was thickened. The lungs showed microscopic changes consistent with pulmonary hypertension. The cause of death was assigned as complications of patent ductus arteriosus, which is a congenital heart disease where there is abnormal circulation of blood between two of the major arteries (the aorta and the pulmonary artery) near the heart. The presence of LV was not found to be a factor in the death, but its presence and concentration may be of significance for other forensic toxicology laboratories that may encounter LV findings.

Keywords: **Gentian Violet, Leucocrystal Violet, Human Blood**

## P34 Fatal Case Report: Postmortem Concentrations of Fentanyl Related to Oral Abuse of Transdermal Patches

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The Food and Drug Administration (December 21, 2007), in response to a number of deaths and other cases of toxicity with life-threatening side effects, issued a second warning concerning the misuse of transdermal skin patches containing the potent analgesic drug fentanyl. Fentanyl is a prescription opioid drug, which includes transdermal skin patches marketed under the brand name Duragesic® as well as four other generic products. The second warning, specific to Duragesic® and generic fentanyl skin patches, emphasizes the strict adherence to patient prescription directions (e.g., dose, frequency) and exact compliance with product application/use instructions. The objective of this presentation is to describe the postmortem toxicological findings for a thirty-eight year old male succumbing to acute fentanyl toxicity as the result of the oral abuse of transdermal skin patches.

The decedent's immediate medical history was remarkable for follow-up treatment for knee pain after initially presenting to the ED three days earlier. The decedent was prescribed Percocet® (12 total). His other medication history was unknown, but previous history indicated depression and suicide ideation. Afterward, the man was found deceased with three fentanyl patches in his mouth. Table 1 summarizes distribution of the drug in blood, urine and gastric contents collected at autopsy.

Table 1. Fentanyl Distribution Results

| | **Blood (heart)** | **Blood (femoral)** | **Urine** | **Gastric*** |
|---|---|---|---|---|
| **Fentanyl (mg/L)** | 0.05 | 0.02 | 0.07 | 14.6 |

*Total Volume of Gastric Contents at autopsy = 120 mL

Conclusion: Herein we describe an oral form of transdermal fentanyl patch abuse with accompanying postmortem fentanyl concentrations reported for heart blood, femoral blood, urine and gastric contents. Chewing on, or sucking, the contents from transdermal fentanyl patches are a popularized route of self-administration of the drug for the purposes of misuse or abuse.

Keywords: **Fentanyl, Oral Abuse, Postmortem**

## P35 Postmortem Distribution of Disopyramide

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Disopyramide (Norpace® and Rythmodan®) is an oral antiarrhythmic medication primarily used to treat ventricular tachycardias. It has been in clinical use since the late 1960s and it is available in immediate and controlled release capsules. The typical disopyramide dosage for adults whose body weight is greater than 110 pounds is 600 mg/day and 400 mg/day for adults whose body weight is less than 110 pounds. The blood level attained with this regimen is 2 to 5 mg/L.

Disopyramide is a Type 1 antiarrhythmic drug, part of a class including procainamide and quinidine. In addition to its antiarryhthmic effects, disopyramide also has anticholinergic effects, resulting in side effects including dry mouth, blurred vision, urinary retention and constipation. Signs of disopyramide toxicity include various heart rhythm disturbances, worsening of congestive heart failure, hypotension, varying kinds and degrees of conduction disturbance, bradycardia and asystole. Rapid treatment of disopyramide overdosage is necessary, even in the absence of symptoms. There is no available antidote for disopyramide intoxication, so traetment is mainly supportive. Hemodialysis or hemoperfusion with charcoal may also assist with lowering serum concentrations of disopyramide.

Case History: A 54 year old male who had no medical history was found lying in his bed. His relatives were concerned that he may have been suicidal after his father's death nine days earlier. On the day of his death, the decedent's relatives contacted him by phone and he asked them not to come by his home for an hour after speaking with them. The decedent was found unresponsive approximately 1 ½ hours later. He was lying supine on his bed with his head resting on the wall. There were over 100 medication bottles found in the home; these medications were prescribed to his parents who shared the residence.

At autopsy, granular sediment was observed in the decedent's stomach; other autopsy findings were unremarkable. Specimens were submitted for toxicological analysis and analyzed for volatiles, acidic/neutral drugs, basic drugs and free morphine. There were no drugs other than disopyramide detected.

Disopyramide was extracted from the matrices with a liquid-liquid extraction for alkaline drugs and quantitated by GC-NPD. A five-point calibration was performed, with ethylmorphine as the internal standard. The distribution of disopyramide in different specimens can be found in the table below.

| Biological Matrix | Disopyramide (mg/L or mg/kg) |
|---|---|
| Heart Blood | 53 |
| Liver | 150 |
| Kidney | 130 |
| Vitreous Humor | 11 |
| Urine | 29 |
| Bile | 95 |
| Gastric Contents | 818 mg total |

Disopyramide was detected in all specimens analyzed. The heart blood concentration was about ten times the therapeutic concentration. The liver concentration was three times that found in blood. The lowest concentration was found in vitreous humor. The medical examiner ruled that the cause of death was disopyramide toxicity and the manner of death was suicide.

Keywords: **Postmortem, Tissue Distribution, Disopyramide**

## P36 Secobarbital Drug Seizure and Fatal Intoxication

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Secobarbital is a short acting sedative hypnotic barbiturate with anesthetic, anticonvulsant, sedative and hypnotic properties. It is a central nervous system depressant used to produce drowsiness and promote sleep. Barbiturates were widely abused in the 1960s and 1970s and were cited as the cause of death in many high-profile suicides during that time. However, in recent years, these incidents of abuse have declined with the preference of much safer sedative hypnotic drugs such as benzodiazepines.

This case report describes an interesting combination of a secobarbital drug seizure by law enforcement and an incident of fatal secobarbital intoxication in approximately the same time period and locality in Southwestern Virginia. The decedent was a 52 year old male who was known to be at a party the night before where unknown drugs were available. He was found dead the next day in the home with red capsules in his personal effects. The body was submitted to the Office of the Chief Medical Examiner, Western District in Roanoke, Virginia for autopsy. That same day law enforcement officers served an arrest warrant on a suspect who was found to be in possession of sixty-six red capsules and thirty-two clear red capsules. The suspect was charged with possession of a controlled substance and the capsules were confiscated and sent to the Drug Chemistry Section of the Department of Forensic Science Western Laboratory in Roanoke for identification.

An autopsy was performed and blood, vitreous, urine, bile and liver were submitted to the Toxicology Section of the Department of Forensic Science Western Laboratory for toxicological analysis. Barbiturates screened positive in the blood using Immunalysis ELISA and secobarbital was identified by GCMS on an acid drug screen. Secobarbital was extracted using a hexane/ethyl acetate liquid-liquid extraction followed by methylation and quantification by SIM GCMS. Cyclopal was used as an internal standard. The linear range was from 1 to 20 mg/L using a 5 point standard curve ($r^2 = 0.999$). Secobarbital was identified in the capsules by full scan GCMS following derivatization with trimethylanilinium hydroxide (TMAH). A small amount of pentobarbital was identified in the capsules upon GCMS analysis. Pentobarbital was also identified in the blood but it was below the 1 mg/L limit of quantification. The pathologist ruled the manner of death as accidental and the cause of death as acute secobarbital poisoning. Pathological findings were pulmonary congestion, hypertensive and atherosclerotic cardiovascular disease and cardiomegaly.

| | Secobarbital |
|---|---|
| Specimen | mg/L or mg/Kg |
| Blood | 15.9 |
| Vitreous humor | 5.2 |
| Urine | 4.9 |
| Bile | 31.2 |
| Liver | 75.2 |

Keywords: **Secobarbital, Postmortem, Tissue Distribution**

## P37 Evaluation of a Hand Held Glucometer for the Determination of Vitreous Humor Glucose Concentrations

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The use of postmortem vitreous humor (VH) is considered a reliable indicator of some antemortem biochemical states, such as diabetic ketoacidosis, dehydration, and renal function. The composition of VH is predominantly water, about ninety-nine percent, while the rest of its constituents include hyaluronic acid and collagen. As with other viscous body fluids like spinal fluid or synovial fluid, the VH can be analyzed by most chemical analyzers. In our laboratory, VH glucose analyses are referred to an outside laboratory. In an effort to have a low cost in house method for the analysis of glucose in VH, we evaluated the use of a hand held glucometer for such measurements.

In this evaluation, comparative measurements of glucose in VH were made on a chemical analyzer and on a hand held glucometer. The reference measurements were performed on a Beckman Coulter DXC 800 model. The hand held glucometer was the One Touch Ultra 2. Samples used in this study were obtained from the Maryland Office of the Chief Medical Examiner during the time between 2006 and 2008.

The VH samples were divided into two groups: group one consisted of cases with a reference VH glucose in excess of 200 mg/dL (n=18) and group two was comprised of cases with a reference VH glucose less than 200 mg/dL (n=48). For the glucometer analysis, the specimens were analyzed with and without hyaluronidase treatment to liquefy the specimen. It was determined that hyaluronidase pretreatment did not alter the glucometer test results. However, most specimens were easily analyzed without liquefying agent treatment.

In our laboratory, a vitreous glucose over 200 mg/dL is generally considered elevated. The hand held glucometer accurately identified elevated vitreous glucose in all cases (n=18) when compared to the analyzer results. The hand held glucometer identified non-elevated vitreous glucose in 44 of 48 cases with a non-elevated reference result. In the four remaining cases from the non-elevated group, the glucometer gave a result greater than 200 mg/dL, while the analyzer result was between 100 and 200 mg/dL. There were no visually identifiable differences between this group of four samples and the remaining 44 samples.

In conclusion, the hand held glucometer was an acceptable screening tool for determination of glucose in vitreous humor. The glucometer correctly identified all specimens with an elevated glucose. However, there were four specimens that the glucometer gave a false positive result for. There were no false negatives in this sample set. While the glucometer would not be suitable as the sole method for vitreous glucose analysis, it would be useful as an initial screening method, with all positive results receiving an additional analysis by a reference analyzer. The use of a glucometer as a screening tool has two prominent advantages; one being low cost, and the other being the rapid turnaround time for results (5 seconds).

Keywords: **Vitreous Humor, Glucose, Postmortem**

## P38 Determination of the Presence of Toluene in Blood Collection Tubes

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Toluene, a volatile compound, is an ingredient often found in paint and industrial solvents. Accidental exposure can occur because of occupational use of toluene and toluene-containing compounds. Toluene can also be inhaled intentionally as a drug of abuse.

Hospital admission samples were received by The Washington State Toxicology Laboratory from a 5 year old male decedent with no anatomical cause of death at time of autopsy. Alcohol/volatile testing was performed using headspace gas chromatography- flame ionization detection (HSGC-FID) and confirmed using gas chromatography-mass spectrometry (GCMS).

In testing the hospital samples for ethanol, the plasma sample from the Vacuette® gel separator tube indicated the presence of toluene. Subsequent testing was performed on concurrently collected admission samples. Hospital blood collected in a lavender top tube tested negative for toluene, as did postmortem blood collected in a grey top tube.

Various Vacuette® and B-D Vacutainer® tubes, both with and without gel separators, were tested for the presence of toluene. Whole blood from 10 volunteers was added to each tube, which was then centrifuged to achieve gel separation. Serum/plasma testing was performed using HSGC-FID and confirmed.

Serum from Vacuette® Z Sep. Serum Clot Activator tubes and plasma from Vacuette® LH Lithium Heparin Sep. tubes tested positive for toluene, based on retention time match from HSGC-FID method, and retention time and mass spectrum matches from GCMS. This demonstrates that the positive toluene is a result of the presence of the gel separators in the tubes.

As a forensic laboratory, it is critical that information provided to submitting agencies is both complete and accurate. Because samples are received in a variety of blood collection tubes, it is important to identify possible false positives due to the manufacturing process or nature of additives in the tube. This experiment was performed in order to determine if the tube was the source of the positive toluene result in this case, as well as to share this important information with other forensic laboratories, decreasing the chance of future false positives being reported.

Keywords: **Toluene, Gel Separators, Volatiles**

## P39 Muscle as an Alternative Post-Mortem Specimen for Drug Screening by Enzyme Linked Immunosorbent Assay (ELISA)

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**Objective**: Forensic toxicologists involved in the provision of post-mortem toxicology services are often presented with unconventional autopsy specimens primarily due to the state in which a cadaver is discovered or the passing of time since death might have occurred. Blood and urine are commonly employed in post mortem drug screening but if a cadaver is decomposed or partially skeletonized, these specimens may be of small volume or may not be available to be collected for analysis. In this study we evaluate the use of human muscle submitted to the toxicology laboratory of the Forensic Laboratory Division of the San Francisco Office of the Chief Medical Examiner as a post-mortem screening specimen using a commercially available enzyme linked immunosorbent assay (ELISA) and we examine the effects, if any, that different time intervals between collection at the autopsy and ELISA analysis and temperature storage conditions may have on the obtained ELISA results.

**Method:** Post mortem specimens were collected at autopsy from five decedents with no known drug history who died within the preceding 48 hrs. From each muscle specimen collected, 13 portions weighing 0.5 g each were cut into separate plastic containers corresponding to intended storage temperature and analysis after overnight storage at -20$^{o}$C. On analysis day one, ELISA was performed on a portion from each muscle specimen as well as on peripheral blood and urine from the same decedent, if collected. All remaining muscle portions were stored into their respective storage temperatures (i.e. room temperature, 4$^{o}$C, or -20$^{o}$C) for the prescribed length of time (i.e. day two, week one, week two, or month one) until being screened by ELISA. In preparation for ELISA, muscle specimens (0.5 g) were homogenized in a 7-mL Pyrex® manual homogenizer in 4.5 mL buffer/rinse solution provided by the ELISA plate manufacturer (Venture Labs, Inc.) Homogenates were centrifuged for 10 minutes at 2800 rpm and a 30-µl aliquot was collected from the resulting middle layer (below the fatty supernatant layer but above the particulate sediment layer) and plated in each well of a row on a Multi-Test Array (MTA™)-12, 96-well, microtiter plate together with two whole blood positive controls and one whole blood negative control. Enzyme conjugate (75-µL) was added to each well and the plate was mixed by gentle tapping and allowed to incubate for 30 minutes at room temperature before being thoroughly washed under cold tap water ten times. The plate was then washed on a Tecan Columbus Pro™ plate washer, rinsed with buffer/rinse solution, and pounded dry on clean paper towels. Substrate solution (100-µl) was added to each well and the plate was allowed to incubate undisturbed at room temperature for 15 minutes before stop solution (50-µl) was added, and the plate was mixed by gentle tapping. At the end of the 15 minute interval the plate was immediately read on a Thermo LabSystems Multiskan EX using Ascent Software.

**Results:** The Venture Labs, Inc. MTA™-12 as used at this facility screens for amphetamine, barbiturates, benzodiazepines, cocaine, fentanyl, methadone, methamphetamine, opiates, oxycodone, phencyclidine, propoxyphene, and tricyclic antidepressants. Using the blood and urine results for comparison purposes, if available, we determined that muscle specimens stored at room temperature should be analyzed by ELISA within 48 hours at the latest in order to minimize the chance of false positive results. In our study, screening muscle specimens by ELISA produced false positive results after storage at room temperature for one week for fentanyl, two weeks for cocaine, propoxyphene, barbiturates and fentanyl and one month for opiates, barbiturates and fentanyl. Specimen refrigeration at 4$^{o}$C improved the accuracy of the ELISA screening as no false positive results were obtained after storage at this temperature for one day, two days, one week or two weeks; false positive results were obtained for opiates and barbiturates after storage at this temperature for one month. Specimen freezing at -20$^{o}$C further improved the accuracy of the ELISA screening as no false positive results were obtained after storage at this temperature for one day, two days, one week, two weeks or one month.

**Conclusions:** Muscle was evaluated as an alternative post-mortem specimen for drug screening using a commercially available ELISA. Our results suggest that some antibodies in the commercially available ELISA plates used (i.e. amphetamine, benzodiazepines, methadone, methamphetamine, oxycodone, phencyclidine, and tricyclic antidepressants) suffered little variation but that others (i.e. cocaine, opiates, propoxyphene, barbiturates and fentanyl) could be prone to varied results depending on the length and temperature of storage as well as the time between death and autopsy as well as the fat content of the analyzed muscle. As this is a study based on a small number of specimens and only employing one manufacturer's ELISA kits, it may be necessary to carry out further work in order to better qualify our preliminary results which appear to suggest that muscle can be a useful analytical specimen in post-mortem toxicology cases where blood or urine are not available or are available in very small volumes which should be saved for confirmatory/quantitative analyses.

Keywords: **ELISA, Muscle, Screening**

## P40 Investigation of the Stability During Storage of Routinely Observed Drugs in Postmortem Blood Over Time Utilizing Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

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During the course of previous method development concordance studies, it was determined that various drugs previously confirmed in case blood samples were no longer present. It was concluded that those drugs had degraded in vitro in the specimens, all of which had been in cold storage (temperature) for a minimum of one year. While this phenomenon is not unknown, there is apparently little systematic and long term data available for drugs aside from common drugs of abuse. This study was performed in an attempt to address this observed phenomenon.

Sixty three commonly encountered drugs were divided into five mixes and prepared at four different concentrations. Zopiclone was prepared in its own solution The postmortem blood used for specimen preparation was screened to confirm that it was negative for detectable levels of study drugs. The sets of spiked samples were then aliquoted at various time intervals and extracted utilizing a previously established protein precipitation method with subsequent analysis with liquid chromatography/tandem mass spectrometry (LC/MS/MS). At each sampling, each specimen was aliquoted twice, and each aliquot extracted and injected twice, totaling four injections per specimen.

Several drugs included in the study which are known to decompose over time, e.g. cocaine, have shown expected reduction in concentrations. Concentrations were compared to the initial value established one day after preparation. The majority of drugs included in this study have not shown a significant reduction in concentration for up to 205 days, however the study is still ongoing. Drugs that have shown significant reduction in concentration include cocaine (-67%), cocaethylene (-47%), benzoylecgonine(-14%), zopiclone (-78%), mesoridazine (-67%), clonazepam (65%), diltiazem (-71%) and bupropion (-62%).

Keywords: **LC/MS/MS, Drug Stability, Postmortem**

## P41 Correction for Drug Loss During Storage: Decomposition of Chlorodiazepoxide and Oxazepam in Whole Blood Under Different Storage Conditions.

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Decomposition of analytes in biological samples during the post-mortem interval or during storage has frequently been investigated, including studies on the instability of chlorodiazepoxide and oxazepam under different storage conditions. Corrections can be made for drug losses and the present study evaluated an approach to the problem based on measurement of the main hydrolysis decomposition product of chlorodiazepoxide and oxazepam, i.e. 2-amino-5-chlorobenzophenone (ACB). The aims of this study were (a) to determine ACB in whole blood as an indicator of the extent of analyte degradation and (b) to investigate the effects of temperature and pH on the stability of chlorodiazepoxide and oxazepam in whole blood for one month.

Blank blood was spiked with chlorodiazepoxide and oxazepam to produce final concentrations of 1000 ng/ml of each analyte. It is known from previous work that these drugs are stable under the usual storage conditions for more than one month. Therefore, for the purposes of this study, degradation of the drugs was accelerated by storing aliquots of blood at high temperature (80°C) and under acidic (pH 2) and basic (pH 12) conditions at room temperature for one month. Samples were taken for analysis in duplicate on days 1, 2, 4, 7, 14 and 30. These were extracted by solid phase extraction and extracts were analysed for chlordiazepoxide, oxazepam and ACB by liquid chromatography-tandem mass spectrometry using methodology developed previously[1].

Recoveries of all analytes were between 83-97%. Calibration curves were linear with correlation coefficients greater than 0.99. Limits of detection and lower limits of quantitation were 0.46-2.3 ng/ml and 1.5-7.5 ng/ml respectively. Intra-day and inter-day precisions were found to be 1-13% and 2-18% respectively. After one month, the chlorodiazepoxide concentration at 80 °C, pH 2 and pH 12 had decreased from the original concentration by 62, 51 and 100 % respectively whereas oxazepam decreased by 100%, 99.7% and 100% respectively. Under all conditions ACB was observed to increase as degradation of the drugs proceeded and could be detected after the parent drugs had completely disappeared. However, ACB itself was further degraded under some of the conditions used. Chlorodiazepoxide was noted to be more stable than oxazepam but both drugs were more sensitive to alkaline pH than to acidic pH or high temperature.

It is concluded that degradation of chlorodiazepoxide or oxazepam in blood under different conditions will produce ACB as the main hydrolysis product, especially when the drug has decomposed due to poor or prolonged storage conditions. ACB can be reliably detected in whole blood and used to correct for drug loss and to indicate the original drug concentration. ACB can also be used to confirm the earlier presence of the drugs in samples after the parent substances are no longer detectable, but cannot identify which drug was initially present.

[1] Correction for drug loss during storage: decomposition of diazepam and temazepam in whole blood under different storage conditions, A.Alfazil, R.A.Anderson,. Presented at the 46th International Meeting of TIAFT, Martinique, 2-8 June 2008.

Keywords: **Benzodiazepines, Degradation, ACB.**

## P42 Genetic Polymorphism of the CYP3A4 Gene May Contribute to Methadone-Related Deaths

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The pharmacokinetics of methadone show extreme inter-individual variability, making the relationship between dose, plasma levels, and effects not clearly defined. These facts make methadone difficult to administer and increase the risk of unexpected death. In a retrospective study of methadone-only deaths from the West Virginia Office of the Chief Medical Examiner (WV OCME), the methadone to 2-ethyl-1,5-dimethyl-3,3-diphenylpyrolidine (EDDP) ratio (an indicator of the amount of methadone metabolized) was found to be higher in individuals who had overdosed compared to individuals successfully undergoing methadone maintenance treatment. The high ratio may be associated with a poor-metabolizer phenotype that could be the result of gene defects associated with or caused by one or more single nucleotide polymorphisms (SNPs) in the CYP3A4 gene. CYP3A4 is a key P450 involved in the metabolism of methadone. To assess this possibility, 93 individuals who had died of a methadone-only overdose were genotyped at four different SNP sites on the CYP3A4 gene. Blood collected during autopsy at the WV OCME was preserved on Whatman Protein Saver Cards. Genomic DNA was isolated from the blood using a Qiagen single tube extraction method for dried blood spots. SNP genotypes were determined by Taqman Allelic Discrimination Analysis. Data were collected and analyzed by ABI 7000 Sequence Detection instrument and software. All four of the loci studied were within Hardy-Weinberg Equilibrium (HWE). Once HWE was confirmed, the frequencies of the genotypes for each SNP were compared to the frequencies expected in the general population based on population statistics from NCBI. In three of four SNP loci studied (rs2246709, rs3735451, and rs4646437); the observed genotype frequencies were not significantly different compared to those expected. However, in the fourth SNP examined (rs2242480), the frequency of individuals who were carriers of the minor allele or homozygous for the minor allele was significantly different than the general population ($p<0.001$). These initial findings indicate that a genetic polymorphism on the CYP3A4 gene may contribute to unexpected death from methadone.

Keywords: **Methadone, CYP3A4, Genetic Polymorphism**

## P43 Pharmacogenomics and Opioid Drug Abuse Therapy: A Case Involving Methadone Toxicity

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Opioid abuse is on the rise in the United States. Approximately 2 to 2.5 million Americans have prescription opioid dependency, but few are receiving effective treatment. The most effective treatment strategies involve pharmacologic and psychosocial approaches to avert drug use. Opioid abuse treatment may be improved by the characterization of the pharmacogenomic profile that determines an individual's ability to metabolize a drug, predicting toxic effects at otherwise normal doses. In this study, we examined if the pharmacogenomics of cytochrome P450 (CYP) drug metabolizing enzyme genes may facilitate opioid abuse treatment by predicting adverse effects in a methadone toxicity case. The decedent was a 49 year old male, with a medical history of heroin and cocaine use. He was undergoing treatment for obstructive pulmonary disease, hepatitis C, and end stage emphysema with oxygen dependency. The decedent was found unresponsive at his home with an inhaler by his leg and drug paraphernalia nearby. Pharmacy records indicated prescriptions for methadone, lactulose, oxycodone, lasix, albuterol, ipratropium, combivent, protonix, spironolactone, zithromax and propoxyphene. Toxicology analysis of the decedent's post-mortem blood sample identified a high level of methadone (1.12mg/L) in subclavian blood. The decedent was genotyped to determine if there was a genetic dysfunction of CYP enzymes related to methadone toxicity. The presence of mutations in CYP 3A4 (*1B), 2D6 (*3, 4, 5, 6, 7, and 8) and 2C19 (*2, 3, and 4) isoforms that metabolize methadone to 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) were examined using pyrosequencing (Biotage Inc.). The decedent's genotype was CYP3A4 *1B/*1B. In conclusion, the genotyping result classified the decedent as a poor metabolizer of methadone, a probable cause of the decedent's high methadone level and ultimate death. Genetic counseling informing an individual of their pharamacogenomic profile and susceptibility to overdose by certain drugs concentrations, may serve to reduce drug diversion and subsequent death.

Keywords: **Pharmacogenomics, Opioid, Toxicity**

## P44 Validation of a 16 Assay ELISA Panel for DUID Investigations

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An ELISA screening procedure was recently validated for both the immunoassay kits and robotic analyzer for the New York State Police Laboratory System. While ELISA testing is not novel for drugs of abuse, the ability to screen for a variety of drugs with a simple dilution was desired. The validation was successful in achieving a single dilution for blood and urine specimens while incorporating cutoff levels from the recently published recommendations for drug impaired driving.[1]

A total of 16 assays from Immunalysis (Pomona, CA) were validated. The kits, their respective calibrators and cutoff values are listed in the table below: All assays were validated in both urine and blood on the Dynex DSX 4 System (Chantilly, VA). Urine samples (125µL) were diluted 1:20 while blood samples (250µL) were diluted 1:10, minimizing sample preparation and amount of sample needed for the analysis. All assays incorporated matrix matched controls: two cutoff controls, two negative controls, and two positive controls at 2x the cutoff value. An exception is made for Carisoprodol's positive control which is analyzed at 10x the cutoff value to achieve adequate discrimination from the cutoff.

The validation protocol included studies of precision, linearity and comparison with previous casework. Intra-assay precision was evaluated at the cutoff and found to be no greater than 15%. Linearity studies were performed to characterize the assay around the cutoff and all assays except carisoprodol exhibited good discrimination. Verification of the method was performed with 100 cases (60 urines and 40 bloods) and all were comparable with the current FPIA screening technique.

With the implementation of ELISA, the cutoffs for many drug assays have been lowered such that the laboratory's screening protocol now addresses the current DUID recommendations. ELISA provides a complementary analytical technique requiring minimum time and specimen to the comprehensive basic drug screening procedure already utilized within the New York State Police Laboratory System.

| Drug or Drug Class | Calibrator | Urine Cutoff ng/mL | Blood Cutoff ng/mL |
|---|---|---|---|
| Amphetamines | d-Amphetamine | 200 | 20 |
| Barbiturates | Secobarbital | 200 | 100 |
| Benzodiazepines | Oxazepam | 100 | 50 |
| Cannabinoids | (-) Carboxy THC | 20 | 10 |
| Carisoprodol | Carisoprodol | 500 | 500 |
| Cocaine/Benzoylecgonine | Benzoylecgonine | 300 | 50 |
| Fentanyl | Fentanyl | 5 | 1 |
| Methadone | Methadone | 300 | 50 |
| Methamphetamine | d-Methamphetamine | 200 | 20 |
| Ketamine | Ketamine | 50 | 10 |
| Opiates | Morphine | 200 | 20 |
| Oxycodone | Oxycodone | 200 | 20 |
| Phencyclidine | Phencyclidine | 25 | 10 |
| Propoxyphene | Propoxyphene | 300 | 50 |
| Tricyclic Antidepressants | Nortriptyline | 200 | 100 |
| Zolpidem | Zolpidem | 50 | 25 |

Keywords: **ELISA, Validation, DUID**

[1] Farrell, L, Kerrigan, S, Logan, B. Recommendations for Toxicological Investigation of Drug Impaired Driving. J Forensic Sci 2007;52(5):1214-8.

## P45 Concentrations of Diazepam, Nordiazepam and Temazepam, in Whole Blood Samples Collected from 354 Drivers in the United Kingdom, Suspected of Driving Whilst Under the Influence of Drugs (DUID)

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The concentration ranges of diazepam, nordiazepam and temazepam in whole blood collected from 354 drivers charged with driving whilst impaired through use of benzodiazepine drugs is presented. Confirmatory and quantitative analyses were undertaken using automated solid phase extraction (SPE) followed by gas chromatography mass spectrometry (GC/MS).

The data is presented as percentage of cases versus whole blood drug concentration. The analysis of data in this format can assist forensic toxicologists to provide interpretative evidence in court, in a manner that can be easily understood by a jury.

| Drug | 25% cases (µg/ml) | 50% cases (µg/ml) | 75% cases (µg/ml) | 100% cases (µg/ml) |
|---|---|---|---|---|
| Diaz with ND | 0.23 | 0.44 | 0.71 | 2.33 |
| Diaz with ND and Tem | 0.63 | 1.13 | 1.79 | 8.13 |
| ND with Diaz | 0.15 | 0.25 | 0.45 | 2.22 |
| ND with Diaz and Tem | 0.31 | 0.68 | 1.26 | 8.88 |
| Tem alone | 0.58 | 0.91 | 1.35 | 3.60 |
| Tem with Diaz and ND | 0.07 | 0.12 | 0.34 | 4.67 |

Diazepam (Diaz), Nordiazepam (ND) and Temazepam (Tem).

Keywords: **DUID, Benzodiazepines, Interpretation**.

**P46 GBL and SFSTs**

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Roadside sobriety tests are often used by police officers to determine if a driver is intoxicated by alcohol or drugs. NHTSA has validated a series of three standardized field sobriety tests (SFSTs) as having the greatest predictive power with respect to impairment: horizontal gaze nystagmus (HGN), one-leg-stand (OLS) and walk-and-turn (WAT). These have been evaluated statistically for their ability to detect both high and low blood alcohol levels, but such evaluations are difficult to do in the case of drugs. If a subject cannot perform the SFST battery, and no alcohol is present, he is most likely under the influence of a depressant drug; however, the responses of subjects under the influence of various drugs to SFSTs have been reported mostly anecdotally.

Gamma-butyrolactone (GBL) is a derivative of gamma-hydroxybutyrate (GHB) and is found in industrial solvents such as floor strippers and wheel cleaners. If ingested, GBL is converted by endogenous lactonases into GHB. There are very few reports of the effects of GBL on driving (Couper & Logan, *J. Anal. Tox*, **28**, 2004; Jones et al, *J. Anal. Tox.* **31**, 2007), and little documentation of the responses of GBL-impaired subjects to field sobriety tests. Presented here is a driver who had ingested GBL, and the results of his performance on a series of SFSTs.

The subject was encountered by police officers while walking along the shoulder after driving his vehicle off the road. He showed visible signs of impairment including slurred speech, confusion, difficulty standing and unsteady gait. A preliminary breath test showed no alcohol present. The officers administered six separate sobriety tests: HGN, OLS, and WAT as well as an alphabet recitation, finger count and number count. The subject either flunked or could not complete five of the tests, passing only the alphabet recitation. A bottle of wheel rim cleaner containing GBL was found in his car. Analysis of his blood showed 70 µg/ml GHB. No alcohol or other drugs were present.

The subject's physical condition and the results of the SFSTs indicated that he was seriously impaired. As it was apparent that he ingested GBL, this case demonstrates that GBL impairs driving ability by internal conversion to GHB, and that GBL's intoxicating effects can be detected by the NHTSA-validated SFST battery.

Keywords**: Gamma-Butyrolactone, Driving, SFST (Standardized Field Sobriety Testing)**

## P47 Alcohol Related Aviation Accidents Involving Pilots with Previous Alcohol Offenses

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**Introduction:** The Federal Aviation Administration (FAA) requires airmen to report legal actions involving ethanol and/or other drugs, including driving while impaired by or while under the influence of alcohol. Pilots are also required to report any administrative action resulting in denial, suspension, cancellation, or revocation of driving privileges or mandatory attendance at an educational or rehabilitation program.

**Objectives:** The purpose of this study was to retrospectivly evaluate fatal civil aviation accidents occuring between the years 2000 and 2007 in which ethanol was present in the pilot, and the pilot had previously documented drug and/or alcohol offenses and/or dependence.

**Methods:** Toxicological and aeromedical findings from pilots were collected for an 8-year period, 2000 - 2007. Case histories, accident information, and the probable cause of the accidents were obtained from the National Transportation Safety Board (NTSB). Toxicological information was obtained from the Civil Aerospace Medical Institute's (CAMI's) Forensic Toxicology Research Laboratory.

**Results:** During the examined time period 215 pilots (9%), of the 2,391 received for analysis, had documented alcohol or drug related offense. Of the 215 pilots, 23 (11%) had consumed ethanol prior to the fatal incident, of these 23 pilots, 16 (~70%) had ethanol concentrations above the FAA's legal limit of 40 mg/dL and 7 (~30%) between 20 and 40 mg/dL. Pilots involved in aviation accidents from 2000 to 2007 whom had a history of drug abuse, dependence, or offense(s) accounted for ~7% of all aviation accidents during that time period.

**Conclusion:** Providing more detailed documentation to Aviation Medical Examiners would aid in the determination of eligibility for medical certification and could potentially save pilots as well as their passengers' lives. Identifying pilots with substance abuse problems is paramount for providing a safe environment to fly but also benefits the pilots whom may not have addressed these issues.

Keywords: **Alcohol, Offenses, Aviaiton**

## P48 Drunk Driving: A Retrospective Study of 440,000 Cases Between 1989 and 2007 in Finland

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Driving under the influence (DUI) of alcohol is considered a serious road safety problem. People with DUI arrests are often associated with alcohol dependence and health problems. The aims of the study were to investigate the incidence, demographics and trends of suspected drunk driving during 19 years between 1989 and in Finland.

The data included all suspected drunk driving cases (N=440,000) in Finland between 1989 and 2007. Cases involving illicit or medical drugs were excluded. The data were limited to motorized road traffic. The annual incidence per 1000 population by sex and age was calculated.

The changes in the number of drunk driving cases paralleled trends of economic development and changes in overall alcohol consumption. These changes were most significant amongst 18 to 19 year olds, whose incidence rate was halved from 20.5 to 10.2 per 1000 population between 1990 and 1994. The proportion of female drunk drivers almost doubled from 6,5 % to 11 % of all cases over the study period. The incidence of drunk driving among those over fifty years increased 2,5-fold. Most incidences occurred during weekend nights.

The proportions of females and the aged drunk drivers has steadily increased. However, young men aged 18 to 19 years are still at the highest risk of committing drunk driving offences. Alcohol consumption shapes drunk driving more among the young than other drivers. Preventative measures in healthcare should be targeted for youths close to the legal age of driving. Traffic control is needed, especially during weekends, national holidays and late hours.

Key words: **Alcohol, Drunk Driving, Road Safety**

**P49 Alcohol Elimination Rates Among Connecticut 2007 DUI Arrestees**.

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In many cases involving driving and alcohol, questions and inferences regarding the rate of elimination of alcohol from the subject may play a significant role in legal proceedings. Challenges to breath-alcohol test validity have been raised in CT based on the difference between the apparent elimination rate, and literature values. While reported values suggest an average elimination rate of ~ 0.018 g/dl/hr for most individuals, the range of this parameter within the drinking/driving population has not been extensively reported. Both genetic factors, and enzymatic induction as a function of drinking history may affect the rate of elimination of alcohol in an individual.

Standard practice for alcohol-DUI arrestees in CT includes two separate breath alcohol tests, collected after the subject has been placed under arrest and following a 15 minute waiting interval, with a 30 minute interval between the two tests. This protocol allows for the determination of the apparent alcohol elimination rate of the subject. We have calculated this parameter for all CT "alcohol DUI" arrestees receiving two breath tests for the year 2007. Apparent elimination rates that were negative (reflective of continued absorptive/distributive processes), and/or <0.008 g/dl/hr (assumed to be at least partly reflective of absorptive/distributive processes) were deleted from consideration, as were values derived from a "between test" interval of less than 20, or more than 90 min, leaving a study group of 5904 subjects. Of this group, 4605 were male, 1299 female. Subject age ranged from 16 to 73 (females) and from 16 to 80 (males). The average BAC at the time of the first test was 0.167 g/dl (sd=0.056), range 0.015 to 0.404 g/dl (0.166 g/dl for males, 0.172 g/dl for females). The average BAC for subjects involved in accidents (n=1337) was 0.181 g/dl (sd=0.058), and for non-accident arrests, (n=4577) was 0.163 g/dl (sd=0.054).

The average elimination rate for males was 0.0238 g/dl/hr (sd=0.0109) and 0.0250 g/dl/hr (sd = 0.0117) for females. The data clearly indicate a significant number of DUI arrestees with alcohol elimination rates greater than might be expected based on literature values. In our data group, 59.7% had alcohol elimination rates $\geq$ 0.02 g/dl/hr, 25.9% $\geq$ 0.03 g/dl/hr, 8.3% $\geq$ 0.04 g/dl/hr, and 2.8% $\geq$ 0.05 g/dl/hr. As expected, individuals arrested with relatively low initial BAC tended to have elimination rates less than those arrested with higher BAC's; the average elimination rate of the lowest 100 BAC subjects (ranging from 0.015 to 0.057 g/dl) was 0.0172 g/dl/hr (sd=0.0048), or similar to what one might expect from a general population. In contrast, the average elimination rate of the highest 500 BAC subjects (ranging from 0.245 to 0.404 g/dl) was 0.0300 g/dl/hr (sd=0.0155).

Because of the inherent variability amongst the subjects and circumstances underlying the generation of this database, no statistically significant correlation was expected amongst the various parameters evaluated. However a trend towards higher elimination rates associated with higher BAC's was apparent in all subgroups evaluated. We would not anticipate that this data would be of any inferential predictive value relative to any specific individual or circumstance.

Conclusion: Average alcohol elimination rates among CT DUI arrestees in 2007 were significantly higher than would have been predicted by literature values.

Key Words: **Alcohol Elimination, Alcohol DUI, Breath Alcohol (BAC)**

## P50 Contribution of Fruit Consumption to Ethanol Breath Measurement

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This paper aims to address the hypothesis that consumption of common fruits will raise an individual's breath alcohol content to legally significant levels. The assertion posed was that an individual that had consumed fruit with alcoholic drinks had an elevated breath alcohol due to 'neo-genesis' or 'auto-brewery syndrome' of ethanol from the fruit reacting with their stomach acid and thus fermenting the fruit

Twelve volunteers were asked to consume greater than typical servings of specific fruits on an empty stomach and two were to consume commercially available fruit juices. The concentration of ethanol was measured on the breath and was followed over a period of two hours to four hours. Breath alcohol concentrations were measured via the evidentiary BAC DataMaster instrument. Ethanol content of the fruit was determined by gas chromatograph head space analysis.

Of the 11 varieties of fruit and two varieties of fruit drinks tested, only three showed any detectable levels of breath alcohol over the four hour period observed post ingestion, with a maximum breath alcohol of 0.004 g/210L. As the initial stage of digestion, mastication and interaction with stomach acid is largely completed within 2 hours, the lack of any indication of significant ethanol observed in the volunteers suggests that fruit digestion does not contribute to a subject's measured breath alcohol as observed with the BAC DataMaster.

Keywords: **Fruit, Breath Alcohol, Auto-Brewery Syndrome**

## P51 Assessment of Response of the Intoxilyzer® 8000C to Volatiles of Forensic Relevance *In Vitro* Part I: Acetone, Isopropanol and Methanol

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The Intoxilyzer® 8000C has recently been approved for evidentiary use in Canada in alcohol-related driving offences. Consequently, the instrument response to a number of potential volatile interferents *in vitro* was examined using a Guth Laboratories PS-590 portable wetbath simulator. The quantitative response to various dilutions of standard alcohol solution (SAS) (target apparent BAC ranging from 10-100 mg/dL) was verified ($m = 1.003$, $R^2 = 0.9995$). The solvents examined were acetone, isopropanol and methanol, and were prepared as aqueous mixtures, or in combination with an appropriate dilution of the SAS to generate a BAC equivalent to 50 mg/dL. Each solvent mixture was examined over a range of concentrations relevant to those encountered in clinical or impaired driving scenarios. The instrument response was measured in replicates of 20 for each mixture and recorded in terms of apparent BAC generated, and whether the "INTERFERENT DETECT" mechanism was actuated.

Aqueous acetone (0-40 mg acetone/dL) resulted in measured apparent BAC values of 0 mg/dL in all cases, with the INTERFERENT DETECT message actuated in 100% of trials at concentrations of 25 mg/dL and greater, and in 0% of trials at a concentration of 5 mg/dL and lower. Aqueous isopropanol (0-100 mg isopropanol/dL) resulted in mean apparent BAC values ranging from 0-43 mg/dL, with the INTERFERENT DETECT message actuated in 100% of trials at concentrations of 30 mg/dL and greater, and in 0% of trials at concentrations of 10 mg/dL and lower. Aqueous methanol (0-100 mg/dL) resulted in mean apparent BAC values ranging from 0-55mg/dL, with the INTERFERENT DETECT message actuated in 100% of trials at the 100 mg/dL concentration only, and in 0% of trials at concentrations of 50 mg/dL and lower. Binary mixtures of acetone and isopropanol in the presence of SAS (diluted to simulate a BAC of 50 mg/dL) were also examined. In cases where the INTERFERENT DETECT message was not actuated in 100% of cases, the maximum overestimation of BAC was 7 mg/dL. Thus, when superimposed with a positive BAC measurement, the potential for true overestimation of BAC in the absence of an INTERFERENT DETECT message will be minimized through the common practice of truncation of breath measurements for purposes of evidentiary reporting, and underestimation through use of a blood-breath ratio of 2100:1 for conversion of measured breath alcohol concentration to a corresponding BAC. The likelihood of significant contributions of methanol to apparent BAC values without associated symptomology related to methanol toxicity is also relatively low.

Keywords: **Breath, Intoxilyzer®, Alcohol, Acetone, Isopropanol, Methanol**

## P52 Detection of Acute Pentobarbital Exposure in Tibial Bone and Marrow by Solid-Phase Extraction, ELISA and Positive Ion Chemical Ionization Gas Chromatography Mass Spectrometry

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Pentobarbital exposure was detected in tibial bone and marrow by ELISA and GC/MS in the positive ion chemical ionization (PICI) mode. Rats (n=15) received sodium pentobarbital acutely (50 mg/kg, i.p.), and were euthanized within 20, 120 or 360 minutes. Drug-free control animals (n=3) were also euthanized. Diaphyseal and epiphyseal fragments were separated from extracted tibiae. Marrow was isolated from the medullary cavity, and mineralized bone fragments were cleaned by ultrasonication in alkaline solution. Pentobarbital was extracted from ground tibial bone by methanolic extraction followed by solid-phase extraction (SPE). Marrow was homogenized in alkaline solution, and then underwent SPE. Extracts were analyzed by ELISA and then by PICI GC/MS following derivatization with TMAH, through selected ion monitoring (SIM, m/z = 255 and 277). The effects of tissue type (marrow, epiphyseal bone and diaphyseal bone) and the delay between drug administration and euthanasia (i.e., the *dose-death interval*) on the ELISA response were examined through determination of binary classification test sensitivity and the relative decrease in absorbance (%DA, drug-positive tissues vs. drug-free controls) in each tissue type. In general, the %DA varied significantly between extracts from different tissues, and decreased in the order of marrow > epiphyseal bone > diaphyseal bone. Binary classification test sensitivity values in marrow, epiphyseal (trabecular) bone and diaphyseal (cortical) bone, obtained with ELISA, were 100%, 0-80% and 0-40%, respectively, at the dose-death intervals examined. Sensitivity values obtained by GC/MS were 60-100%, 0-60% and 0-40%, respectively, at the dose-death intervals examined. These results suggest that acute pentobarbital exposure may be detected in skeletal tissues, and that the tissue type and position within a given bone are important considerations in the choice of substrate for skeletal barbiturate screening.

Keywords: **Bone Marrow, Penotbarbital Immunoassay, GCMS**

## P53 Effects of Tissue Type and the Dose-Death Interval on the Detection of Acute Ketamine Exposure in Bone and Marrow with Solid-Phase Extraction and ELISA with LC/MS/MS Confirmation

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Ketamine exposure was detected in skeletal tissues by ELISA and LC/MS/MS. Rats (n=9) received ketamine hydrochloride acutely (75 mg/kg, i.p.), and were euthanized within 15, 30 or 90 minutes. Drug-free control animals (n=3) were also euthanized. Extracted femora were separated into epiphyseal and diaphyseal fragments, with marrow isolated from the medullary cavity. Bone was ground and incubated in methanol. Extracts were dried and reconstituted in phosphate buffer (0.1 M, pH 7.3), marrow was homogenized in alkaline solution; both underwent solid phase extraction (SPE). Extracts were then assayed by ELISA, with data expressed in terms of relative decrease in absorbance (%DA, drug-positive tissues vs. matrix-matched drug-free controls) and binary classification test sensitivity (*S*). Generally, %DA decreased in the order of marrow > epiphyseal bone > diaphyseal bone, and was negatively correlated with dose-death interval (DDI). Measured *S* values were 100% in ELISA analysis of extracts of all tissue types. Sensitivity values were computed from LC/MS/MS data using a 5 ng/mL cutoff. Measured values were 100%, 0-100% and 0%, for ketamine and 0-66%, 0-66% and 0%, for norketamine, respectively, over the three DDIs investigated. These results suggest that the tissue type sampled and DDI may influence the sensitivity of detection of ketamine exposure in skeletal tissues.

Keywords: **Bone Marrow, Ketamine, LCMS**

## P54 Sleepless Preacher in SoCal

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Determining fault in a two vehicle accident is never as straight forward as indicated in DUID impairment literature. This is a case of a preacher who, after finishing his sermon, was driving home at night and apparently fell asleep or nodded off at the wheel on a two lane highway. His vehicle veered off to the right side shoulder on the side of the roadway, and according to three witnesses, the car returned to the roadway, overcorrected and crossed the centerline of the highway. The preacher's car impacted a semi-trailer tractor and he was killed instantly.

The responding police officer was not a certified DRE and did not perform sobriety testing but he did state in his report that he did not recognize any signs or symptoms of impairment in the truck driver and he did not appear to be under the influence of drugs.

The police report included three witnesses who all concurred that the preacher appeared to be impaired or falling asleep at the wheel prior to the accident. They all related that he drove off the roadway, appeared to regain control, and then shot across the center-line, colliding broadside with the semi-truck. All agreed that the truck driver did not have sufficient time to react.

Toxicology testing was performed on both drivers. The peripheral post-mortem blood of the causing driver (the preacher) was tested for ethanol and other volatiles by headspace GC and the central post-mortem blood was screened by ELISA for cocaine metabolites, amphetamines, benzodiazepines, cannabinoids and fentanyl. Both blood specimens had no drugs detected. The semi truck driver was tested for ethanol by GC, screened for amphetamines, benzodiazepines, cannabinoids, cocaine metabolites, opiates and PCP. It confirmed positive for both methamphetamine and amphetamine by LCMSMS and was reconfirmed at a second laboratory by GCMS.

In spite of the toxicology results, the police report concluded that the primary cause of the accident was the decedent driver crossing the centerline and impacting the truck. The presence of drugs in the semi-truck driver was considered to be minor and not implicated in the accident because the police report concluded that there was insufficient time for the semi-tractor trailer to stop. Recent reports of "sleepy driving" have received significant attention and it appears this is relevant in this case. If California had a per se law for illicit drugs in drivers, would this case have had a different outcome?

Keywords: **Sleepy Driving, Methamphetamine, DUID**

## P55 Analysis of Drug-Induced/Related Deaths Using a Comprehensive Forensic Drug Database

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Toxicological findings in drug deaths are difficult to interpret due to possible interactions, varying patient characteristics/co-morbidities, and other factors. This project's objectives were to: 1) develop an online drug induced/related deaths database (Forensic Drug Database [FDD]) that allowed for direct data entry by remotely located medical examiners; and 2) describe and analyze drug toxicity characteristics/patterns for involved drugs or drug combinations, the concentrations of drugs and metabolites seen in single and multi-drug fatalities, and the influence of pre-existing medical conditions, co-morbidities, and patient factors such as gender, age, and BMI on toxicity.

The West Virginia University School of Pharmacy's Center for Drug and Health Information and the West Virginia Office of the Chief Medical Examiner (WV OCME) collaborated in this project, with work progressing from January 2005 to present. The information in the FDD includes: decedent age, gender, BMI, location (by zip code); body condition; drug(s)' role in death; death certificate information; co-morbidities present; autopsy findings; drug/metabolite concentrations at specific sampling sites, and how drug was obtained, drug dosage form, route of administration, amount taken, and brand name (if known); postmortem time interval; and other noncontributory drugs taken, among other data. The FDD was programmed using PHP language and became operational in mid-2007.

The Forensic Drug Information (FDI) web site (http://www.forensicdi.org) provides a password protected, secure interface to the FDD. Data from the drug-induced or drug-related death cases handled by the WV OCME from January 2005 to mid-November 2007 (1199 cases) have been entered thus far. The FDD data can be downloaded from the central server at any time as an Excel file. Preliminary descriptive and statistical data analyses have been performed. Some of the key results follow.

The most commonly involved drugs were methadone (27.8% of cases), diazepam (24.3% of cases), ethanol (23.9% of cases), cocaine (18.3% of cases), and oxycodone (18.0% of cases). This pattern changed from 2005 to 2007 with benzodiazepines most commonly implicated in 2007. Approximately 36% to 39% of all drug deaths in 2005 and 2006 involved 3 or more drugs, compared to 50% of the 2007 deaths. Methadone, the drug most commonly identified as a cause of or contributor to the death cases in 2005 and 2006, dropped to #5 in 2007. Oxycodone and morphine involvement in the death cases slowly but steadily increased during this time. However, the most dramatic increases occurred with diazepam and alprazolam. Diazepam was the most frequent contributor to these types of deaths in 2007. Alprazolam was the 3$^{rd}$ most common drug cause/contributor to the 2007 drug deaths, an 89% increase over the previous year. Alprazolam concentrations decreased as the number of concurrent drugs increased. Similar findings were observed with diazepam concentrations, which decreased from 0.29 $\pm$ 0.49 mg/L (mean $\pm$ SD) when diazepam was identified with 1 or 2 other drugs (179 cases) to 0.12 $\pm$ 0.09 mg/L (mean $\pm$ SD) when identified with 4 or more drugs (32 cases). Diphenhydramine was implicated as a cause/contributor to death in ~ 11% of female decedents compared to 4% of male decedents. Citalopram was a more common cause/contributor to death in female compared to male decedents, 11.1% (44 cases) vs. 2.6% (21 cases), respectively. The proportion of female decedents who were obese (BMI $\geq$ 30) was significantly higher than that for males (40.9% vs. 30.3%, respectively), and 51% of female decedents in whom alprazolam was an implicated drug were obese. Many further analyses of drugs, including co-morbidities, are planned.

In conclusion, the FDD allows for the characterization and analysis of any drug(s) or combinations, patient characteristics, co-morbidities, or cause/manner of death of interest and can be accessed from any location worldwide. As the data expand, many important research questions will be addressed.

Keywords: **Database, Concentrations, Forensic Toxicology**

## P56 Statistical Study Between OMEC Technology and GC/MS for Cocaine, Opiates, and Cannabinoids

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The use of illicit drugs creates numerous health, safety, and productivity concerns among employers. As a consequence, many employers are requiring random drug screening from their employees. The objective of this study was to determine if positive results obtained with Optimized Multiplied Enzymatic Chemistry (OMEC) Technology can be confirmed using GC/MS. From January to December 2007, the Institute of Forensic Sciences of Puerto Rico received 6,641 samples to perform drug screening for cocaine, THC, and opiates. Of these, 55 tested positive for cocaine, 40 for THC, and 16 for opiates using the OMEC Technology. All samples that tested positive for cocaine, 93% that tested positive for THC and 19% that tested positive for opiates were confirmed by GC/MS. These data show a high correlation between both methodologies when testing for cocaine and THC but not for opiates. The apparent discrepancy may be due to the fact that our GC/MS methodology for opiates screens only for codeine, morphine, and the heroin metabolite (6-MAM) whereas the OMEC technology relies on monoclonal antibodies. Opiates comprised a large group of drugs, such as oxycodone, hydrocodone, and hydromorphone that are commonly prescribed as painkillers. It is possible that the monoclonal antibodies may recognize a wider range of opiates. This is not a concern when screening for cocaine and THC, since their use as therapeutic agents is negligible. It is important to emphasize that OMEC technology is a qualitative and semiquantitative method based on the competitive binding principle of the monoclonal antibody. In summary, our data shows that OMEC technology is a sensitive and reliable drug screening methodology for cocaine and THC.

Keywords: **O.M.E.C., GC/MS, Drugs of Abuse**

## P57 The Outcome of Drugs Use/Abuse on Violent Deaths in Italy: Eight Years of Observations (2000-2007)

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**Introduction**: A period of eight years (2000-2007) was evaluated for an epidemiological study on violent deaths which occurred in two Italian regions: the provinces of Perugia and Rome. The laboratory of forensic toxicology analyzes biological samples, collected at autopsy, for the detection of xenobiotics and this data is used to understand the trend of drug use/abuse. In this paper authors included demographic data from all decedents in the period examined. Specific attention was devoted to traffic fatalities and lethal intoxication when drugs of abuse were detected.

**Methods**: A total of 2777 violent deaths registered in Rome and 1316 in Perugia in the period considered, were studied for demographic data. Toxicological examinations were performed when intake of drugs was presumed (15% of deceased in Rome, 21% in Perugia). The incidence of drugs of abuse and alcohol was investigated in all traffic fatalities and overdoses. Moreover, lethal intoxications were studied in detail for the substances directly involved in the death.

**Results**: A prevalence of males (about 70% in Rome and in Perugia), a significant presence of foreign people (about 16% in Rome, 15% in Perugia), and significant data about the age (in Rome more than 50% of deaths were younger than 50 years old, as opposed to Perugia where 55% of deaths were older than 50 years old), were observed. In the population examined, an important cause of death is represented by traffic fatalities (about 30% in Rome and 28% in Perugia in the entire period); we observed a preponderance of male in the range 19-40 years in Rome, and in the range > 50 years in Perugia. When alcohol determination was performed, 39% were positive with concentrations from 0.02-0.6 g/100mL. In those cases where toxicological data was available, 10% were positive for cocaine and 12% were positive for cannabis. Eight percent of violent deaths observed in Rome involved drug intoxications; the demographic evaluations showed that 79% were males and about 48% of them were 31-40 years old. Female (21% of total) exhibited a similar age trend. Twenty-seven per cent (27%) of overdoses were due to morphine alone with a range of concentration of 100-2900 ng/ml. Twenty-six per cent (26%) were a combination of morphine and alcohol with morphine concentrations in the range of 50-4400 ng/ml and alcohol in the range 0.02-0.4 g/100mL. Morphine and cocaine combination deaths occurred in 10% of cases; and 10% of deaths were ascribed to alcohol alone. Five percent of the deaths were methadone related. Only 3% of deaths were due to cocaine alone (150-23000 ng/ml cocaine, 50-18000 ng/ml benzoylecgonine). In Perugia 89% of overdoses were males and 61% of them were in the age 19-40; 27% were due to morphine alone (ranging from 27 to 2381 ng/ml); 22% were due to morphine and alcohol with morphine concentrations ranging from 12-2985 ng/ml and alcohol ranging from 0.032-0.248 g/100 mL). Thirty-two percent (32%)were combination morphine and cocaine death with morphine from 57-1800 ng/ml and cocaine from 170-72000 ng/ml. Four per cent (4%) were due to cocaine alone and 13% were morphine, cocaine and alcohol combined intoxications. Methadone was recovered in 2% of overdoses, in association with morphine.

**Conclusion**: The present study suggests the usefulness of periodic review of mortality phenomena related to drug intoxications, both as direct consequence or indirect effects. The authors observed in the period examined some particular phenomena. Traffic fatalities are frequently caused by the use of alcohol and illicit drugs, even though Italian legislation requires total abstinence from drugs and requires a limit of 0.05 g/100mL ethanol in blood. Morphine appears to be the primary cause of death in intoxications. The low frequence of cocaine related-death in Rome doesn't correspond to the seizures of drugs observed on the illicit market in the same period; this may suggest an underestimation of cocaine related death, like in the case of sudden heart related death.

Keywords: **Violent Death, Epidemiology, Lethal Intoxication**

## P58 The Stability of Ethyl Glucuronide (EtG) in Hair Used as a Biological Marker to Demonstrate Alcohol Abstinence or Consumption

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Ethyl glucuronide (EtG) is a biological marker in hair used to indicate abstinence or excessive alcohol consumption. The main aim of this paper is to evaluate the stability of EtG in hair samples as it can potentially be affected by normal hygiene, and affect interpretation of the results.

EtG was measured by GC-MS/MS in 102 hair samples, which were sectioned in three monthly sections when available to produce 291 sections of hair (Sectioned set) and 468 hair samples (Not Sectioned set), where the most recent centimeter was analysed

The 95% percentiles of the EtG levels detected in the Not Sectioned set and in the First Section of the Sectioned set were 0.23 ng/mg (N=468) and 0.22 ng/mg (N=102), respectively. The 95% percentiles of the levels detected in the Second Sections and in the Third Sections were 0.15 ng/mg (N=102) and 0.10 ng/mg (N=87), respectively. Levels were below cut-off (0.01 ng/mg) in 61% in the Not Sectioned set and 67% in the Sectioned set. Of the samples where EtG was detected, the Second Section samples showed mean EtG levels 74% lower the levels detected in the First Section. The mean levels detected of EtG in Third Section were 62% the levels of the Second Section and 47% the levels detected in the First Section. Analysis of variance showed the levels of the Third Section significantly lower ($p<0.05$) than the First Section.

The results of this study suggest that normal hair hygiene might wash out EtG from the hair. The recommendation is therefore that only the most recent month must reliably monitor abstinence or chronic alcohol abuse using head hair and that data should be evaluated in conjunction with other biochemical tests and clinical evaluation.

Keywords: **Hair, Drug Stability, Ethyl Glucuronide (EtG)**

## P59 Positive Prevalence Rates Based on Various Cutoff Concentrations of Ethyl Glucuronide in a Large Population of Unpreserved Random Urine Specimens

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**OBJECTIVES:** Since 2006, Redwood Toxicology Laboratory has been testing for ethyl glucuronide (EtG), a minor but important metabolite of ethanol, in urine. Over the last few years, we have analyzed tens of thousands of specimens. The purpose of this study was to examine the data from a statistically significant high number of positive specimens to determine positivity rates based on various proposed EtG cut-offs. Since EtG testing is relatively new and limited information is available, this epidemiological data is presented to the scientific community to aid in suggesting an analytical cutoff level for EtG and to add valuable information to the existing literature.

**METHODS:** All specimens submitted to the laboratory for urine EtG testing were screened for EtG using a fully validated LC/MS/MS method with a cut off of 100ng/mL. A separate aliquot of all screened EtG positive specimens was extracted and subjected to separate LC/MS/MS analysis for confirmation. Recently our laboratory has started testing for ethyl sulfate (EtG) along with EtG in the confirmatory method, but only EtG data was used for this study. A Shimadzu Prominence liquid chromatograph with rack changer connected to API 3200 triple quadrupole mass spectrometer from Applied Biosystems, operating in negative electrospray mode was used for analysis. Separation was performed on a Synergi Polar-RP 4µ column (150 x 2 mm) at a flow rate of 0.6mL/min with 20µL injection. The mobile phase was 0.1% formic acid in de-ionized water (A) and 0.1% formic acid in de-ionized water with 0.2% isopropanol (B). EtG peak eluted in the isocratic part (100% A) of the run and the column was flushed with a gradient (10% A, 90% B) after the peak eluted. The MS/MS ion transitions monitored were 221/85 (quantifier) and 221/74.8 (qualifier) for EtG and 226/85 for EtG-D5 (internal standard). The ratio of the quantifier to the qualifier was automatically calculated and was required to be within ±20% of the calibrator ion transition ratios. The method was linear from 50-500,000ng/mL for EtG with inter and intra assay CV's < 7.4%. No carry-over at 500,000ng/mL and no endogenous/exogenous interference observed.

**RESULTS & a** 10 month period. 20076 specimens (12.3%) were positive and 142603 (87.7%) were negative for EtG using a cutoff of 100ng/mL. The positives results (divided into 6 categories) are presented in the table. Assuming the positivity rate to be 100% with an EtG cut off of 100ng/mL, a cutoff of 250ng/mL detects 91.4% of the positives, 500 ng/mL cutoff detects 78.1%, 1000ng/ml cutoff detects 63.8% and a cutoff of 2000ng/mL detects 51.1% of the positives in a given population. This is in agreement with previously published data (Quest 2004-2006 data, *Clinical Toxicology News*); however, care must be taken while trying to determine a cutoff. A higher cutoff value will result in significant number of undetected cases and a lower cutoff may result in "innocent positives". There is little doubt that the analytical sensitivity and specificity of the LC/MS/MS methods for the detection of EtG/EtS have been firmly established. However, effective EtG testing cutoff levels to differentiate "innocent exposure" from ethanol consumption cannot be determined until diagnostic sensitivity and specificity studies have been performed.

| ***EtG Conc. (ng/mL)*** | ***# of Pos.*** | ***Pos. %*** |
|---|---|---|
| 100-250 | 1740 | 8.6 |
| 251-500 | 2668 | 13.3 |
| 501-1000 | 2870 | 14.3 |
| 1001-2000 | 2548 | 12.7 |
| 2000-10000 | 4452 | 22.2 |
| >10000 | 5810 | 28.9 |

Keywords: **Ethyl Glucuronide, Cutoff, Positivity Rate**

## P60 Urinary MDMA, MDA, HMMA, and HMA Excretion Following Controlled MDMA Administration to Human Subjects

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**Background:** 3,4-Methylenedioxymethamphetamine (MDMA), commonly known as ecstasy, is an illicit drug ingested for its stimulatory and hallucinogenic effects. MDMA produces a euphoric/loving feeling with greater self-confidence, self-acceptance, lowered defenses, and enhanced communication, empathy and/or understanding. MDMA is excreted primarily as unchanged drug in urine, with additional N-demethylation to MDA, O-dealkylation and O-methylation to 4-hydroxy-3-methoxymethamphetamine (HMMA) and 4-hydroxy-3-methoxyamphetamine (HMA), respectively.

**Objective:** To simultaneously determine MDMA and metabolites' disposition and detection windows in urine.

**Methods:** The NIDA Institutional Review Board approved this research and 16 participants (18-27 yrs, 6 females & 10 males) provided written informed consent. The randomized, balanced, double blind, within-subject drug administration study administered placebo, low (1.0 mg/kg), and high (1.6 mg/kg) oral MDMA. All urine specimens were collected individually *ad libidum* throughout the study. Urine specimens (n = 944) were hydrolyzed with concentrated hydrochloric acid, subjected to solid phase extraction (Clean Screen® ZSDAU020, United Chemical Technologies), and derivatized with heptafluorobutyric acid anhydride. Heptafluorobutyryl derivatives of MDMA, MDA, HMMA, and HMA were quantified by GCMS in selected ion monitoring mode. GCMS was performed on an Agilent 6890 gas chromatograph interfaced with a 5973 mass-selective detector and equipped with a DB-35MS capillary column (15m x 0.32mm i.d.; 0.25µm-film thickness). Limit of quantification was 25 ng/mL for all analytes.

**Results:** Maximum urine concentrations ($C_{max}$) ± SD for low-dose MDMA were 14,932 ± 7,599 (5,438 – 31,777), MDA 1,302 ± 747 (454 – 3,256), HMA 808 ± 348 (270 – 1,716), and HMMA 16,893 ± 10,623 (5,698 - 46,876) ng/mL. Mean times of maximum concentration ($T_{max}$) ± SD were 11.9 ± 4.3, 15.0 ± 6.1, 17.3 ± 6.3, and 10.9 ± 7.0 h, respectively. After the high MDMA dose, urine $C_{max}$ ± SD for MDMA, MDA, HMA and HMMA were 25,424 ± 12,015 (12,292 - 48,947), 2,491 ± 1,262 (1,064 – 5,135), 1,121 ± 792 (465 – 3,217), and 20,393 ± 8,962 (7,399 - 36,492) ng/mL, respectively. Mean $T_{max}$ ± SD were 15.6 ± 9.2, 19.6 ± 7.4, 22.1 ± 6.2, and 12.2 ± 8.8 h for MDMA, MDA, HMA, and HMMA, respectively. Mean times of last detection (≥250 ng/mL) in 12 subjects following the high dose were 52.6 ± 11.9, 44.1 ± 11.6, 48.9 ± 12.3, 71.8 ± 18.6 h for MDMA, MDA, HMA and HMMA, respectively.

**Conclusions:** This controlled MDMA administration study provides pharmacokinetic data on urinary disposition of MDMA and MDA, HMMA, and HMA metabolites to improve interpretation of workplace, drug treatment, criminal justice, and military urine drug tests. Measurement of urinary HMMA has the longest duration of detection of MDMA exposure.

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

Keywords: **MDMA, MDA, Urine Pharmacokinetics**

## P61 Cannabichromene Potentiates the Antinociceptive Effect of $\Delta^9$-Tetrahydrocannabinol

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The antinociceptive effects of $\Delta^9$-tetrahydrocannabinol (THC) have been demonstrated in many different animal species. THC has been more effective than opioids or NSAIDS in relieving neuropathic pain in some studies. Recently, a formulation of THC and cannabidiol (CBD) was approved in Canada for relieving neuropathic pain related to multiple sclerosis. While THC is recognized as the primary pharmacologically active cannabinoid in marijuana, 69 other cannabinoids, such as CBD or cannabichromene (CBC), may also have pharmacological properties of their own, like antinociception or they may modulate the antinociceptive effects of THC.

In the present study, we hypothesized that CBC has antinociceptive properties of its own and could modulate the antinociceptive effect of THC. ICR mice were evaluated in a time-course study to determine the antinociceptive effects of a maximum effective dose of THC in combination with various doses of CBC or CBC administered alone. Mice were intravenously administered a dose of vehicle (veh), 3, 10, 30, or 100 mg/kg CBC followed by a second dose of veh or 10 mg/kg THC. The mice were then evaluated at 20 minutes post-drug administration for tail-flick latency via radiant heat exposure as a measure of antinociception. Compared to veh + veh administered mice, veh + THC significantly increased antinociception up to 2 hours while veh + 100 mg/kg CBC significantly increased antinociception up to 20 minutes. Also, 30 and 100 mg/kg CBC in combination with THC significantly increased antinociception up to 12 hours compared to control group mice.

Blood and brain concentrations of THC and CBC (in a separate set of mice from the previous experiment) were evaluated for pharmacokinetic parameters. Mice received intravenous doses of veh, 3, 10, 30, or 100 mg/kg CBC followed by a second dose of veh or 10 mg/kg THC. The mice were sacrificed and blood and brain tissue were harvested at 20 minutes and 6 hours post-drug administration. There was a significant increase at 20 minutes in THC concentrations when 30 and 100 mg/kg CBC were dosed in combination with THC compared to veh + THC. At 6 hours, both cannabinoid concentrations had dropped significantly in these dose groups and probably did not contribute to the potentiated antinociceptive time-course effect observed at 6 hours for those respective dose groups.

The initial concentrations of cannabinoids that were present in blood and brain tissue may have allowed for a continuous short term ligand-CB1 receptor interaction that signals with greater intensity to maintain changes in intracellular mechanisms compared to either drug administered alone. These intracellular mechanisms may have continued to function for hours after the ligand-receptor interaction was essentially not significant. Future studies will investigate changes in intracellular signaling mechanisms that may be potentiating the antinociceptive effect of these drugs in combination compared to each administered separately.

Keywords: **Cannabichromene, Cannabinoids, Tetrahydrocannabinol**

## P62 Novel Biomarkers of Prenatal Methamphetamine Exposure and Correlations with Self-reported Trimester, Frequency and Route of Administration

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**Background:** Effects of methamphetamine (MAMP) on the developing fetus are poorly understood. The Infant Development, Environment, and Lifestyle (IDEAL) study is the largest study investigating prenatal MAMP exposure on neonatal neurobehavioral and physical outcomes. Self-reported use or positive MAMP or amphetamine (AMP) finding in meconium defined "exposed" infants. Only 40% of immunoassay (IA)-positive screens were confirmed by GCMS for MAMP and/or AMP at 5 ng/g.

**Objective:** To determine if novel MAMP metabolites, *p*-hydroxymethamphetamine (pOHMAMP), *p*-hydroxyamphetamine (pOHAMP), and/or norephedrine (NOREPH) could improve identification of MAMP-exposed neonates and if meconium biomarker concentrations correlate to timing, frequency, and/or route of MAMP administration.

**Methods:** 83 meconium specimens were re-analyzed by a recently developed LCMSMS method quantifying MAMP, AMP, pOHMAMP, pOHAMP, and NOREPH.

**Results**: Among IA-positive specimens, no additional neonates were identified as exposed by pOHMAMP, pOHAMP or NOREPH. 25% of IA-negative meconium (<500 ng/g cutoff) contained low biomarker concentrations; 2 contained only pOHMAMP at 14.4 and 23.0 ng/g. NOREPH (N=20) and pOHAMP (N=1) were always detected with MAMP and AMP. There were more positive meconium results for neonates exposed in late (3rd trimester) than early (1st and/or 2nd) pregnancy (60% vs. 10% IA; 83.3% vs. 13.3% LCMSMS). MAMP and AMP concentrations were greater in late pregnancy-exposed neonates' meconium as compared to early exposure (median 1,455.4 vs. 33.4 ng/g MAMP; 321.4 vs. 28.7 ng/g AMP); however, no statistical difference was observed for pOHMAMP (139.1 vs. 26.0 ng/g) and NOREPH (14.9 vs. 14.9 ng/g). Neither frequency of 3rd trimester MAMP use (<1/week or ≥1/week) or administration route (smoked, insufflation, or multiple routes) affected positive screening rates, MAMP biomarker detection by LCMSMS or concentration distributions.

**Conclusions:** Including novel MAMP biomarkers did not increase confirmation rates in IA-positive meconium. Endogenous meconium compounds, other sympathomimetic amines or as yet uncharacterized MAMP metabolites might contribute to positive amphetamines IA screens. MAMP biomarkers were detected in 25% of IA-negative meconium specimens with positive maternal self-report, albeit at low concentrations; pOHMAMP alone was detected in two specimens. Although theoretically able to determine 2nd and 3rd trimester maternal drug use, meconium analysis appears to better reflect 3rd trimester exposure. While no correlations between the frequency of MAMP consumption and meconium concentration were observed, meconium concentrations may better reflect the amount of MAMP consumed. LCMSMS analysis allows sensitive and specific detection of MAMP biomarkers in meconium and may improve identification of MAMP-exposed neonates.

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Keywords: **Methamphetamine, Meconium, *In Utero***

## P63 A Five Year Analysis of Oral Fluid Drug Testing Results from a Medical Review Officer (MRO) Data Source, 2003-2007

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**Background:** The use of oral fluid (OF) in non-federally regulated workplace drug testing has continued to increase over the last five years. Currently, there are no federal guidelines regarding the use of OF in workplace drug testing programs nor is this matrix approved for use in any federally regulated testing programs. To date, there are no systematic data available for examining the relationship between laboratory reported confirmed positive OF drug test results and subsequent MRO review in the workplace setting.

**Objective:** To compile a cumulative database of laboratory-tested OF workplace drug test results and to determine the relationship between laboratory reported OF drug test results and MRO verified results reported to employers.

**Methods:** Records for nearly 650K oral fluid specimens collected during the 5-year period from 2003-2007 were obtained from a large MRO data source. The records include donor demographics, employer information, collection site information, laboratory results, and MRO determinations but do not include employer blind quality control samples. The majority of the specimens were analyzed by two large laboratory systems. All records were handled in compliance with the Department of Health and Human Services Human Subject protection criteria.

**Results:** The analysis of the 2003-2007 data indicates a significant increase in OF drug testing in the last year with more than half of the results collected in 2007. The aggregate laboratory confirmed positive specimen rate for the 5-year period was 4.28%. Approximately 8.6K specimens (1.33%) were reported as 'non-negative' (e.g., invalid, QNS**,** etc.). Table 1 illustrates the percentage breakdown of laboratory confirmed positive drug test results, MRO verified positives, MRO reversals, and the relative prevalence of verified drug results by drug class. Marijuana (60.42%) and cocaine (24.14%) were the predominant drugs detected. MRO reversal rates ranged from 0.00 to 56.74% by drug class with amphetamine and opiates having the highest rates.

| **Aggregate OF Data 2003-07** | %Laboratory Confirmed + | % MRO Verified + | % MRO Reversed | % of Total MRO Verified + |
|---|---|---|---|---|
| # Specimens Tested = 648,771 | **4.28** | **95.57** | 4.43 | |
| Amphetamine | 0.20 | 52.70 | **47.30** | 4.28 |
| Methamphetamine | 0.30 | 99.53 | 0.47 | 6.42 |
| MDMA or MDA | 0.01 | 100.00 | 0.00 | 0.29 |
| Cocaine | 1.12 | 99.94 | 0.06 | **24.14** |
| Marijuana | 2.81 | 99.89 | 0.11 | **60.42** |
| Opiates | 0.18 | 43.26 | **56.74** | 3.93 |
| Phencyclidine | 0.02 | 100.00 | 0.00 | 0.51 |

**Conclusions:** The analysis of nearly 650K OF specimens collected during the last 5 years indicated an overall laboratory confirmed positive rate of 4.28% for the drug classes tested. Laboratory confirmed positive rates and the percentage of positives by drug class appear to be comparable to the urine test result rates typically observed in the non-regulated workforce. The overall MRO verified positive rate of 95.57% was higher than typically observed with urine test results. The MRO reversals were due primarily to a legitimate medical explanation for the presence of opiates (56.74%) and amphetamine (47.30%); these rates are lower than observed with urine results.

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

## P64 Analysis of 2006-2007 Urine Drug Testing Results from a Medical Review Officer Data Source

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**Background:** Drug testing indices which are based only on laboratory confirmed positive results may not accurately represent illicit drug use rates since they include blind quality control samples and results later reversed after Medical Review Officer (MRO) determination of valid medical explanations for the test results.

**Objective:** To evaluate the relationship between laboratory reported drug test results and MRO verified results reported to employers in federally regulated (FR) and non-regulated (NR) workplaces and to evaluate NR synthetic opioid positive drug test results.

**Methods:** Records for 2.52M FR and NR urine specimens collected during 2006-2007 from employees in more than 5,000 companies were obtained from a large MRO data source. The database includes donor demographics, employer information, collection site information, laboratory results, and MRO determinations but does not include agency or employer blind quality control samples. Virtually all drug test results (99.9%) were from SAMHSA-certified laboratories. All records were handled in compliance with the Department of Health and Human Services Human Subject protection criteria.

**Results:** Laboratory confirmed positive rates ranged from 1.38% (2006 FR specimens) to 4.19% (2006 NR specimens), and MRO reversal rates ranged from 9.46% (2006 FR specimens) to 27.72% (2007 NR specimens). About 35% of the NR specimens were tested for synthetic opioids; synthetic opioids account for almost half of all NR opioid (both natural and synthetic) laboratory confirmed positive results. MRO review determined that a majority of FR and NR opioid positive drug tests were due to legitimate prescription drug use.

| **Specimens Tested** | Federally Regulated[a] | | | | | | Non-Regulated[b] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | % Laboratory Confirmed + | | % MRO Verified + | | % MRO Reversed | | % Laboratory Confirmed + | | % MRO Verified + | | % MRO Reversed | |
| **Year** | 2006 | 2007 | 2006 | 2007 | 2006 | 2007 | 2006 | 2007 | 2006 | 2007 | 2006 | 2007 |
| Total All Drugs | **1.38** | 1.43 | 90.54 | 85.07 | **9.46** | 14.93 | **4.19** | 4.11 | 75.94 | 72.80 | 24.06 | **27.72** |
| **Amphetamines** | 0.14 | 0.19 | 62.26 | 41.35 | **37.74** | **58.65** | 0.33 | 0.39 | 43.08 | 31.09 | **56.92** | **68.91** |
| **Cocaine** | 0.45 | 0.37 | 99.40 | 99.86 | 0.60 | 0.14 | 0.58 | 0.47 | 99.75 | 99.92 | 0.25 | 0.08 |
| **Marijuana** | 0.70 | 0.79 | 99.11 | 99.55 | 0.89 | 0.45 | 2.11 | 2.17 | 99.54 | 99.50 | 0.46 | 0.50 |
| **Phencyclidine** | <0.01 | <0.01 | 100.00 | 100.00 | 0.00 | 0.00 | 0.01 | 0.01 | 99.17 | 100.00 | 0.83 | 0.00 |
| **Opioids**[c] | 0.10 | 0.13 | 30.09 | 17.80 | **69.91** | **82.20** | 0.51 | 0.55 | 26.91 | 23.20 | **73.09** | **76.80** |
| Hydrocodone | | | | | | | 0.67 | 0.70 | 19.02 | 14.43 | **80.98** | **85.57** |
| Hydromorphone | | | | | | | 0.36 | 0.42 | 21.25 | 16.73 | **78.75** | **83.27** |
| Oxycodone | | | | | | | 0.30 | 0.35 | 34.93 | 26.61 | **65.07** | **73.39** |
| Oxymorphone | | | | | | | 0.33 | 0.46 | 34.42 | 32.41 | **65.58** | **67.59** |
| **Barbiturates** | | | | | | | 0.34 | 0.33 | 19.73 | 19.24 | **80.27** | **80.76** |
| **Benzodiazepines** | | | | | | | 0.58 | 0.67 | 29.55 | 27.51 | **70.45** | **72.49** |

[a] Number of FR specimens = 223,053 in 2006 and 199,966 in 2007.

[b] Number of NR specimens = 1,062,367 in 2006 and 1,037,473 in 2007.

[c] FR opioid data includes codeine, morphine and 6-acetylmorphine; NR opioid data also includes the synthetic opioids hydrocodone, hydromorphone, oxycodone, and oxymorphone.

**Conclusions:** Comparing year 2007 to 2006 for all drug classes combined, MRO verified positive rates decreased; hence, MRO reversals increased. In FR testing, there was an increase in the reversal rates for natural opioids and amphetamines. In the NR data, increased MRO reversal rates were found in the amphetamine, natural and synthetic opioid, barbiturate, and benzodiazepine drug classes.

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

## P65 Recurrence of Xylazine as an Adulterant of Heroin in Puerto Rico

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Illicit drugs such as heroin are often adulterated (cut) with other pharmacological agents to either enhance or diminish the drug effects and to increase the weight and volume of the drug, thus increasing the dealer's profits. Many different substances are used to cut heroin. In 2007, a total of 663 street heroin samples were analyzed by the Controlled Substances Section at the Institute of Forensic Sciences of Puerto Rico. Some of the more common non-opiate cutting agents with pharmaceutical effect encountered were: caffeine (23%), procaine (3%), cocaine (5%), quinine (10%), lidocaine (3%) and the most frequently detected xylazine (36%). Other cutting agents were found less frequently (< 3%) when compared to the aforementioned compounds. The xylazine results in the street heroin samples for 2007 (36%) are similar to the year 2006 (36%), showing a recurrence of xylazine as the major adulterant used with heroin.

Xylazine is marketed as a veterinary drug, used as a sedative, analgesic and muscle relaxant for large animals, such as deer, ruminants and horses. Xylazine is not approved for human use because it has been proven harmful to humans. To our knowledge, 27 cases of toxicity caused by xylazine consumption have been documented in humans.

Both drugs, heroin and xylazine, are definitively dangerous to humans, therefore xylazine may be fatal when used in combination with heroin. Literature shows some similar pharmacologic effects between xylazine and heroin in humans. Both drugs cause bradycardia, hypotension, central nervous system depression and respiratory depression. Due to these similar pharmacologic effects, synergistic effects can occur in humans.

The aim of this study is to present the most frequently identified heroin combinations in street heroin and to alert law enforcement as well as public health agencies about the potential abuse of xylazine in Puerto Rico.

Keywords: **Xylazine, Heroin Aadulterants, GC/MS**

## P66 Performance Evaluation of Three Liquid Chromatography Mass Spectrometry Systems for Broad Spectrum Drug Testing

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**Introduction:** In the forensic and clinical toxicology laboratory, traditional methods for comprehensive drug screening are being replaced by new technologies, specifically liquid chromatography mass spectrometry (LC-MS). Unlike traditional gas chromatography mass spectrometry (GC-MS) methods, LC-MS techniques are capable of detecting polar, non volatile and thermally labile compounds and do not require extensive sample preparation. However, there are many barriers that laboratories have to overcome in order to utilize these technologies, including, difficulties with method optimization and the limited availability of LC-MS libraries and data analysis software with reliable library search algorithms. This multi-center study was designed to characterize the sensitivity and specificity of three LC-MS systems for broad spectrum drug screening compared to traditional LC-UV and GC-MS techniques, and to identify common problems associated with the methodology and data review process for each system.

**Methods:** Sixty patient urines were run on five systems including, REMEDi HS®-LC-UV (Bio-Rad), full scan EI GC-MS (Agilent), ZQ™ Mass Detector (LC-Q-MS) with MassLynx™ software (Waters), 3200-QTRAP® (LC-QTRAP®-MS/MS) with Cliquid® Drug Screen and Quant software (Applied Biosystems) and Finnigan™ LXQ™ Linear Ion Trap (LC-LIT-$MS^n$) with ToxID™ software (Thermo). Precision, limit-of-detection, ion suppression and carryover studies were performed on the LC-MS systems. LC parameters, sample preparation (extraction for LC-Q-MS and LC-LIT-$MS^n$**,** dilution for LC-QTRAP®-MS/MS) and MS acquisition modes were different for each LC-MS system. Sample preparation for GC-MS did not include hydrolysis or derivatization. The LC-MS systems were initially compared based on their ability to detect a subset of 60 drugs, all of which were included in the three LC-MS lists of detectable drugs.

**Results:** In the 60 patient samples, the total number of drugs identified by each system after data review were 110 (REMEDi), 157 (GC-MS), 157 (LC-Q-MS), 200 (LC-QTRAP®-MS/MS) and 217 (LC-LIT-$MS^n$). For the identified drugs 99.1% (REMEDi), 99.4% (GC-MS), 92.4% (LC-Q-MS), 97.0% (LC-QTRAP®-MS/MS) and 85.3% (LC-LIT-$MS^n$) were also identified by at least one other system or medical record review, strengthening the possibility that those drugs were true positives. The LC-MS/MS systems (LC-QTRAP®-MS/MS and LC-LIT-$MS^n$) identified more drugs than the traditional LC-UV and GC-MS techniques. It was necessary to establish data review criteria for each LC-MS method to eliminate false positives identified by the search algorithms and to find drugs missed by the search algorithms. The common causes of false positives with the LC-MS systems included, carryover, endogenous substances matching library spectra and nonspecific matching of sub-optimally fragmented drugs to library spectra or acquired spectra to sparse library spectra (<3 ions). Out of the total number of drugs identified with each system before data review, 47.2% (LC-Q-MS), 30.2% (LC-QTRAP®-MS/MS) and 40.2% (LC-LIT-$MS^n$) were identified as false positives upon review. The common causes of false negatives with the LC-MS systems included, early- or co-eluting drugs and dynamic range limitations. Out of the total number of drugs identified with each system after data review, 11.8% (LC-Q-MS) and 50.7% (LC-QTRAP®-MS/MS) were not identified by the search algorithms but rather upon manual data review. This data is currently not available for the LC-LIT-$MS^n$.

**Conclusions:** The LC-MS/MS systems (LC-QTRAP®-MS/MS and LC-LIT-$MS^n$) detected a broader menu of drugs; however, it is essential to establish data review criteria for all LC-MS drug screening methods to eliminate the large number of false positives identified and false negatives missed by the current search algorithms. Search algorithms will need to be improved to eliminate lengthy review processes. This study indicates that consensus guidelines are needed for the evaluation of LC-MS/MS full scan product ion

spectra. LC tandem MS does not provide sufficient specificity to be used in isolation in the forensic laboratory. Use of two broad spectrum screening methods identified more drugs than any single system alone. An EI-GC-MS and ESI-LC-MS/MS combination may be optimal due to the orthogonal nature of the chromatographic and ionization techniques.

Keywords: **General Unknown Screening, LC/MS (Liquid-Chromatography Mass Spectrometry), Method Comparison**

## P67 A Quick LC/MS/MS Method for the Analysis of Common Benzodiazepines and Opiates

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**Objective:** The objective of this paper is to develop a fast method for analysis of common opiates and benzodiazepines in urine.

**Methods:** Analytes included in this method are: 6-Monoacetyl Morphine (6-MAM), Codeine, Morphine, Oxycodone, Hydrocodone, Hydromorphone, Desalkylflurazepam, Alprazolam, α-Hydroxyalprazolam, Diazepam, Nordiazepam, Lorazepam, Oxazepam, Temazepam, Triazolam, 7-Aminoclonazepam and Clonazepam. Deuterated analogs of each analyte were used as internal standards.

Urine samples were hydrolyzed, centrifuged for 2 minutes and diluted 1:5 with LC mobile phase. LC/MS/MS analysis was performed on a Shimadzu Prominence LC stack interfaced to an Applied Biosystems hybrid triple quadrupole/linear ion trap mass spectrometer. Injection-to-injection analytical run time was 6.5 minutes. Two MRM transitions per analyte were monitored and one transition per internal standard. The Scheduled MRM™ algorithm was use for optimal method performance for this multi-analyte method.

**Results:** Results showed that all analytes were successfully detected in the 6.5 minute run time utilized. The LLOQs for most analytes was around ≤5 ng/mL and all analytes had an LLOQ ≤50 ng/mL. Precision and accuracy were both within 10% except at or near the LLOQ, where both precision and accuracy were within 15%. The linear dynamic range was at least three orders of magnitude for all analytes.

**Conclusion:** An LC/MS/MS method was developed to quickly analyze common benzodiazepines and opiates in urine. The minimal sample preparation, combined with short LC/MS/MS run time drastically decreased sample turnaround time and increased throughput without compromising sensitivity or selectivity. Additionally, the ability to combine two assays into one quick LC/MS/MS run further decreased analysis times and costs.

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

## P68 Evaluation of Inter-Instrument Transferability of LC/MS/MS Methods

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**Objective:** The objective of this paper is to develop an LC/MS/MS method in one lab and directly transferring that method to other laboratories which have the same make/model of LC/MS/MS system without further optimization. Data are analyzed to compare variations in data quality across instruments and labs.

**Methods:** An LC/MS/MS method was developed to detect and quantify several different drug compounds across various drug classes. The drugs included : codeine, methamphetamine, benzoylecgonine, phencyclidine, amitryptyline, haloperidol, and diazepam. After method development, the method was directly transferred to 4 different laboratories with the same model of LC/MS/MS instrument; no additional tuning or optimization of the system was performed. The inter-instrument data was analyzed and the consistency of the data evaluated.

**Results:** Data analysis showed that direct transfer of an LC/MS/MS method between different instruments was possible. When sensitivity of the method was evaluated, all instruments showed peaks intensities and area counts were within a factor of three The biggest variable was retention time of the analytes, as it is necessary to consider several factors, such as tubing length, mobile phase consistency, and column-to-column reproducibility.

**Conclusion:** This study showed that it is feasible to develop methods and directly transfer these methods to other instruments of the same model without significant variations in sensitivity or other aspects of data quality. The ability to transfer methods without optimizing each instrument can save substantial time in method set-up.

Keywords: **LC/MS/MS, Drug Screening, Interlaboratory Validation**

## P69 Evaluation of a GC/MS Drugs of Abuse Confirmation Kit Based Around New GC/MS Software

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Presented is a description of the evaluation of a kit which allows for fast start up for common GC/MS confirmation assays. Also included is an evaluation of a new GC/MS software package which is included in the kit and is intended to streamline method development, batch setup, data acquisition and review, and report generation.

In addition to the new GC/MS operating software, the kit includes preloaded validated methods for the extraction and analysis of carboxy-THC, PCP, BE, amphetamines and opiates in a urine matrix. Also contained in the kit are many of the items needed for method re-validation and daily operation of the instrument. These items include necessary consumables, a quick start guide, and standard operating procedures.

In addition to the description of the software and other items will be a discussion of the method techniques and validation procedures used to evaluate the kit. This will include data showing the performance for method linearity, precision and specificity of the five drug classes.

The methodology and contents in the kit proved to be a useful tool to quickly validate these assays on a new instrument and gave excellent quantitative performance during the validation process. The following is example data from linearity and inter-day precision studies for the methods contained in the kit:

| Drug | LOD (ng/mL) | LOQ (ng/mL) | ULOL (ng/mL) | Precision at Cutoff | Precison @ 40% QC | Precision @ 125% QC |
|---|---|---|---|---|---|---|
| **THCA** | 1.5 | 1.5 | 1,000 | 2.9% | 1.3% | 4.3% |
| **BE** | 15 | 15 | 12,500 | 0.7% | 1.1% | 2.3% |
| **PCP** | 5 | 5 | 5,000 | 1.6% | 1.1% | 1.9% |
| **AM** | 25 | 25 | 50,000 | 1.6% | 1.9% | 1.3% |
| **AM (200 ng/mL)** | | | | 2.2% | 1.2% | 2.1% |
| **MA** | 25 | 25 | 25,000 | 4.3% | 4.6% | 4.4% |
| **Codeine** | 60 | 60 | 50,000 | 1.4% | 1.4% | 3.3% |
| **Morphine** | 100 | 100 | 50,000 | 1.8% | 1.0% | 5.5% |

Keywords: **Toxicology, Validation, Software**

## P70 Utilization of Comprehensive Two-Dimensional Gas Chromatography Time-of-Flight Mass Spectrometry (GCxGC-TOFMS) in Forensic Toxicology Applications

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Comprehensive two dimensional gas chromatography in combination with time-of-flight mass spectrometry detection (GCxGC-TOFMS) was used for drugs of abuse analysis in urine. Methamphetamine, cocaine, diacetylmorphine, codeine, oxycodone, ecstasy, acetylcodeine, monoacetylmorphine, and LSD were identified in this research. This poster will present experimental data from the forensic application conducted by GCxGC-TOFMS.

Non-derivatized 5mL urine aliquots spiked with a multiple drug standard mixture were prepared from Sigma-Aldrich drug standards and a hexachlorobenzene (HCB) internal standard at 500ng/mL was added to each. Each sample was extracted by solid phase microextraction (SPME fiber- 50/30µm DVB/Carboxen™/PDMS Stableflex™, Supelco, Bellefonte, PA). Drugs of abuse standards were spiked in urine from a 9 component stock standard mixture in the concentration range of 10 to 1500ng/mL. Solid phase microextraction (SPME) was performed on non-derivatized samples at room temperature with constant stirring for 30 minutes prior to SPME sample injection into the GCxGC-TOFMS system. GCxGC analysis was performed using a nonpolar stationary phase in the first dimension, (30m x 0.25mm x 0.25µm film thickness, Rxi-5ms, Restek Corp.) and a midpolarity column in the second dimension (1.5m x 0.18mm x 0.20µm film thickness, Rtx-200, Restek Corp.) followed by TOFMS detection.

The benefits of enhanced chromatographic separation and analyte detectability are featured with emphasis on the increased peak capacity and resolution gained by two dimensional gas chromatography. The advantage of time-of-flight mass spectrometry (TOFMS) to acquire full mass range data at fast acquisition rates necessary for multidimensional chromatography will be described. TOFMS provides simultaneous non-skewed mass spectral information required for accurate deconvolution of overlapping peaks and the data density needed to allow deconvolution algorithms to correctly identify poorly resolved chromatographic peaks which are buried in heavy sample matrices. Examples of deconvolution will be illustrated along with experimental research data from the drugs of abuse in urine analysis.

This data will be illustrated as well as examples of deconvolution showing the identification of target compounds in sample matrix. The use of SPME applied to non-derivatized samples combined with GCxGC-TOFMS will demonstrate that this is a favorable technique for quantitative and qualitative analysis of drugs in urine.

Keywords: **GCxGC-TOFMS, Deconvolution, Drugs of Abuse**

## P71 Development and Validation of a LC-MS/MS Method for Analysis of Acetaminophen-Protein Adducts in Serum

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Acetaminophen (APAP) protein adducts can be detected following exposure to acetaminophen at therapeutic doses and overdose. The purpose of the current study was to develop and validate a sensitive and specific liquid chromatography-tandem mass spectrometric method (LC-MS/MS) to detect serum APAP protein adducts. This method was also compared to a previously reported HPLC method with electrochemical detection (HPLC-ECD) (Muldrew, et al. *Drug Metab Dispos* 30: 446, 2002).

Serum samples were prepared by the following procedures: 1) Dialysis (MWCO: 3,500) against 10 mM sodium acetate pH 6.5 to remove APAP, non-protein adduct APAP- cysteine (APAP-CYS) and other small molecules; 2) protease digestion to release APAP that is adducted to cysteine in protein (APAP-CYS); 3) acetonitrile protein precipitation to remove residual protein, followed by supernatant evaporation; 4) the final residual was reconstituted in the HPLC initial mobile phase and injected to the LC-MS/MS.

Calibrators were prepared in 100 µl of serum with a dynamic range of 0.01 to 10 µM. Norbuprenorphine-$D_3$ was used as an internal standard. The APAP-CYS and norbuprenorphine-$D_3$ were detected by measuring transitions of *m/z* 271→ *m/z* 140 and *m/z* 417 →*m/z* 417, respectively, on a TSQ Quantum triple quadrupole mass spectrometer with ESI source and Surveyor HPLC system. Chromatographic separation was achieved on a YMC ODS-AQ column (2.0 × 50 mm, 3µM, Waters Corporation, Milford, MA). The mobile phase was MilliQ $H_2O$ with 0.1% formic acid (A) and methanol (B), the gradient elution was 98% A for 6 min, decreased to 50% A at 7 min, held for 6 min, then increased to 98% at 14 min. A 1/X weighted quadratic curve was used for quantification of APAP-CYS and coefficient of determination was obtained $r^2$>0.99.

By determination of fortified samples at three quality control concentrations (0.03, 0.3 and 7.5 µM), intra- and inter-run imprecision (CVs) were <5.6% and accuracy is within 8.7% of target concentration. In serum, the mean recovery of added APAP-CYS was 94.2%. Dialyzed serum fortified with APAP-CYS was stable after 3 freeze-thaw cycles and at ambient temperature for 24 h; the extracted samples were stable up to 144 hours at -20ºC and 48 hours on the autosampler. The methanolic stock solution of APAP-CYS was stable for 17 hours at room temperature and for 210 days at -20ºC. Comparison of APAP-CYS protein adduct concentrations in 18 mouse serum samples collected 2 h after administration of APAP i.p. at 75, 150 and 300 mg/kg measured with our LC-MS/MS assay and the previously reported HPLC-ECD method showed good correlation, with a coefficient of determination ($r^2$) of 0.998. The mean concentrations of APAP protein adduct in mouse serum are 0.25, 14.16 and 40.50 µM at 75, 150 and 300 mg/kg dose, respectively.

The LC-MS/MS method is valid and reliable for the quantitation of APAP-CYS that originate as acetaminophen protein adducts in serum.

Research supported by McNeil Consumer Healthcare

Keywords: **Acetaminophen, Protein Adduct, LC-MS/MS**

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## P72 Detection and Quantitation of Drugs of Abuse in Oral Fluids by LC/MS/MS

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The aim of the present study was to develop and validate a method for the detection and quantitation of drugs of abuse in oral fluids. Fortified oral fluid samples (made in-house) and samples from donors collected with Quantasil oral fluid collection kits from Immunalysis were screened on an Olympus 5400 using reagents purchased from Immunalysis. Amphetamines (AMPs: amphetamine, methamphetamine, MDA and MDMA), opiates (codeine, morphine, hydrocodone, hydromorphone, oxycodone and oxymorphone), phencyclidine (PCP), cocaine and its metabolite benzoylecgonine (BE) in oral fluids were quantitated by Applied Biosystems 3200 QTRAP LC/MS/MS. AMPs and PCP were extracted from samples using liquid/liquid extraction whereas opiates, cocaine and BE were extracted by solid phase extraction. The extracts were separated on a Shimadzu high performance liquid chromatography (HPLC) prior to the MS/MS analysis. For each drug, two MRM (multiple reaction mode) transitions (Table 1) were monitored using positive electrospray ionization (ESI) coupled to an MS/MS detector. Corresponding D3, D5, D6 and D11 internal standards were used to quantitate the results. The method was verified by participating in the North America Oral Fluid Proficiency Testing administered by Research Triangle Institute (RTI) and by analyzing real samples from donors. In conclusion, LC/MS/MS provided a simple way to analyze and quantitate drugs of abuse in oral fluids.

**Table 1. Details of MRM Transitions, LOD/LOQ, Precision and Accuracy Studies**

| Name | MRM Transitions | LOD/LOQ (ng/mL) | Precision (% CV) | Accuracy (%) |
|---|---|---|---|---|
| Amphetamine | 136→91; 136→119 | 10 | 6.1 | 94 |
| Methamphetamine | 150→91; 150→119 | 10 | 3.1 | 98 |
| MDA | 180→163; 180→105 | 10 | 8.2 | 90 |
| MDMA | 194→163; 194→105 | 10 | 5.1 | 96 |
| Codeine | 300→152; 300→115 | 10 | 3.1 | 87 |
| Morphine | 286→152; 286→165 | 10 | 7.6 | 90 |
| Hydrocodone | 300→199; 300→128 | 10 | 3.9 | 96 |
| Hydromorphone | 286→185; 286→157 | 10 | 2.9 | 95 |
| Oxycodone | 316→298; 316→241 | 10 | 4.9 | 87 |
| Oxymorphone | 302→284; 302→227 | 10 | 3.7 | 82 |
| Cocaine | 304→182; 304→82 | 2 | 4.6 | 85 |
| Benzoylecgonine | 290→168; 290→105 | 2 | 2.8 | 93 |
| Phencyclidine | 244→91; 244→86 | 2 | 5.0 | 95 |

Precision and accuracy studies were carried out by analyzing 10 samples for each drug at 40% of cutoff.

Keywords; **LC/MS/MS, Oral Fluids, Drugs of Abuse**

## P73 Modifications to the NIST Reference Measurement Procedure (RMP) for the Determination of Serum Glucose by Isotope Dilution Gas Chromatography/ Mass Spectrometry (ID-GC/MS)

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There are many examples in toxicology where measurement of blood glucose is vital. One of the most common uses for blood glucose measurements is in the diagnosis and treatment of diabetes. However, there are other instances where accurate measurements of blood glucose are important. In recent years there has been an increase in the number of cases reporting the onset of hyperglycemia in patients being treated with atypical neuroleptic drugs, namely olanzapine and clozapine. Meatherall et al. reported on a case history of a 31-year-old male schizophrenic patient whose death was attributed to hyperosmolar nonketotic diabetic coma while taking a prescribed does of olanzapine. The risk associated with olanzapine/ clozapine-induced hyperglycemia is just one example of the variety of toxicological cases where it is vital to have accurate measurements of blood glucose levels in these patients.

The National Institute of Standards and Technology (NIST) has a long history of supporting accuracy in health care measurements. NIST has been providing a standard reference material (SRM) glucose in serum for decades. We discuss here an adaptation of the method associated with serum glucose measurements using a modified ID-GC/MS method. The modified method demonstrates both good precision and accuracy. In addition the sample preparation is less extensive than the previous definitive method (DM), with the preparation time being reduced by approximately a day and half. NIST has used this method to certify the concentrations of glucose in SRM 965b, Glucose in Human Serum and SRM 1950, Metabolites in Human Plasma, which can provide an accuracy base to which routine methods for glucose cans be compared.

Keywords**: Glucose, Serum, Isotope Dilution, GC/MS, Toxicology, Quality Control**

## P74 Simultaneous Quantification of Amphetamine and Methamphetamine in Meconium Using ISOLUTE® HM-N Supported Liquid Extraction Columns and UPLC/MS/MS.

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A procedure for the rapid extraction and quantification of amphetamine and methamphetamine from meconium using ISOLUTE HM-N supported liquid extraction columns and ultra performance liquid chromatography coupled with tandem mass spectrometry (UPLC/MS/MS) is described. Due to the matrix complexity of meconium samples, extraction and sample preparation prior to instrumental analysis can prove difficult and time consuming. The present study introduces a novel sample preparation technique for the simultaneous quantification of amphetamine and methamphetamine in meconium using UPLC/MS/MS. Ultra performance liquid chromatography (UPLC) is an emerging analytical technique which draws upon the principles of chromatography to run separations at higher flow rates for increased speed, while simultaneously achieving superior resolution and sensitivity. Extraction of both analytes was achieved using ISOLUTE HM-N supported liquid extraction columns containing a modified form of diatomaceous earth. Subsequent separation and quantification using UPLC/MS/MS was achieved in less than 3 minutes. Limits of detection for amphetamine and methamphetamine were 3 ng/g and 750 pg/g respectively. The lower limit of quantitation (LLOQ) was 15 ng/g. Linearity was achieved over the range 15 ng/g to 1500 ng/g. The methodology showed excellent intra run precision with %CV values ranging from 1-9% for amphetamine and 1-6% for methamphetamine. Inter run precision experiments produced %CV values ranging from 3-7% for amphetamine and 1-6% for methamphetamine. The reported methodology proved suitable for the accurate quantification of amphetamine and methamphetamine in meconium samples and greatly reduced sample preparation time normally required for traditional solid phase extraction. Development and validation of such analytical methodologies will prove beneficial for the identification of prenatal substance abuse which is an ongoing concern across socioeconomic lines.

Keywords: **Meconium, UPLC/MS/MS, Supported Liquid Extraction**

**P75 Detection and Quantification of Cocaine and Benzoylecgonine in Meconium Using Solid Phase Extraction and UPLC/MS/MS**.

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A methodology for the selective determination and quantification of cocaine and its major metabolite benzoylecgonine in meconium using ultra performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) is described. Past studies indicate that up to 40% of neonates dying within two days of birth with no apparent cause of death have cocaine and/or benzoylecgonine in their blood, and rates of infants exposed to cocaine prenatally has been estimated to be between 2.6% and 11% of all live births[1]. Ultra performance liquid chromatography (UPLC) is an emerging analytical technique which draws upon the principles of chromatography to run separations at higher flow rates for increased speed, while simultaneously achieving superior resolution and sensitivity. Extraction of both analytes was achieved using a preliminary protein crash followed by solid-phase extraction (SPE) employing UCT Clean-screen columns with reversed phase and ion-exchange retention mechanisms. The column was conditioned with sequential washes of methanol, DI water and 0.1M phosphate buffer (pH 6.0). Samples were then loaded onto the columns and following washes with DI water, 1M HCl and methanol, analytes were eluted with 3 mL of dichloromethane: 2-propanol: ammonium hydroxide (78:20:2) elution solvent. Samples were dried down under a gentle stream of nitrogen and reconstituted in 200µL of DI water: acetonitrile (75:25). Limits of detection for both analytes were 3 ng/g and the lower limit of quantitation (LLOQ) was 30 ng/g. Linearity was achieved over the range 30 to 250 ng/g. The methodology showed excellent intra run precision with % CV values ranging from 1-11% for cocaine and 1-16% for benzoylecgonine. Inter run precision was evaluated and experiments produced % CV values ranging from 3-5 % for cocaine and 4-9% for benzoylecgonine. The increased speed and separation efficiency offered by UPLC, allowed for the separation and subsequent quantification of both analytes in less than 2 minutes. Dramatic increases in separation speed such as those afforded by UPLC translate into increased samples per unit time in high throughput toxicology laboratories. Development of sensitive analytical methodologies capable of detecting low levels of such drugs in meconium will prove beneficial for the identification of prenatal substance abuse.

[1]Birchfield, M., J. Scully, and A. Handler, *Perinatal screening for illicit drugs: policies in hospitals in a large metropolitan area.* J Perinatol, 1995. **15**(3): p. 208-214.

Keywords: **Cocaine, Meconium, UPLC-MS/MS**

## P76 Simultaneous Quantitative Determination of Ethyl Glucuronide and Ethyl Sulphate in Human Urine Using UPLC®/MS/MS

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**Objective:** To develop and validate a simple and rapid UPLC/MS/MS method for the simultaneous quantitative determination of ethyl glucuronide (EtG) and ethyl sulphate (EtS) in human urine which can be used to detect recent ethanol intake within a clinical, forensic or workplace setting.

**Methods:** Human urine samples (50µL) were prepared following centrifugation (12000rpm for 10 minutes) by a simple dilution (1:20) using 0.1% formic acid and the addition of deuterated internal standards (EtG-D5 and EtS-D5). Chromatography was performed using a Waters ACQUITY UPLC® system; analytes were separated on a Waters ACQUITY UPLC HSS C18 (2.1 x 150mm, 1.8µm) column using a gradient elution over 4 minutes with a mixture of 0.05% formic acid in water (A) and acetonitrile (B). A Waters® TQ detector (tandem mass spectrometer) was used for analysis with electrospray ionisation in negative mode (ESI-). Two MRM transitions were used for each compound (m/z 221 > 85, 221 > 75 for EtG and m/z 125 > 97, 125 > 125 for EtS) and each transition was optimised to achieve maximum sensitivity.

**Results:** For both compounds, responses were linear over the investigated range (0.25–100mg/L for EtG, 0.05-20mg/L for EtS). Intra and inter-assay precision and accuracy was assessed at four spiked QC levels (0.75, 2.5, 7.5 and 50mg/L for EtG, 0.15, 0.5, 1.5 and 10mg/L for EtS) and was found to be good, with precision CV's < 10% and accuracy between 97-112%. The cut-offs applied for EtG and EtS analysis were 0.5 and 0.1mg/L with limits of detection being 0.15 and 0.04mg/L, respectively. Possible matrix effects were assessed by spiking blank prepared patient samples ($n$ = 6) with both compounds and comparing the responses against the equivalent concentration of solvent standard solution. The average matrix effects were found to be acceptable (-16% for EtG and -7% for EtS). Both compounds were shown to be stable in prepared samples over 24 hours when stored on the ACQUITY UPLC at 5°C. The method was applied to the analysis of forensic samples ($n$ = 39) which were previously analysed for EtG only, by Microgenics DRI® EtG Enzyme Immunoassay and all samples showed good correlation ($r^2$ = 0.977). The quantifier/qualifier ion ratios for both compounds were monitored for all calibrators, QC's and samples and were found within ±20% of the desired ion ratios.

**Conclusion:** This method provides a simple solution for the simultaneous quantification of EtG and EtS in 5 minutes. The developed method has shown to be accurate, precise and linear over the desired analytical range. The method was successfully applied to the analysis of forensic samples.

Keywords: **Ethyl Glucuronide, Ethyl Sulphate, UPLC/MS/MS**

## P77 Improved Analysis of Benzoylecgonine in Urine Using a Modified Elution Solvent and Direct Elution

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Urine extracts often show the presence of substances which may interfere with GC/MS SIM analysis of drug metabolites such as benzoylecgonine (BZE). This study evaluated several different elution solvents to find the solvent mixture that would greatly reduce or eliminate interfering peaks and still provide good recovery of the BZE derivative. GC/MS analysis was performed on urine specimens spiked with deuterated internal standard ($d_8$-BZE), subjected to solid-phase extraction, and derivatized with pentafluoropropionic anhydride (PFPA) and pentafluoropropanol (PFPOH). A change in the composition of the methylene chloride:methanol:ammonium hydroxide elution solvent from 70:25:5 to 85:10:5 was shown to decrease of presence of several of these substances that interfered with the fast GC/MS SIM analysis of pentafluoropropyl (PFP) derivatives of BZE.

Direct elution was used during the extraction procedure to decrease extraction time by eliminating the need to transfer the derivatized extract from the receiver tube to the autosampler vial. The PFP ester derivative of BZE was identified and quantified by GC/MS utilizing a rapid GC temperature ramp method. The modified extraction method produced results that were linear from 12.5 to 10,000 ng/mL of BZE with a correlation coefficient ($r^2$) of 0.9999. The intra-assay precision of a 100 ng/mL BZE standard (n = 19) yielded an average concentration of 100.23 ng/mL and a coefficient of variation (CV) of 1.26%. The inter-assay precision of 13 sets of 50 and 125 ng/mL BZE controls had CVs less than 4.0%. No interferences were observed when the extraction method was challenged with cocaine, ecgonine, ecgonine methyl ester and cocaethylene.

Seventy-two specimens containing BZE, including specimens that had been in frozen storage for one year were analyzed using both elution solvents. Regression analysis showed comparable GC/MS quantitations ($r^2$ = 0.996). Eleven specimens with concentrations ranging from 45-200 ng/mL had interfering peaks when extracted with the 70:25:5 elution solvent that were not present when extracted with the 85:10:5 elution solvent. A regression analysis was performed on GC/MS results from 16 Armed Forces Institute of Pathology (AFIP) proficiency specimens that were extracted using the two elution solvents ($r^2$ = 0.9999).

Internal standard recoveries from four paired sets of specimens showed that the procedure employing the 85:10:5 mixture had a 19% CV versus a 37% CV with the 70:25:5 mixture.

In conclusion, the use of the 85:10:5 elution solvent coupled with direct elution reduced extraneous peaks which provided better resolution at shorter retention times and less variability in the recovery of the internal standard.

Keywords: **Extraction, GC-MS Analysis, Benzoylecgonine**

**P78 Analysis of 20-Hydroxyecydsone and a Metabolite in Male and Female Human Urine**

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Historically, athletes have used anabolic-androgenic steroids (AAS) in an effort to increase lean muscle mass and enhance athletic performance. More recently, nutritional supplements containing other constituents, such as 20-hydroxyecdysone (20E), are becoming popular alternatives to AAS. Supplements containing 20E are promoted to increase muscle growth and decrease fat mass, without androgenic, thymolytic, or antigonadotropic side effects. Limited data regarding the quantitative excretion of 20E in human urine is available. Therefore, the purpose of this study was to investigate the excretion of 20E after single-day (SD) and multiple-day (MD) administration, as well as to determine any changes to the endogenous steroid profiles. After obtaining informed consent, male and female subjects (n=9) received both SD (600mg/day) and MD (600mg/day for 5 days) 20E in a cross-over design. Urine was collected 0-4hrs; 4-8hrs; 8-12hrs; 12-24hrs and every 24hrs for up to 7 days after the final dose of 20E. A liquid chromatography-quadrupole-time-of-flight mass spectrometry (LC-Q-TOF) method was developed and validated for the detection and quantitation of 20E and its metabolite, 2-deoxy-20-hydroxyecydsone (2d), in human urine, following solid phase extraction. The product was also screened for the presence of banned substances of abuse using a previously validated GC-MS method. Data for males and females were combined, as no differences were found between the two groups. The urine concentrations (mean ± SEM) of unconjugated 20E and 2d were 5,313.9 ± 1,092.4 μg/day and 70.2 ± 18.6 μg/day, respectively, 0-24hr after dosing in the SD protocol. In the MD protocol, the mean concentrations of 20E and 2d ranged from 3,269.4 ± 976.8 to 5,471.3 ± 2,093.1 μg/day and 43.7 ± 23.8 to 76.8 ± 30.8 μg/day, respectively, during the 5 days of dosing. The mean urine 20E concentrations returned to baseline by 48hrs for both protocols. Urine 2d concentrations remained detectable up to 168hrs for both protocols, for several subjects. Hydrolysis of the patient urine was investigated in order to explore the possibility of conjugated urinary metabolites of 20E, however no differences were found between the hydrolyzed and non-hydrolyzed urine. An additional metabolite, with an identical m/z and similar retention time to 2d was detected in all patient samples. This metabolite could not be quantified due to lack of reference material, but its peak intensity ranged from approximately 7 to 11 times greater than that for 2d. No changes to the total daily urinary excretion of the endogenous steroids, testosterone, epitestosterone, androsterone, and etiocholanolone were found. This data demonstrates that 20E and its metabolite, 2d, as well as an unidentified metabolite, are excreted in human urine for up to 48 hours after administration. This data also indicates that consumption of a 20E-containing supplement does not change endogenous urinary steroid profiles.

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Keywords: **20-Hydroxyecdysone,LC-TOF-MS (Liquid Chromatography-Quadrupole-Time-of-Flight Mass Spectrometry), Nutritional Supplements**

## P79 Detection and Quantification of Common Pain Drugs in Urine by LC/MS/MS

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**Objective**: To develop a simple, quick method for analysis of urine to detect and quantify over 40 common pain medications.

**Methods:** Sample preparation consisted of Beta Glucoronidase enzyme hydrolysis followed by centrifugation and subsequent 1:4 dilution of the urine sample with LC mobile phase consisting of Water and Methanol, with 0.1% Formic Acid. 25 µL of sample was injected for analysis and a (2.1mm x 50mm) C18 column was used for separation using a gradient starting at 2% to 95% MeOH with 0.1% Formic acid. Total analysis time was <8.5 min from injection to injection and over 40 drugs were analyzed in a single injection

**Results:** The Limits of Quantitation differed for each drug but ranged from <5 ng/ml to 200ng/ml when extracted using a 1:4 dilution of urine samples. The linearity for each drug spiked into urine exceeded R correlation of 0.98. Each drug was analyzed using two transitions and the LOQ was based on the least sensitive of the two transitions.

**Conclusion:** An LC/MS/MS method was developed to analyze over 40 drugs in a single analytical run. It was possible to use minimal sample preparation, basically "dilute and shoot", and still maintain adequate sensitivity and selectivity. This minimal sample preparation, combined with a short LC/MS/MS run time, resulted in an easy, fast method.

Keywords: **LC/MS/MS, Drug, Pain**

## P80 A Sensitive Method for Quantitation of Flunitrazepam and Five Metabolites in Urine Using Solid Phase Extraction and LC-MS-MS

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The objective of this paper was to develop a method for quantitation of trace amounts of flunitrazepam and its metabolites in urine. Flunitrazepam is a low dose sedative known to be used in connection with sexual assaults or robbery to incapacitate the victim. Blood and urine samples in connection with sexual assaults are commonly obtained for toxicological analysis, however, they are often taken a considerable time after the assault and present with negative results. To achieve a sensitive method we used solid phase extraction followed by LC-MS-MS. The method included flunitrazepam (Flu), desmethylflunitrazepam (dmFlu), 7-aminoflunitrazepam (7AFlu), 7-aminodesmethylflunitrazepam (7AdmFlu), 7-acetamidoflunitrazepam (7AAFlu), and 7-acetamidodesmethylflunitrazepam (7AAdmFlu).

The analysis was performed on an API4000 MSMS coupled to a Waters Acquity solvent delivery system. Chromatography was done as a linear gradient using 10mM ammoniumformiate as A-phase and acetonitrile as B-phase on a BEH C18 column (50x2.1, 1.8 µm particles). All analytes were baseline separated using a linear gradient 90 % B over 3 minutes. Two transitions were measured for each analyte and the area-ratio between them should be within 30% of the target value.

To 1 mL of urine were added internal standard, 40 µL of β-glucuronidase and 1 mL of acetate buffer (pH 6), and the samples were incubated at room temperature for 30 minutes. Bond Elut Certify Columns were then pretreated with 2 mL of methanol and 2 mL of 50mM acetate buffer (pH 6) before the urine samples were added to the columns. The columns were washed with 2 mL of MilliQ-water followed by 1 mL of 1M acetic acid and dried for 20 minutes. Finally the columns were washed with 1 mL of methanol and dried for 1 minute before elution of the analytes with 2 mL of dichloromethane: 2-propanol (80:20) with 2% ammonia. The eluate was evaporated and reconstituted in 200 µL of 20mM ammoniumformiate buffer: acetonitrile (80:20). Calibrators were prepared as authentic samples. at 0.25, 0.5, 5.0, 20, 50, 75, 100, 150 and 200 ng/mL.

The between-day precision and accuracy are presented in the table. The within-day precision (n = 5) varied between 2.1 and 13.4 % at the same levels as described in the table.

| **Analyte** | **Betweeen-day precision** (n = 15) | | | | | |
|---|---|---|---|---|---|---|
| | **Low** (1.0 ng/ml) | | **High** (100 ng/ml) | | **Authentic sample** | |
| | **mean** ng/ml | **CV %** | **mean** ng/ml | **CV %** | **Mean** ng/ml | **CV %** |
| Flu | 1.04 | 13.8 | 104 | 12.5 | <0.5 | -- |
| dmFlu | 1.14 | 16.0 | 110 | 14.3 | 0.797 | 23.2 |
| 7AFlu | 0.97 | 7.7 | 94.8 | 5.5 | 138 | 6.4 |
| 7AdmFlu | 1.03 | 12.9 | 102 | 7.3 | 19.6 | 12.8 |
| 7AAFlu | 0.91 | 14.9 | 88.6 | 10.8 | 2.53 | 15.2 |
| 7AAdmFlu | 0.92 | 10.6 | 87.9 | 8.6 | 13.1 | 13.2 |

Recovery was tested at two levels, 1.0 ng/ mL and 10 ng/ mL for all analytes. Recovery ranged between 67 to 95 % at the low level and between 59 to 115 % at 10 ng/ mL. The method has been used in a controlled study of urinary excretion of flunitrazepam metabolites and shown to detect metabolites up to ten days after ingestion of 0.5 mg flunitrazepam.

We conclude that the developed method has sufficient sensitivity and analytical performance to be used in e.g. sexual assaults even if samples are obtained several days after the event.

Keywords: **Flunitrazepam, Solid Phase Extraction, LC-MS-MS**

## P81 Detection and Quantitation of Opioids in Whole Blood by LC/MS/MS Using Simple Precipitation

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Ion suppression due to the matrix effects of whole blood samples can make it difficult to detect early eluting compounds such as common opioids by certain liquid chromatography –electrospray ionization tandem mass spectrometry (LC/MS/MS) methods. A method was developed for identification and quantitation of morphine, hydromorphone, codeine, hydrocodone, and oxycodone to overcome the challenges posed by ion suppression. Reproducible results were observed for both detection and quantitation of these compounds by using a quick and simple precipitation procedure, developed initially for enzyme immunoassay. This procedure is explained in a previous publication; modifications to this method are sample reconstitution using a 97% mobile phase A (ammonium formate buffer) and 3% mobile phase B (acetonitrile) solution (1). Identification was performed using a 1mL aliquot for the extraction and screened by LC/MS/MS with an EPI (enhanced product ion) scan for full mass spectrum identification to a library match. Quantitation was performed separately using a 200 µL aliquot for the extraction and detecting only parent to fragment MRM (multiple reaction monitoring) transitions yielding no structural data. All analyses were performed with an Applied Biosystems 3200 QTrap LC/MS/MS, and chromatographic separation was performed on a Phenomenex Synergi Polar-RP (150x2.00mm) column with a gradient elution system.

| Time (min) | Mobile Phase A (%) | Mobile Phase B (%) |
|---|---|---|
| 0 | 97 | 3 |
| 3 | 84.5 | 15.5 |
| 12 | 82 | 18 |
| 16 | 5 | 95 |
| 18 | 5 | 95 |
| 18.1 | 97 | 3 |
| 20 | 97 | 3 |

Experiments were executed to determine the limits of qualitative identification (LOI). Quantitations were conducted over a calibration range of 12.5-200 µg/L with linear quantitation curves yielding variabilities less than 20 % for all samples.

| **Compound** | **Parent Ion** | **Product Ion** | **Internal standard** | **RT (min)** | **Qualitative LOI** | **Quantitative Variability (%)** |
|---|---|---|---|---|---|---|
| morphine | 286.1 | 201 | morphine-d6 | 5.93 | 6 µg/L | 17 |
| hydromorphone | 286.1 | 185 | mepivacaine | 6.38 | 3 µg/L | 12 |
| codeine | 300.2 | 152 | codeine-d6 | 7.28 | 6 µg/L | 18 |
| oxycodone | 316.1 | 241 | mepivacaine | 8.51 | 4 µg/L | 12 |
| hydrocodone | 300.2 | 199 | mepivacaine | 9.54 | 4 µg/L | 14 |

[1] George L. Herrin, H. Horton McCurdy, William H. Wall. Investigation of an LC/MS/MS (QTrap®) Method for the Rapid Screening and Identification of Drugs in Postmortem Toxicology Whole Blood Samples. Journal of Analytical Toxicology 2005;29(7):599-606.

Keywords: **LC/MS/MS, Opiates, Ion Suppression**

## P82 A Rapid LC-MS/MS Method for Fentanyl in Post Mortem Blood

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A liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed for the analysis of fentanyl in post mortem blood specimens. The results from this recently developed LC-MS/MS method were compared with those obtained by gas chromatography-mass spectrometry (GC-MS).

The LC-MS/MS assay was calibrated from 0.5 to 25 ng/mL, and quality control samples were prepared in blank blood at 2.5 and 10 ng/mL. In addition, seven post mortem blood specimens were analysed in duplicate by LC-MS/MS and GC-MS. $d_5$-Fentanyl at 5 ng/mL was utilized as an internal standard.

The extraction of fentanyl was performed using conditioned SPE cartridges (Varian, Inc. BOND ELUT 130 mg) with a positive pressure manifold. Following appropriate washes, fentanyl and $d_5$-fentanyl were eluted with elution solvent (isopropanol:ammonium hydroxide:methylene chloride; 20:2:78 v/v/v). Extracts were dried under a nitrogen stream at 40°C and reconstituted in methanol:0.1 mM ammonium acetate buffer (1:3 v/v).

LC-MS/MS analyses were performed on a Varian Prostar Quadrupole LC-MS/MS system equipped with a 3 µm Pursuit XRs C8 column (50 mm x 2.0 mm) and a Metaguard 2.0 mm Pursuit XRs C8 guard column (Varian, Inc). Mobile phases include A (0.1 mM ammonium acetate) and B (methanol) with a flow rate of 0.3 mL/min. During analysis, mobile phase B was ramped from 75% to 100%. Multiple reaction monitoring was performed with electrospray ionization in the positive mode and m/z 336.8 and 342.2 were selected as the parent ions for fentanyl and $d_5$-fentanyl, respectively. The product ions m/z 188.1 and 105.0 were selected for fentanyl and $d_5$-fentanyl, and m/z 188.1 was used as the quantitative ion for both analytes.

The assay run time was 5 minutes with a retention time of approximately 2 minutes. The range of linearity was 0.5-25 ng/mL ($R^2 > 0.99$) utilizing a minimum of four calibrators. The mean result of all analyses were within ±15% of the target concentration and intra-assay precision (%CV) was <3.1% for control samples prepared at 2.5 ng/mL and <4.9% for control samples prepared at 10 ng/mL. Inter-assay %CVs were <3.8 % for both control samples concentrations based on triplicate analyses on three separate days. Results from a direct comparison of authentic post mortem specimens using the LC-MS/MS method with a routine GC-MS method was within ±17%, and the correlation coefficient was greater than 0.99.

An LC-MS/MS method for fentanyl in post mortem blood has been developed and validated. The assay is linear, highly accurate and precise, and readily adaptable to post mortem blood specimens. Finally, results obtained from the LC-MS/MS method are comparable to those obtained by GC-MS.

Keywords: **Fentanyl, Blood, LC-MS/MS**

## P83 Automated Extraction, Derivatization and GC/MS Analysis of Tetrahydrocannabinol and Metabolites in Whole Blood Using Disposable Pipette Extraction

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The analysis of tetrahydrocannabinol (THC) and its metabolites, hydroxy-THC (OH-THC) and carboxy-THC (COOH-THC), is one of the most tedious and time consuming methods in forensic toxicology. This analytical challenge is because the limits of detection need to be very low, preferably 1 ng/mL. In addition, most solid-phase extraction (SPE) methods require separate chromatographic analysis of THC from COOH-THC due to possible interfering sample matrix components, making the extractions and chromatographic analyses even longer than most methods.

Recently, Disposable Pipette Extraction (DPX) has been found to be a rapid and efficient SPE method for the analysis of THC and metabolites in whole blood. 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. Also, less sorbent is required for elution which means faster concentration times. Using reversed phase DPX extraction mechanisms (DPX-RP), the GC/MS analysis has been shown to be efficient for the simultaneous analysis of THC and its metabolites.

Using 0.5 mL of blood, the samples were first protein precipitated with 0.75 mL acetonitrile, centrifuged, and then transferred to clean sample tubes. Then 1 mL of DI water and 50 µL of 0.1 M HCl were added to each tube, which were then placed on the GERSTEL MPS-2 sample tray. Automated DPX was performed using 1 mL DPX-RP (reversed phase) tips and multiple extractions (3 total) of approximately 0.7 mL each. After one wash step and one elution step into a clean GC vial, a large volume injection (50 µL) was made into the GC inlet (GERSTEL CIS) along with 20 µL 50% BSTFA in acetonitrile. The extraction of each sample was performed in about 6 minutes, and the on-column derivatization was performed using the GERSTEL CIS during the injection process. This short time enables extractions and derivatizations of THC and metabolites to be completed within the chromatographic run times using fast GC/MS.

The GC/MS (Agilent Technologies 6890 GC with 5975 MSD) used selective ion monitoring with 20 ms dwell times for THC (386, 371, 303), d3-THC (389, 374), COOH-THC (473, 371, 488), d3-COOH-THC (476, 374), OH-THC (371, 372, 474), and d3-OH-THC (374, 477). The limits of detection and quantitation were less than 1 ng/mL for THC, COOH-THC and OH-THC with coefficients of variation of less than 5% for all analytes.

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

**P84 An LC-MS-MS Method for Quantitative Measurements of Plasma Buprenorphine and Norbuprenorphine in 100 Microliter Samples**

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Analysis of pediatric, forensic, and laboratory animal samples is often limited to small volumes. Our current methods for buprenorphine and metabolites required 1 mL sample to achieve LLOQs of 0.1 ng/mL (1,2). This study describes modifications of a previously published LC-MS-MS method (1) aimed at enhancing sensitivity of detection and perfecting chromatographic separations to make it suitable for small sample analysis. For norbuprenorphine, the acetonitrile adduct ion at m/z 455 is collision induced dissociated to $MH^+$ ion of 414 as described by Chang et al. (3). For buprenorphine, the survival $MH^+$ ion at m/z 468 passing through the collision chamber at collision gas pressure of 2.5 mtorr and collision energy at 25 eV was monitored. These choices of the ion scanning modes increase detection sensitivities for both analytes by approximately 10-fold when compared with use of the transition from 414 to 414 for norbuprenorphine or the 468 to 386 for buprenorphine. Use of a YMC ODS-AQ 5 µm 120 A 2.0 x 100 mm column provided excellent separation of the analyte peaks from the background interferences. A simple liquid-liquid extraction procedure of 0.1 mL plasma was used allowing greater than 74% recovery of the analytes. In 4 clinical batch runs, the %diff (differences from the target) and the %RSD of the calibrators (0.1, 0.25, 0.5, 0.75, 1, 2.5, 5, 8, and 10 ng/mL) were all within 15% and the correlation coefficients $R^2$ of all calibration curves were greater than 0.99. The %CV for norbuprenorphene calculated from N=8 (2 per run) were 6.0 at 0.25, 14.4 at 2, and 2.3 at 7.5 ng/mL; for buprenorphine were 8.0 at 0.25, 10.4 at 2.0, and 9.5 at 7.5 ng/mL. The %target for norbuprenorphine was 102.7 at 0.25, 100.8 at 2.0, and 100.4 at 7.5 ng/mL. The %target for buprenorphine was 99.0 at 0.25, 101.4 at 2, and 97.9 at 7.5 ng/mL. The %CVs measured at the LLOQ of 0.1 ng/mL were 7.02 for norbuprenorphine and 4.42 for buprenorphine. Application of this method is demonstrated in another publication concerning use of buprenorphine to treat the neonatal abstinence syndrome (4).

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Keywords: **LC-MS-MS, Buprenorphine/Norbuprenorphine, Small Volume Sample**

## P85 Rapid Quantification of Duloxetine in Blood and Urine by LC-ESI-MS/MS

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The objective of this study was to validate a liquid chromatographic-mass spectrometric-mass spectrometric (LC-MS/MS) method for the quantification of duloxetine in blood and urine using SPE as the mode of extraction.

Duloxetine and the internal standard (ethyl morphine) were extracted from buffered (pH 6) whole blood/ urine samples (1 mL) utilizing Clean Screen™ SPE cartridges (UCT, Inc.). After washing the columns, DI water, acetic acid, and methanol (3 mL, 2 mL, 3 mL, respectively), samples were eluted with $CH_2Cl_2$/ IPA/ $NH_4OH$ (3 mL) and evaporated to dryness. To the dry eluates, 200 µL of 0.1 % aqueous formic acid was added prior to chromatographic analysis.

Liquid chromatography was performed using Imtakt™ $C_{18}$ column (50 x 2.1mm, 5 µm), at 0.5ml/min flow using a gradient program. The mobile phase program: (A) 0.1% aqueous formic acid / (B) acetonitrile containing 0.1% formic acid was started at 5% (B) for 0.5 min, increasing to 90% (B) over 4 min, before returning to 5% (B) and equilibrated for 1 min. The total chromatographic run time: 6 minutes including equilibration. MS/MS analysis was conducted using an Applied Biosystems 3200 QTrap instrument equipped with ESI source in the positive ion mode and operated with multiple reaction monitoring (MRM) under the following conditions: curtain gas 15, collision gas medium, ion spray voltage 5000V, temperature 650 °C, ion source gas(1) 50 psi, ion source gas (2) 50 psi. The following transitions were monitored (quantification ions underlined): m/z 298.1 → <u>44.1</u> and 154.3 for duloxetine, and m/z 314.2 → <u>152.2</u> and 128.3 for ethyl morphine.

Linearity ($r^2$ >0.99) was achieved from 0.1 to 50 ng/mL, and the limits of detection and quantification were 0.05 and 0.1 ng/mL, respectively. Recovery values for blood and urine (target value: 4.0 ng/mL) were > 92%. Intra and inter-day imprecision was less than 6% and 10%, respectively. Ion suppression studies revealed that suppression of monitored ions was less than 8%.

This method of analysis provides a simple, sensitive, and reproducible quantitative method and should be of great assistance to those analysts actively involved with the LC/MS/MS analysis of this drug in biological matrices, and shows an efficient method of extraction as well.

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

**P86 In Vitro Determination of Oxycodone Metabolism in Human Liver Microsomes and Supersomes by Liquid Chromatography Tandem Mass Spectrometry**

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Oxycodone is an opioid agonist used in the treatment of moderate to severe pain. Oxycodone undergoes N-demethylation to noroxycodone and O-demethylation to oxymorphone. In order to better characterize the cytochrome P450 (CYP) isoforms involved in the oxidative metabolism of oxycodone in vitro, our previously presented highly sensitive method in plasma was cross-validated to measure oxycodone and its metabolites, noroxycodone and oxymorphone, in human liver microsomes. Deuterated oxycodone, noroxycodone and oxymorphone were used as internal standards. Liquid / liquid extraction was applied for sample preparation using n-butyl chloride: acetonitrile (4:1) as organic solvent. High performance liquid chromatography interfaced by electrospray ionization to a tandem mass spectrometric detector (HPLC-ESI-MS/MS) was used for quantitation. A YMC ODS-AQ S 5 µm 2.0 x 100 mm column (Waters Corporation, Milford, MA) was used for separation. The mass spectrometer was a Thermo Scientific model TSQ Quantum. The calibration range was from 0.2 to 250 ng/mL with the calibration curve constructed as quadratic with 1/X weighting. Specificity for oxycodone, noroxycodone and oxymorphone was determined from analysis of microsomes fortified with internal standard only (3 replicates) and with lower limit of quantitation (LLOQ) concentration (0.2 ng/mL) (1 replicate) in six different sources of human liver microsomes. The primary evaluation was to compare mean peak area ratio of any signal at retention time of the analytes to its internal standard for each source of microsomes with the mean peak area ratio of the six LLOQ samples. Mean ratios relative to mean LLOQ ranged from 3.18% to 17.3% with a mean of less than 13.9% for each analyte. Intra-run accuracy of the LLOQ was within 5.0% of target with intra-run precision within 11.7%. Intra -run precision and accuracy were also evaluated at 0.6, 10 and 200 ng/mL. The intra-run accuracy was within 6.0% of target with intra-run precision within 11.8%. Oxycodone was incubated with a panel of recombinant human CYPs (1A2, 2A6, 2B6, 2C8, 2C9, 2C18, 2C19, 2D6, 2E1, 3A4, 3A5 and 3A7). Supersomes that coexpressed cytochrome $b_5$ were used where available (not for 1A2, 2C18 and 3A5). CYP2C18 and CYP3A4 displayed the highest activity for oxycodone N-demethylation whereas CYP2D6 showed highest activity for O-demethylation. Oxycodone was also incubated with three human liver microsomes, the preliminary results showed that the N-demethylated metabolites were formed to a greater extent than the O-demethylated metabolites which is consistent with other studies.

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

## P87 Obtaining Symmetrical Peak Shape and Reproducibility When Analyzing Amphetamines by GC

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Analyzing amphetamines by GC/MS can be challenging whether they are derivatized or underivatized. Underivatized amphetamines can chromatograph as irregular and unsymmetrical peaks which are difficult to integrate and reproduce. On the other hand, chromatographing derivatized amphetamines results in symmetrical peaks that are accurately integrated, but can cause contamination problems leading to increase system maintenance and elevated baselines that interfere with analyzing the target compounds.

In this study, the effects of several sample pretreatment methods were evaluated including no pretreatment, converting the salt forms into free bases, derivatizing with HFAA (heptafluorobutyric acid anhydride), and derivatizing with HFAA followed by a clean up. Our first objective of the study was to obtain symmetrical peak shapes. Our second objective was to ensure a "clean" baseline. And, our last objective was to maintain low column/system bleed from injection to injection.

Results indicate that analyzing untreated and free base amphetamine & methamphetamine produces irregular peak shapes. Symmetrical peak shapes are obtained by derivatizing the amphetamines with HFAA (heptafluorobutyric acid anhydride). However, derivatization by-products (acidic) led to noisy baselines and high background. Even though there is an increase in contamination in the system, this method results in good peak area reproducibility (i.e. accurate integration). By incorporating a post clean-up procedure into the derivatization method, acidic by-products are removed and the chromatography is maintained reducing system maintenance. The post clean up procedure included mixing the sample with a phosphate buffer (pH=7.0) before dilution, removing the butylchloride layer, then diluting the sample just before injecting into the GC. This method ensures accurate area count reproducibility, a clean GC system and a stable baseline.

Keywords: **Amphetamines, Sample Preparation, Gas Chromatography**

## P88 Reproducible Headspace Analysis for Fast Screening and Confirmation of Gamma-Hydroxybutyrate (GHB) in Urine

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For more than ten years, gamma-hydroxybutyrate (GHB) and its related products (1,4-butanediol and gamma-butyrolactone (GBL)) have been identified as abused substances in cases of driving under the influence and drug facilitated sexual assault. Currently, GHB is regulated as a federally controlled Schedule I drug. Traditional analytical methods for GHB employ gas chromatography/ mass spectrometry (GC/MS) for quantification and confirmation. In its native state, GHB is extremely difficult to chromatograph, so it is usually either analyzed as a TMS (trimethylsilyl) derivative or converted to GBL for analysis using a liquid injection. The derivatization by-products and sample matrix can contribute to contamination of the injector and column, requiring added maintenance and downtime.

The methodology described here is a headspace GC/FID screening procedure followed by confirmation and quantification by headspace GC/MS using a total vaporization sample introduction technique (TVT). The headspace screening instrumentation used was the Tekmar HT3 headspace loop/trap autosampler connected to a Shimadzu GC 2010 equipped with an FID. The confirmation instrumentation included an Agilent 5890 equipped with a 5971 mass spectrometer. Samples were prepared using a modified version of the FBI Chemistry Unit's extraction and conversion procedure using sulfuric acid. This solvent extraction method was applied to both liquid injection and headspace sample introduction methods.

Since the methodology for analyzing GHB employs sample introduction through a headspace technique, the need for injector and column maintenance is reduced. The reduction in maintenance time is dependent on the number of samples analyzed per day. However, headspace analysis minimizes the amount of matrix components that can be transferred to the GC system and is considered a clean sample introduction technique. Faster throughput also results from elimination of the derivatization step by using the solvent extraction method, which saves approximately 30 minutes per analytical batch. Headspace injection results using solvent extraction showed GHB reproducibility and linearity from 10 to 300 µg/mL in matrix with a coefficient of variation equal to 6.2 for the midpoint of the calibration curve and an $R^2$ of 0.9911. The solvent extraction and conversion procedure was also applied to liquid injection and resulted in a coefficient of variation of 3.0 for the midpoint of the calibration curve and an $R^2$ of 0.9984.

Based on this work, the sample extraction and analysis method presented here is suitable for quantitative analysis of GHB and GBL. This method is very versatile, and is suitable for headspace injection or liquid injection, as well as analysis by GC/MS or GC/FID on commonly used blood alcohol and drug analysis GC systems. This allows analysts to use their existing laboratory equipment to quickly analyze urine samples for both GHB and GBL.

Keywords: **GHB, Headspace, Gas Chromatography**

## P89 Advantages of Mass Spectral Deconvolution Software

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Deconvolution software is purported to improve efficiency and accuracy for GC-MS screening. In that regard, the Agilent mass spectral deconvolution software was validated and put into use for screening forensic toxicological cases by a large reference laboratory. Deconvolution software has the ability to detect compounds in complex mixtures even when there is poor chromatographic resolution. For example, a postmortem blood, urine, or tissue sample with poly-drug findings can routinely be screened by deconvolution in about 2 minutes versus 10 minutes by traditional GC-MS methods. By employing this software, our laboratory throughput has increased dramatically – anywhere from 50-100% as measured by turn around time and total number of cases screened by traditional methods.

This notable increase in productivity is largely due to the software's ability to focus on user-delineated library searching. Deconvolution software utilizes full scan mass spectral data to isolate "components" in a Total Ion Chromatogram (TIC) and then apply an algorithm to generate an AMDIS (Automated Mass spectral Deconvolution and Identification Software) match factor. The isolated or "de convoluted" components are then compared to spectra in the user created database. The greater the match factor, the greater the confidence for an accurate mass spectral identification.

Retention time locking is also employed as an additional quality measure. Deconvolution Reporting Software (DRS) is beneficial because of its automated process, thereby eliminating the need for manual peak-by-peak library searches. A potential disadvantage of this version of DRS is its inability to detect trace amounts of compounds, although identification appears to be better for some compounds compared to our traditional GC-MS screening method. Even so, the increases in productivity, certainty of mass spectral identification, and simple use make this indispensable in the forensic toxicology laboratory.

Keywords: **Deconvolution, AMDIS, Advantages**

## P90 On-line Extraction Coupled to LC-MS/MS for the Determination of Drugs of Abuse

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**Objectives:** A new quantitation method for the determination of drugs of abuse (opiates, amfetamine and derivatives, cocaine, methadone and metabolites) in serum by using on-line extraction coupled to LC-MS/MS has been developed. It offers an alternative to the traditional off-line extraction methods, reducing sample preparation time, resources and systematic errors.

**Method:** The on-line extraction uses two extraction columns simultaneously: the sample is loaded with aqueous solvent in one of the columns, while the analytes retained in the other one are eluted to the analytical column by an organic gradient, which separates them. The extraction and the analytical columns are Restek Allure PFP (extraction column: 10x2.1 mm, 5µm, analytical column: 30x2.1mm, 3 µm) with a high capacity factor (k') for basic compounds. For the sample preparation, serum (0.1 mL) is spiked with a mixture of deuterated analogues of the drugs. After protein precipitation (efficiency was measured by UV detection) with 0.2 mL of a solution of methanol/zinc sulfate (0.1 M) (4:1, v/v), centrifugation, evaporation and reconstitution with 0.1 mL formic acid (0.1 %), the sample (50 µL) is injected into the LC-system. The quantitation is based on the analysis of two MRM transitions per drug, one as quantifier and the other one as qualifier, and one for each deuterated standard.

**Results:** The recovery of the protein precipitation step is over 80 % for all analytes. Matrix effects were also examined. Especially polar analytes like morphine (-30 %), morphine glucuronide (-50 %) and codeine glucuronide (-50 %) were suppressed. Amfetamine and its derivatives, benzoylecgonine, codeine and methadone did not show ion suppression effects in serum samples. Linear regression was performed with an external calibration in the range of 10-1000 ng/mL for all drugs, except for cocaine (2-200 ng/mL) and benzoylecgonine (25-2500 ng/mL). The regression coefficient of the calibration curve of each analyte is over 0.994.

The developed method was used to quantify basic drugs in samples from DRUID cases. The results were compared with those obtained by using SPE-GC-MS. The measured concentrations were comparable (e.g., MDMA 178 and 172 ng/mL, benzoylecgonine 316 and 324 ng/mL and codeine 216 and 195 ng/mL, LC-MS/MS and GC-MS, respectively).

**Conclusion:** This new method allows the quantitation of the studied drugs of abuse in serum samples. It is selective and sensitive and it has been successfully applied to the routine analysis of serum samples. Its application to urine samples is currently under investigation.

Keywords: **On-Line Extraction, Drugs of Abuse, LC-MS/MS**

## P91 A Novel Method for the Determination of Guanfacine in Urine by GC/MS

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Guanfacine, (Tenex®) an antihypertensive available since 1975, has recently been indicated for the treatment of attention deficit hyperactivity disorder (ADHD) in children (Intuniv ™). Due to this new usage, a gas chromatography/mass spectrometry (GC/MS) method was developed and validated for the determination of guanfacine in urine. Guanfacine and 100 ng of protriptyline (internal standard) were extracted from 1.0 ml urine with 0.5 ml of saturated carbonate: bicarbonate buffer and 2 ml of ethyl acetate. The solvent extract was evaporated and derivatized with heptaflurobutyric anhydride (HFBA) in n-butyl chloride. Chromatographic separation was achieved using a DB-5 capillary column (30 m x 0.32 mm, 0.25 um). Ions monitored for guanfacine were 86.1, 272.1, 274.1 m/z and for protriptyline, 191.1 and 189.1 m/z. Concentrations were determined using calibrators over the range of 0.1 - 2.0 mg/L. The linear regression for all calibration curves had $r^2$ values $\geq 0.99$. The limit of detection (LOD) was 0.05mg/L; limit of quantitation (LOQ) was 0.1mg/L; and upper limit of linearity was 10.0 mg/L. Percent recovery of guanfacine at 0.1 and 2.0 mg/L was 93% and 71%, respectively. The method was found acceptable for routine quantitative analysis of guanfacine in urine. In addition to validation studies, over 100 random post-diagnostic urine samples from children were tested for the presence of guanfacine. Guanfacine was detected in three urine samples at the following concentrations, < 0.1, 0.27 and 0.93 mg/L.

Keywords: **Guanfacine, Tenex®, Intuniv, GC/MS**

## P92 Rapid Analysis of Benzodiazepines by UHPLC/MS/MS

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The method developed in this study shows the increased sample throughput possible using Ultra High Pressure Liquid Chromatography (UHPLC), a selective stationary phase chemistry and highly sensitive tandem mass spectrometric (MS/MS) detection. With optimized method conditions, the rapid analysis of 27 benzodiazepines and three related receptor agonists, Zolpidem, Zaleplon and Zopiclone, is possible. The UHPLC/MS/MS methodology discussed provides the forensic community with a high speed approach for analyzing benzodiazepines at low concentrations.

UHPLC is a powerful tool for today's practicing chromatographer, as it can significantly increase the efficiency and speed of a chromatographic separation. Although small particles have made fast separations possible, the ultimate goal behind chromatography is still resolution. UHPLC does maximize efficiency (e.g. theoretical plates), but selectivity is still the first and most critical consideration when attempting to resolve mixtures of compounds. A pentafluorphenyl propyl (PFP propyl) phase was selected for the analysis of benzodiazepines because of the enhanced selectivity that it offers for organohalogens or other compounds containing basic or electronegative functionalities.

This study includes the development of a UHPLC/MS/MS method for analyzing 27 benzodiazepines, as well as Zolpidem, Zaleplon and Zopiclone. To reduce the analysis time, the stationary phase chemistry, mobile phase composition, and LC column geometry (particle size, length, and ID) were all optimized. Results show that choosing a column with enhanced selectivity towards this class of compounds permits rapid resolution from the sample matrix, while still maintaining peak integrity and sensitivity. Based on this work, a PFP Propyl UHPLC column coupled to a highly sensitive Quadrupole-Linear Ion Trap MS/MS allows for rapid identification and quantitation of the benzodiazepines. For forensic urine samples, quantitation and confirmation of all compounds at levels less than 10ng/mL is possible. Matrix effects can be minimized or eliminated by a 10-fold dilution of the urine sample. The earliest eluting compounds, such as 7-Aminoclonazepam, had the strongest matrix effects. Using the dilution procedure, an LOQ of 0.5ng/mL was determined.

Keywords: **Mass Spectrometry, HPLC, Benzodiazepines**

## P93 Determination and Quantitation of Noroxycodone in Human Urine Samples using High Performance Liquid Chromatography-Electrospray Ionization-Tandem Mass Spectrometry

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After attending this presentation, attendees will gain information about an analytical method for determination of noroxycodone, a metabolite of oxycodone, and will understand the metabolic pattern for oxycodone.

Oxycodone (4,5-epoxy-14-hydroxy-3-methoxy-17-methylmorphinan-6-one), is an analgesic, semi synthetic opioid derived from thebaine. Oxycodone is commonly prescribed for significant pain management typically associated with cancer, and has been used clinically for this purpose in the United States for the past eighty years. It has been a "drug of abuse" for nearly 50 years.

Oxycodone is metabolized in the body by two isoenzymes Cytochrome P450 (CYP) 3A4 and CYP2D6. CYP3A4-mediated metabolism of the compound yields N-demethylated metabolites noroxycodone, noroxymorphone, and α and β noroxycodol. CYP2D6-mediated metabolism produces O-demethylation of oxycodone to oxymorphone and α and β noroxymorphol, and 6-keto-reduction to α and β oxycodol.

Human urine samples, collected as part of another study to determine the elimination rate of oxycodone, were used as test samples for the detection and quantitation of noroxycodone. A method developed for the simultaneous quantitation of several opiates, including codeine, hydrocodone, hydromorphone, oxycodone, oxymorphone, and morphine, was modified to also incorporate noroxycodone as one of the compounds using selected ion monitoring (SIM). This method was utilized on a 4-channel multiplexing HPLC system interfaced with triple quadrupole mass spectrometer. Limit of quantitation, as well as between day accuracy and precision (%deviation and %CV) of noroxycodone was established at 100 ng/mL (3.9% and 24.9%).

Urine samples were collected over a period of a week from ten individuals given one of three different concentrations of oxycodone, along with a naltrexone blockade (50 mg per day). Concentrations of noroxycodone, oxycodone, and oxymorphone resulting from the analysis of an individual dosed with 80 mg tablets of oxycodone have shown noroxycodone to be the primary metabolite (70.8%±4.7) followed by oxycodone (18.5%±5.2) and oxymorphone (10.8%±2.1). Results for samples from other individuals will be tabulated and presented. These concentration results indicate that CYP3A4 mediation is the predominant metabolic pathway of oxycodone in humans.

Keywords: **Noroxycodone, HPLC (High Performance Liquid Chromatography), Tandem Mass Spectrometry**

## P94 Detector Response and Intensity Cross-Contribution as Contributing Factors to the Observed Nonlinear Calibration Curves in GC-MS Analysis

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It is a common knowledge that *detector fatigue* causes a calibration curve to deviate from the preferred linear relationship at the higher concentration end. With the adaptation of an isotopically-labeled analog of the analyte as the internal standard, *cross-contribution* (CC) of the intensities monitored for the ions designating the analyte and the IS can also result in the non-linear relationship at both ends (Liu et al., Anal. Chem. 74:618A; 2002). A novel approach (Chen et al., J. Am. Soc. Mass Spectrom. 19:598; 2008) developed to assess the accuracy of CC values is hereby applied to evaluated whether detector fatigue is a contributing factor to the observed nonlinear relationship in a calibration curve.

Contributions of detector fatigue and the CC to the above-stated phenomenon are illustrated by data derived the secobarbital (SB)/$^2H_5$-SB system (as methyl-derivatives) as shown in Table 1. Comparing the non-linear nature of calibration data that are empirically observed and theoretically calculated (incorporating the adjustment of CC values derived from three methods) indicates: both factors contribute significantly to the observed non-linear nature of the calibration curve based on ion-pair *m/z* 111/116; while detector fatigue is the dominating contributor to the observed non-linear nature of the calibration curve based on ion-pair *m/z* 196/201.

**Table 1.** Deviation of calibration data from a linear relationship: comparison of empirically observed and theoretically calculated data derived from ion-pairs with different levels of CC — SB/$^2H_5$-SB (as methyl-derivatives) example

| | | | Theoretically calculated with CC derived from | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Empirically observed | | Raw ion intensity data | | Normalized ion intensity data | | | |
| Theor. conc. | Ion int. ratio | Observed conc. (% deviation) | Ion int. ratio | Calculated conc. (% deviation) | Ion int. ratio | Calculated conc. (% deviation) | Ion int. ratio | Calculated conc. (% deviation) |
| *m/z* 111/116 and CC values | | | | 10%/0.11% | 8.6%/0.14% | | 12%/0.01% | |
| 25 | 0.2954 | 45.7 (82.8) | 0.2750 | 42.5 (70.2) | 0.2592 | 40.1 (60.4) | 0.2974 | 46.0 (84.0) |
| 50 | 0.4557 | 70.5 (41.0) | 0.4206 | 65.1 (30.1) | 0.4071 | 62.9 (25.9) | 0.4397 | 68.0 (36.0) |
| 100 | 0.7643 | 118.2 (18.2) | 0.7115 | 110.1 (10.1) | 0.7026 | 108.7 (8.68) | 0.7241 | 112.0 (12.0) |
| 200 | 1.293[b] | 200.0 (Calibrator) | 1.293 | 200.0 (0.00) | 1.293 | 200.0 (0.00) | 1.293 | 200.0 (0.00) |
| 800 | 4.891 | 756.5 (–5.4) | 4.768 | 737.6 (–7.80) | 4.818 | 745.3 (–6.84) | 4.705 | 727.8 (–9.03) |
| 1,800 | 9.444 | 1,461 (–18.8) | 10.51 | 1,626 (–9.68) | 10.63 | 1,644 (–8.67) | 10.39 | 1,607 (–10.7) |
| 2,400 | 11.91 | 1,842 (–23.2) | 13.93 | 2,154 (–10.3) | 14.08 | 2,177 (–9.28) | 13.79 | 2,134 (–11.1) |
| 3,600 | 16.13 | 2,495 (–30.7) | 20.69 | 3,200 (–11.1) | 20.89 | 3,231 (–10.3) | 20.60 | 3,187 (–11.5) |
| 4,800 | 19.73 | 3,052 (–36.4) | 27.37 | 4,233 (–11.8) | 27.59 | 4,267 (–11.1) | 27.40 | 4,238 (–11.7) |
| *m/z* 196/201 and CC values | | | 1.7%/0.0081% | | 1.4%/0.0097% | | 2%/0.01% | |
| 25 | 0.1743 | 28.6 (14.4) | 0.1705 | 27.9 (11.9) | 0.1681 | 27.6 (10.3) | 0.1737 | 28.5 (14.0) |
| 50 | 0.3267 | 53.6 (7.20) | 0.3203 | 52.6 (5.11) | 0.3182 | 52.2 (4.42) | 0.3231 | 53.0 (6.00) |
| 100 | 0.6507 | 106.8 (6.76) | 0.6199 | 101.7 (1.70) | 0.6185 | 101.5 (1.47) | 0.6217 | 102.0 (2.00) |
| 200 | 1.219[b] | 200.0 (Calibrator) | 1.219 | 200.0 (0.00) | 1.219 | 200.0 (0.00) | 1.219 | 200.0 (0.00) |
| 800 | 4.898 | 803.6 (0.51) | 4.813 | 789.6 (–1.29) | 4.821 | 790.9 (–1.13) | 4.801 | 787.8 (–1.53) |
| 1,800 | 9.797 | 1,607 (–10.7) | 10.79 | 1,772 (–1.57) | 10.82 | 1,775 (–1.38) | 10.77 | 1,767 (–1.86) |
| 2,400 | 11.99 | 1,967 (–18.0) | 14.39 | 2,360 (–1.65) | 14.42 | 2,365 (–1.45) | 14.34 | 2,353 (–1.94) |
| 3,600 | 15.22 | 2,497 (–30.6) | 21.56 | 3,537 (–1.74) | 21.60 | 3,545 (–1.55) | 21.49 | 3,526 (–2.06) |
| 4,800 | 19.05 | 3,126 (–34.9) | 28.73 | 4,713 (–1.81) | 28.78 | 4,722 (–1.63) | 28.63 | 4,697 (–2.14) |

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

## P95 Determination of Drugs of Abuse in Foods by Using Ultra High Performance Liquid Chromatography with Mass Spectrometric Detection (UHPLC/MS)

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**Objective:** To update gas chromatographic methods for identifying drugs of abuse with liquid chromatographic methods that are simpler and more robust. UHPLC offers a threefold benefit compared to GC; simpler sample prep, no derivatization, and less time wasted baking out or cleaning the instrument.

**Methods:** Methods were developed in a working forensic laboratory to analyze real samples of forensic importance. Samples were obtained from forensic evidence archived after adjudication. The samples were extracted with methanol, filtered, and 1 µL of filtrate was injected onto an ultra high performance liquid chromatograph (UHPLC). With no derivatization necessary, analytes were separated by using simple water and solvent gradients on Hypersil GOLD 1.9 µm, 2.1 x 100 mm columns. Analytes were detected by using a single quadrupole mass spectrometer operating in either full scan or selected ion monitoring mode, with spray voltage of 4.5 kV, probe temperature of 450 Celsius degree and dwell time of 0.03 s.

**Results:** We were able to positively identify cannabinoids in chocolate brownies and cookies, psilocin and psilocybin in mushrooms and chocolate, and lysergic acid diethylamide (LSD) in sugar candies after a simple methanol extraction and no chemical derivatization. The chromatogram of a 14 drug standard is shown in Fig 1. The drugs are separated within 8 minutes on a Hypersil GOLD 1.9 µm, 2.1 x 100 mm column. For a 1 µL sample injection, the linear dynamic range of all fourteen drugs spanned 1-2000 ng/mL, i.e., 1-2000 pg on-column ($r^2 \geq 0.998$), with limits of detection (LOD) around 1 ng/mL.

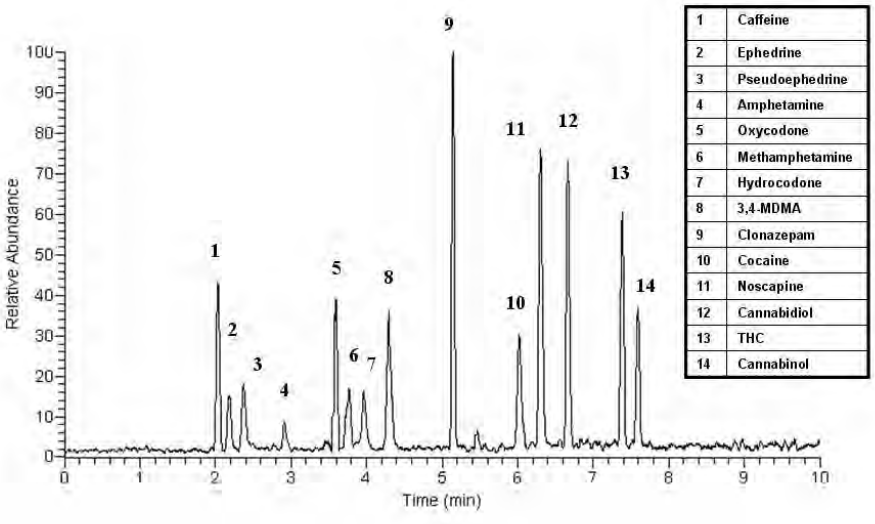


Figure1. UHPLC/MS chromatogram of 14 illicit drugs separated with ternary gradient.

Keywords: **Cannabinoids, Psilocin, Lysergic Acid Diethylamide, UHPLC/MS**