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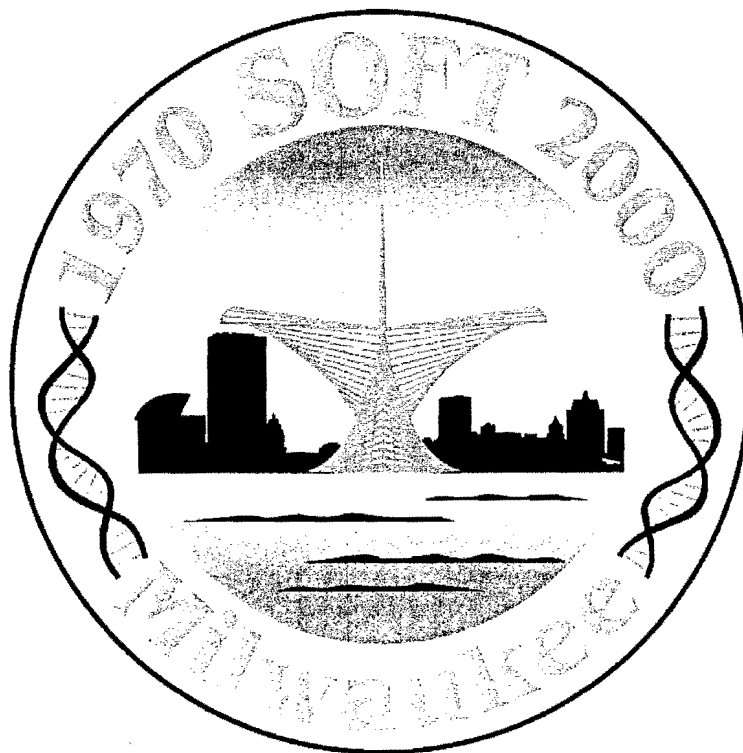
2000

Society of Forensic Toxicologists

## Program and Abstracts

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*Launching thirty years of forensic toxicology  
into the next millennium*



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# Scientific Abstracts

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## Genotyping CYP2D6 by Conventional and Real Time PCR

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Cytochrome P450 (CYP) mixed-function mono-oxygenases consist of more than 30 different forms of enzymes, intimately involved in the metabolism of a large variety of drugs. Of interest to forensic toxicology are the antidepressants, antipsychotics and selected analgesic opioids such as methadone which is increasingly used as an effective analgesic for pain management. With the advent of genomics, there has been rapid development in understanding the role of genetic polymorphism in drug metabolism - as a basis for rational drug therapy, and for explanation of drug toxicity and interaction. The CYP450 2D6 family of enzymes metabolizes some drugs in the groups listed above. This preliminary study demonstrated genotyping by a conventional PCR for CYP450 2D6\*3 and 4, and by a real-time PCR protocol for CYP 2D6\*4 using fluorescent hybridization probes. The conventional PCR protocol involved DNA extraction from peripheral blood using PureGene DNA Isolation kit. Then, specific sequences of CYP450 2D6 including the \*3 and \*4 mutations were amplified by PCR, followed by digestion with restriction endonucleases Msp1 and Mva1, respectively. Electrophoresis of the resulting fragments for each mutation was visualized by ethidium bromide staining. Poor metabolizers (PM) homozygous for the \*4 mutation were identified by a single 355 bp band, and PM homozygous for the \*3 mutation showed 168, 82 and 20 bp bands. Using these protocols with samples taken from 22 individuals, the prevalence of PMs exhibiting the \*3 and \*4 mutations were 0 and 3, respectively. This is similar to the prevalence in the general population. A preliminary feasibility study using fluorescent hybridization probes and a Lightcycler™ for real-time PCR, showed that a one step assay for the \*4 mutation could be achieved in about 45 minutes. LCRed-640 labeled probe formed an A:C mismatch with the mutant-type allele. The single base mismatch between the probe and mutant allele resulted in a lower melting temperature ( $T_m=52.2^{\circ}\text{C}$ ) than with the wild type ( $T_m=64.2^{\circ}\text{C}$ ). The heterozygote exhibited the characteristic  $T_m$  of both the homozygote and the wild type. Real-time PCR results correlated well with conventional PCR results. These methods provide rapid and reliable means of genotyping CYP450 2D6, which may be useful for understanding clinical efficacy of drug therapy.

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Keywords: Genotyping CYP450 2D6, antidepressants/antipsychotics/methadone, Conventional PCR and Lightcycler™ (real-time PCR)

## Detection of 7-Aminoflunitrazepam and Flunitrazepam in Hair After a Single Dose of Rohypnol<sup>®</sup>

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The objective of this paper was to determine whether the hypnotic benzodiazepaine flunitrazepam (FN) and its major metabolite 7-aminoflunitrazepam (7AFN) could be detected in hair collected from 10 healthy volunteers after receiving a single 2 mg dose of Rohypnol<sup>®</sup> (FN). Such data would be of great importance to law enforcement agencies trying to determine the best time interval for hair collection from a victim of drug-facilitated sexual assault in order to reveal drug use. The aim of this study was to develop and validate highly sensitive NCI-GC-MS method for the simultaneous quantitation of FN and its major metabolite 7AFN in hair and to analyze hair samples collected from volunteers who received a single dose of FN. Ten healthy volunteers (8 women and 2 men, 21-49 years old) participated in the study. The following hair samples were collected from each volunteer: one before FN administration, and 1, 3, 5, 14, 21 and 28 days after. All hair samples were pulverized and 50 mg aliquots were sonicated in methanol and digested with 0.1 N HCl at 55<sup>o</sup> C for 18-24 hours. Both extracts were combined and extracted using HClX solid phase extraction columns. Standard curves for FN (2.5-15 pg/mg) and 7AFN(0.5-20 pg/mg) were prepared by spiking aliquots (50 mg) of negative hair. Internal standards, d<sub>5</sub> diazepam (600 pg/mg) was used. Dried extracts were derivatized using HFBA (50 μl). A Hewlett-Packard GC-MS system (6890 GC and 5973 MSD) operating in negative ion chemical ionization mode was used for the analysis. The monitored ions for 7AFN derivative were m/z 459 and 441, for FN m/z 313 and 297, and for d<sub>5</sub> diazepam m/z 289. All method validation parameters were within acceptable limits. In five volunteers 7AFN was detected 24 hours after FN administration and remained in hair throughout the entire 28-day study period (0.6-8.0 pg/mg). In two cases 7AFN appeared in hair 21 days after FN intake (0.5-2.7 pg/mg), and in two subjects 14 days later (0.5-5.4 pg/mg). In one volunteer 7AFN was detected on day 14 and 21 but concentrations were below quantitation limit (BQL). FN was detected in only a few samples. All concentrations were BQL (0.5-2.3 pg/mg).

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Keywords: Date-Rape Drugs, Flunitrazepam, Solid Phase Extraction, NCI-GC-MS

## Irreversible Association of (3H) Nicotine, (3H) Flunitrazepam and (3H) Cocaine with Melanin During Generation from Tyrosine: A Model of Drug Deposition in Pigmented Hair

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To further investigate the chemical nature of drug deposition in hair, we examined the tyrosinase-mediated formation of melanin from tyrosine *in vitro* in the presence of three radiolabeled drugs: [3H]nicotine (3H-NIC), [3H]flunitrazepam (3H-FLU) and [3H]cocaine (3H-COC). Polymerization of tyrosine with tyrosinase leads to the formation of insoluble melanin polymers similar to the eumelanin pigments found in the keratinocytes of pigmented hair. Addition of 3H-COC to the polymerization incubation at 10 or 100 ng/ml resulted in virtually complete removal of the 3H-COC from solution and incorporation into the melanin pellet. Only 20% of the pellet-associated radioactivity could be released upon extended treatment with 6M HCl. Polymerization in the presence of higher concentrations of 3H-COC resulted in a smaller percentage of label being lost to the melanin precipitate (78% at 10  $\mu$ g/ml; 66% at 100  $\mu$ g/ml). Polymerization in the presence of 10 or 100 ng/ml of 3H-FLU resulted in the removal of apx. 95% of the radioactivity from solution and incorporation into the melanin pellet. Only 28% of this radioactivity could be released upon treatment of the pellet with 6M HCl. Larger concentrations of 3H-FLU resulted in less radioactivity being lost to the pellet (80% at 10  $\mu$ g/ml; 46% at 100  $\mu$ g/ml). Polymerization in the presence of 3H-NIC (10-100 ng/ml) resulted in 90-95% of the radioactivity lost from solution to the melanin pellet. Larger concentrations on 3H-NIC resulted in less radioactivity lost to the pellet (80% at 10  $\mu$ g/ml; 58% at 100  $\mu$ g/ml). The amount of 3H-NIC-associated radioactivity which could be released from the pellet by 6M HCl was apx. 12% for all concentrations of 3H-NIC. Nicotine-associated radioactivity in the melanin pellet was subject to much greater quenching than was 3H-COC or 3H-FLU, suggesting a much tighter association with the melanin. The results suggest an irreversible, perhaps chemical, association of these drugs with melanin pigments derived from L-tyrosine, consistent with our recent histological demonstration of the selective deposition of these drugs with melanosomes in pigmented hair. Supported by NIH grant DA09545.

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Keywords: Hair, Cocaine, Flunitrazepam

## Driving Under the Influence of GHB

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Recreationally, GHB and its precursor drugs gamma-butyrolactone and 1,4-butanediol, are abused by body builders as an alternative to anabolic steroids to enhance muscle growth, and by others for its euphoric, sedating and intoxicating effects. In Washington State, there have been a number of subjects taking GHB being arrested for impaired driving. Their corresponding GHB concentrations, concurrent drug use, clinical symptoms, circumstances and driving behaviour are discussed.

GHB was analysed by GC-MS, following extraction from blood using ethyl acetate and subsequent derivatization using BSTFA/TMCS. Blood specimens additionally underwent alcohol analysis, an EMIT screen for drugs of abuse and several prescription drug classes, and a GCMS screen for acidic, neutral and basic compounds.

GHB was identified in the blood of eight subjects arrested for impaired driving. GHB concentrations ranged from 26 to 127 mg/L, (mean 72 mg/L; median 81 mg/L). In six subjects, GHB was the only drug detected and signs of impairment were consistent with those of a central nervous system (CNS) depressant, including erratic driving (weaving, swerving, ignoring road signs), confusion, incoherent speech, unresponsiveness, lack of balance, unsteady co-ordination, horizontal gaze nystagmus, poor performances on field sobriety tests, and varying states of wakefulness. The symptoms, adverse reactions and behaviour of the subjects are consistent with observations made in previous reports.

Other drugs detected were carboxy-tetrahydrocannabinol and ethanol (0.06 g/100mL) in one subject, and carboxy-tetrahydrocannabinol and dextromethorphan in another driver. Symptoms and behaviour observed in these two drivers were very similar to those observed in the GHB only drivers.

Given the ability of GHB to induce sleep and unconsciousness, it is evident from these cases that recreational use of the drug is clearly capable of causing impairment in driving skills. GHB should be considered and tested for when drivers exhibit symptoms of CNS depression not accounted for by alcohol and other drugs.

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Keywords: GHB, driving impairment, CNS depression

### Intra- and Interindividual Variations in Urinary Levels of Endogenous GHB

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In recent years, there has been an increase in the recreational abuse of GHB and related products. Further, GHB's natural presence in the body, powerful sedative property, short half-life, and strong amnesiac effect has made it a favored drug in drug-facilitated sexual assaults. These cases often require interpretation of GHB levels to differentiate between endogenous and exogenous concentrations of GHB in biological specimens. This study was designed to determine variations in the urinary levels of endogenous GHB over a one-week period. Specifically, to ascertain whether endogenous concentrations fluctuate to such an extent that they may be misinterpreted as due to GHB ingestion.

The NIDA Institutional Review Board approved the protocol, and subjects provided informed consent and resided on the closed research ward under continuous medical surveillance. Every urine void produced by three GHB-free male subjects over a one-week period was individually collected and analyzed for the presence of endogenous GHB. Quantitative analysis was carried out using a previously published headspace GC/MS analytical procedure. The results are summarized in the table below:

	<b>n</b>	<b>Mean ± Std Dev:</b>	<b>CV:</b>	<b>Range:</b>
Subject #1:	42	0.63 ± 0.34 µg/mL	53.9%	< 0.19 - 1.65 µg/mL
Subject #2:	52	3.02 ± 1.52 µg/mL	50.3%	0.62 - 6.62 µg/mL
Subject #3:	35	0.56 ± 0.31 µg/mL	55.4%	< 0.19 - 1.94 µg/mL
	<b>129</b>	<b>1.58 ± 1.55</b> <b>µg/mL</b>	<b>98.1%</b>	<b>&lt; 0.19 - 6.62</b> <b>µg/mL</b>

The results of our study indicate that while there are significant intra- and interindividual variations in the urinary levels of endogenous GHB, the concentrations do not fluctuate to levels that should be misinterpreted as an indication of GHB ingestion (>10 µg/mL).

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Keywords: GHB, endogenous, drug-facilitated sexual assault

## Simultaneous GC/MS Analysis of GHB and Other Low Boiling Acidic Drugs Using a Water Scavenger Technique

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A procedure is presented for the analysis of gamma-hydroxybutyrate (GHB) and other low boiling drugs, such as acetaminophen, salicylates, ibuprofen and valproic acid, which is based on a water scavenger technique previously reported for the analysis of ethylene glycol. This procedure employed the use of 2,2-dimethylpropane (DMP), which reacts with water to produce volatile methanol. Also, dimethylformamide had been employed as a means to "trap" ethylene glycol before evaporating to dryness, a step we found not to be necessary.

During the course of development of adapting this procedure for use in our laboratory, we also found it to be also generally applicable for the analysis of GHB and other low molecular weight acidic drugs with only minor modifications. For the analysis of GHB and other analytes including ethylene glycol, we employed the use of a single internal standard: 2-hydroxy-3-methylbutyric acid.

For the analysis of GHB, the procedure is to combine a mixture of 80 mL of water and 100 mL of acetic acid/acetonitrile (1:10) containing 240 mg/L of internal standard with 100 mL of blood or urine. Acetonitrile (200 mL) is added to each tube, and then vortexed and centrifuged. The supernatant (150 mL) is combined with 500 mL of DMP/DMF (80:20) reagent and centrifuged. The clear supernatant is decanted to a clean test tube and evaporated to dryness using a Turbo-Vap. Ethyl acetate (50 mL) and 50 mL of MBSTFA are added and then transferred to an autosampling vial. We employed a rather novel heating technique of placing the sealed vial in a 70°C water bath for approximately 1 hour followed by direct analysis using GC/MS.

We have found this procedure to produce remarkably clean extracts for GHB. We have successfully applied this technique for the analysis of GHB and ethylene glycol in postmortem and driving-under-the-influence cases. The results of several of these cases will be presented.

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Keywords: GHB, Ethylene Glycol, Water Scavenger



## Analysis of Gamma-hydroxybutyrate (GHB) in Whole Blood and Urine by GC/MS

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In recent years, GHB and related precursors such as gamma butyrolactone and 1,4-butanediol have become widely abused for their euphoric and sedative properties as well as their purported body-building effects. A sensitive and selective assay for GHB without conversion to GBL was developed for small volumes of whole blood and urine. This method has also been applied to other biological specimens such as vitreous humor and bile.

100  $\mu$ l aliquots of whole blood were spiked with  $d_6$ -GHB as an internal standard and extracted with 99:1 methanol:ammonium hydroxide. After complete evaporation, samples were then reconstituted in DMF and extracted with DMF-saturated hexane. The DMF layer was collected and evaporated. Samples were then reconstituted with acetonitrile and derivitized with BSTFA containing 1% TMCS at 70<sup>0</sup> C for 30 minutes. One  $\mu$ l of the derivitized sample was analyzed by GC/MS (Fisons MD800). Each sample was injected twice. The first injection was analyzed in the SIM mode for quantitation, and the second injection analyzed in the full scan mode for spectral confirmation. The following ions were monitored: GHB (m/z 233, 234, 235) and  $d_6$ -GHB (m/z 239, 240, 241). Calibration was linear over the range of 1-500  $\mu$ g/ml.

A similar method was also developed for urine specimens. The urine method simply adds a solid phase extraction step (United Chemical Technologies CLEAN SCREEN<sup>®</sup> ZSGHB020 columns) prior to the previously mentioned liquid-liquid extractions to filter excess urea. Remaining steps are identical. Calibration was linear over the range of 1-1000  $\mu$ g/ml.

In addition, we present data on forensic cases including 11 cases of driving under the influence of GHB (ranging from 81-360  $\mu$ g/ml in the blood and 780-2380  $\mu$ g/ml in the urine), one fatal overdose (211  $\mu$ g/ml GHB and 0.12 gm/dl ethanol in vitreous humor) and one non-fatal overdose (13  $\mu$ g/ml and 272  $\mu$ g/ml GHB in blood and urine, respectively, 9 hrs after ingestion of 1,4-butanediol).

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Keywords: Gamma-hydroxybutyrate, GHB, GC/MS

## **Data Management in Forensic Toxicology: An Inexpensive Solution with MS Access©**

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The database to be presented offers a means of tracking case demographics, evidence receipt, storage and disposal, results and casework activities in MS Access©. This database offers a means of reporting findings and retrieving results in a searchable dialog format that can be operated from a single desktop or laptop computer or a networked server. This database does not link to nor does it import raw data from instruments. Rather, it is a low-cost manual data entry alternative to a full LIMS. Features available include:

- Case demographics including case type, subject/suspect age, cause of death if applicable and dates received and reported
  - Submitting individual and agency address and case identification
  - Evidence inventory including specimens type(s), containers, volumes, dates received and disposed, in-house storage location and automated disposal lists
  - Results of individual analyses for individual specimens including analytes, quantifications, methods and footnotes
  - Casework activities expended in completing individual cases
  - Technical and administrative review documentation
  - Report generation
  - Periodic caseload and activity reports
  - Searchable single and multiple drug queries
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Keywords: Database, Results management, Evidence management

## Preliminary Findings on the Ingestion of Hemp Seed Oil and Urine Cannabinoid Immunoassay Screening Results

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The increasing availability of  $\Delta^9$ -Tetrahydrocannabinol (THC) food products, such as hemp seed oil, and the possibility of a positive urinalysis for cannabinoids by standard workplace urine drug testing have necessitated the need for a controlled study of hemp oil ingestion. The NIDA Institutional Review Board approved this study and each subject gave informed consent. During the study, all subjects resided on the NIDA IRP research ward, under continuous medical surveillance. The study was designed as a randomized, double blind, double dummy, placebo-controlled within-subject protocol. The participants were dosed three times a day for five consecutive days followed by a ten-day washout period before the next dosing session began. Three (N=3) healthy volunteers with a history of marijuana abuse ingested commercially available hemp seed oils of differing THC concentrations: 0, 9, 92, 347  $\mu\text{g/g}$ , in liquid or capsule form, for total THC dose per day of: 0, 0.385, 0.472, and 14.8 mg; 7.5 mg of Dronabinol was used as a positive control. Dronabinol, Marinol<sup>®</sup>, is a synthetic THC therapeutic drug and was administered in a 2.5 mg capsule. All urine specimens were collected from the participants and analyzed by the Syva EMIT II<sup>®</sup> Cannabinoid Immunoassay at cutoffs of T-20, T50, and T-100.

A total of 1434 urine specimens were screened, this does not include urine specimens collected during the initial wash out period. The following data refers only to results from urine specimens screened at the T-50 cutoff concentration. No samples screened positive after the 0.385 mg/day dose. Only 3 samples assayed positive out of 311(1.0%) after receiving the 0.472 mg/day dose. The first positive urine for the .472 mg/day dose occurred at 100 hours after the first dose and no positive urine specimens occurred after the final dose. At the 7.5 mg/day dose, 126 of 236(53.4%) specimens assayed positive and 119 of 268(44.4%) samples were positive for the 14.8 mg/day dose. The first positives for the 7.5 and 14.8 mg/day doses were at 18 and 11 hours, respectively, and 19 and 25 hours after the last dose, respectively.

These data demonstrate a consistent relationship between THC dose and positive urinalysis results. The study also shows that ingestion of hemp oil containing 347  $\mu\text{g/g}$  THC, when consumed per manufacturer's direction of three doses per day, may produce positive urine tests for up to 68 hours using the Syva EMIT II<sup>®</sup> Cannabinoid Immunoassay at a T-50 concentration cutoff.

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Keywords: Marijuana, Hemp Oil, Urine

**Excretion of  $\Delta^9$ -Tetrahydrocannabinol (THC), 11-hydroxy- $\Delta^9$ -THC (11-OH-THC) and 11-nor-9-carboxy- $\Delta^9$ -THC (THCCOOH) in Human Urine Following Administration of Smoked Marijuana**

*Abraham T. Wtsadik, William D. Darwin, Eric T. Moolchan, Jonathan M. Oyler and Marilyn A. Huestis. Chemistry and Drug Metabolism Section, IRP, NIDA, NIH, Baltimore, MD*

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There are limited quantitative data available on the timecourse of excretion of inhaled cannabinoids into urine. This study examined the timecourse of THC, 11-OH-THC, and THCCOOH in urine for 48 h following smoked marijuana. Subjects (N=5) were healthy volunteers with a history of marijuana use. The NIDA Institutional Review Board approved the protocol and all subjects provided informed consent. Subjects resided on the closed NIDA IRP research ward under continuous medical surveillance. Subjects smoked two marijuana cigarettes (2.64% THC) separated by 4 hrs according to a paced dosing design. Subjects inhaled 8 puffs per cigarette with ad libitum puff duration and 60 second inter-puff interval. All urine specimens were collected throughout the study and frozen at -20°C until analysis. Specimen preparation included enzymatic followed by base hydrolysis, solid phase extraction (CleanThru DAU, UCT), derivatization with BSTFA (1% TMCS) and GC/MS analysis. The mean time (h) to peak concentration  $\pm$ SEM (range) was  $7.4 \pm 5.6$  (1.3-24.3),  $9.0 \pm 4.7$  (1.3-24.3), and  $14.6 \pm 4.5$  (1.3-27.1), respectively for THC, 11-OH-THC, and THCCOOH. Mean peak concentration, ng/mL  $\pm$  SEM (range), was  $9.1 \pm 4.8$  (1.2-21.9),  $87.5 \pm 47.3$  (5.3-206.7), and  $133.7 \pm 68.2$  (12.5-392.8), respectively for THC, 11-OH-THC, and THCCOOH. Mean  $\pm$  SEM (range) detection times were  $14.9 \pm 6.4$ ,  $32.9 \pm 4.7$ , and  $34.3 \pm 4.5$ , respectively for THC, 11-OH-THC, and THCCOOH. These cannabinoid urine excretion data following controlled clinical drug administration may be useful in the interpretation of urine cannabinoid results.

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Keywords: THC and metabolites, Smoked marijuana, GC/MS.

## Detection of Cocaine/Cocaine Metabolite in Vitreous Humor by Cloned Enzyme Donor Immunoassay

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The usual specimens submitted by the medical examiner for toxicological analysis include blood, urine, bile, vitreous humor, and solid-organ tissue. The detection of drugs in these specimens typically involves a combination of techniques including colorimetric, immunoassay, and gas chromatographic. Although many laboratories rely on urine for the detection of drugs of abuse by immunoassay, these assays may be applied to other specimen types. An evaluation of Microgenics Corporation's cloned enzyme donor immunoassay (CEDIA<sup>®</sup>) was conducted in order to evaluate its use in the detection of cocaine/cocaine metabolites in vitreous humor specimens.

During a 14-month period (2200 total cases), 392 vitreous humor specimens were analyzed by the CEDIA Cocaine Assay (Microgenics Corporation). Instrument parameters were set according to published manufacturer's guidelines. All positive vitreous humor immunoassay screen results prompted confirmatory testing by gas chromatography/mass spectrometry (GC/MS) of corresponding specimens including blood. The CEDIA assay produced 23 positive screen results, 22 of which were confirmed by GC/MS. The only case which could not be confirmed, elicited a screen value near the cutoff limit. Routine analysis by gas chromatographic-nitrogen phosphorous detection revealed the presence of cocaine/cocaine metabolites in only 7 of the 22 confirmed cases. The concentration range of cocaine and benzoylecgonine in the blood specimens was none detected to 337 ng/mL and 17 to 8598 ng/mL, respectively. Cocaethylene was not detected in any of the cases.

Analysis of vitreous humor specimens by CEDIA improved the detection rate of cocaine/cocaine metabolites by 0.7% in the cases submitted to our laboratory during the 14-month period.

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Keywords: Cocaine, Vitreous Humor, Immunoassay Analysis.

### Cocaine and Metabolite Concentrations after Swallowing Illicit Cocaine

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Vendors of crack cocaine, some of whom store the drug in their mouths, will swallow it if approached by the authorities. Depending on how well secured the packages are, if they are packaged at all, the drug can dissolve in the GI tract, introducing a large bolus dose of cocaine. Depending on whether they are packaged, this can occur several hours to days after the ingestion, during which time the subject will often be in police custody. We consider the clinical presentation, and cocaine and metabolite concentrations in a series of 25 cases who ingested unpackaged crack cocaine to avoid arrest.

Analysis was performed by gas chromatography/mass spectrometry, using an extractive alkylation method. The mean (SD) concentrations (mg/L) were; cocaine 0.6 (0.09), benzoylecgonine 1.6 (0.27), ecgonine methyl ester 0.38 (0.07), ecgonine 0.4 (0.1), norcocaine 0.03 (0.04), and cocaethylene 0.02 (0.01, n=5). Common presenting symptoms in the 25 patients included tachycardia, hypertension, tremors, agitation, disorientation, and diaphoresis. In some cases patients complained of chest pain and one patient seized. One patient was admitted to the hospital, and none had a poor outcome or died.

We compare the findings and outcome of these cases with a description of the circumstances of two in-custody death cases. The 2 death cases had cocaine and metabolite concentrations as follows; cocaine 0.08, and <0.05mg/L, benzoylecgonine 0.63 and 0.74mg/L, and ecgonine methyl ester, 0.35 and 0.75mg/L. GCMS analysis was performed using a conventional extraction, and formation of TMS derivatives, and ecgonine and norcocaine concentrations were not available. Ethanol and cocaethylene were negative in both cases. The circumstances strongly suggest cocaine excited delirium in both cases, although both were also complicated with police restraint issues, and significant underlying cardiac pathology. Deaths and toxidromes from this pattern of ingestion appear quite common, and may be characterized by delayed onset. Particular consideration is given to the significance of ecgonine methyl ester concentrations which often appear elevated following this route of administration.

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Keywords: cocaine, oral ingestion, post mortem, in custody-deaths

**Postmortem Examination of Liver and Brain for the Presence of Methylecgonidine (MED), Ecgonidine (ED), Cocaine, and Benzoylecgonine (BZE) by Selected Ion Monitoring (SIM) GC/MS**

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The presence of MED, a pyrolysis product of cocaine, has been suggested as an indicator of cocaine smoking. Detection of MED and ED (metabolic product of MED) in tissue could assist medical examiners in developing a more complete picture of cases involving cocaine. This method describes the extraction and GC/MS detection of MED, ED, cocaine and BZE in postmortem liver and brain tissue. Together with NaF, a 1g portion of liver or tissue was homogenized in phosphate buffer, pH 6.0, with deuterated-MED, ED, cocaine, and BZE as internal standards. After centrifugation, the supernatant was passed through mixed mode (C8/benzene sulfonic acid) SPE columns, where MED, cocaine, eluent (containing ED) was adjusted to pH 2.0 and extracted on a second set of SPE columns. The GC/MS analysis was performed on underivatized MED and cocaine. The tert-butylmethylsilyl derivative of ED was prepared prior to GC/MS analysis and BZE was derivatized on-column as the propyl derivative using DMF-DPA.

Liver and brain specimens were tested from postmortem cases screened positive for cocaine and/or BZE. Detectable levels (12-45ng/g) of MED were observed in 4 out of 15 liver specimens, while ED was observed in all specimens, ranging from 90-3274 ng/g liver. Levels of MED in brain (4 out of 6) ranged from 19-115 ng/g brain, while ED was detected in 4 out of 6 brain specimens, ranging from 35-69 ng/g brain. Cocaine and BZE levels in liver ranged from 18-1028 ng/g and 45-4217 ng/g, respectively, and levels in brain ranged from 11-1346 ng/g and 293-2952 ng/g, respectively.

In liver specimens, ED may be a more useful indicator of cocaine smoking than MED, since ED was detected in all specimens at a wide range of concentrations. Since higher levels of MED were found in 4 out of 6 brain specimens, examination of brain specimens for MED directly or indirectly through ED may both be options in brain specimens.

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Keywords: Methylecgonidine, ecgonidine, cocaine smoking

**Cardiomyopathy in Young Cocaine Abusing Patients Presenting to an Inner City  
Emergency Department**

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Cardiomyopathy and congestive heart failure are becoming more prevalent in young cocaine abusing patients. We report the clinical findings and outcome in 33 patients with congestive heart failure associated with cocaine use who presented to the emergency department. Cocaine use was confirmed by either history and/or urine drug screen and/or blood cocaine metabolites. The most common route of administration was crack smoking (N=XX). All patients had a history of cocaine use for a mean of 4 years. The age ranged from 32-56 years, the mean was 47 ±7 years. The majority (N=23) were males and 10 were females. All patients except 1 presented to the ED for treatment of symptoms associated with heart failure. Nine were admitted, 24 were treated and discharged. Blood cocaine concentrations were measured by an extractive alkylation/mass spectrometry procedure in 4 patients. The mean cocaine concentration was 0.12 mg/L (0-0.387 mg/L). The results of ECHO cardiograms were available from 10 patients. Common findings included depressed systolic function with ejection fractions ranging from 15-45%, increased heard size, global hypokinesis and valvular regurgitation. We believe cocaine use is a significant cause of heart failure in relatively young patients from large urban centers.

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Keywords: Congestive heart failure, cocaine, drug abuse



**Changing Demographics of Cocaine Abusing Patients Presenting to an Inner City  
Emergency Department**

Kari Blaho\* and Stephen Winbery. Department of Emergency Medicine, UTMG, Memphis, TN  
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We report an increase in the number of patients over the age of 40 years presenting to the emergency department with recent cocaine use. We have previously reported the mean age of the cocaine abuser presenting to our ED to be  $30 \pm 10.6$  years. However, in the past 12 months the mean age has increased to  $39 \pm 8$  years (N=786) while the number of cocaine abusers presenting to the ED has remained approximately the same. All patients, either self reporting cocaine use or who had positive urine drug screens were included in this study. Demographics and outcome were documented. The oldest patient with a positive cocaine drug screen was 63 years. Crack smoking continues to be the most common route of self-administration (48%). Seventy eight percent of patients co-abused other drugs, the most common were tobacco and ethanol. The incidence of co-morbid disease or severe outcomes increases with age. In patients less than 30 years, the incidence of sequelae is 4.4%, 49% of those 30-39 years of age had adverse outcomes, 48% of those 40-49 years and 97% of those over the age of 49 had adverse outcomes. Patients over the age of 30 had a higher incidence of heart failure, myocardial infarction, exacerbation of chronic disease, strokes and renal failure than those less than 30 years of age. Case examples will be presented.

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Keywords: Cocaine abuse, drug abuse, adverse drug events

**Comparative Analysis of Sweat Patches for Cocaine and Metabolites by Gas Chromatography/Mass Spectrometry and Radioimmunoassay**

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Sweat patches have been advocated for monitoring illicit drug use during therapy for drug dependence. A gas chromatography / mass spectrometric (GC/MS) method for determination of cocaine, ecgonine methyl ester (EME) and benzoylecgonine (BE) and a radioimmunoassay (RIA) method for determination of cocaine immunoequivalents in extracts of sweat patches in extracts of sweat patches have been validated. Acceptable precision and accuracy of controls were found during validation. Both methods were used to analyze 879 patches worn by subjects in treatment for cocaine dependence. Cutoffs were evaluated at 4, 5 and 10 ng cocaine/patch. Six hundred and sixty one, 632 and 584 patches were GC/MS positive at cocaine cutoffs of 4, 5 and 10 ng/patch, respectively. In positive patches the maximum concentrations of cocaine, EME and BE were 31900, 2280 and 3460 ng/patch, respectively. When the RIA was used solely to determine if cocaine was present at or above the cutoff (ie., qualitative), good agreement was found between the two methods. Using receiver-operating characteristic curve analysis, an optimal cutoff of 5 ng/patch was found for both methods where the RIA had a sensitivity (determination of positives) of 92.1% and a specificity (determination of negatives) of 88.7%. The RIA was not consistently acceptable for quantitative analysis. For the runs where quantitation of controls were acceptable, however, a good correlation ( $r = 0.989$ ,  $n = 139$ ) was found between GC/MS and RIA results. RIA was useful for screening of sweat patch extracts and determining if dilution would be required during GC/MS quantitation. (Supported by NIDA Contract NO1DA-7-8074).

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Keywords: sweat patch, cocaine, GC/MS50

**Effects of Low Dose GHB and EtOH, Administered Alone and in Combination, on a Memory Task in Rats: Comparison with Scopolamine (SCOP)**

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The present studies determined whether GHB or a combination of GHB and EtOH can produce memory impairment in a rat model for anterograde amnesia; SCOP was used as a reference. Male Sprague Dawley rats were used. Memory training and testing was conducted over a 5-day period. On Day 1 the water was removed. On Day 2 (24 hr water deprived) the rats were placed in a standard rodent testing chamber with a water tube recessed into one wall. All animals found the drinking tube and were allowed to drink freely for 200 licks. On Day 3 (48 hr water deprived) the rats received drug treatments and passive avoidance training. The drug treatments were administered intraperitoneally (15-20 min pretreatment) and were as follows: No treatment, vehicle (DW), 1.0 mg/kg SCOP, 2.0mg/kg SCOP, 100 mg/kg GHB, 500 mg/kg EtOH, and a combination of 100 mg/kg GHB and 500 mg/kg EtOH. For passive avoidance (memory) training the rat was allowed 50 free licks of water, but on the 51st lick and all subsequent licks a shocker (200 uAmp) was activated. After passive avoidance training the rats were given water overnight. On Day 4 the water was removed. On Day 5 (memory testing day) the rats were returned to the chamber to see if they would return quickly to the water tube or if they would "remember" the shock and avoid drinking. Thus, the measure of memory in this paradigm was the ability to refrain from drinking on Day 5. "Memory" was significantly impaired by both doses of SCOP. In contrast, GHB and EtOH, administered alone or in combination, did not produce memory impairment. This is somewhat surprising because EtOH and, in anecdotal reports, GHB produce memory impairment. The EtOH and GHB doses in the present study were relatively low. Unfortunately, higher doses (eg., 200 mg/kg GHB) produced profound sedation such that the rats could not be trained. The memory disrupting effects of GHB reported in humans are more frequently associated with higher GHB levels. A modification of the passive avoidance task will be necessary to allow for testing higher doses of GHB.

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Keywords: GHB, Ethanol, Memory Impairment

### Free and Total Morphine Concentrations in Postmortem Blood

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Interpretation of postmortem blood morphine concentrations can be difficult as morphine concentrations in heroin related deaths overlapped with those found in non-drug related deaths. The free to total morphine ratio may provide information regarding which opiate was ingested and the time interval between opiate exposure and death. This study was undertaken to determine the relationships between postmortem free and total morphine levels in morphine and heroin related deaths. Free and total morphine concentrations were measured by gas chromatography-mass spectrometry in 87 medical examiner cases obtained over 20 months. The means for total morphine concentration, free morphine concentration and percent free morphine for all cases were 2.3 mg/L (SD 5.2 mg/L), 0.5 mg/L (SD 1.6 mg/L), and 19.4% (SD 22.8%), respectively. Regression analyses showed weak correlation between total and free morphine over the total morphine range (0 to 36.6 mg/L,  $r=0.603$ ) and over subset range of 0 to 1.0 mg/L ( $r=0.369$ ). 23 out of 56 cases (41%) tested positive for 6-monoacetylmorphine (6-MAM). The results showed lower total and free morphine concentrations and higher percent free morphine in individuals with detectable 6-MAM. Comparing mean blood concentrations for cases with and without 6-MAM present demonstrated total morphine: 0.9 mg/L, 2.1 mg/L ( $p=NS$ ); free morphine: 0.3 mg/L, 0.4 mg/L ( $p=NS$ ); percent free morphine: 34.7%, 13.7% ( $p<0.003$ ). Our findings demonstrate higher free to total morphine ratios in individuals with detectable 6-MAM than individuals without 6-MAM.

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Keywords: free morphine, total morphine, 6-monoacetylmorphine

## Development of a Generic Method to the Solid Phase Extraction of Acidic Compounds from Complex Matrices

*Diane M. Boland\*<sup>1</sup>, Michael F. Burke<sup>1</sup>, Tracy Mitchell<sup>2</sup>, Paul Madley<sup>2</sup>, Claire Desbrow<sup>2</sup>, and Richard Calverley<sup>2</sup>. <sup>1</sup>University of Arizona, Department of Chemistry, Tucson, AZ; <sup>2</sup>International Sorbent Technology, Hengoed, UK*

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The importance of isolating and purifying acidic compounds from biological fluids is evident in many fields including pharmaceuticals, environmental science, and forensic chemistry. In most cases, the extraction of acidic analytes is effected by acidifying the sample and treating them as neutrals i.e. using a purely non-polar retention mechanism (e.g. C4, C8, C18). Often times this approach works, but in some cases the molecules are too polar, and hydrophobic interactions are not sufficient enough to retain the analyte. Another flaw with the hydrophobic methods is lack of selectivity, as the extracts may contain a large amount of non-polar co-extracted material, which can interfere with the subsequent analysis. Because the selectivity for ionizable analytes can be increased by ion-exchange sorbents, the factors that influence the retention and recovery of acidic analytes using pure anion exchange vs. mixed mode hydrophobic / anion exchange retention mechanisms will be addressed.

In order for successful extraction of acidic analytes, an understanding of the affinity that competitive anions have for an ion-exchange site is necessary. Strong anion exchange sorbents containing a quaternary amine group with a permanent positive charge must have a counter-ion present to balance the charge. Initial studies show that different counter-ions have different affinity for the positively charged group i.e. lower selectivity counter-ions (eg. propionate, acetate) are more easily displaced from the sorbent by the analyte than higher selectivity counter-ions (dihydrogen phosphate, bicarbonate).

In comparison to a pure anion exchange retention mechanism, mixed mode columns containing both hydrophobic and ion-exchange sorbents provided higher recoveries (>90%) by HPLC analysis. It is believed that retention on the ionic sites allows a rigorous interference elution regime, leading to very clean final extracts, as many non-polar interferences (retained by hydrophobic interaction alone) can be selectively eluted, prior to the drug of interest.

Data will be presented illustrating that in anion exchange solid phase extraction, correct choice of counter-ion can lead to dramatic improvements in recovery for certain analytes. Studies also show that the mixed-mode approach to extraction of acidic drugs leads to robust methodology, high recoveries, and clean extracts. Furthermore, optimized ion-exchange capacity in mixed-mode solid phase extraction allow rigorous interference elution without analyte loss.

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Keywords: Solid Phase Extraction, Ion-Exchange, Acidic Compounds

**Disposition of Methamphetamine and Its Metabolite, Amphetamine, In Urine  
Following Controlled Drug Administration**

*Jonathan M. Oyler, Raf J-F. Schepers, Eric T. Moolchan and Marilyn A. Huestis. Chemistry and Drug Metabolism, IRP, NIDA, NIH, Baltimore, MD*

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The stimulant methamphetamine (MAMP) has marked abuse liability, and its abuse is on the rise. This report describes the disposition of MAMP and its major metabolite, amphetamine (AMP), in urine following controlled drug administration. Subjects (n=3) were administered 4 daily low (10 mg) and high (20 mg) oral doses of (d)-methamphetamine HCl (Desoxyn Gradumet tablets). Urine specimens were collected ad libitum prior to and for 36 h following drug administration. Copolymer solid phase extraction (Clean-Thru<sup>®</sup> DAU columns) was employed to isolate analytes followed by GC-PCI-MS analysis employing a dual silyl derivatization method. LOD's and LOQ's were  $\leq 5.0$  ng/mL, and standard curves were linear from 5-1000 ng/mL. MAMP was initially detected in the first urine specimen collected (1-4 h) following drug administration. Peak concentrations occurred within 1.5-12 h (2<sup>nd</sup>-5<sup>th</sup> void) following drug administration and were  $>3000$  ng/mL following both low and high doses. AMP peak concentrations generally occurred in the void collected immediately following peak MAMP excretion, and concentrations ranged from 10-20% of those observed for MAMP. Accumulation of MAMP and AMP in urine following multiple oral administrations of MAMP was also observed. At 36 h following the fourth administration of both high and low doses, MAMP was still detectable (100-700 ng/mL), and the concentration ratio of AMP to MAMP had increased to 30-60%. This increase implies a longer half-life for AMP than its precursor MAMP and indicates that the rate-limiting step in the clearance of AMP is excretion rather than formation. Preliminary data indicates that urinalysis for MAMP and AMP provides a detection window of  $>36$  h following this multiple dosing regimen with relatively low doses.

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Keywords: Methamphetamine, Urinalysis, GC/MS

## Secretion of Methamphetamine and Amphetamine in Sweat Following Controlled Drug Administration

*Marilyn A. Huestis, Raf J-F Schepers, Robert E. Joseph, Jr., Diana M. Lafko, Ann E. Basham, Eric T. Moolchan and Jonathan M. Oyler. Chemistry and Drug Metabolism Section, IRP, NIDA, NIH, Baltimore, MD*

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Diverse biological matrices provide unique pharmacokinetic information and differing windows of drug detection; however, limited data are available to provide a scientific database for interpretation of drug testing results. The objective of this study is to characterize the disposition of methamphetamine (MAMP) & amphetamine (AMP) in sweat. Seven subjects received four daily oral 10 & 20 mg (d)-methamphetamine HCl formulated in Desoxyn Gradumet tablets. Subjects provided informed consent and resided on the IRP research ward for 10-weeks. Sweat was collected with the Pharmchek Sweat Patch (PSP) and the Torso (TFP) and Hand-held Fast (HFP) Patches. Fast Patches employ heat to increase sweat production and were applied for 30 min. PSP collected sweat over days to weeks. Samples were analyzed by GC-MS following sodium acetate buffer elution, solid phase extraction & derivatization by BSTFA (MAMP) & MTBSTFA (AMP). The LOQ for MAMP & AMP in sweat was 2.5 ng/patch. The time to peak concentration was determined only for the first administration of the low or high dose; C<sub>max</sub> is the maximum drug concentration observed within each dosing session. MAMP was first detected in sweat collected with PSP, TFP and HFP from 2 to 23 h, 2.5 to 4.5 h, and 2.5 to 4.5 h, respectively, following 10 mg and 2-4 h, 2.5-4.5 h, and 2.5 h, respectively, following 20 mg dose. Mean  $\pm$ SEM (range) C<sub>max</sub> MAMP during low dose (N=6) were 78.7 $\pm$ 25.2 (20.8-71.2), 36.2 $\pm$ 6.8 (5.2-97.7), and 49.7 $\pm$ 13.2 (16.8-175.3) ng/patch for PSP, TFP and HFP, respectively. After the high dose (N=3), mean C<sub>max</sub> MAMP were 315.4 $\pm$ 116.4 (36.7-606.5), 106.6 $\pm$ 15.3 (64.1-154.0), and 215.0 $\pm$ 32.9 (158.0-560.3) ng/patch for PSP, TFP and HFP. C<sub>max</sub> in PSP were worn for 6 to 8 days, while TFP and HFP sweat collections were made in 30 min. Sweat AMP generally followed a similar pattern of secretion as the parent drug, albeit at much lower concentrations. Our study demonstrates the usefulness of three different methods of sweat collection for monitoring methamphetamine abuse. Thus, as additional methods for identifying drug use are needed, PSP, TFP, and HFP may provide unique alternatives for identifying MAMP exposure in drug treatment, criminal justice, workplace and military drug testing programs.

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Keywords: Sweat, Methamphetamine, Sweat Patch

## Disposition of Methamphetamine and Amphetamine in Saliva and Plasma Following Controlled Oral Drug Administration to Human Volunteers

*Raf J-F. Scheper, Jonathan M. Oyler, Robine E. Evans, Robert E. Joseph, Jr., Eric T. Moolchan and Marilyn Huestis. CDM Section, IRP, NIDA, NIH, Baltimore, MD*

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Numerous articles report the disposition of cocaine, opiates, cannabinoids and other drugs of abuse in saliva. However, only limited data are available characterizing the salivary pharmacokinetics of methamphetamine (METH) and its primary metabolite, amphetamine (AMP), following controlled drug administration in human volunteers. This study is designed to elucidate the disposition of METH and AMP in plasma and saliva after oral METH administration. Volunteers received 4 daily oral 10 mg (n=7) followed 3 weeks later by 4 daily oral 20 mg (n=4) dextro-methamphetamine hydrochloride tablets (Desoxyn Gradumet). Saliva specimens were obtained by two different methods: by expectorating in polypropylene tubes – the production of saliva is stimulated by candy containing citric acid – or by placing a cotton swab in the subject's mouth and extracting the liquid by centrifugation. During the first session of each dosage regimen, blood samples were collected simultaneously with saliva specimens for up to 24 h. Specimens were frozen at -20°C until analysis by solid phase extraction and GC-MS. The limit of quantitation for both analytes was 2.5 ng/mL. Standard curves were linear across a concentration range of 2.5-50 ng/mL and 50-500 ng/mL with correlation coefficients  $\square$  0.993. METH was detected in saliva and plasma within 0.5-2 h after administration of the first dose of METH, and drug concentrations in both matrices peaked within 2-8 h. Both methods of saliva collection produced similar quantitative results. C<sub>max</sub> measures for METH following the first low dose ranged from 25.7-312.2 ng/mL in saliva and from 14.5-33.8 ng/mL in plasma. Four volunteers received high doses of METH; C<sub>max</sub> measures following the high dose were 58.4 to 315.2 ng/mL in saliva and 15.6 to 36.1 ng/mL in plasma. AMP was detected in saliva collected from 2-4 h after dosing and in plasma collected from 2-8 h. After the 4<sup>th</sup> 20-mg dose of METH, METH and AMP could still be detected in saliva 71.8 h after the drug administration. A saliva to plasma ratio for METH >1 was obtained immediately after the appearance of measurable drug concentrations in both matrices. However, this ratio can differ greatly in the same subject over time and between the different volunteers. Advantages of measurement of methamphetamine and amphetamine in saliva as compared to plasma include higher analyte concentrations, ease and non-invasiveness of specimen collection and similar or longer windows of drug detection. Thus, saliva testing may be a useful alternative to plasma testing for monitoring methamphetamine abuse.

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Keywords: Saliva, Methamphetamine, Plasma



## **Methamphetamine Movement into Memphis Tennessee: Experiences of an Inner City Emergency Department**

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Memphis, TN*

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In the past 9 months, the number of confirmed urine drug screens for amphetamine has increased. The most common drugs of abuse in inner city Memphis are cocaine and ethanol. However, stricter laws in neighboring states limiting certain chemicals identified as methamphetamine precursors has resulted in an increase in the number of chemical exposures during theft and the number of patients presenting acutely intoxicated with methamphetamine. Sixty-eight patients in the past 9 months have presented with clinical findings consistent with CNS stimulant intoxication including tachycardia, hypertension, agitation/combatative behavior, and dysphoria. The clinical findings were indistinguishable from cocaine intoxication. All but one patient was Caucasian, the mean age was 23 ±4 years. Fifty eight percent of patients co-abused other drugs including ethanol, tobacco, opioids and benzodiazepines. Ten patients presented with respiratory distress secondary to anhydrous ammonia exposure. No patient had required hospital admission, although 70% were treated with benzodiazepines. No patient died or had significant morbidity.

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Key words: Methamphetamine, anhydrous ammonia, CNS stimulant

**Simplified GCMS SIM Screen and Confirmation Analysis for  
Methylenedioxyamphetamine (MDA) and Methylenedioxymethamphetamine (MDMA) in  
Air Force Member Specimens**

*Vincent M Papa, James Kuhlman, Edward Hubster, James Swaby. Air Force Drug Testing Laboratory, Brooks AFB, TX*

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A simplified and economical method of testing for amphetamine analogues employing our GCMS amphetamine test procedure both as screen and confirmation for the identification and quantitation of MDA and MDMA was established. The method consists of a liquid/liquid back extraction, derivitization with heptafluorobutyric acid and GCMS Electron Impact (EI) analysis. The GCMS was an HP5973 with a DB-1, 12.5m column and the injector and interface temperature set at 270°. The temperature program was 100-260° with a 20:1 injection split ratio. Ions monitored were m/e 375, 162 and 135 for MDA; m/e 254, 162 and 210 for MDMA. The internal standard ions for d5 MDA were m/e 380 and 167, and 258 and 213 for d5 MDMA.

Retention time results were 5.52 and 6.13 minutes for each analyte. Linearities were examined using 0, 50, 100, 500, 1,000, 5,000, 8,000, 10,000 ng/ml concentration range for each analyte. Upper limit of detection was determined by analyzing ten replicate specimens of 10,000 ng/ml showing %CV of 4.32 and 4.69. LOD/LOQ was determined by analyzing n =10 replicates of 100 and 50 ng/ml of MDA, MDMA, resulting in SD of 5.28 and 1.28 with 4.85 and 3.87 %CV, respectively. Interference by ephedrine, phenylpropanolamine, pseudoephedrine, phenethylamine and phentermine was evaluated at 100,000, 500,000 and 1,000,000 ng/ml. Studies were conducted with the drug at cutoff and in the absence of the drug with or without periodate treatment. There was no interference with methamphetamine or designer analogues detected. Precision for analogues was determined by analyzing n=10 concentrations of 500 ng/ml for each analogue with 1.80 and 2.65 %CV.

The laboratory has reported 20 positive military member specimens during the first quarter of 2000 compared to 21 for all of 1999. We have successfully employed this method for the last three years and will continue to monitor positive rates in member specimens.

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**Keywords:** Methylenedioxyamphetamine, Methylenedioxymethamphetamine, GCMS

### Comparison of Six Immunoassays for the Detection of Amphetamines and Ecstasy

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The detection of ecstasy (XTC) by some immunoassays for (met) amphetamine is poor. We compared a new CEDIA immunoassay for XTC and a CEDIA cocktail assay for amphetamines and ecstasy (A+X) to fluorescence polarization immunoassay (FPIA), EMIT II monoclonal amphetamine/methamphetamine, CEDIA DAU amphetamines (CEDIA A) and KIMS (Roche Cobas Integra). The CEIDA and EMIT assays were performed on a Hitachi 917. Confirmation was performed by GC-MS, with solid phase extraction and HFBA derivatization, using deuterated internal standards. The samples included 200 patient samples and 24 quality assurance samples. Ninety-nine samples were positive: 50 for (met) amphetamine, 35 for MDMA, MDEA or MBDB and metabolites and 14 for both (met) amphetamine and MDMA. The sensitivity and specificity at a screening cut-off of 500 ng/mL, area under the ROC curve (for the detection of either amphetamine or XTC and for the detection of XTC, with the exclusion of amphetamine containing samples) of the assays is shown in the table below.

Assay	Amphetamine and/or XTC (n=224)			XTC (n=158)
	Sensitivity	Specificity	AUC ROC	AUC ROC
CEDIA A	78%	90%	0.946	0.956
CEDIA A+X	86%	92%	0.959	0.998
CEDIA XTC				1.000
EMIT	83%	94%	0.943	0.934
FPIA	95%	99%	0.996	0.995
KIMS	88%	86%	0.920	0.936

The new CEDIA XTC assay allows an excellent detection of XTC. The CEDIA A+X cocktail assay performs better than the traditional assay EMIT and KIMS, but has more false positives than FPIA.

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Keywords: Amphetamine, Ecstasy, Immunoassay

## Amphetamine – beta-Phenethylamine Analysis by Gas Chromatography/Time-of-flight Mass Spectrometry

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A problem frequently encountered by forensic toxicologists is the analysis of the sympathomimetic amine class of compounds in decomposed biological samples. This has traditionally been problematic due to the chemical similarities between the compounds within this class of chemicals and decomposition products. Drug screening of decomposed samples typically produces positive immunoassay “amphetamine” results with inconclusive GC/MS confirmation. Further complicating this problem is the chromatographic coelution of the decomposition product and the controlled substance (Figure 1), with the decomposition product being present typically at vastly greater concentrations than the drug of interest.

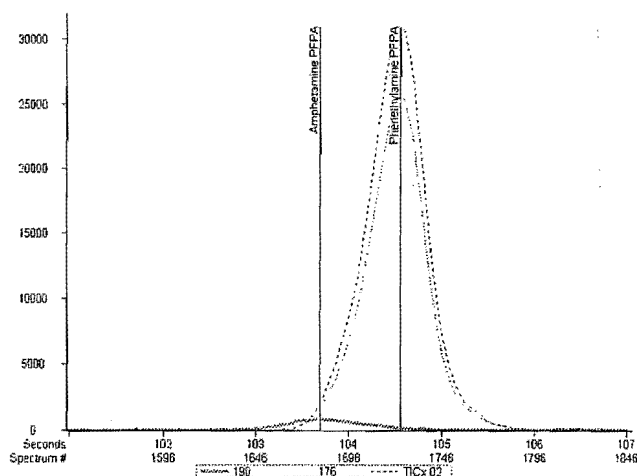


Figure 1: Total Ion Chromatogram and Unique Ion Profiles of PFPA derivatized Amphetamine (m/z 190) and Phenethylamine (m/z 176).

Data will be presented discussing the analytical approach used to solve this problem by Gas Chromatography/Time-of-Flight (TOF) mass spectrometry coupled with mass spectral Deconvolution (Figures 2 and 3).

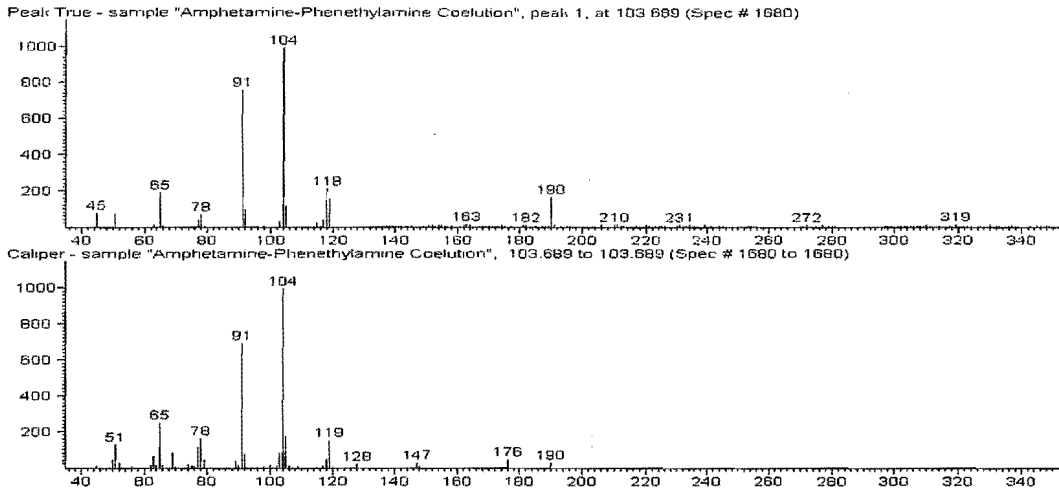


Figure 2: Amphetamine PFPA deconvoluted spectra (top). Manual spectra display (bottom).

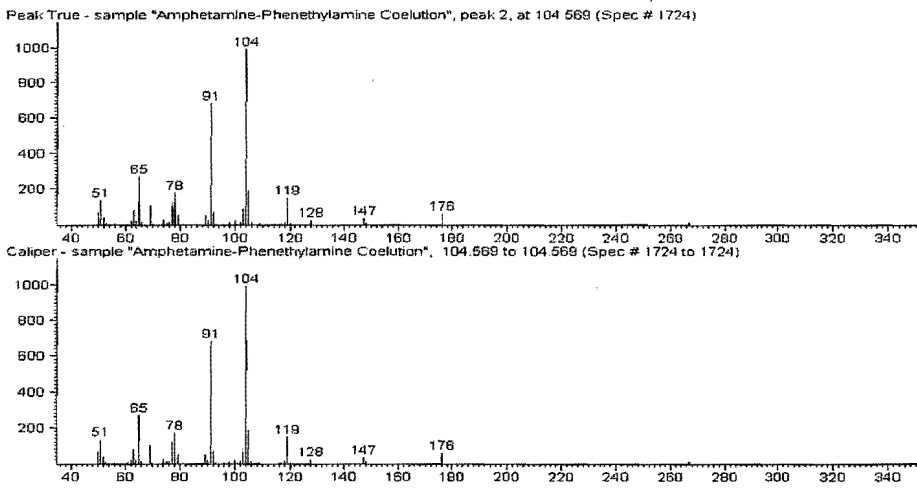


Figure 3: Phenethylamine PFPA deconvoluted spectra (top). Manual spectra display (bottom).

Successfully resolving this issue will provide investigators with a more comprehensive case-solving result.

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 Keywords: time-of-flight Mass Spectrometry, coelution, deconvolution

## Buprenorphine Glucuronide Plasma Concentrations by LC-MS-MS after High Dose Intravenous and Sublingual Buprenorphine Administration

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The evaluation of the metabolic profile of a drug, including glucuronide conjugates, in biological samples is of great practical importance in interpretive forensic and clinical toxicology. Different factors, such as the development of metabolic tolerance, pharmacokinetic interactions with other substances, pharmacogenetic differences, and metabolic pathologies, may modify a drug's metabolic profile and as a consequence, the intensity and/or the type of biological response. Furthermore, parent drug-to-metabolite ratios may be useful in the assessment of the time elapsed after administration. An LC-MS-MS method for the simultaneous determination of buprenorphine (BUP), norbuprenorphine (NBUP), and buprenorphine-3-glucuronide (BUPG) was developed to characterize the time course of appearance and peak concentrations of BUPG in plasma after high dose (12 mg) IV (n=4) and SL (n=3) administration. Plasma samples collected in a controlled BUP administration study and previously examined for free BUP and NBUP concentrations were analyzed. Samples were submitted to SPE with Varian LRC C-18 cartridges. Binary gradient RP-LC separation was achieved using a Microsphere C18 Chrompack (100 x 3mm ID, 3 μm) column. Electrospray MS-MS analyses were carried out on a PE-Sciex API-3000 tandem mass spectrometer by monitoring the m/z 644 → m/z 468 transition for BUPG and the surviving parent ions for BUP, NBUP and their deuterated analogs. The LOQ was established at 0.1 ng/mL for each analyte. Mean peak concentrations of BUP, NBUP and BUPG were 108.8 ng/mL, 3.3 ng/mL, and 7.0 ng/mL, respectively, after IV and 14.2 ng/mL, 1.0 ng/mL, and 1.8 ng/mL after SL administration. BUPG was detected in the first plasma specimen drawn 10 min after IV and by 15 min following SL drug. BUPG concentrations peaked 15 min after IV and 0.25-7 h after SL administration. BUPG was detectable up to 48 h after SL and was still detectable at 72 h after IV administration in 3 of 4 subjects. The BUP/BUPG ratio peaked at 3 h after IV and SL administration (23.4 and 22.6, respectively) and decreased below 10 by 24 h and 5 h, respectively. By monitoring the m/z 590 → m/z 414 transition, norbuprenorphine glucuronide (NBUPG) was tentatively identified in plasma samples. This highly sensitive and specific LC-MS-MS procedure for the simultaneous determination of free and conjugated BUP metabolites in plasma proved to be useful for pharmacokinetic monitoring of high dose buprenorphine administration.

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Keywords: Buprenorphine Glucuronide, plasma, IV and SL administration.

## Analysis of Capsaicinoids in Extracts of Fresh Pepper, Oleoresin Capsicum, and Pepper Spray Products Using LC/MS

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Liquid chromatography-mass spectrometry was used to identify and quantify the predominant capsaicinoid analogues in extracts of fresh peppers, oleoresin capsicum (OC), and pepper spray products. The concentration of capsaicinoids in the extracts of fresh peppers was dependent upon the relative pungency of the pepper and ranged from  $0.0018 \pm 0.0002$  to  $510 \pm 27$   $\mu\text{g/g}$ . Significant differences were also observed when the same type of pepper, but from a different geographical origin, was analyzed. The differences in capsaicinoid concentration in peppers as a result of individual variability and geographical origin were manifested in OC. Analysis of three OC samples obtained from independent manufacturers, all having a Scoville Heat Unit (SHU) rating of  $1.0 \times 10^6$ , exhibited a range in capsaicinoid concentration from  $63 \pm 1$  to  $71 \pm 2$   $\mu\text{g}/\mu\text{L}$ . As expected, significant variability in the capsaicinoid concentration of pepper sprays was also observed. Canisters labeled as having identical properties (ie. the same SHU value and OC content) were found to vary in their capsaicinoid concentration by as much as 17-fold, ranging from  $0.95 \pm 0.03$  to  $16.0 \pm 0.4$   $\mu\text{g}/\mu\text{L}$ . Two identical pepper sprays obtained from different product lots were found to differ in their concentration of capsaicinoids from  $2.5 \pm 0.2$  to  $13.5 \pm 0.1$   $\mu\text{g}/\mu\text{L}$ . Collectively, these data indicate that commercially available OC and pepper spray products that are classified by SHU are not standardized for capsaicinoid concentration. Variability in the capsaicinoid concentrations in OC-based self-defense weapons could alter the potency and efficacy and may jeopardize the safety and health of users and assailants.

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Keywords: Pepper Spray Products, LC/MS, and Capsaicinoids

### A Luminescent Screen for Carisoprodol

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Carisoprodol (Soma) and meprobamate (Miltown) are N-methylcarbamate (NMC) compounds prescribed as muscle relaxants. Because they are not federally scheduled, they are commonly abused for their sedative/euphoric effects. Therapeutic plasma levels of carisoprodol and meprobamate are 2-20  $\mu\text{g/ml}$ , making them straightforward to detect and confirm by mass spectrometry (MS). However, there is at present no screening assay available. The increase in popularity of these compounds makes it desirable to have such a test to identify potentially positive samples. We present here a luminescent screening assay for carisoprodol and meprobamate in blood adapted from a commercially available test for NMC insecticides in aqueous samples. The method is based upon inhibition of a luciferin-linked esterase by NMC compounds from acetone/ethyl acetate extracts of aqueous samples. Our assay will detect carisoprodol and meprobamate in blood extracts at concentrations of  $\leq 100 \text{ ng/ml}$ .  $> 92\%$  of samples screened positive for NMC were confirmed for carisoprodol/meprobamate by MS. Little or no interference was seen from other common drugs of abuse at concentrations up to 3  $\mu\text{g/ml}$ , although the presence of several classes of non-NMC drugs in some cases gave a positive result. We conclude that this assay would be useful in screening putative negative samples for the presence of the NMC compounds carisoprodol and meprobamate.

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Keywords: carisoprodol, meprobamate, Soma



## The Analysis of Ketamine and Norketamine in Humane Urine Specimens using Liquid Chromatography/Selected Ion Monitoring Mass Spectrometry

*Kevin L. Klette\**, Gregory K. Poch, and Cynthia J. Anderson. Navy Drug Screening Laboratory, San Diego, CA

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A new method has been developed for the quantitative determination of ketamine and a known ketamine metabolite (norketamine) in human urine. Urine samples were extracted using a rapid three-step liquid-liquid basic extraction procedure. Identification and quantitation was accomplished, without derivatization, using Liquid Chromatography/Mass Spectrometry (LC/MS) in the Selected Ion Monitoring (SIM) mode with a Eclipse ® XDB-C18 column and Atmospheric Pressure Chemical Ionization (APCI). Ketamine and norketamine were positively identified by comparison with reference standards using the following ions: 238, 220 and 207  $m/z$  (ketamine) and 224, 207, and 179  $m/z$  (norketamine). A validation study of the assay including analyte recovery, linearity, inter and intra assay performance, Limit of Detection (LOD), and Limit of Quantitation (LOQ) will be presented. Additionally, a blind trial consisting of 20 negative urine specimens spiked with varying concentrations of ketamine and norketamine was conducted to confirm the validity of the assay. The LC/MS method produces excellent chromatography and sensitivity without the need for derivatization and provides an alternative to the Gas Chromatography/Mass Spectrometry (GC/MS) analysis of ketamine and norketamine.

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Keywords: Ketamine, Liquid Chromatography/Mass Spectrometry, Norketamine

### Case Report: A Polypharmacy Suicidal Overdose Involving Loratadine (Claritin®)

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Loratadine (Claritin®) is a long-acting tricyclic antihistamine utilized in the treatment of allergic rhinitis and urticaria. At therapeutic doses of 10mg/d, this second-generation H1 receptor antagonist is effective without causing adverse effects such as sedation and interference of cognition, psychomotor performance or mood. Limited information has been published on loratadine toxicity. Presently, there are no reported deaths in which the causative agent(s) did not include loratadine nor have any postmortem fluid and tissue concentrations been documented in the literature.

This report involves a 49 y.o. woman who was found dead at home with an accompanying note suggesting suicide. The decedent had a recent history of marital problems. Scene investigation uncovered loose capsules and one cut-opened capsule of pharmacy-brand "allergy formula capsules" (Diphenhydramine HCL 25mg). Autopsy findings were notable for cerebral swelling and urinary bladder distention. In addition, no obvious pill content was noted in the gastric content. Heart blood, liver, urine and gastric contents were collected for toxicological analysis based on the death scene investigation and autopsy findings. Initial toxicological screening of the heart blood included volatiles, cocaine, opiates, and acidic basic, and neutral drugs. The presence of loratadine was detected in postmortem specimens by an n-butyl chloride basic extraction procedure with screening by gas chromatography with nitrogen phosphorus detection (GC-NPD) and confirmation of identity by gas chromatography/mass spectrometry (GC/MS). A four-point calibration curve with a linear range of 0.25 to 4.0 mg/L was used to quantitate loratadine by GC-NPD using alphaprodine as the internal standard. Other procedures were used to quantitate acetaminophen, guaifenesin, and propoxyphene.

The following drugs and their respective concentrations were detected in heart blood: loratadine at 0.46 mg/L, acetaminophen at 248 mg/L, propoxyphene at 0.97 mg/L, norpropoxyphene at 1.4 mg/L, guaifenesin at 49 mg/L, and diphenhydramine at trace amounts. Liver concentrations included loratadine at 22 mg/kg, propoxyphene at 20 mg/kg, and norpropoxyphene at 66 mg/kg. Concentrations detected in the gastric contents included a loratadine of 3.4 total mg, propoxyphene of 4.0 total mg, and norpropoxyphene of 0.57 total mg. Loratadine was not detected in the urine. These postmortem concentrations indicated that an acute dose of loratadine, propoxyphene, acetaminophen, and guaifenesin had been ingested prior to death. After toxicological investigation, the manner of death was ruled a suicide and the cause of death were attributed to an overdose of multiple agents including loratadine.

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Keywords: loratadine, postmortem fluids and tissue, GC-NPD, GC/MS32-62

### Metabolism of Lysergic Acid Diethylamide (LSD) to 2-oxo-3-hydroxy LSD (O-H-LSD) in Human Liver Microsomes and Cryopreserved Human Hepatocytes

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The metabolism of lysergic acid diethylamide (LSD) to 2-oxo-3-hydroxy lysergic acid diethylamide (O-H-LSD) was investigated in liver microsomes and cryopreserved hepatocytes from humans. Previous studies have demonstrated that O-H-LSD is present in human urine at concentrations 16 times greater than LSD, the parent compound. Additionally, these studies have determined that O-H-LSD is not generated during the specimen extraction and analytical processes or due to parent compound degradation in urine samples in aqueous urine samples. However, these studies have not been conclusive in demonstrating that O-H-LSD is uniquely produced during *in vivo* metabolism. Phase I drug metabolism was investigated by incubating human liver microsomes with LSD. Phase I/Phase II drug metabolism was examined by incubating cryopreserved human hepatocytes with LSD. The reaction was quenched at various time points and the aliquots were extracted using liquid partitioning and analyzed by Liquid Chromatography/Mass Spectrometry (LC/MS). O-H-LSD was positively identified in all human liver microsomal and human hepatocyte fractions incubated with LSD. Additionally, O-H-LSD was not detected in any microsomal or hepatocyte fraction not treated with LSD and in LSD specimens devoid of microsomes or hepatocytes. This study provides definitive evidence that O-H-LSD is produced as a metabolic product following metabolism of LSD in humans.

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Keywords: Metabolism, LSD, Liquid Chromatography/Mass Spectrometry

**Determination of Methadone, EDDP and EMDP in Meconium by GC-PCI-MS**

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Methadone is currently the drug of choice for treating opiate dependent pregnant drug abusers. Methadone is extensively metabolized to EDDP and EMDP. Most current methods analyze for methadone and EDDP only. Meconium is an excellent matrix to examine fetal exposure to illicit substances due to ease and non-invasiveness of collection and its long window of drug detection. The detection of methadone and its metabolites has been difficult due to the low concentration of these compounds in meconium. We have developed an assay for the detection of methadone, EDDP and EMDP in meconium using solid phase extraction (SPE) followed by GC-MS analysis in the positive chemical ionization mode (PCI). Approximately 1 g of meconium was mixed with 2 mL of methanol. Sodium acetate buffer (pH 4.0, 0.2 M) and internal standards were added. Analytes were eluted with methylene chloride:2-propanol:ammonium hydroxide (80:20:2 v:v:v) from Clean Screen DAU cation exchange/hydrophobic columns. Eluates were dried under a stream of nitrogen without heating, and reconstituted in 40 $\mu$ L of acetonitrile. Extraction recoveries were 83%, 74% and 95% for methadone, EDDP and EMDP, respectively. Correlation coefficients for the calibration curves were  $\geq 0.98$  with a linear range of 5-1000 ng/g for all analytes. Subsequent to method development and validation, we analyzed 5 meconium samples from methadone in utero-exposed neonates. Specimens contained high concentrations of methadone (>1000 ng/g), EDDP (>2000 ng/g) and to a lesser extent EMDP (10-20 ng/g). It appears this method may have application in the identification of neonatal exposure to methadone.

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Keywords: Methadone and metabolites, meconium and GC/MS.

## Detection of Nefazodone (Serzone) in Whole Blood by Gas Chromatography/Mass Spectrometry

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Nefazodone (Serzone) is a relatively new drug for treatment of depressive disorder. It is reportedly safer than traditional antidepressants, and is relatively non-sedating in normal use. The objective of this study was to develop a modification to our routine basic drug screening procedure, to disclose the presence of nefazodone and its metabolite m-chlorophenylpiperazine (m-CPP) in whole blood. The method has been applied to series of impaired driving and postmortem cases from Washington State.

Nefazodone's primary mechanism of action is its inhibition of the postsynaptic 5-HT<sub>2</sub> (serotonin-2) receptor, however it also inhibits the re-uptake of norepinephrine. There is therefore a potential for interaction with other serotonergic drugs such as the SSRI's or MAOI's. Metabolism of nefazodone is mediated via cytochrome P4503A4, and its metabolism may be affected by other substrates for that enzyme. Nefazodone is typically prescribed at doses of 200-600mg/day. Following a single 200mg dose, peak plasma concentrations are in the range 0.25 to 0.45mg/L. Steady state concentrations after seven days of dosing averaged 2mg/L for nefazodone.

Nefazodone and m-CPP were extracted into butyl chloride at pH 9, and by gas chromatography/mass spectrometry in electron impact mode (HP 6890/5973). The temperature program was increased from 150°C, at a rate of 20°C/min to a final temperature of 300°C. The retention times were 5.3 minutes for m-CPP metabolite, and 26 minutes for nefazodone. Ions monitored include 209, 274 and 303 (quantitative) for nefazodone and 156, 196 and 154 (quantitative) for m-CPP. The LOD and LOQ were both established at 0.005 mg/L for m-CPP and 0.10 and 0.25 mg/L respectively for nefazodone.

We report whole blood nefazodone and/or the m-CPP metabolite levels in eight deaths and two driving cases. The mean blood nefazodone and m-CPP metabolite concentrations were 0.82 (median 0.80, range <0.10-1.91) and 0.11 (median 0.17, range <0.01-0.34) mg/L, respectively. None of these deaths was directly attributed to nefazodone. We conclude that the method as presented is useful for the detection and measurement of nefazodone concentrations within the normal therapeutic range. Toxicologists should be aware of the potential for pharmacological interaction between nefazodone and other serotonin acting drugs and should employ caution when interpreting findings when these drugs are present together.

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Keywords: Nefazodone, analysis, forensic toxicology

### Comparative Postmortem Stability of Pentobarbital in Brain, Liver, Skeletal Muscle and Hair Follicles of Eumelanin-pigmented and Albino Mice

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Drug redistribution is a potential complicating factor in the assessment of antemortem toxicology from postmortem tissue drug concentrations. In order to assess the hair follicle as a potentially stable site of postmortem drug storage relative to other tissues, an animal model was investigated. Balb/c (albino) and C57-B1 (eumelanin-pigmented) mice were injected i.p. with pentobarbital (PB) (90 mg/Kg), and sacrificed by asphyxiation with CO<sub>2</sub> one hour later. Blood PB concentrations were measured at the time of death. Hair follicles, brain, liver and skeletal muscle were removed from 3 animals of each strain at 0,1,4,8,12,24 and 36 hours postmortem. The tissues were weighed, then homogenized in 100 mM phosphate buffer (pH 4.5) containing PB-d3 as internal standard. Following liquid extraction the PB content of the tissues (ng/g wet weight) was determined by gc/ms. In albino mice, PB tissue concentrations demonstrate a multiphasic relationship over time, substantially decreasing at 24 hours, then rising 36 hours postmortem, a relationship possible related to water loss of the tissues over time. Over this same time period, hair follicle PB concentrations remained much more stable at apx. 30% of initial blood concentration. PB tissue concentrations in C57B1 mice demonstrated a much more complex, but quite reproducible, relationship over the 36 hour postmortem interval. However hair follicle concentrations remained much more stable, at apx. the same concentration as the initial PB concentration in blood, and apx. 3-fold greater than that observed in follicle of albino mice.

The data demonstrate the potential utility of the hair follicle as a reasonably stable site for postmortem drug determination. Supported by NIH grant DA09545.

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Key words: Hair follicle, pentobarbital, postmortem drug stability

### Quetiapine Related Fatalities

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Quetiapine is a new atypical antipsychotic used to treat schizophrenia and is classified as a dibenzothiazepine with a high affinity for the 5-HT<sub>2</sub> receptors and to a lesser extent the D<sub>1</sub> and D<sub>2</sub> dopamine receptors. Blood concentration range from 0.044 to 0.091mg/L for doses of 300 to 600 mg. This study reports three cases involving quetiapine.

Comprehensive drug screens and quantitative analyses were performed. The first case involved a Caucasian female, 26, receiving treatment for depression and bipolar disorder. Drugs identified in iliac blood: sertraline 0.94, desmethylsertraline 0.20, ephedrine 0.10, pseudoephedrine 0.04, olanzapine 0.24, and quetiapine 0.22 mg/L. Gastric contents showed: 0.18 sertraline, 0.82 olanzapine, and 0.52 mg quetiapine. The second case involved a Caucasian male, 46, diagnosed with manic depressive disorder but not currently under medical supervision. Drugs identified in iliac blood: propoxyphene 5.2, diphenhydramine 0.14, acetaminophen 55, sertraline 0.11\*, and quetiapine 2.9 mg/L (\*subclavian blood). The third case involved a Caucasian male, 36, diagnosed as a paranoid schizophrenic. Only quetiapine was identified: 170 mg/L in cavity blood, and 190 mg/Kg in liver, and 27 mg as gastric content. The cause of death-manner of death were: Case 1-mixed drug overdose (sertraline, olanzapine and quetiapine)-undetermined. Case 2- mixed drug overdose (propoxyphene, and quetiapine,)-suicide. Case 3-quetiapine toxicity-accident. To the best of our knowledge, the third case is the first reported case where quetiapine was the sole agent involved in fatal quetiapine toxicity.

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Keywords: Quetiapine, case report, overdose

### Death by Strychnine – A Case for Postmortem Redistribution

*Kristin M. Rossum\*, Glenn Holt and Michael D. Robertson. Office of the Medical Examiner, San Diego, CA*

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Strychnine, a potent and naturally occurring alkaloid, has been attributed to many cases of death due to toxicity. Strychnine is a very lipophilic and rapidly acting toxin producing post-synaptic inhibition of the central inhibitory neurotransmitter glycine, resulting in central nervous system stimulation, convulsions, and death. Documented postmortem blood concentrations of strychnine range from 0.5 mg/L to 60 mg/L with some associated documented tissue concentrations for brain liver, kidney urine and gastric. Currently there is no data with regards to vitreous or site-specific blood concentrations.

This study investigated the concentrations of strychnine in a recent death in San Diego. The decedent was a 31-year-old caucasian male with a long history of depression who was found in a hotel room with a suicide note. Found at the scene were some OTC medications and a container of "Quick Action Gopher Mix" containing 0.50% strychnine as the active ingredient. A small amount of rum was consumed and within two plastic cups were moist unidentified seeds. Two plastic bottles of Coca-Cola were also located at the scene.

Gastric contents, blood, liver, urine and vitreous were collected for toxicological investigation. The gastric contents consisted of 310 grams of solid seed-like matter consistent with the "Gopher Mix", and were found to contain strychnine. Analyses detected ethanol at a concentration of 0.03%, diphenhydramine and caffeine in central blood. Strychnine was identified in the central blood by GC-MS and quantified by GC-NPD. Concentrations in the central and peripheral blood samples were 8.8 mg/L and 0.59 mg/L respectively. Concentrations in liver, vitreous humor and urine will also be presented.

This case demonstrates that strychnine concentrations in blood following ingestion vary depending on site of collection. In this case the variation is most likely due to ongoing postmortem diffusion from the gastric contents into the central blood vessels.

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Keywords: strychnine, postmortem, toxicology



**Telazol®: A Dissociative Animal Anesthetic Agent Found In Two Fatalities**

*Norman A. Wade\*, Charles E. Spies and Susan M. Cooley. Office of the Medical Examiner, Phoenix, AZ*

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Telazol® is a nonnarcotic, nonbarbiturate, injectable anesthetic agent for dogs and cats that is found in most veterinary clinics. Chemically it is a combination of equal parts by weight of base of tiletamine hydrochloride (an arylamino-cycloalkanone dissociative anesthetic) and zolazepam hydrochloride (a nonphenothiazine dizepinone having minor tranquilizing properties). This product is supplied in sterile vials. The addition of five milliliters of diluent produces a solution containing the equivalent of 50 mg of tiletamine base, 50 mg of zolazepam base and 57.7 mg of mannitol per milliliter. This solution has a pH of 2 to 3.5 and is recommended for deep intramuscular injection.

Two cases are presented in which Telazol® was identified in cases investigated by the Office of the Medical Examiner, Phoenix, Arizona. Tiletamine and zolazepam were identified by gas chromatography-nitrogen-phosphorous detection (GC-NPD) following routine alkaline extraction and analytical separation on a HP Ultra 2 column using no derivitization. Confirmation was achieved by full scan electron impact gas chromatography - mass spectrometry (EI GC/MS) with tiletamine giving a base peak of m/z 166 and prominent ions at m/z 195, 110 and 123 and zolazepam giving a base peak at the molecular ion of m/z 285 and prominent ions at m/z 257 and 267. Both drugs were quantitated by GC-NPD using internal standards, a four-point calibration curve and appropriate specimen dilutions.

In case 1, which was ruled an accidental drowning, norchlorcyclizine (0.2mg/L) and ethanol (0.03 g/L) were found in the femoral blood in addition to the tiletamine (0.09 mg/L) and zolazepam (1.0 mg/L). Case 2, which was ruled an accidental overdose, was found to likewise have fentanyl (2.7 ng/mL), ketamine (0.1mg/L) and atropine in addition to tiletamine (<0.01 mg/L) and zolazepam (0.3 mg/L) in the femoral blood. Both subjects were known drug users, had recent antecubital injection sites, apparent suicide ideation and both worked in a veterinary clinic at least part-time and therefore had access to this medication.

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Keywords: tiletamine, zolazepam, anesthetic

### □-Thujone and Absinthe: Structural and Metabolic Aspects of Neurotoxic Action

*Karin M. Höld, Nilantha S. Sirisoma, and John E. Casida. Environmental Chemistry and Toxicology Laboratory, Department of Environmental Science, Policy and Management, University of California, Berkeley, CA*

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□-Thujone is the principal active ingredient of wormwood oil and, except for ethanol, of the emerald-green licorice-flavored liquor absinthe. It is also an insecticide. The goal of this study was to elucidate the mechanism of □-thujone neurotoxicity and identify its major metabolites and their role in the poisoning process.

Three observations suggest that □-thujone acts as a GABA-gated chloride channel blocker; First, the poisoning signs are similar to those of the classical antagonist picrotoxinin; Second, it inhibits [<sup>3</sup>H]ethynylbicycloorthobenzoate binding to mouse brain membranes; Third, a strain of *Drosophila* specifically resistant to chloride channel blockers is also tolerant to □-thujone. □-thujone is quickly metabolized by cytochrome P450 forming at least three monohydroxylated derivatives, which are also detected by GC-CI-MS in the brain of thujone-treated mice. Vincent van Gogh in the past with absinthe and current aficionados of □-thujone in herbal medicines subject themselves to a rapidly acting and readily metabolized neurotoxicant acting as a noncompetitive blocker of the GABA-gated chloride channel. Grant support: NIH R01 ES08419

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Keywords: convulsant, metabolism, EBOB

**Nicotine and Nicotine Metabolite Adducts of Melanin Demonstrated by Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) Mass Spectrometry**

*David J. Claffey\*, Donna L. Dehn, Mark W. Duncan and James A. Ruth. University of Colorado Health Sciences Center, Denver, CO*

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Pigmentation may be a key component in the incorporation of selected drugs into hair. In an attempt to elucidate a potential mechanism of melanin-drug interaction, melanin was synthesized *in vitro* in the presence of nicotine, a drug known to have an affinity for melanin, and cotinine, the primary nicotine metabolite. L-DOPA, a eumelanin precursor, was oxidized and oligomerized with tyrosinase in DI water at 37°C under a stream of oxygen. Nicotine, cotinine and/or their deuterated analogs were added at the start of the incubation period in a 10:1 ratio of L-DOPA to drug. A black precipitate formed 90-120 minutes later. Aliquots were removed from the incubation mixture at 60, 120 and 360 minutes, mixed with a vast molar excess of the MALDI matrix ( $\alpha$ -cyano-4-hydroxycinnamic acid, CHCA), then applied to the MALDI target. The mixture was allowed to crystallize at room temperature and pressure. A Voyager STR MALDI-TOF mass spectrometer operating in reflectron mode was used for all analyses. Multiple mass determinations (n=10) were made on each sample to provide a mean and standard error for the masses of interest. Internal calibration employing matrix ions that bracketed the masses of interest allowed accurate mass assignments on the products. A careful comparison of the spectra of samples prepared both with and without drug indicated the presence of (exact) masses corresponding to the protonated drug, the forming melanin oligomers and the melanin-nicotine or -cotinine adducts. These assignments are consistent with the formation of a covalent adduct between melanin and the drugs under investigated. Further confirmation of the melanin-drug adducts was provided by the use of deuterated drug. Supported by NIH grant DA09545.

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Keywords: Melanin-drug adducts, drugs in hair, nicotine

### Criteria for Conducting Hair Analysis for Drugs of Abuse

*Thomas Cairns\*, John Irving, Michael Schaffer and William Thistle. Psychomedics Corporation, Culver City, CA*

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The objectives of this presentation is to discuss the unique critical elements required for scientific compliance in the execution of the analysis of hair samples for the detection of drugs of abuse for forensic purposes:

- The sample should be taken using an observed collection under strict chain of custody procedures
- The preferred sample site is anywhere along the vertex of the head, although body hair can be used as an alternative
- A very sensitive first initial screen, such as RIA, should be used to separate the negative samples from those which are presumptively positive
- An exhaustive wash procedure should be employed, which uses hair swelling solvents, to remove contamination from the external surfaces of the hair. As scientifically appropriate for each drug, additionally an extensive wash procedure is augmented with testing of the wash, and metabolic profiling, ie., cocaine, benzoylecgonine, cocaethylene/ethyl cocaine and norcocaine.
- A strong method validation package for the structural confirmational analysis by Mass Spectrometry (GC or LC) to include, linearity, LOD/LOQ, carryover, specificity and precision.
- Strong QA and QC review of the process, and auditing of steps along the entire process of sample collection, accessioning, initial testing confirmatory testing, and review of results to reporting and issuance of the final result.
- Review of test result by a qualified Medical Review Officer or other responsible qualified individual.

This presentation will discuss the exact sequence of steps involved in the analysis with particular emphasis on the detailed forensic requirements of the test, the screening and confirmatory methods used, the criteria employed for reporting a positive result, and the importance of the washes and their concentration levels relative to the amount detected in the washed hair digest. Results from the practical experience of analyzing samples relative to the distribution of drugs and their concentrations will be discussed.

In summary, the data will clearly demonstrate the need for a rigorous protocol involving certain basic requirements for hair testing.

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Keywords: Hair, Drugs of Abuse, Mass Spectrometry

**HAIR ANALYSIS: Correlation Between Original Analysis and Subsequent Follow-Up Test**

*Thomas Cairns\*, John Irving and Michael Schaffer. Psychomedics Corporation, Culver City, CA*  
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One of the unique properties/advantages of hair testing for drugs of abuse is the ability to re-sample the donor soon after the original test results become available. This ability permits a direct challenge by the donor /client by providing a wholly new follow-up sample.

This system is similar to the split sample technique used by DOT, in urine testing, but goes a step further, since it is a witnessed collection, and would resolve any issues of sample mix-up at the collection site or within the testing laboratory.

The second collection should be from the same site as the first, and there must be governing policies to ensure that the donor does not take any intervening actions to alter or destroy the specimen, i.e. shaving their head.

Since the average growth rate of head hair is approximately 0.5 inches per month, the total time window represented by the 1.5 inches used for analysis can be a close approximation to the time window represented by the original sample, which in most cases is approximately 90 days. Within the population of 2 million hair tests performed by this laboratory a small percentage (<0.1%) have been subjected to follow-up testing. Data will be presented for two major drug groups (cocaine and marijuana). Evidence will be presented to illustrate the high correlation between the original analysis and the subsequent follow up test. Results generally fall within a correlation factor of 0.95. Furthermore, these follow-up tests can be subjected to segmental analysis whereby the 1.5 inches used for analysis is divided into three 0.5 inch segments to provide the Medical Review Officer with a monthly window on drug abuse. Examples provided will illustrate the habitual user versus the occasional user. The advantages provided by followup testing and segmental analysis can be employed by the MRO to monitor rehabilitation, or continued abuse. In a forensic environment such follow-up tests can definitively address the arguments often advanced by users who question the first positive result.

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Keywords: Hair, Follow-up Sampling, Segmental Analysis

## **An Evaluation of Two Wash Procedures for the Differentiation of External Contamination vs. Ingestion in the Analysis of Human Hair Samples for Cocaine**

*Michael I. Schaffer, Wen-Ling Wang and John Irving. Psychemedics Corporation, Culver City, CA*

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In order to evaluate the effectiveness of Psychemedics' wash procedures as compared to a methanol wash procedure reported in an earlier citation<sup>(1)</sup>, an experiment was designed using Psychemedics' current protocol after soaking normal human hair in cocaine contaminated aqueous solutions. Fourteen normal human hair samples were soaked in a solution of cocaine HCl (1,000 ng/mL) at room temperature for one hour, then rinsed with distilled water and dried at room temperature. Using Psychemedics extensive wash procedures (15" isopropanol wash, followed by three 15 " phosphate buffer washes and then two 60" washes) as compared to the methanol wash procedure, in no case would any of the samples be reported out as positive under present workplace criteria. Using the other procedure, eight of the 14 methanol washed samples, could have been reported out as positive, using workplace reporting, ie., cutoff of 5 ng/10 mg hair. It is important to recognize that the methanol wash procedure used hair cut into approx. 5 mm segments, and we do not recommend the cutting of hair, as this makes washing problematic. Psychemedics' extensive washing was shown to be a far more efficient procedure for removal of external contamination as compared to the methanol wash procedure reported earlier. In all cases, the protocol developed by Psychemedics would allow the differentiation of ingestion vs. external contamination, as defined by this soaking experiment.

All samples were extracted using SPE columns and derivatized followed by LC/MS/MS analysis. Analysis was performed on a triple quadrupole API 2000 Perkin Elmer Sciex MS equipped with an atmospheric pressure ionization source via an ionspray interface. The MS operated in the positive CI Multiple Reaction Mode.

<sup>(1)</sup>W.L. Wang and E.J.Cone. Testing of human hair for drugs of abuse IV, Environmental cocaine contamination and washing effects. *Forens. Sci. Int.* 70:39-51 (1995)

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**Keywords:** Hair Testing, External Contamination, LC/MS/MS

**pH Dependent Organic-Aqueous Partitioning does not Occur with Non-conjugated 11-nor- $\Delta^9$ -tetrahydrocannabinol-9-carboxylic acid (THCA) but does Occur With Conjugated THCA**

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Ethyl acetate and water were used as models of lipid and aqueous body compartments to examine pH-dependent distribution of THCA.

THCA in water, 60 ng/ml, was prepared with unconjugated THCA.

A THCA positive urine specimen was diluted to 69 ng/ml THCA.

Triplicate 5 ml aliquots were buffered with 1 ml 0.15 M phosphate to various pH. Ethyl acetate was added and tubes were inversion-mixed for 10 minutes then centrifuged. Portions of aqueous and organic phases were dried, reconstituted with water and assayed for THCA.

Analysis was alkaline-hydrolysis, extraction by SPE, derivatization with BSTFA, and GC/MS SIM using deuterated THCA internal standard. The assay limit of detection was 2 ng/ml.

Aliquot pH	THC Acid Concentration ng/ml: Mean (CV)					
	Conjugated		Unconjugated			
	Aqueous	Organic	Aqueous	Organic	Aqueous	Organic
3	0	47 (0.05)	0	30 (0.19)	0	35 (0.40)
4	0	47 (0.05)	0	35 (0.40)	0	51 (0.24)
5	6	(0.17)	37 (0.03)	0	51 (0.24)	51 (0.24)
6	16	(0.17)	2 (0.14)	0	47 (0.09)	47 (0.09)
7	38	(0.03)	7 (0.34)	0	36 (0.24)	36 (0.24)
8	39	(0.02)	5 (0.33)	0	58 (0.04)	58 (0.04)
9	33	(0.17)	7 (0.32)	0	52 (0.08)	52 (0.08)

Conclusion: Conjugated THCA demonstrates pH dependent water - ethyl acetate partitioning at pH 3 to 9. Unconjugated THCA does not.

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Keywords: THCA, partitioning, pH-dependent

## Modulation of Oxidizing Agents Adulteration by Manipulation of Urinary pH Values

*Jane S Tsai\*, Mahmoud ElSohly, Shiow-Fen Tsai and Salvatore J. Salamone. Roche Diagnostics Corp., Indianapolis, IN*

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Oxidizing adulterants such as Urine Luck (Pyridinium Chlorochromate), Bleach (Sodium Oxychloride), Klear (Potassium Nitrite) and Whizzies (Sodium Nitrite) can react with 11-nor- $\Delta^9$ -tetrahydrocannabinol-9-carboxylic acid (THC-COOH) to produce compounds and render the THC-acid non-detectable in routine urine drug testing. The reaction can be influenced by urine sample matrix, especially the urinary pH values, and the duration of exposure to the oxidizing agents. We demonstrated the urinary pH-dependency of the effect of nitrite adulteration with forty confirmed clinical "THC positive samples" at various time intervals after nitrite spiking by Abuscreen ONLINE cannabinoids immunoassay. Significant decreases in the immunoassay results could be observed within 1 to 4 hours of nitrite treatment in the majority of samples with acidic urinary pH values regardless of their original concentration of THC-COOH. In contrast, the immunoassay results of samples with neutral or basic pH values were less or not affected by nitrite exposure in the same studies. The same urinary pH-dependency was also observed with Pyridinium Chlorochromate. Since it has been hypothesized that the reaction of nitrite and THC-acid under acidic condition may be terminated through sample pre- or post-treatment with a basic buffer (Lewis et al., Forensic Sci. 44:951, 1999), we investigated this hypothesis by changing the urinary pH of 10 clinical samples. Each of the THC-positive clinical samples was adjusted to four pH levels: 5-6, 6-7, 7-8 or 8-9. Regardless of the original urinary pH, the effectiveness of nitrite adulteration is dependent on the final adjusted pH value. Therefore, adjusting acidic sample to basic pH can prevent the nitrite-induced loss of THC-acid while the decrease of pH values in basic samples resulted in the quick loss of THC-COOH.

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Keywords: Oxidizing adulterants, Urinary pH, THC-COOH



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## Program and Abstracts

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**A Five Year Statistical Review of Toxicological Findings in Motor Vehicle Fatalities in Cuyahoga County**

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Alcohol use among motor vehicle fatalities has been well documented in the USA. However, the incidence of other drug use is largely unknown. The objective of this study was to examine drug prevalence in motor vehicle fatalities (MVF) in Cuyahoga County, OH over a five year period. Review of data compiled at the Cuyahoga County Coroner's Office in Cleveland, OH for the years of 1995 through 1999 indicated a total of 788 motor vehicle fatalities. Demographic data collected included accident location, and the race, age, and sex of the deceased. Accidents which occurred outside the County but with the death occurring inside the county (eg. at an area hospital) were also integrated into the study.

Review of the data showed that in 33% of the cases the accident occurred in the city of Cleveland while 35% occurred in the 60 surrounding cities, villages and townships, and 31.5% occurred outside the county. Fatalities in the age group 16-20 years accounted for more than 10% of the total cases. The percentage of male and female fatalities were 68.9% and 31.1% respectively. Fatalities were subdivided into six vehicular modes (with percentage incidence): drivers (51.4%), passengers (21.3%), pedestrians (15.6%), motorcyclists (8.9%), bicyclists (1.8%), and motorcyclist passengers (0.3%). Of the 788 fatalities, 174 (22%) cases were positive for drugs, 173 (22%) were positive for alcohol with 36 (4.6%) cases involving combined drug and alcohol use. The incidence of prescription drugs such as antidepressants was most prevalent (66.7%) followed by illicit (27.9%), and over the counter (3.6%) medications. Forty-eight percent of alcohol related vehicular fatalities occurred when the concentration of alcohol in the blood (BAC) was between 0.10 and 0.19%. In approximately 30% of the alcohol positive cases, the BAC was greater than 0.20%. The driver was the most likely individual to be positive for alcohol (57.8%) and drugs (54.0%). Analysis of blood specimens demonstrated that the most prevalent illicit drug was marijuana (53.2%) followed by cocaine (29.9%), and PCP (14.3%). The major prescription drugs detected in MVF were analgesics (51.1%), followed by antidepressants (17.9%), benzodiazepines (17.4%) and antihistamines (9.2%). In cases involving both drugs and alcohol, 61.1% of the fatalities were drivers, 16.6% were pedestrians, and 13.8% were passengers. These data indicate that in this midwest metropolitan area, motor vehicle fatalities are most likely to be young males; the accident is most likely to occur in the city; and approximately 44% of decedents will be positive for alcohol and /or drugs.

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Keywords: Vehicular Accidents, Drug Use, Statistics

**Paired Measurements of Creatinine and Specific Gravity after Water Loading**

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Additional information on the paired measurements of urine creatinine and specific gravity after water loading became necessary after criteria, developed by HHS/SAMHSA identifying a specimen as “substituted” (ie., creatinine  $\leq 5$  mg/dL and specific gravity  $\leq 1.001$  or  $\geq 1.020$ ), became subject to debate (eg., relevant studies had limited number of paired creatinine and specific gravity data points; data were biased toward male subjects).

Participants volunteered to consume at least 80 oz. (approximately 2370 mL) of fluid spread evenly over six consecutive hours. Urine specimens were collected prior to the start of the six-hour test period, at the end of each hour in the period, on awakening the morning of the test day, and the following day (nine specimens were requested from each participant).

Creatinine was measured using a modified Jaffé reagent. Specific gravity was measured using an electronic refractometer.

Fifty-four participants provided a total of 480 urine specimens. Forty participants were female; 13 were male. Two participants were unable to consume the minimum amount of fluid originally intended. Eleven participants (5 men and 6 women) consumed over one gallon of fluid. None of the specimens were identified as “substituted” based on the aforementioned criteria. However, 103 of the specimens did meet the HHS criteria for a “dilute” specimen (ie., creatinine  $< 20$  mg/dL and specific gravity  $< 1.003$ ).

The examination of paired values of creatinine and specific gravity from specimens collected after water loading supports the HHS criteria.

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Keywords: creatinine, specific gravity, workplace drug testing

**Precision and Accuracy of Positive Urine Drug Testing Confirmation Results**

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Identification of a positive urine specimen requires a positive immunoassay screen and positive GC/MS confirmation. Considering that a positive screen is not always confirmed and a positive confirmation result is usually based upon a single aliquot analyzed once, it is important to demonstrate accuracy and reproducibility of positive confirmation results.

Confirmation results were evaluated for intra and inter-run precision and accuracy using external and internal quality control specimens. Intra-run precision and accuracy were assessed by performance on monthly AFIP open proficiency surveys for amphetamine (AMP), methamphetamine (METH), benzoylecgonine (BE), codeine (COD), morphine (MOR), lysergic acid diethylamide (LSD), phencyclidine (PCP), and 9-carboxy-tetrahydrocannabinol (THC). Additional intra-run precision studies utilized THC internal blind quality controls. Internal standard pipetting precision was evaluated for each of six technicians using unextracted THC standards. Inter-run confirmation precision was assessed by analysis of internal BQC data. Mean, SD and CV were calculated for all of the above precision studies.

Analysis of AFIP proficiency results for intra-run precision showed the following mean CVs and CV ranges: AMP 1.4 (0.7-2.6), METH 1.3 (0.3-3.4), BE 1.3 (0.2-4.2), COD 1.4 (0.6-3.8), MOR 1.2 (0.3-3.1), LSD 3.2 (1.4-8.5), PCP 1.2 (0.4-2.1), THC 2.2 (0-7.4). The two LSD and three THC CV values >5% contained below and slightly above cutoff concentrations respectively. All survey results were within +/- 20% of the group mean. Intra-run precision studies of THC BQCs demonstrated CV values ranging from 1.0-2.2. Intra-run CV values for unextracted THC standard ranged from 0.8-2.0. Analysis of BQC data revealed the following inter-run CVs: AMP 1.9, METH 2.2, BE 3.4, COD 6.6, MOR 5.9, LSD 7.0, PCP 4.8, THC 3.3.

Based upon the above analyses, this laboratory's confirmation results are reproducible and accurate within generally accepted drug testing standards.

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Keywords: Confirmation, Accuracy, Precision

**Identification of Gasoline Components Present in Brain Tissue by DFLEX, Diffuse Flammable Liquid Extraction**

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Deaths resulting from "huffing" of volatile organic solvents and fuels often present challenging analytical problems. Before toxicants can be quantified, they must first be identified in the target tissue matrix. This study reports a novel approach to identification of multiple analytes in brain following death as a result of "huffing" the complex mixture, gasoline. A sample of brain tissue from a suspected "huffer" was placed in a 100mL beaker, and then placed into a 1 quart metal can with one DFLEX<sup>®</sup> Diffusive Flammable Liquid Extraction charcoal strip, and heated overnight at 45 degrees C. The charcoal strip was then removed from the can, placed in a GC vial with carbon disulfide, which extracts the volatile components from the charcoal strip, and the supernatant is analyzed on the GCMS. Specific toxicants were identified by comparison with known mass spectra. The initial screen, using the DFLEX method, indicated the presence of toluene, trimethylbenzene, o-xylene, p-xylene, and m-xylene. In comparison, the more conventional analysis of brain sample using headspace gas chromatography demonstrated the presence of toluene only. Moreover, the relative amounts of the five analytes identified in brain, using the DFLEX<sup>®</sup> method was consistent with the relative composition of these analytes contained in gasoline. The DFLEX<sup>®</sup> method used in this study thus appeared to be superior to previous methods used for detecting "huffing agents" in target tissues. Further evaluation of the DFLEX<sup>®</sup> method will be performed as cases present themselves.

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Keywords: Gasoline, huffing, DFLEX(r)P4-41

**Quantitation of Cocaine and Metabolites in Hair via API-ES LC/MSD**

*R. Bu. Paulsen, D. G. Wilkins, M. H. Slawson, K. Shaw, and D. E. Rollins. Center for Human Toxicology, University of Utah, Salt Lake City, UT*

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The analysis of drugs of abuse in hair requires sensitive and reliable methods for quantitative measurement. We have developed a procedure for the analysis of cocaine (COC) and its major metabolites (norcocaine NOR), benzoylecgonine (BE), cocaethylene (CE), and ecgonine methyl ester (EME) in hair using electrospray ionization liquid chromatography/mass spectrometry. Calibration standards were prepared by fortifying human hair with known concentrations of the analytes. Hair from rats dosed with COC was used for additional method validation. Deuterated internal standards were added to 20 mg samples prior to digestion in 1 mL 0.1M HCl and extraction via solid phase extraction. Dried residues were reconstituted in 90:10 0.1% formic acid: methanol. Chromatographic separation was accomplished with a MetaSil Basic 3u, 100x3mm column (0.250mL/min, 75% 0.1% formic acid, 25% methanol) on a HP series 1100 LC/MSD. The linear range was 0.05-50 ng/mg for COC and EME, 0.02-50 ng/mg for BE and CE, and 0.05-10 ng/mg for NOR. Intra-assay precision (n=5) for all five analytes was <4% at 0.5 ng/mg and <3% at 10 ng/mg. Intra-assay precision was <5% at 25 ng/mg for EME, COC, BE and CE. Inter-assay precision was <5% at 0.5 ng/mg and <8% at 10 ng/mg for all five analytes (n=25), and <6% at 25 ng/mg for EME, COC, BE (n=25 each), and CE (n=24).

Four simple laboratory hair wash procedures (n=5 each) of: (a) methanol, (b) 0.1 M phosphate buffer pH=6, or (c) pH=8, and (d) isopropanol and 0.1 M phosphate (pH=5.5) buffer were evaluated on pooled rat hair. The hair was collected from the backs of rats that were dosed with COC and housed in individual hanging wire cages to minimize external drug contamination. For all four washes, quantitation of the analytes in the washed rat hair yielded significant differences (p(0.005) from controls for some or all of the detectable analytic (COC, BE, NOR, EME). These data suggest that wash procedures must be considered when comparing quantitative results between independent laboratories. Supported by NIH grant DA09096.

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Keywords: Hair analysis, cocaine, API-ES LC/MSD

**The Utility of Ofloxacin as a Reference Marker for Hair Analysis**

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It has been proposed that administration of a reliable marker substance to human subjects may enhance the ability to identify drug use and treatment compliance in drug treatment programs. The goal of this study was to determine if the antibiotic ofloxacin (OFLX) could be used as a "marker" substance to establish reference points with respect to time in hair. Male and female subjects (n=31) between 18-40 years of age received an 800-mg oral dose of OFLX. Subjects were restricted from cutting their hair or performing chemical treatments. Hair was collected (by cutting) before, and at weeks 4, 5, 6 and 7, after drug administration. Subjects were classified as having black (n=5), brown (n=12), blonde (n=8) or red (n=6) hair. Hair was segmented into 3-cm segments prior to digestion, extraction and analysis by HPLC. At 7 weeks, the mean OFLX concentrations (+- 1SD) in the first 3-cm of hair closest to the scalp were: black (30.6 +-8.5), brown (6.0 +-1.8), blonde (3.5 +-1.6), and red (1.4 +-0.3). A similar pattern was found in hair collected at weeks 4-6. Eumelanin (EUM) hair concentrations for each subject were determined via HPLC. A strong relationship between OFLX concentration at 7 weeks and EUM was noted ( $r^2=0.728$ ). In 6 subjects, we also determined the intra-subject variability of OFLX incorporation into individual hair strands. Five strands from each subject were segmented into 2-mm segments and analyzed. OFLX appeared in segments #1-10 at week 5 (the first cm of hair). OFLX appeared in segments #2-20 at week 7 (the first cm of hair). This was consistent with a growth rate of about 1.2 cm/month, although considerable inter-subject variability was found. No axial diffusion of OFLX along the hair shaft was noted. Despite a significant effect of hair color, these data suggest that OFLX may be a suitable marker substance for hair, allowing a subject to serve as their own "control". Future studies will explore whether drug use, treatment compliance or recidivism in clinical drug-abuse studies can be determined with the aid of OFLX. Supported by NIDA grant DA09096.

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Keywords: Ofloxacin, hair analysis, DAU

**An Evaluation of the OnLine II Immunoassay for Opiate 300/2000 ng/mL Cutoffs:  
Comparison to EMIT II and CEDIA DAU for Cross-Reactivity of Prescription  
Medications or Common Adulterants**

*Huiru Zhao\*, Davina C. Ananias, Sienna L. Yarbrough, Alan J. McNally, and Salvatore J. Salamone. Roche Diagnostics Corporation, Indianapolis, IN*

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A study was conducted using a Roche/Hitachi 717 analyzer to compare the performance of the OnLine® II Opiates immunoassay to EMIT II®, and CEDIA DAU®. These commercially available immunoassays were tested in a semi-quantitative mode at cutoffs of both 300 and 2000 ng/mL for cross-reactivity with some commonly prescribed medications and for effectiveness in the presence of adulterants. The OnLine II immunoassay is based on the kinetic inhibition of the agglutination reaction between antibody-coated microparticles and soluble drug-conjugate by free drug in a sample. Both EMIT II and CEDIA DAU assays are enzyme-based immunoassays.

For the present study, solutions of adulterants were independently prepared to give final morphine concentrations of 300 ng/mL or 2000 ng/mL. The adulterated samples were then analyzed to determine morphine recovery at the cutoff concentration. All three assays were unaffected by the designer adulterants Clear Choice, Urine Luck, and KLEAR (500 mg potassium nitrite). The assays were most affected by adulteration with household ingredients, showing under-recovery for Bleach, Drano, or 5% liquid hand soap solutions at both 300 and 2000 ng/mL cutoffs. Prescription medications which were tested included amitriptyline, clomipramine, desipramine, erythromycin, imipramine, mianserin, naloxone, naltrexone, ofloxacin, and promazine. Stock solutions of these medications were prepared at concentrations that produced responses closely below and above the 300 and 2000 ng/mL cutoff concentrations. CEDIA showed the highest cross-reactivity to prescription medications, with clomipramine recovering 7.7% for the 300 ng/mL cutoff and 11.1 % for the 2000 ng/mL cutoff. Overall, the OnLine II and EMIT assays were least affected by either prescription medications or commercial adulterants.

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**Key Words:** Opiates, Adulteration, Immunoassay



**Study of Heroin Metabolism Pattern in the Urines of Addicts**

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The purpose of this study is to identify the pattern and the range of individual variance of metabolism of the constituents in heroin by analyzing and monitoring the metabolite components from the urines of heroin addicts, so that the findings can be used as a forensic basis for differentiating heroin addicts.

The methods that were used in the study included (1) SPE/HPLC and (2) REMEDI HS. The method of SPE/HPLC was used to measure the pattern and variance of morphine and morphine-3-glucuronide (M-3-G) from the urines of heroin addicts. REMEDI HS was used to monitor the metabolism pattern of 6-monoacetyl-morphine (6-MAM), morphine and codeine.

The urine samples were collected from (1) sixty heroin addicts in detoxification treatment centers and (2) voluntary contribution of two drug abusers. The findings included (1) 6-MAM can only be detected in the urine of most abusers within 6 hours of ingestion, however it can be detected in the urine of heavy users up to 10 hours after ingestion, (2) there is a positive correlation of morphine and M-3-G with the urine excretion time, and (3) the heroin that is seized in this area contains an impurity of 6-monoacetylcodeine, and it matches well with the metabolite pattern in the urine among the arrested heroin addicts, including 6-MAM, morphine, codeine, N-dimethylmorphine and N-demethylcodeine.

This study indicated that the findings could provide a rapid, easy and accurate differentiation of heroin abusers from medical usage of morphine.

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Keywords: Heroin, SPE/HPLC, REMEDI HS

## Analysis of Biological Specimens for Opiates by Solvent Extraction and Derivatization with $d_6$ -Acetic Anhydride: Application to a Case of Morphine Poisoning

*Madeline Montgomery\**, *Rebecca A. Jufer* and *Marc A. LeBeau*. *Federal Bureau of Investigation Laboratory, Washington, DC*

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In a recent case submission to the FBI Laboratory, biological specimens were received from two critically ill hospital patients that were suspected to have died as a result of intentional fatal morphine administration. Both patients were administered morphine by the suspect, who was a nurse at the hospital where they were being cared for. Patient A survived for approximately one day following morphine administration, while Patient B died shortly after receiving morphine. Neither patient was reported to have received chronic morphine therapy. The specimens received were analyzed for opiates by solvent extraction followed by chemical derivatization and GC-MS analysis. Specimens that required analysis for "total" morphine were hydrolyzed with  $\beta$ -glucuronidase prior to solvent extraction.  $d_3$ -morphine was used as an internal standard. Following a three step solvent extraction, the final extract was evaporated to dryness under nitrogen at 50°C. The residue was reconstituted in 100 $\mu$ L pyridine and 100 $\mu$ L  $d_6$ -acetic anhydride and heated at 80°C for 45-60 minutes. After derivatization, the samples were evaporated to dryness then reconstituted in 10 $\mu$ L chloroform. Samples were analyzed by full scan electron ionization GC-MS. Both submitted cases were positive for morphine. The analytical results are tabulated below.

Matrix	Patient A Free/Total Morphine	Patient B Free/Total Morphine
Blood	0.07 / 0.34 $\mu$ g/mL	0.40/ 0.65 $\mu$ g/mL
Urine	NR*	NR
Liver	0.12 / 0.54 $\mu$ g/g	NR
Gastric Contents	0.19 $\mu$ g/mL / NP** (9.5 $\mu$ g total)	NR
Spinal Fluid	0.05 $\mu$ g/mL / NP**	NR
Spleen	0.13 / 0.39 $\mu$ g/g	NR
Kidney	0.09 / 0.30 $\mu$ g/g	NR
Heart	0.33 / 2.3 $\mu$ g/g	NR
Brain	0.11 / 0.22 $\mu$ g/g	NR
Lung	0.11 / 0.59 $\mu$ g/g	NR
Muscle	0.05 / 0.22 $\mu$ g/g	NR

\*NR = specimen not received \*\*NP = test not performed

These results were consistent with a lethal injection of morphine in Patient B. The results from Patient A were difficult to interpret due to the delay between morphine administration and the time of death. Ultimately, this case resulted in a confession from the nurse who was suspected of poisoning the patients.

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**A Retrospective Study of Oxycodone Monitoring in Pain Management**

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Oxycodone (oxy) is a synthetic opioid used for pain management for oncology and other patients. Cmax and trough concentrations for 5 mg q6h were and 7 and 7 ng/mL respectively. This study was concerned with the efficacy of oxy therapy and monitoring of enrolled patients in a pain management clinic. These patients were medicated with opioid analgesic which may or may not include oxy as part of the regimen. Daily dosages ranged from 10 to 250 mg. As part of the routine clinical management protocol, urinary drug screening was assigned on a routine, unannounced basis. Urine samples were collected clinically with supervision, but without direct observation (in the same clinic) and sent to a reference laboratory for drug screening by thin layer chromatography, and confirmation by gas chromatography/mass spectrometry - with cut-off limits of 300 ng/mL for both assays. This retrospective study, over a twelve months period, involved the analysis of 129 patients. Overall test sensitivity and specificity for both assays were 72.7 and 84.2%.

Groups	n	Sensitivity	Specificity:
≤20 mg	17	76.4	84.2
20-50 mg	26	76.9	84.2
50-150 mg	58	68.9	84.2
>150 mg	9	77.8	84.2

The persistent sensitivity of 68.9 to 77.8% may be indicative of non-compliance as well as possible oxy urinary concentrations below cut-off limits. This retrospective study indicated that urinary monitoring of oxy might be a useful patient management protocol along with patient history.

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Keywords: Oxycodone, pain management, urine screen and confirmation

**Detection of Illicit Heroin Markers in Urine with Liquid Chromatography - Atmospheric Pressure Chemical Ionization Mass Spectrometer**

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**Objective:**

Recent introduction of heroin prescription program in Germany created a need of differentiation between illicit and prescribed diamorphine use. Acetylcodeine (AC), is metabolites codeine (C) and codeine 6-glucuronide (C6G), papaverine (P) and noscapine (N) <sup>(1-3)</sup> were chosen as markers of illicit heroin. In order to check the diagnostic values of these markers, their occurrence of these drugs were investigated in urine samples of heroin addicts. Also, typical heroin markers: diamorphine (DAM) and its metabolites: monoacetylmorphine (MAM) and morphine (M) were determined.

**Material and Method:**

Urine samples collected from heroin abusers (road traffic offenders and overdosed patients) were examined. Drugs were extracted from urine samples using solid phase extraction (C18) using standard cartridges and 96-well microplates. Standard extraction procedure for basic drugs was applied <sup>(4)</sup>. The extracts were examined with LC-APCI-MS (positive ionization) in two isocratic systems. Selected ion monitoring procedures were applied for quasi-molecular ions and fragments of drugs involved.

**Results:**

The LOD's were in the range of 0.5-1 ng/ml urine. N and C were found in all urine extracts, followed by P and AC. This reflects the concentration of detected substances in illegal heroin samples on European market.

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**Validation of Fully Automated Microplate Enzyme Immunoassays for Opiates, Methamphetamine, Benzodiazepines, Benzoyllecgonine (BE), Phencyclidine (PCP) and Marijuana in Blood and Urine**

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A fully automated robotic analyzer (Tecan Genesis RMP, Tecan, NC) was used to perform microplate enzyme immunoassays for common drugs of abuse. The validation study involved rigorous investigation of the analytical limitations of the enzyme immunoassays and the instrumentation itself. Limits of detection, binding characteristics, sensitivity, specificity, intra and interassay precision, interferences and carryover were determined. The study included comparison of immunoassay and GC/MS results using forensic case samples.

Limits of detection for morphine, *d*-methamphetamine, nordiazepam, BE, PCP and THCA in blood and urine (in brackets) were <1 (<1), 2 (<1), 15 (4), 2 (<2), <1 (<1) and 6 (4) ng/mL respectively. Dose-response curves were used to estimate the binding efficiency of each of the assays. There were no significant differences in precision between blood and urine. Intraassay CVs (n=8) for morphine (10 ng/mL), *d*-methamphetamine (100 ng/mL), BE (150 ng/mL), nordiazepam (100 ng/mL), PCP (10 ng/mL) and THCA (30 ng/mL) in blood were 8.9, 3.5, 5.7, 9.8, 11.2 and 8.5%. Interassay CVs (n=8) were 19.0, 8.7, 6.5, 20.1, 12.4 and 11.8% respectively for assays performed on different days.

Confirmatory analyses using GC/MS allowed the sensitivity, specificity, positive and negative predictive value of each assay to be determined at different cut-off concentrations. Discordant results were investigated and additional cross-reactivity studies were performed for commonly interfering substances including ephedrine. Statistical tests were used to determine the limitations of the instrument in terms of carryover of drug from one sample to the next and to determine the extent of interference caused by common additives including fluoride, citrate, oxalate and EDTA.

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Keywords: Automation, Enzyme Immunoassay, Drugs

**Simultaneous Assay for  $\Delta^9$ -Tetrahydrocannabinol (THC), 11-Hydroxy- $\Delta^9$ -Tetrahydrocannabinol (11-OH-THC), and 11-Nor-9-Carboxy- $\Delta^9$ -Tetrahydrocannabinol (THCCOOH) in Human Urine by GC/MS**

*William D. Darwin\*, Abraham T. Wtsadik, Jonathan M. Oyler, Raf J. P. Schepers, and Marilyn A. Huestis. Intramural Research Program, NIDA, NIH, Baltimore, MD*

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THC is extensively metabolized in man to 11-OH-THC, THCCOOH, various glucuronides, and other metabolites. A simultaneous assay for multiple THC components has been difficult to achieve due to ineffective isolation techniques, the different physicochemical properties of THC and metabolites, and the complex matrix created by enzymatic hydrolysis. We developed a simultaneous assay for THC, 11-OH-THC, and THCCOOH that can be used with clinical urine specimens. Urine samples were hydrolyzed by two methods in series. The first hydrolysis utilized 5000 units of  $\beta$ -glucuronidase/mL of urine, Type IX-A bacterial from *E. coli*, with incubation at 37° C for 16 hrs. Base hydrolysis of the sample was then achieved with 80  $\mu$ L of 10N NaOH heated at 60° C for 15 min, neutralized with 50  $\mu$ L of glacial acetic acid, and adjusted to pH 6.8. After the addition of 3 mL of acetonitrile to precipitate the enzyme, vortexing, and centrifuging, the supernate was decanted into a clean tube. The 200-mg Clean-Thru<sup>®</sup> DAU solid phase extraction column was used to extract THC, 11-OH-THC, and THCCOOH from urine. The extraction conditions were as follows: add deuterated internal standards and sodium acetate buffer (pH 4.0, 2N); condition the columns with methanol and water; after the samples are introduced to the column, wash with water and 0.2N HCl; and elute with methylene chloride: 2-propanol: ammonium hydroxide (80:20:2) and hexane:ethyl acetate (80:20). The extracts were concentrated under a stream of nitrogen, derivatized with BSTFA (with 1% TMCS), and analyzed by GC/MS in the SIM mode. Extraction efficiencies were ~92% for all three analytes. Correlation coefficients of the calibration curves were ~0.98. Responses were linear across a concentration range of 2.5-100 ng of drug/mL of urine. The limit of detection (LOD) is 1.0 ng/mL for THC and 11-OH-THC and 2.5 ng/mL for THCCOOH. This assay is applicable to pharmacokinetic studies of THC and its metabolites in urine. Further refinement of this assay should make it applicable for analysis of THC and its metabolites in other biological matrices.

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Keywords: THC and metabolites, simultaneous assay, GC/MS.

**Development of a Rapid Screening Method for Cannabinoids in Post Mortem Blood**

*Rachel D. Fontenot\* and Amanda J. Jenkins. The Office of the Cuyahoga County Coroner, Cleveland, OH*

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The detection of marijuana exposure by assaying urine specimens is easily achieved with current immunoassay technology. However, detection of cannabinoids in post mortem blood is typically conducted by liquid-liquid or solid phase extraction followed by gas chromatographic mass spectrometric (GC/MS) analysis. This procedure is costly and time consuming. The use of a rapid reliable screening method for the detection of marijuana use in blood would provide savings in analyst time, resources and instrument use. The objective of this study was to develop such an assay for use in post mortem blood using the current immunoassay technology available in the laboratory.

Specimens were prepared by dilution with buffer at pH 4 followed by extraction with hexane/ethyl acetate (9:1). The organic layer was evaporated to dryness at 40°C under nitrogen. The extracts were reconstituted with EMIT® d.a.u.™ buffer, vortexed and assayed using an ETS Plus analyzer (Behring Diagnostics, Cupertino, CA) and EMIT® d.a.u.™ Cannabinoid 50 ng Assay (#3M019 50ng, lots L and M).

Initial method development involved determination of an assay cutoff by spiking drug free blood with THC-COOH to give concentrations of 1, 5, 10, 25, 50, 75, 100, 150 and 300 ng/ml. The assay was linear throughout this range with an R>0.99. A separation of ≥ 25 from the drug free blood was considered satisfactory for distinguishing positive and negative samples. This was achieved using a cutoff of 10 ng/ml. Concentrations of parent THC and 11-OH-THC metabolite at 50 ng/ml demonstrated no cross reactivity.

The procedure was initially validated by assaying 25 postmortem blood specimens by this procedure (using lot#L) and also by a GC/MS method with a limit of quantitation of 5 ng/ml for THC, 11-OH-THC and THC-COOH. Data analysis showed that 14 of the specimens were negative by both procedures and 9 specimens were positive by both procedures. There were 2 false positives and no false negatives. Additional case specimens were assayed to further validate the procedure but using a new lot of reagents (#M). The assay failed using this new lot as adequate separation between the drug free blood and cutoff calibrator was not achieved. It appeared there was an increase in the background response due to the blood matrix. No information was available from the manufacturer regarding possible changes in the reagent constituents. Development of the procedure continues in an attempt to explain and resolve this problem. Areas of investigation include additional pre-treatment of samples by filtration, centrifugation or organic extraction.

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Key Words: Immunoassay, Cannabinoids, Drug Screening

**A Rapid Instrumented Fluorescence Immunoassay for the Detection of Phencyclidine in Oral Fluid**

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A fluorescence immunoassay using a patented flow fluorescence immunoassay system for the detection of Phencyclidine (PCP) in oral fluid has been developed. The assay device consists of a micro-column filled with chromatography matrix coupled with mouse anti-PCP monoclonal antibody-PCP-cy5 complex. When a sample solution containing PCP flows through the micro-column, PCP in the sample solution displaces PCP-cy5 conjugate at the antibody binding site. The displaced PCP-cy5 conjugate produces a fluorescence signal in the solution, which is proportional to the quantity of PCP in the sample solution. The assay is conducted with an automated laminar flow fluorescence assay system developed at LifePoint, Inc.

The assay, with a limit of detection for saliva PCP of 5ng/ml, takes small sample volume (50µl) and a total reaction time of five minutes. The assay is ideally suited for use in on-site testing of saliva PCP by law enforcement, in the workplace, and emergency room.

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Keywords: Fluorescence immunoassay, Phencyclidine, oral fluid



**A Simple Method for Derivatizing the Surface of Silica Gel with Aldehyde Groups by Employing a New Alkoxy Aldehyde Silane**

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Attachments of biologically active molecules to solid supports have many practical applications such as analysis of drugs and metabolites in biological fluids, affinity chromatography, cell separation, diagnostics and molecular biology products. A common protocol for ligand attachment is activation of an amine-functionalized surface with glutaraldehyde, creating aldehyde groups. The aldehydes will then react readily with primary amines found on most ligand surfaces resulting in covalent immobilization. Typical problems of this protocol are: 1) glutaraldehyde is an unstable and difficult to purify compound; 2) the coupling forms two Schiff bases that are hydrolytically labile; 3) the process is labor intensive. A unique method for obtaining an aldehyde-functionalized surface while avoiding many problems of glutaraldehyde activation will be described. It is a simple procedure, which involves organically modifying silica gel with a triethoxy aldehyde silane, basic laboratory equipment and reagents. Alkoxy silanes under acidic conditions will react with the active surfaces found on most siliceous, glass and modified plastics. United Chemical Technologies, Inc. has introduced a new line of alkoxy silanes called BIO-CONEXT™ that have aldehyde groups at their termini. Reactive silanols generated in situ from hydrolysis of alkoxy silanes were covalently attached to the gel's surface to form a dense cross-linked aldehyde functionalized silicone polymer coating. Surface polymerization was confirmed by organic loading tests and results were in the vicinity of 8 percent. Protein A was then used as a model ligand and was successfully immobilized on the aldehyde functionalized silica gel. Protein A coating value was determined by monitoring the change in absorbance at 280nm. The activity and performance of the protein A support was confirmed with a whole molecule rabbit IgG-horseradish peroxidase enzyme assay.

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Keywords: Crosslinker, Immunoassay, Method

**Optimization of Drugs of Abuse Assays on the Abbott AEROSET<sup>®</sup> Clinical Chemistry Analyzer**

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Optimized abused drug assays for Methamphetamine/Amphetamine, Benzodiazepine, Cocaine Metabolite, Cannabinoids, Opiates, Methadone, Phencyclidine, Propoxyphene, and Barbiturates are being developed for the Abbott AEROSET<sup>®</sup> clinical chemistry analyzer. These assays use a homogeneous enzyme immunoassay method that is optimized for the AEROSET<sup>®</sup> analyzer. All reagents and calibrators are liquid, ready-to-use. These tests require a sample volume of 2-8  $\mu\text{L}$ , a R1 volume of 120 –140  $\mu\text{L}$ , and R2 volume of 50-60  $\mu\text{L}$ . These tests qualitatively determine drug concentrations in urine samples in less than ten minutes and are designed as screening assays. Performance was characterized by evaluating precision, onboard stability, and interference by testing endogenous substances or additives. Method comparison was performed against both the Syva 30R<sup>®</sup> and the Abbott AxSYM<sup>®</sup> analyzers. NCCLS protocols (EP5, EP6, EP7, and EP9) were followed. Within run precision for all assays yielded CVs less than 1.53%. Total precision for all assays yielded CVs less than 1.68 %.

These nine drugs of abuse assays, as adapted to the Abbott AEROSET<sup>®</sup> analyzer, provide accurate qualitative results, are not affected by significant levels of endogenous substances or additives, and compare well with the most common analytical methods for these analytes. These assays are acceptable for routine analysis by clinical and forensic laboratories.

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**Keywords:** Drug of Abuse, Immunoassay, Drug Screening

**A Liquid Chromatography Screening Method Using a Polymer Column with an Alkaline Mobile Phase for Detection of Drugs of Abuse**

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Basic drugs constitute the majority of drugs of abuse. They are a challenge for HPLC screening techniques due to interactions between charged amino groups and active sites on silica-based columns. These interactions lead to low column efficiency and asymmetrical peaks. Some reports have shown improved chromatographic performance when basic drugs were separated on polymer-based columns with alkaline mobile phases. Improved performance was observed due to both the use of a polymer-based packing and separation of the drugs in an unionized state. In this study, chromatographic performance was determined for 20 common drugs of abuse and their metabolites: morphine, codeine, methamphetamine, hydrocodone, 6-monoacetylmorphine, acetaminophen, benzoylecgonine, tramadol, heroin, cocaine, phenobarbital, butalbital, carbamazepine, nortriptyline, amitriptyline, alprazolam, methadone, lorazepam, clonazepam, and diazepam. First, a gradient with an acidic mobile phase, pH 3, was optimized for separation of the drug mixture on a silica-based column. The separation of the mixture was then optimized on a polymer column with an acidic mobile phase, pH 3, and finally, with an alkaline mobile phase adjusted to pH 12. The chromatographic performance of most of the drugs showed significant improvement on the polymer column at pH 12 that was evident by reduced peak width and asymmetry. Thus, the use of polymer-based columns with highly alkaline mobile phases may improve sensitivity and specificity of HPLC drug screening techniques.

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Keywords: drugs of abuse, drug screening, liquid chromatography

## Improved Liquid Chromatographic Resolution of Drugs on a Polymer Column with an Alkaline Mobile Phase

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The benefit of using polymer-based over silica-based columns for HPLC analysis of drugs was investigated. The chromatographic performance of six common drugs (amitriptyline, nortriptyline, diphenhydramine, verapamil, flunitrazepam, and diazepam) was determined on an octadecylsilyl (ODS) column over a pH range of 2 to 8, and an Asahipak ODP-50 polymer column over a pH range of 2 to 12. Column efficiency and peak asymmetry were determined. Basic drugs on the ODS column showed improvement in both asymmetry and efficiency at pH 2, followed by a decline at pH 4 and 6, and improvement again at pH 8. Whereas acidic drugs showed gradual improvement as the pH increased from 2 to 8. A similar pattern was observed on the polymer column for both basic and acid drugs of a pH range of 2 to 8. For all of the drugs, efficiency and asymmetry were higher on the ODS column compared to the polymer column at all pH levels. Over the pH range from 8 to 12, efficiency and asymmetry improved with increasing pH for all drugs on the polymer column. At pH 12, asymmetry ranged from 2.0 to 1.1 and efficiency ranged from an N of 2300 to 4700 for all six drugs. The values achieved on the ODS column for basic drugs at pH 2 were asymmetry from 3.4 to 1.7 and N from 1500 to 1800. Results for the acid drugs at pH 8 ranged from 2.5 to 2.4 for asymmetry and 2500 to 2700 for N. These results show a significant improvement of in chromatographic performance of both acidic and basic drugs on a polymer column with a highly alkaline mobile phase.

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Keywords: drugs of abuse, drug screening, liquid chromatography

**Detection of  $\Delta^9$ -THC in Human Saliva by GC/MS and A Continues Flow Immunoassay**

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The detection of  $\Delta^9$ -THC in human saliva was investigated with GC/MS and a flow immunoassay. The LifePoint™ flow immunoassay relies on the displacement of fluorescence labeled antigen by the corresponding antigen in the sample being tested. It can be expected that the interaction between  $\Delta^9$ -THC and chemical components in solution could affect both GC/MS and immunoassay sensitivity and specificity.

GC/MS results showed that salivary  $\Delta^9$ -THC exhibited quite different behavior from aqueous buffered  $\Delta^9$ -THC. Severe partitioning of  $\Delta^9$ -THC in saliva was observed while it was stored at 4°C overnight. Additionally, we have observed that polar solvents can significantly improve liquid extraction efficiency and recovery yield of  $\Delta^9$ -THC from saliva. An excellent linear calibration curve between 1ng/ml and 150ng/ml of  $\Delta^9$ -THC has been achieved using 1ml of saliva. Under optimum extraction conditions, the recovery yield of THC from saliva and 0.2%BSA/PBS was 61% and 84%, respectively.

Furthermore, buffer composition not only affects GC/MS assay of  $\Delta^9$ -THC, but also influences the flow immunoassay of  $\Delta^9$ -THC. Using automated LifePoint™ immunosensor combined with appropriate buffer composition, the lower detection limit for  $\Delta^9$ -THC in saliva is below 10ng/ml.

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Keywords: GC/MS, Drugs of abuse, Flow Immunoassay

**Side by Side Comparison of Microplate Enzyme Immunoassays for Opiates, Methamphetamine, Benzodiazepines, Benzoylcegonine (BE), Phencyclidine (PCP) and Marijuana in Blood and Urine**

*Sarah Kerrigan\*, and William H. Phillips. California Dept. of Justice, Toxicology Laboratory, Sacramento, CA*

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Microplate enzyme immunoassays for six commonly abused drug classes were compared in a side by side study. Analytical performance including binding characteristics, dose-response curves, limit of detection, intra and interassay precision, as well as performance of the assays using forensic case samples were investigated. All assays were performed manually. Dose-response curves using morphine, *d*-methamphetamine, BE, nordiazepam, PCP and *l*-11-nor- $\Delta^9$ -carboxy-tetrahydrocannabinol using Immunalysis assays indicated improved binding at low drug concentrations. These binding characteristics were consistent with lower limits of detection in blood and urine using Immunalysis assays. However, both intra and interassay CVs were substantially improved using STC assays. The principal differences in analytical performance were evident in terms of binding characteristics and precision: Immunalysis offered improved LOD's and STC offered improved precision.

A total of 855 forensic case samples were screened for opiates, methamphetamine, benzodiazepines, BE and marijuana using cut-off concentrations of 10, 100, 100, 150 and 30 ng/mL respectively. Average presumptive positive rates for all toxicological submissions decreased in the order marijuana (36%) > methamphetamine (33%) > opiates (21%) > benzoylcegonine (15%) > benzodiazepines (8%). The concordance of results using Immunalysis and STC assays was determined. Of the 855 samples in the study, there were a total of 92 discordant results (44 marijuana, 15 opiate, 15 methamphetamine, 11 benzodiazepines and 7 BE). The majority (96%) of these discordant results fell within 50% of the cut-off calibrator, reflecting differences in cross-reactivity between the assays. Investigation using GC/MS indicated a total of 4 unconfirmed positive results using Immunalysis (1 FP opiate, 1 FP marijuana, 1 FN marijuana) and STC (1 FN marijuana).

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Keywords: Enyme Immunoasay, Blood, Urine.

**Urinary Analytes that define the Limits of Renal Dilution**

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Verifying the validity of the random urine specimen submitted for workplace drugs of abuse analysis is a challenge for the forensic toxicologist. Determining whether the submitted specimen is consistent with human urine is best accomplished through the performance of specific laboratory tests (*e.g.*, creatinine, specific gravity and osmolality.)

This paper characterizes the dilutional limits of the kidneys through a review of urinary analyte data derived from published scientific literature. Relevant studies include normal random urine reference range studies, clinical studies involving the analysis of random urine specimens, theoretical dilutional limits, medical conditions resulting in overhydration and water loading studies.

Seventy references with case study data for urinary creatinine, specific gravity and/or osmolality are summarized. The lowest reported individual urinary results were creatinine - 4 mg/dL, specific gravity - 1.000 and osmolality - 18 mOsm/kg in the non-drug study population.

The data are consistent with urinary creatinine  $\leq 5.0$  mg/dL and specific gravity  $\leq 1.001$ , criteria defined by HHS/SAMHSA as characteristic of a specimen considered inconsistent with normal human urine.

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Keywords: Urine dilution, Workplace drug testing, Validity testing.

**Countering the Effect of Stealth Adulterant on the Analysis of Morphine and Codeine**

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Stealth is an adulterant used to avoid detection of drug use. The product does have a significant effect on the ability to detect several drugs, including the opiates morphine and codeine. At concentrations of morphine near the cutoff, a sample may test negative by immunoassay. At higher concentrations, the sample may screen positive and thus be sent to confirmation.

Two different urine samples were spiked with 6,000 ng/mL of both codeine and morphine glucuronide then split into separate aliquots. One sample had a pH of 5.4 and the other 7.0. One aliquot of each was adulterated with Stealth following package directions. The samples were then tested by immunoassay and GC/MS. The unadulterated and adulterated aliquots were positive by immunoassay. GC/MS analysis following standard procedures using deuterated internal standards proved to be unsuccessful. Neither the drugs nor internal standards were recovered from the Stealth adulterated samples despite repeated attempts, while the unadulterated aliquots posed no problem with recovery.

This effect was reversed by addition of 2.5 mg/mL sodium disulfite to the aliquots prior to extraction. Triplicate analysis of the samples showed the concentration of morphine and codeine decreased by 25 and 15% respectively in the pH 7.0 sample. The pH 5.4 sample saw reductions of 13 and 4% for morphine and codeine respectively. While these represent only two samples, it demonstrates that the addition of sodium disulfite allows recovery of drug and internal standard in Stealth adulterated samples that otherwise could not be properly assayed. Since these samples are likely to be immunoassay positive, it is important to consider this procedure as an option for samples that screen positive but internal standards can not be recovered for GC/MS analysis.

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Keywords: Adulteration, Stealth, Opiates



**Application of CEDIA Reagents for Drug Screening Dilute Urine Specimens**

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Water induced diuresis is perhaps the easiest and most common technique available to drug testing subjects to avoid positive test results. At the request of a client who was experiencing a significant quality of "dilute specimen" reports, a new analytical protocol was developed to identify dilute specimens that contained amounts of drugs/metabolites below regulatory cutoff concentrations.

This paper describes an analytical method to detect sub-threshold drug concentrations in diluted urine samples using CEDIA reagent on a Hitachi 717. Urine specimens having a creatine level of  $\leq 20$  mg/dL and a specific gravity of  $\leq 1.003$ , were subjected to sub-threshold initial data review and then rescreen according to the new method. CEDIA dilute cutoff concentrations have been established: cocaine metabolite – 15 ng/mL, codeine – 120 ng/mL, morphine - 120 ng/mL, phencyclidine – 5 ng/mL, oxazepam – 50 ng/mL, temazepam – 50 ng/mL and d-methamphetamine – 100 ng/mL.

For the last 5 months this new method has been run alongside of the GC/MS method. There have been 133 samples in this timeframe that have been creatine and specific gravity positive. Of these 133 samples there were 94 cocaine metabolite presumptive positives, 2 phencyclidine presumptive positives, 7 oxazepam or temazepam presumptive positives and 33 codeine or morphine presumptive positives. The agreement between the CEDIA screening and the GC/MS was as follows: 93% for cocaine metabolite, 85% for codeine or morphine, 100% for phencyclidine and 42% for oxazepam or temazepam.

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Keywords: creatine, dilute, CEDIA

**Chromate Adulteration in Employment Related Drug Screens**

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Adulteration of urine samples poses a challenge to all drug-testing laboratories. Samples adulterated with high concentrations of the commercial adulterant Urine Luck, may produce negative immunoassay screening results. GC/MSD confirmation assays for morphine and the marijuana metabolite, 11-nor- $\Delta^9$ -tetrahydrocannabinol-9-carboxylic acid (THCA), may also be affected in that the recovery of analyte and internal standard are decreased. The active ingredient in Urine Luck has been identified as pyridinium chlorochromate. However, the active ingredient may now be another chromate substance. Thus, chromium analysis is useful in identifying this adulterant in its various formulations. Using an atomic absorption spectrometer equipped with a graphite furnace, a method was developed to accurately quantitate the amount of chromium in samples that were suspected of being adulterated with Urine Luck. Because all samples are not routinely screened for chromate adulteration, the majority of the samples selected had screened positive for cannabinoids using Syva<sup>®</sup> EMIT reagents but the deuterated internal standard was not recovered when the samples were subjected to GC/MSD confirmations. In total, 57 samples (approximately 2% of all samples undergoing confirmations for THCA) were analyzed for their chromium content. The mean and median chromium concentrations of the samples analyzed were 357,076  $\mu\text{g/L}$  and 232,330  $\mu\text{g/L}$  respectively with values ranging from 82,150 to 1,182,400  $\mu\text{g/L}$ . All 57 samples were reported as adulterated. In addition, the mean pH value of these samples was 5.77 and ranged from 3.69 to 7.48. Correlation analysis revealed that urine sample pH was not a significant predictor of chromium concentration ( $r^2 = 0.0151$ ;  $p = 0.3625$ ). A vial of Urine Luck purchased commercially in July 1999 was found to have a pH value of less than 2.0 and had a chromium concentration of 1,314,800  $\mu\text{g/L}$ . The specificity and sensitivity of atomic absorption spectrometry makes it a valuable tool as a confirmation procedure for the detection of chromate adulteration.

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Keywords: Adulteration, Cannabinoids, Chromium

**Development of Peroxidase-Detect™ Test for Automated Chemistry Analyzers to Screen Urine Samples Adulterated with STEALTH™**

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Chemical agents such as Klear™ and Urine Luck™ have gained popularity as adulterants because of their effectiveness as oxidizing agents. STEALTH™ adulterant is an oxidizing agent and is sold as a package containing two vials, a powder 'catalyst' and a liquid 'activator'. The powder has been identified as Horseradish Peroxidase (HRP) and the liquid as Hydrogen Peroxide (unpublished results by Microgenics Corp.).

The objective of this study was to develop a test to detect peroxidase-containing adulterants like STEALTH in urine samples. HRP catalyzes hydrogen peroxide oxidation by transferring electrons from a donor to the peroxide. The donors can be dye substrates, which yield colored products or oxidizable drugs such as THC. Based on this principle we have developed a Peroxidase-Detect test to determine adulteration of urine samples by STEALTH. The Peroxidase-Detect test kit contains ready-to-use liquid reagents containing TMB as substrate and calibrators (negative and 100 ng/mL peroxidase). The performance data were collected on the Hitachi 717 analyzer. The within-run and between-run CVs for the low (50 ng/mL) and high (200 ng/mL) controls range from 1.0 to 1.7%. The test has linearity ranging from 10 to 1500 ng/mL and the Limit of Detection (LOD) is 4.1 ng/mL. No significant interference was observed with endogenous substances such as hemoglobin and bilirubin except for ascorbic acid at 4 mg/dL. Accuracy of the test was determined by spike recovery (91.3-103.2%) and method comparison with HPLC.

The Peroxidase-Detect Test is a simple and specific method, which can be applied to most automated chemistry analyzers.

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Keywords: STEALTH™, Peroxidase, TMB

**Evaluation of Adulteration Effects on OnLine, EMIT II, FPIA, and CEDIA DAT Technologies**

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The addition of adulterants in an attempt to mask the presence of drug, can produce different effects in different Drugs of Abuse Testing (DAT) assay systems. The adulterants themselves can be classified into two groups. "Classic" adulterants can be defined as consumer products (eg., soaps, bleach, vinegar, etc.) commercially available and often found in the locations urine specimens are procured. In contrast, "designer" adulterants can be defined as chemicals or chemical combinations specifically formulated to interfere with the drug testing assays, or to destroy the drug or drug metabolites in situ. These are most commonly available from the Internet sites or from periodicals popular within the subculture of drug users. Less is known about the comparative efficacy of these "designer" adulterants on the assay systems commonly used in today's high volume DAT laboratories. This study was designed to observe the effects of both types of adulterants on the predominant assay systems in use today at high volume DAT laboratories.

The information contained in this Report describes the findings from 12 "classic" and 5 "designer" adulterants. OnLine reagents produced, or tied, the fewest adulteration effects in seven of the ten cases. FPIA was the best, or tied for best, in four cases. CEDIA was best, or tied for best, in only one case. EMIT II fared the best in no case. THC, opiate and LSD were the assays to most frequently show adulteration effects across all methodologies.

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Keywords: Adulteration, Screening Immunoassays, Method Comparison

Long Term Stability of Alcohol in Blood and Urine

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Blood (with preservatives) and unpreserved urines were spiked with ethanol and stored refrigerated at 4°C. Samples were analyzed up to 7 years for blood and 11 months for urines by Gas Chromatography (GC). Ethanol concentrations ranged from 24 to 325 mg/dL for blood and 25 to 300 mg/dL for urine. Least squares analysis of spiked blood values (x), in comparison to the first analysis (y), gave a slope (m) of 0.964, y-intercept (b) of -2.27 and correlation coefficient (r) of 0.99 ( $y = 0.96x - 2.27$ ,  $r = 0.99$ ). Retesting of blood samples over a 7 year period gave the following results:  $y = 0.64x - 21.6$ ,  $r = 0.66$ . Least squares analysis of urine samples gave the following results where (x) is the expected concentration and (y) is the found concentration. At six months,  $y = 0.83x + 8.84$ ,  $r = 1.0$ , at seven months,  $y = 0.84x + 5.1$ ,  $r = 0.98$ , and at eleven months,  $y = 0.88x + 5.5$ ,  $r = 1.0$ . No statistically significant differences were observed in urines stored for eleven months. Analytical differences were observed in blood samples, which are more than 2 years old. Stability of alcohol samples stored at 4°C is less than 2 years for blood and 11 months or more for urine.

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Keywords: stability alcohol, blood, urine

## Influence of Sodium Fluoride (NaF) Concentration on Quantitative Analysis of Ethanol by Headspace Gas Chromatography (HSGC) in Urine Samples

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Presence of high concentrations of NaF in blood and aqueous solutions containing ethanol is one of several factors believed to adversely affect the accuracy of measurement and interpretation of alcohol results. Minnesota State urine evidence collection kits include a 100 mL plastic bottle containing 1000 mg NaF. Evidentiary urine samples collected in these bottles vary from 1 mL to 100 mL. Accordingly, the NaF concentrations in these samples would vary from 10-1000 mg/mL. Whether such variation in NaF concentrations affects the quantitative analysis of ethanol in urine samples was examined. Aqueous solutions containing known concentrations of ethanol diluted (1:5) in *n*-propanol internal standard solutions containing zero or 30 mg/mL NaF and subjected to quantitative analysis of ethanol by HSGC resulted in identical measurements of alcohol concentrations. Further, urine samples containing known concentrations of ethanol first treated with 0–200 mg/mL NaF, incubated at room temperature for up to 336 hr and then the liquid phase of such urine samples subjected to quantitative analysis of ethanol by HSGC, lead to the following observations. 1) Ethanol concentrations measured in the urine samples treated with low concentrations of NaF (<20 mg/mL) were identical to those in urine samples not treated with NaF, irrespective of the length of incubation at room temperature. 2) Ethanol concentrations measured in the urine sample treated with high concentrations of NaF (>40 mg/mL) were significantly lower ( $p \leq 0.004$ ) than those in urine samples treated with 0–20 mg/mL NaF irrespective of the length of incubation at room temperature. 3) Irrespective of the NaF concentration in the urine sample the quantitative analysis of ethanol in the liquid phase as per the HSGC procedure employed was unaffected. Thus, the reduction in ethanol concentrations measured in urine samples containing >40 mg/mL NaF was due to salting-out effected during their incubation at room temperature.

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Keywords: Ethanol, Salting-out effect, Sodium fluoride.

**Performance of the Microgenics Ethyl Alcohol Assay on the Boehringer Mannheim Hitachi 717 Analyzer**

*Weixing Luo, Amoolya Peapally, Lakshmi Anne\*. Microgenics Corporation, Fremont, CA*

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The objective of this study was to evaluate the performance of the Microgenics Ethyl Alcohol Assay (EtOH) on the Hitachi 717. This Assay has been successfully applied to other automated chemistry analyzers such as Olympus AU800, Hitachi 747, and Hitachi 911. The assay consists of stable, ready-to-use liquid reagents and calibrators. The assay principle is based on the high specificity of Alcohol Dehydrogenase (ADH) for ethyl alcohol. In the presence of ADH and nicotinamide (NAD), ethyl alcohol is oxidized to acetaldehyde and NADH. The conversion of NAD to NADH results in an absorbance change, which can be measured at 340 nm.

The intended use of this assay is for the quantitation of ethyl alcohol in urine, plasma, serum and treated whole blood. The within-run and between-run precision CV's for controls at 50 mg/dl, 75 mg/dL and 300 mg/dL is <2.0%. The assay offers an analytical range of 5-1400 mg/dL. Recovery of Ethanol spiked samples ranged from 93 to 102%. The minimum detection limit is 0.44 mg/dL with 95% confidence. The assay demonstrated 30 days on-board reagent stability and calibration stability. The reagents are stable for over two years at 2-8°C. No significant interference was observed with various structurally related compounds such as isopropanol, methanol, endogenous substances such as hemoglobin and bilirubin, and commonly used anticoagulants. Method comparison with a commercially available enzymatic method using urine/serum samples gave excellent correlation ( $y=1.02x+0.854$ ,  $r=0.999$ ).

In conclusion, the Microgenics Ethyl Alcohol Assay is a convenient, precise and reliable method for the determination of ethyl alcohol in urine, plasma, serum and treated whole blood samples.

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Keywords: Ethyl Alcohol, ADH, NAD

### Comparison of Ethyl Alcohol Concentrations in Simultaneously Collected Blood, Breath and Urine Samples: A Field Study

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We have previously reported the comparison of post-absorptive phase alcohol concentrations measured in breath, blood and urine samples collected simultaneously and under controlled conditions (Sreerama, et. al., *Proc. Am. Acad. Forensic Sci.*, 6:280-281, 2000). This study compares alcohol concentrations in these three media on subjects under actual field conditions. Annually ~30,000 evidentiary breath tests - Intoxilyzer 5000EN - are administered to subjects suspected to be under the influence of alcohol. Blood and/or urine samples are some times collected because of breath test refusals, breath test interferences and/or suspicion of drug use. We have reviewed breath, blood and urine alcohol test records collected from April 1997-April 2000 and found 265 subjects from whom breath, blood and/or urine samples were collected within 30 min of each other. Among the selected subjects 217 were males and 48 were females with mean±SD (range) age of 34.1±11.6 (17-67), and 35±10.5(18-62), years, respectively. The mean±SD and median (range) alcohol concentrations in breath (n=250), blood (n=103) and urine (n=177) samples were 0.17±0.05 and 0.17 (0.04-0.36), g/210L, 0.18±0.06 and 0.18 (0.07-0.37), g/100 mL and 0.16±0.05 and 0.15 (0.04-0.29), g/67 mL, respectively. The ratios, mean±SD and median (range; n), of alcohol concentrations of blood/breath, urine/breath and urine/blood were 1.03±0.08 and 1.03 (0.87-1.26; 88), 0.95±0.09 and 0.95 (0.71-1.41; 162), and 0.90±0.11 and 0.90 (0.71-1.08; 15), respectively. Regression analysis revealed a strong correlation between breath and blood ( $r^2=0.95$ ; slope =1.03), urine and breath ( $r^2=91$ ; slope=0.94), and urine and blood ( $r^2=91$ ; slope=0.86), alcohol concentrations. As in the previous study, data presented herein clearly demonstrates that the differences in alcohol concentrations between breath and blood are minimal. However, the urine alcohol concentrations were lower than those of the breath or blood.

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Keywords: Intoxilyzer 5000EN, Ethyl Alcohol and Breath.



**Amphetamine, Clobenzorex and 4-hydroxyclobenzorex Levels Following Multi-Dose Administration of Clobenzorex**

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Clobenzorex (Asenlix) is an anorectic drug used as part of a weight management program. The drug is metabolized by the body to amphetamine, thus causing difficulty in interpretation of amphetamine positive drug tests. Previous studies have shown the parent drug and several metabolites are excreted in urine. Clobenzorex itself has been detected for as long as 29 hours following administration of a single dose. However, the parent drug was not always detected in samples that contained amphetamine at  $\geq 500$  ng/mL, the administrative cutoff for a positive amphetamine result. Consequently, the parent compound clobenzorex is not ideal for ascertaining if the drug was the origin of the amphetamine positive result. In a previous study of urine samples provided following administration of a single 30 mg dose of this drug, 4-hydroxyclobenzorex could be detected in all samples that were positive for amphetamine.

The present study extends that work to assess the presence of the metabolite following repeated administration of clobenzorex. Samples from a controlled multi-dose administration (30 mg of clobenzorex daily for seven days) were assayed for the presence of 4-hydroxyclobenzorex. The analytical procedure used acid hydrolysis followed by liquid-liquid extraction and GC/MS analysis with monitoring of ions at m/z 125, 330 and 364 for 4-hydroxyclobenzorex and its 3-Cl regioisomer which was used as the internal standard. Peak concentrations of 4-hydroxyclobenzorex ranged from approximately 17,786 – 99,044 ng/mL. Most importantly, however, this study showed all samples that contained amphetamine at  $\geq 500$  ng/mL also contained detectable amounts of this hydroxy metabolite (LOD 10 ng/mL) making it a valuable tool in differentiating use of clobenzorex from that of amphetamine.

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Keywords: Amphetamines, Clobenzorex, 4-hydroxyclobenzorex

## Optimizing Sympathomimetic Amine Analysis using a Dual Derivatization Technique

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The analysis of amphetamine, methamphetamine and other related sympathomimetic amines can be complicated by the presence of illicit drugs and over the counter medications that have similar chemical structures. Testing for these compounds is routinely performed after derivatizing reactive amine or hydroxyl functional groups in the molecule.

Typically, acid anhydrides like HFBA have been used as derivatizing reagents to form fluoroacyl derivatives of the amine functional groups. But HFBA exhibits very little capacity for derivatizing any hydroxyl groups in the same molecule. Heptafluorobutyryl derivatives can also be formed using the imidazole derivatizing reagent HFBI. Trimethylsilyl derivatives of the hydroxyl functional group can also be prepared using another imidazole reagent, TMSI. When used in the sequence of TMSI first followed by HFBI, selective derivatization of the hydroxyl group with a trimethylsilyl group is followed by derivatization of the amine group with a heptafluorobutyryl group.

The resulting chromatographic performance and mass spectral data yield significantly different performance for compounds that contain a reactive hydroxyl functional group. Ephedrine, pseudoephedrine, phenylpropanolamine and phenylephrine all exhibit longer retention times that can be used to improve their resolution from other sympathomimetic amines. In addition, unique high mass fragments are produced with this derivatization protocol that assist in positively identifying these compounds.

Since derivatization with imidazole reagents can be accomplished at room temperature, a simple reconstitution/derivatization procedure will be presented along with retention time data for derivatized compounds on two stationary phases. Mass spectral data of dual derivatized compounds will also be shown along with proposed fragmentation patterns.

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Keywords: Amphetamines, Derivatization, GC/MS

**CEDIA® Ecstasy Drugs Assay for Urine Drug Testing**

*Bob T. Ramage, Jeff E. Shindelman\*, Mike Leos, Riaz Rhohani, Rao Kammula, William A. Coty and Yuh-Geng Tsay. Microgenics Corporation, Fremont, CA.*

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We have developed a homogeneous enzyme immunoassay for Ecstasy drugs based on the CEDIA® technology. The name Ecstasy drugs is commonly used to identify a group of amphetamine analogues that include MDA, MDMA and MDEA. The current method of detection for these drugs is use of commercial amphetamines immunoassays which are relatively insensitive and non-specific. The CEDIA Ecstasy assay allows specific detection of Ecstasy drugs while maintaining sensitivity with a cutoff of 300 ng/mL of MDMA.

In CEDIA technology, the enzyme  $\beta$ -galactosidase is split into two inactive fragments: a large fragment (EA) and a smaller (ED), which can recombine to form active enzyme. Analyte is covalently attached to the ED molecule and enzyme formation can be inhibited by a highly specific monoclonal antibody to the analyte. Analyte present in a sample competes with the conjugate for binding to the antibody. Thus the amount of enzyme formed is proportional to the analyte concentration.

The CEDIA Ecstasy assay is sensitive to a variety of Ecstasy drugs. The following cross-reactivity at cutoff was found: MDMA (100%), MDEA (110%), MDA (72%), MBDB (35%), and BDB (52%). The assay is specific with little cross-reactivity to other critical cross-reactants: amphetamine (0.05%), methamphetamine (0.06%), ephedrine (<0.01%), pseudoephedrine (<0.01%), and phenylpropanolamine (<0.01%).

Studies to date have been performed on the Hitachi 911 and Hitachi 717 analyzers. A group of samples (n=48) containing either MDA, MDMA or MDEA were evaluated using the Ecstasy assay. All 48 of the samples positive for Ecstasy by REMEDI also tested positive by the CEDIA assay. In a separate study, negative urine samples (n=443) for Ecstasy drugs were evaluated. The CEDIA Ecstasy correctly evaluated all 443 samples.

Thus the CEDIA Ecstasy assay is a convenient and effective method for the detection of Ecstasy drugs in urine. The assay can be applied to high throughput automated analyzers, and will improve the sensitivity for detection of Ecstasy drugs.

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**Keywords:** MDMA, Ecstasy Drugs, Immunoassay

**CEDIA® Amphetamines Assay with Improved Recognition of Ecstasy Drugs for Urine Drug Testing**

*Bob T. Ramage, Jeff E. Shindelman\*, Mike Leos, Riaz Rhouhani, Rao Kammula, William A. Coty and Yuh-Geng Tsay. Microgenics Corporation, Fremont, CA*

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Commercially available assays for Amphetamines have limited recognition of the Ecstasy drugs. The CEDIA Amphetamines assay has been improved to include recognition of all Ecstasy drugs with over 100% cross-reactivity for the more commonly abused MDMA and MDEA. Recognition of amphetamine and methamphetamine will remain the same at 100%.

In CEDIA technology, the enzyme  $\beta$ -galactosidase is split into two inactive fragments: a large fragment (EA) and a smaller (ED), which can recombine to form active enzyme. Analyte is covalently attached to the ED molecule so that enzyme formation is not affected; however, binding of the conjugate by a highly specific monoclonal antibody to the analyte inhibits re-association of the enzyme fragments. Analyte present in a calibrator or sample competes with the conjugate for binding to the antibody. Thus the amount of enzyme formed is proportional to the analyte concentration.

Studies to date have been performed on the Hitachi 911, Hitachi 717, and Olympus 640 analyzers. The improved CEDIA Amphetamines assay uses a 500 ng/mL methamphetamine cutoff calibrator and has a range of 0 to 3000 ng/mL. The assay has enhanced sensitivity to a variety of Ecstasy drugs. The following cross-reactivity at cutoff was found: MDMA (147%), MDEA (167%), MDA (100%), MBDB (105%), BDB (123%).

A group of samples (n=48) containing either MDA, MDMA or MDEA were evaluated. All 48 of the samples positive for Ecstasy drugs by REMEDI also tested positive in the improved CEDIA Amphetamines assay. In a separate study, negative urine samples (n=1170) for either amphetamines or Ecstasy drugs were evaluated. The improved CEDIA Amphetamines correctly identified 1166/1170 samples compare to 1165/1170 for the current CEDIA Amphetamines assay.

Thus the improved CEDIA Amphetamines assay is a convenient and effective method for the detection of both Amphetamines and Ecstasy drugs in urine. The assay can be applied to high throughput automated analyzers, and will improve the sensitivity for detection of Ecstasy drugs.

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**Keywords:** Ecstasy Drugs, Amphetamines, Immunoassay

**OnTrak TesTstik Methamphetamine Assay for On-Site Urine Drug Testing**

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OnTrak TesTstik for Methamphetamine is a rapid, onsite immunoassay intended for the qualitative detection for methamphetamine at a 500 ng/mL cutoff level. In this presentation we report the evaluation of 3 lots of the OnTrak TesTstik Methamphetamine devices. Assay precision was determined using urine standards containing six drug concentrations. For each standard level, 20 replicates were tested each day for 5 days. A total of 1800 TesTstiks were interpreted by 3 readers, blind as to the origin of the samples. For all lots evaluated, 100% correlation was observed with drugs at 125 ng/ml, 250 ng/ml, 625 ng/ml and 750 ng/ml. The validity of the TesTstik Methamphetamine test result over a period of time was assessed by reading the results at 0, 30, and 60 minutes after the test was completed. Clinical performance of the TesTstik Methamphetamine assay was assessed using 70 positive specimens (GC/MS-confirmed according to the 200/500 ng/ml amphetamine/methamphetamine guideline) and 135 specimens that had been screened negative at a clinical laboratory. The specimens were also tested with Syva Emit II assay. The Emit calibrators (d-methamphetamine) were reconstituted following the manufacturer's instruction. The 500 ng/mL calibrator was prepared to serve as a customized cutoff reference for distinguishing "positive" from "negative" samples. The clinical correlation for all the positive and negative specimens was 97%. The approximate percent cross-reactivity of structurally related and unrelated compounds was expressed as the amount of the compound capable of producing of result equivalent to the specific assay cutoff. For the 3 lots evaluated, the range of cross-reactivity was as follows: d-amphetamine (1-1.5 %), l-methamphetamine (14.3-16.7%) and MDMA (33.3-50%). Stability studies demonstrated that the OnTrak TesTstik Methamphetamine remained stable for six months at 45°C and 55°C. In summary, the OnTrak TesTstik assay can serve as a useful tool for onsite screening of methamphetamine abuse.

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Keywords: Onsite Immunoassay, TesTstik, Methamphetamine

**Case Report: Postmortem Tissue Distribution of Gamma Hydroxybutyrate (GHB) and Gamma Butyrolactone (GBL) in a Single Fatality**

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Gamma Hydroxybutyrate (GHB) is a drug that is currently abused by the RAVE crowd and has been implicated in drug facilitated rape. Over the past several years, the Los Angeles County Coroner's Toxicology Laboratory has analyzed biological specimens for GHB. To date, our laboratory has had only one case in which GHB was implicated as the sole cause of death. Tissue distribution for both GHB and GBL were determined in this case and the results are presented here.

The analysis of GHB from postmortem specimens (0.50 milliliters or grams sample size) consisted of a two part sampling and extraction process utilizing chloroform. The first extraction was for GHB, isolated and identified as GBL after acid hydrolysis ('total GBL'). The second extraction was for gamma butyrolactone (GBL) from an unhydrolyzed sample ('intact GBL'). The difference between the 'total' GBL and 'intact' GBL levels were reportable GHB concentrations. Identification and quantitation of GBL was on a gas chromatograph-mass spectrometer using the selected ion monitoring (SIM) mode. The GBL ions monitored were **42**, 56, and 86 m/Z and the internal standard, gamma valerolactone (GVL), were **56**, 41, and 85 m/Z (quantitation ion in **bold**). The linear range of the intact GBL and total GBL assays were 1.0 mg/L to 20 mg/L and 2.0 mg/L to 50 mg/L, respectively.

The postmortem tissue distribution of GHB in the single fatality was: Heart blood 1,473 mg/L, femoral blood 761 mg/L, vitreous 771 mg/L, liver 1,578 mg/kg, bile 1,440 mg/L, urine 407 mg/L, and gastric 0.78 g total. The cause of death was GHB toxicity and the mode of death was ruled an accident.

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Keywords: Gamma Hydroxybutyrate, Fatality, Tissue Distribution

**GHB Overdose Case: Examination of Blood, Brain and Hair**

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Blood, brain and hair from a suspected gamma-hydroxybutyrate overdose case were received for analysis. Samples were extracted using solid phase extraction columns (Worldwide Monitoring ZSGHB020) with methanol/ammonium hydroxide (99/1) as the elution solvent. Sample clean-up was performed using dimethylformamide in hexane. Samples were derivatized with bis(trimethyl-silyl)-trifluoroacetamide with 1% trimethylchlorosilane. Analysis was performed using an ion-trap GC/MS (Varian Saturn 3) monitoring ions 233, 234, and 235.

The submitting unit had found 648 ng/ul of GHB in the heart blood. Our determination of the peripheral blood found 330 ng/ul GHB. Control postmortem blood contained 15 ng/ul GHB (avg, n=10) and control antemortem blood contained 2 ng/ul of GHB (avg, n=5).

Frontal cortex brain contained 221ng/mg GHB. Control brain had non-quantitatable amounts of GHB.

The exposed head hair had no detectable amounts of GHB but the hair root bulbs had considerable amounts of drug. The plucked root bulbs with the outer root sheath attached contained 2,221ng/mg GHB and after washing the root bulb with 1% sodium dodecyl sulfate and water, the outer root sheath was removed and the root bulb analysis yielded 47.4 ng/mg GHB.

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Keywords: GHB, blood/brain/hair, GC/MS

### A Death from Ingestion of 1,4-Butanediol, a GHB Precursor

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Gamma hydroxybutyrate (GHB) is a central nervous system depressant with effects that include sedation, dizziness, nausea, seizures, respiratory depression and coma. While the sale of GHB has been banned, several products are available that contain 1,4-Butanediol (BD) which is converted *in vivo* to GHB. Recently a healthy forty-year-old female purchased several bottles of a commercially available product containing BD and began using the product on a regular basis. However, one morning her husband found her unresponsive on the couch with foam present around the nose and mouth. She was declared dead at the scene by medical personnel. At autopsy, the pulmonary parenchyma was soft, spongy, dark purple to black, severely congested and edematous. Routine toxicology screening revealed only the presence of caffeine and nicotine metabolite. No alcohol was found in either the blood, urine, or vitreous fluid. GHB and BD were assayed by GC/MS.

	Blood	Vitreous	Urine	Bile
1,4-Butanediol	7.6 µg/mL	12.3 µg/mL	146 µg/mL	ND
<b>GHB</b>	280 µg/mL	324 µg/mL	6171 µg/mL	218 µg/mL

An open container of the commercial product at the scene was analyzed by GC/MS and found to contain BD with no GHB detected. The blood GHB level is consistent with other GHB-related fatalities. Here we report for the first time a GHB fatality occurring from the apparent ingestion of the GHB precursor, 1,4-Butanediol.

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Keywords: 1,4-Butanediol, GHB, Overdose.



## Performance Characteristics of 2H5- and 13C4-Butalbital in Serving as Internal Standards for the Quantitation of Butalbital

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As part of a broader study on various aspects pertinent to the use of isotopic analogs as internal standards (IS), this report presents some performance characteristics of 2H5- and 13C4-bitalbital in serving as the IS for the quantitation of butalbital. Butalbital/2H5-bitalbital and butalbital/13C4-bitalbital pairs are evaluated to determine (a) the extent of cross-contribution of these isotopic analogs to the intensities of ions designated for the counter-analog in the pair; (b) the effects of cross-contribution and other factors on the effectiveness of these two labeled analogs in serving as IS.

Standard solid-phase extraction and methylation procedures are used prior to selected ion monitoring GC-MS analyses. A "standard addition" procedure is used to evaluate the cross-contribution data. Observed butalbital concentrations in a series of standard solutions are calculated using ion pairs with various degrees of cross-contribution. Deviations from their respective theoretical values are compared to inter-compound (butalbital vs. 2H5-bitalbital vs. 13C4-bitalbital) and inter-system (butalbital/2H5-bitalbital vs. butalbital/13C4-bitalbital) cross-contribution data. Changes in intra-molecular ion intensity ratios in a series of standard solutions are also examined to determine these ions' relative susceptibilities to cross contribution caused by the presence of the counter-analog.

Both labeled analogs cause more significant cross-contribution to ions designated for butalbital than butalbital to the labeled analogs. Compared to 2H5-bitalbital, 13C4-bitalbital appears to cause less cross-contributions to ions designated for butalbital. Cross-contributions between the following ion pairs are minimal and can be used effectively for quantitation: m/z 200/196, 199/195, 185/181 (13C4-bitalbital as IS); m/z 201/196 (2H5-bitalbital as IS). Ion pair intensity ratios for the butalbital/2H5-bitalbital system increase as the volume of the solvent used to reconstitute the extraction-derivatization product is increased. This phenomenon is not observed for the butalbital/13C4-bitalbital system.

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Keywords: Quantitation, Internal Standard, Butalbital.

## Chromium (VI) Reduction Mediated by Cytochrome *b*<sub>5</sub> in Human Hepatic Microsomes Leads to Hydroxyl Radical Formation and Oxidative Damage

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The reduction of chromium(VI) to Cr(III), via reactive intermediates, is a key component in the cytotoxicity, genotoxicity, and carcinogenicity of Cr(VI) compounds. In order to better understand Cr(VI) toxicity, our lab is examining the roles of human microsomal enzymes in the reductive transformation of Cr(VI). The NADPH-dependent Cr(VI) reduction rate more than doubled when purified recombinant human cytochrome *b*<sub>5</sub> was added to human microsomes. This increase could not be attributed to cytochrome *b*<sub>5</sub> acting alone, thereby implicating cooperation between cytochrome *b*<sub>5</sub> and its natural electron donor (P450 reductase). To address the role of cytochrome *b*<sub>5</sub> in NADPH-dependent Cr(VI) reduction, proteoliposomes containing cytochrome *b*<sub>5</sub> plus P450 reductase were constructed. Using this reconstituted system, the NADPH-dependent Cr(VI) reduction rate was compared to that of human microsomes. When these rates were normalized to equivalent cytochrome *b*<sub>5</sub> concentrations, the NADPH-dependent Cr(VI) reduction rate mediated by human microsomes was essentially identical to that of proteoliposomes containing cytochrome *b*<sub>5</sub> plus P450 reductase. Proteoliposomes containing only P450 reductase or cytochrome *b*<sub>5</sub> exhibited poor Cr(VI)-reducing activity.

Electron paramagnetic resonance (EPR) measurements provided evidence for the formation of transient Cr(V) intermediates and hydroxyl radicals during the reduction of Cr(VI) by proteoliposomes containing cytochrome *b*<sub>5</sub> plus P450 reductase. The hydroxyl radicals were detected by EPR spin trapping using 5-Diethoxyphosphoryl-5-methyl-1-pyrroline-*N*-oxide (DEPMPO) as a spin trap. In addition, the levels of Cr(VI)-induced oxidative stress were evaluated by malonaldehyde (MA) formation. Increased levels of MA were detected by HPLC during human microsomal Cr(VI) reduction. Overall, these studies show that cytochrome *b*<sub>5</sub>, in cooperation with P450 reductase, likely accounts for the majority of the NADPH-dependent Cr(VI) reduction seen in human microsomes. The reduction of Cr(VI) by cytochrome *b*<sub>5</sub> also leads to hydroxyl radical formation and lipid peroxidation which may play a significant role in the pathogenesis and toxicity associated with exposure to Cr(VI).

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Keywords: Chromium, Cytochrome *b*<sub>5</sub>, Lipid peroxidation.

**Citalopram Distribution in Postmortem Cases**

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Citalopram is a highly selective serotonin reuptake inhibitor used therapeutically as an antidepressant. This is a report of the analytical findings in 13 cases investigated by either the Office of the Chief Medical Examiner, State of Maryland or the Bexar County Medical Examiner's Office where citalopram was identified. Citalopram was quantitated in fluid or tissue specimens by gas chromatography and confirmed by full scan electron ionization gas chromatography/mass spectrometry.

In 8 of the 9 cases in which both blood and urine specimens were received, the urine citalopram concentration exceeded the blood concentration, indicating that urine is an appropriate specimen for screening citalopram use. The average liver to blood citalopram concentration ratio was 6.5 (range 3.1-13, n=6). A higher concentration of drug in the liver is consistent with most antidepressants.

Three cases had blood concentrations less than 0.24 mg/L, which is in the reported antemortem therapeutic range of the drug. Eleven cases had blood concentrations less than 1.3 mg/L; in each of these cases, citalopram was determined to be an incidental finding to the ultimate cause of death. Quantitation of citalopram and the metabolite desmethylcitalopram in these cases yielded an average parent to metabolite ratio of 6.4. The higher concentration of parent in relation to the metabolite in these "therapeutic" postmortem cases is consistent with data collected with clinical blood specimens.

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Keywords: citalopram, postmortem, selective serotonin reuptake inhibitors

### Distribution of Citalopram in Postmortem Tissues

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Citalopram, a second generation antidepressant, is a selective serotonin reuptake inhibitor. Due to limited information about citalopram in postmortem specimens, we found it of interest to investigate the distribution of the drug in two postmortem cases.

Citalopram was extracted from various postmortem by alkaline extraction at pH 9 and back extraction into an organic solvent. The organic phase was dried under nitrogen and reconstituted into ethyl acetate for quantitative analysis by gas-chromatography mass-spectrometry. The results of concentration of Citalopram are given below.

<u>Specimen</u>	<u>Case 1</u>	<u>Case 2</u>
<u>Blood</u>	<u>0.29 mg/L</u>	<u>0.82 mg/L</u>
<u>Stomach Content</u>	<u>0.04 mg/Total Content</u>	<u>Not Detected</u>
<u>Liver</u>	<u>7.0 mg/kg</u>	<u>4.76 mg/kg</u>
<u>Vitreous Humor</u>	<u>0.34 mg/L</u>	<u>0.2 mg/L</u>
<u>Bile</u>	<u>2.0 mg/L</u>	<u>2.2 mg/L</u>

Case one was a 51-year old male who had a history of depression and alcohol abuse. Case two was a 35-year old female who had a history of depression. The cause of death was ruled to be gunshot wound of the head, and pulmonary thromboembolus due to deep venous thrombosis, respectively. Citalopram in the presented cases was considered to represent therapeutic levels in postmortem cases.

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Keywords: Citalopram, postmortem tissues, gas-chromatography mass-spectrometry

**Detection Period for Cocaine Metabolite in Urine Following Topical Ophthalmic Cocaine Administration for Testing Horner's Syndrome**

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**Objective:** To determine the duration of positive benzoylecgonine (BZE) assay following topical ophthalmic administration of cocaine for testing the presence of Homer's syndrome. Initial immunoassays and GC-MS confirmatory tests were conducted using cutoff concentrations specified by SAMHSA/DHHS Guidelines for Federal workplace drug testing.

**Methods:** Two drops of freshly prepared cocaine 10% were applied to each eye of 50 normal volunteer adult subjects. Urine samples were collected 4-6, 24, 48, 72, and 96 hours later. Each sample was assayed for BZE using a *screening* competitive enzyme donor immunoassay (+ cutoff, 300ng/ml) followed by a *confirmatory* gas chromatography-mass spectrometry assay (+ cutoff, 150ng/ml).

**Results:** Positive screening results were obtained in 94% of subjects 4-6 hours, 70% 24 hours, and 2% 48 hours after topical application of cocaine. None of the samples tested positive beyond 48 hours. Positive confirmatory tests (> 150 ng/mL) were obtained in 98% 4-6 hours, 78% 24 hours, and 16% 48 hours after cocaine application. The following would have been reported positive for cocaine metabolite: 47 of 50 (94%) at 4 hrs, 35 of 50 (70%) at 24 hrs, 1 of 50 (2 %) at 48 hrs, 0 of 50 beyond 48 hrs. Although not tested, similar results would be expected if non-instrumented initial immunoassays were used.

**Conclusion:** Patients should be informed that their urine may test positive for cocaine, if cutoff levels for federal workplace drug testing programs are applied, for up to two days after undergoing testing for Homer's syndrome. Medical Review Officers can use 48 hours as the limit for invoking ophthalmic administration of cocaine as a reason for a positive drug test.

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**Keywords:** Cocaine, Horner's Syndrome, detection period

**Mirtazapine in 16 Postmortem Cases**

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Mirtazapine (mirt) is a tetracyclic piperazinoazepine, classified as a noradrenergic and specific serotonin antidepressant. This study summarized 16 mirt cases. Drug screening of blood and urine was performed: mirt detected in urine by TLC, and in blood, by GC/flame ionization detector. Confirmation was by GC/mass spectrometry.

Cases	Mirt.mg/L	EtOH	Cause of Death	Manner Death
1	0.04	ND	Asphyxia	Suicide
2	0.04	ND	Anoxic encephalopathy	Natural
3	0.05	0.19	Carbon monoxide poisoning	Undetermined
4	0.06	ND	Sudden death/cocaine	Accident
5	0.06	ND	Gun shot wound to head	Suicide
6	0.06	ND	Smoke inhal./self immolation	Suicide
7	0.08	ND	Morphine overdose	Accident
8	0.08	ND	Carbon monoxide poisoning	Suicide
9	0.09	ND	Bronchopneumonia	Accident
10	0.1	ND	Mixed drug overdose	Suicide
11	0.16	ND	Acute bronchopneumonia	Accident
12	0.25	0.22	Bilateral pulmonary emboli	Natural
13	0.29	ND	Bilateral pulmonary thromb.	Natural
14	0.74	0.05	Mixed drug overdose	Undetermined
15	4.21	ND	Asphyxia by plastic bag	Suicide
16	4.75	ND	Carbon monoxide poisoning	Suicide

For cases 15 and 16 with high mirt concentrations possibly due to acute ingestion, COD and MOD were not attributable to mirt ingestion alone.

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Keywords: Mirtazapine, case report, mixed drug overdose

**Tissue Distribution of Paroxetine (Paxil®) in Fifty-six Postmortem Cases**

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Paroxetine (Paxil®) is an antidepressant that was first prescribed in the United States in 1992 for the treatment of depression, obsessive compulsive disorder, panic disorder, and social anxiety disorder in 1999. Paroxetine is a phenylpiperidine derivative that inhibits the re-uptake of serotonin. During 1998 and 1999, the Los Angeles County Department of Coroner Forensic Toxicology Laboratory detected paroxetine in fifty-six postmortem cases. Due to the limited information available in the literature, the postmortem tissue distribution of paroxetine was determined. Presented are the case histories, cause and manner of death, and postmortem tissue distribution of paroxetine, all designed to aid the forensic toxicologist with the interpretation of their own casework.

The analysis of paroxetine from postmortem specimens consisted of a basic extraction procedure with screening and quantitation by GC/NPD. Linearity was achieved from 0.05 mg/L to 3.0 mg/L, with the limit of quantitation at 0.05 mg/L. Confirmation of paroxetine was determined using a GC/MS by comparison with an analytical standard.

The tissue distribution of paroxetine are in the following concentration ranges with the number of cases associated: heart blood +<0.05-3.4 mg/L (55), femoral blood +<0.05-1.4 mg/L (48), liver +<0.05-290 mg/g (32), gastric 0.05-103 mg total (23), bile 0.18-70 mg/L (24), urine +<0.05-7.6 mg/L (24), vitreous +<0.05-0.45 mg/L (13), kidney 0.36-12 mg/g (8), spleen 0.73-118 mg/g (4), and brain 0.61-76 mg/g (5).

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Keywords: Paroxetine, Tissue Distribution, Postmortem

## Semi-Automated Assay of Tacrolimus (FK506) Concentrations in Blood by Liquid Chromatography–Electrospray Ionization Mass Spectrometry

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Tacrolimus, CAS 104987-11-3, is the active agent in Prograf™ (Fujisawa Healthcare, Deerfield, IL) and is widely used to suppress rejection response in patients who have received solid organ transplants. Monitoring tacrolimus concentrations in these patients is necessary because of potential nephrotoxicity and neurotoxicity when greater-than-therapeutic blood concentrations are achieved. We have developed a clean, rapid assay for tacrolimus in blood using semi-automated solid-phase extraction coupled with liquid chromatographic (LC) separation and electrospray ionization (ESI)-mass spectrometric (MS) detection. In our procedure, blood is mixed with a protein precipitation reagent containing internal standard (ascomycin). Tacrolimus and internal standard are extracted from the supernatant on a Gilson ASPEC XL4 instrument using Empore SDB-XC sorbent disk cartridges. The instrument is programmed to: 1) condition the sorbent; 2) apply sample to the sorbent; 3) elute and discard extraneous adsorbed compounds from the sorbent; 4) elute and transfer the analytes into autosampler vials. The vials are transferred to the autosampler module of an Agilent 1100 LCMS system. Ten microliter aliquots are injected onto a 30mm x 3.2mm C18 column held at 75°C. Mobile phase, consisting of 90 parts acetonitrile, 10 parts water, is supplied by a binary pump at a rate of 0.5 mL/min. Detection is by selected monitoring of positively charged tacrolimus and ascomycin sodium adduct ions ( $m/z$  826.5 and 814.5, respectively). Fragment ions of tacrolimus and ascomycin ( $m/z$  616.4 and 604.4, respectively) are monitored as qualifiers. Retention times are 0.756 min for tacrolimus and 0.766 min for ascomycin. Total run time is 1.00 min. per injection. Advantages of this procedure are rapid turn-around-time, low detection limit, extended range of linearity, and excellent accuracy and precision.

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Keywords: tacrolimus, electrospray, LCMS



**Analysis of Urine for Anionic Surfactants**

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Surfactants may be added to urine at the time of collection in an attempt to avoid drug abuse detection. In order to assess the validity of specimens when adulteration by a surfactant is suspected, an analytical procedure for surfactants is required. A semi-quantitative method was developed and validated for the analysis of urine for anionic surfactants. The method described was adapted from a technique utilized by the wastewater industry and is based on methylene blue active substances (MBAS).

Methylene blue is a water-soluble, organic, cationic dye which when paired with a MBAS will become soluble in an organic solvent, lending its blue color to the solvent. The color intensity in the organic phase is a measure of MBAS concentration. Most anionic surfactants are MBAS.

Urine was analyzed for MBAS by mixing it with a solution of methylene blue and an alkaline buffer. The mixture was then extracted with chloroform and the chloroform back-washed with an acid to eliminate positive interferences. The intensity of the blue color in the chloroform was measured with a spectrophotometer at 580 nm.

The method was linear from 10 to 120  $\mu\text{g/mL}$  of MBAS. The coefficients of variation for the intra- and inter-assay precisions at 100  $\mu\text{g/mL}$  (suggested cutoff) were 2.3% and 8.3%, respectively. Samples negative for anionic surfactants gave the chloroform a pink, clear, or pale blue color. Samples positive for anionic surfactants produced a deep blue chloroform layer.

In conclusion, a method has been adapted to analyze urine samples for gross concentrations of anionic surfactants seen in cases of adulteration. Samples that contain inherent MBAS can, with confidence, be distinguished from samples that have been adulterated.

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Keywords: Surfactant; Adulteration; Methylene Blue Active Substances

**Characterization of the Effects of Stealth Adulterant on Drugs of Abuse Testing**

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Stealth is an adulterant that is advertised as being undetectable by standard adulteration tests. It has been shown to be peroxidase and peroxide which when added to urine samples is supposed to prevent a positive drug test. Characterization of the effect of Stealth on urine samples and immunoassay results was undertaken to assist in the identification of this adulterant.

Stealth was added to a number of urine matrices and various characteristics of the urine were measured, including pH, specific gravity, color, creatinine, chloride, urea, blood, glucose and nitrite.

Results of these analyses showed that Stealth did not cause the urine sample to exceed any parameter routinely used in drug testing laboratories that would indicate adulteration of a sample. It did however, cause samples positive for THC-COOH, LSD and opiate (morphine) at 125 - 150% of cutoff to screen negative by immunoassay. Similarly, adulterating an authentic positive sample provided by a marijuana user caused that sample to screen negative.

The presence of the peroxidase in the sample could be detected using commercially available reagents for monitoring peroxidase activity. These tests can be conducted manually or on an autoanalyzer. Normal urine has no peroxidase activity, however, samples that contain blood have pseudoperoxidase activity caused by the presence of hemoglobin. Some samples have peroxidase activity owing to the presence of some forms of bacteria that produce peroxidase. To assess the potential for these samples to test positive by this assay, clinical samples that tested positive for blood and/or bacterial contamination were tested with the assay. Several of these samples showed activity in the assay but could be differentiated from those adulterated by Stealth.

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Keywords: Adulteration, Stealth

**Clinical Implications and Identification of Designer and Drugs of Abuse on Urine Samples**

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The purpose of the study is to determine the most cost effective way to provide timely, detailed information about drug presence in patient samples to enhance diagnosis and treatment. Drugs are assayed to monitor therapeutic levels, dosing and compliance and to differentiate prescribed drugs from drugs of abuse. The Chemistry department of a 500 bed community hospital uses three drug testing systems: Triage, EMIT II on the Beckman CX4, and the Remedi HS. Triage identifies seven illicit drugs classes by enzyme immunosorbent methods. EMIT utilizes EIA. Remedi HS utilizes HPLC to identify specific parent compound and metabolite(s). The advantages and limitations of each methodology, and the cost per reportable result of each system are compared. Bayesian Analysis and Cohen's kappa are used to analyze results obtained from the three systems. Analysis shows no significant difference between the three systems. Remedi HS identifies a greater number of drugs. The combination of the Remedi Hs and EMIT II results in a faster turn around times as well as fewer false positives. The cost comparison reveals that this combination is the most cost effective.

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**pH Interference on Dry Chemistry Test Strip Specific Gravity Assays**

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A study was conducted to determine the effects of pH interference on solid-phase dry chemistry test strip Specific Gravity (SG) assays. Twenty urine specimens were collected from healthy volunteers. These were assayed for pH, SG and creatinine before and after dilution with water.

Samples were assayed on a Clinitek 50 using Multistix™ 10 SG strips from Bayer (Elkart, IN), and a Chemstrip UA utilizing Chemstrip™ 10 UA strips from Roche (Indianapolis, IN). Urine creatinine assays were performed on a Hitachi 911 with reagents obtained from Chimera/Dade Behring (Asheville, NC). Specific gravity and pH values for all specimens were confirmed by refractometer and pH meter, respectively.

Undiluted urines with a pH of 5.0, 6.0, 7.0, and 8.0 showed good agreement between refractometer and dipstick SG readings. These samples were diluted to less than 10 mg/dl of creatinine. Test strip readings correlated with refractometer values of 1.000 – 1.001. However, the Roche test strips missed diluted specimens at pH 7. These samples showed normal SG values of 1.005.

Urines were pH adjusted to 3.0, 4.0, 9.0, and 10.0. SG dipstick readings correlated with refractometer measurements for both dilute and undiluted samples.

This study and literature references have shown that pH can interfere with dry chemistry strip SG assays. The conclusion here is that creatinine testing is more reliable in assessing whether or not a urine sample has been diluted.

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Keywords: Specific Gravity, pH, creatinine

**Applicability of the New Federal Opiate "Cutoffs" to Opiate Intoxication Cases**

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Recently, the Departments of Health and Human Services (HHS) and Defense (DOD) have modified the requirements for testing urine specimens for morphine and codeine (M/C). Specifically, both have raised the screening cutoff from 300 to 2000 ng/mL. Also, DOD has raised the confirmation cutoff for M/C from 300 ng/mL to 4000 ng/mL (HHS confirmation=2000 ng/mL). If these cutoffs are reached, additional testing for 6-AM at a cutoff of 10 ng/mL is required. All cutoffs must be exceeded before a specimen is reported as "positive" for opiates.

As a way of assessing the impact of these new cutoffs in detecting opiate intoxication cases, 50 consecutive cases reported as "narcotic intoxication" by the Chief Medical Examiner (Maryland) were studied. Urine specimens were quantitated for total morphine and 6-AM by GC/MS. Concentrations are:

Total Morphine Concentration (ng/mL)	Total
<50	7
50-299	3
300-1999	5
2000-3999	7
>4000	28

Forty-two of 48 specimens were positive for 6-AM at the 10 ng/mL cutoff; 1 of the "negative" 6-AM specimens had a total morphine concentration >2000 ng/ml.

Twenty percent of the specimens would have been called "negative" under the old confirmation cutoffs. Under the current HHS guidelines, 32 % of the cases would be called "negative". Forty-six percent would be "negative" under current DOD guidelines. This study emphasizes that the opiate cutoffs currently used in forensic urine drug testing are inappropriate for detecting opiate intoxication cases.

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**Keywords:** Opiate intoxication, Forensic urine drug testing, Urine opiate values

**Development of a Simple and Universal Extraction Procedure for the SAMSHA Drugs of Abuse (DOA) Using the Waters Oasis® MCX Extraction Cartridge and the Zymark RapidTrace® SPE Robotics Instrument**

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Waters Oasis® MCX cartridges contain a novel, polymeric, water-wettable, sorbent, stable from pH 0 to 14. This polymer has no free silanols to complicate retention, a 1meq/g cation exchange capacity and a reverse phase with more than three times the capacity of a C18 sorbent. The mixed bed sorbent of the MCX provides for the efficient extraction of acidic, neutral and basic drugs. Using the Oasis® MCX Cartridges our objective was to develop a simple and universal procedure for extraction of the SAMSHA DOA from urine that minimized the use of organic solvents and buffers. The following procedure was developed: After loading (1 to 3 mls Urine) onto the Oasis® MCX Cartridge, the Cartridge is washed sequentially with NaOH, Water and Hexane. No pretreatment of the Cartridge is required prior to Loading. The Cartridge is eluted with an IPA/Methylene Chloride mixture, evaporated to dryness with Nitrogen, and derivatized (except for PCP) prior to GC/MS analysis. This universal 4-step extraction procedure was validated for all of the SAMSHA DOA using the Zymark RapidTrace SPE Robotics Instrument. The Controls in the Validation Protocol were set at 40% of SAMSHA Cutoff (Low), SAMSHA Cutoff (Medium) and 2X or greater of the SAMSHA Cutoff (High). (The Cutoff for Morphine & Codeine was 300 ng/ml.) The Validation Study demonstrated CVs < 10% at the Low Control and < 5% at the Medium and High Control for all of the SAMSHA DOA. This procedure, using the Oasis® MCX Cartridge, was demonstrated to provide an efficient and highly reproducible universal extraction procedure for all of the SAMSHA DOA.

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Keywords: SAMSHA Drugs of Abuse, Waters Oasis® MCX, GC/MS

**A Comparison of Selection Criteria in Meconium Drug Testing**

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Drug abuse during pregnancy has been associated with a high incidence of premature birth, low birth weight, and physical/developmental deficiencies. While history of drug abuse is often used in identifying at-risk infants, analysis of meconium for drugs of abuse has also been used as criteria for establishing drug use during pregnancy. We have compiled a database of meconium drug testing results from three criteria. These criteria are (1) specimens from infants of low birth weight (< 2500 grams) and head circumference (< third percentile for gestational age) (Growth Deficient Infants), (2) specimens per request of the physician (Physician Requested), and (3) specimens submitted from all consecutive births at 6 separate birthing institutions (No Selection Criteria). The results from the meconium analysis were used to evaluate selection criteria for predicting drug use during pregnancy. The drugs/drug classes included in this study were amphetamines (amphetamine, methamphetamine), cannabinoids, cocaine, and opiates (codeine, morphine and hydrocodone). All meconium specimens were screened by Radioimmunoassay (RIA) and presumptive positives were confirmed by Gas Chromatography/Mass Spectrometry. Specimens (total 842) submitted from the No Selection Criteria group demonstrated a 4.2% positive rate. This group was also tested for cotinine and 6.9% of the specimens were found to be positive. A positive rate of 12.6% was found in the specimens from the GDI group (total 222). The Physician Requested group (total 758) demonstrated a 28.1% positive rate. This finding suggests that while growth deficiency may be useful in predicting drug abuse during pregnancy, the physician's evaluation is more often predictive.

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Keywords: Meconium, Drug test, Epidemiology

**Drugs of Abuse Testing in a Wisconsin Correctional System Facility Using the Olympus AU640**

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Drugs of abuse urine (DAU) testing has been effectively used in criminal justice systems and correctional facility as an adjunct for assessing the role of drug intake and compliance to abstinence of using illicit drugs. Testing may be performed by point-of-care testing devices using various forms of immunoassays, or by centralized laboratories using high-through put analyzers. Due to the application of these results in judicial systems, the urine samples are usually collected in secured settings, following some form of limited chain of custody. Once received in the central laboratories, these samples are processed in a similar manner as in a clinical laboratory. Quality assurance is maintained by adhering to well established quality control guidelines, subscription to proficiency survey, and laboratory accreditation in accordance with CLIA 88 regulations. Recently, the laboratory changed the analyzer from SYVA – 30R to Olympus 640 using the newly available EMIT II Plus reagents for most of the DAU testings. This study summarized the initial experience of the change by comparing the performance of the two analyzers. Drug(s) or drug groups with their respective cut-off concentrations (ng/mL) are: alcohol 0.1(mg/dL), amphetamines 1000, barbiturates 200, benzodiazepines 200, cannabinoids 50, cocaine metabolite 300, methaqualone 300, methadone 300, opiates 300, phencyclidine 25, and propoxyphene 300. In checking the clinical efficacy of AU 640, 40 to 50 clinical and survey samples were assayed by both instruments. At the above stated cut-offs, sensitivity and specificity were 100%. Further, the CAP survey results and the experience of the past three months have verified the ease of use of the EMIT II Plus reagents, reliability of the instrument, and the efficacy of the quality control programs.

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**Keywords:** DAU testings, correctional systems, immunoassay analyzers



**Integrity of THC-COOH in Polypropylene and Polyethylene Containers**

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The loss of THC-COOH from urine stored in polypropylene and polyethylene containers at 4°C and 25°C was examined. All samples were analyzed by GC/MS after sampling at various times over a 1-week period. Data were analyzed by one-way ANOVA and fitted with a first order kinetic equation. Rapid loss of THC-COOH was seen at 4°C from both polypropylene (14% maximal loss,  $t_{1/2}=0.53$  minutes) and polyethylene (17% maximal loss,  $t_{1/2}=5.77$  minutes). A small loss (<5%) was observed for urine stored in polypropylene at 25°C and no significant loss was seen for urine stored in polyethylene. All losses stabilized within one hour and no further losses were seen over one week. The results indicate that THC-COOH binding may be due to decreased solubility of THC-COOH at lower temperatures and subsequent association of THC-COOH with the more lipophilic plastic. The results also indicate that polypropylene and polyethylene do not bind THC-COOH to such an extent as to compromise the integrity of samples.

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Keywords: THC-COOH, storage, polypropylene and polyethylene containers