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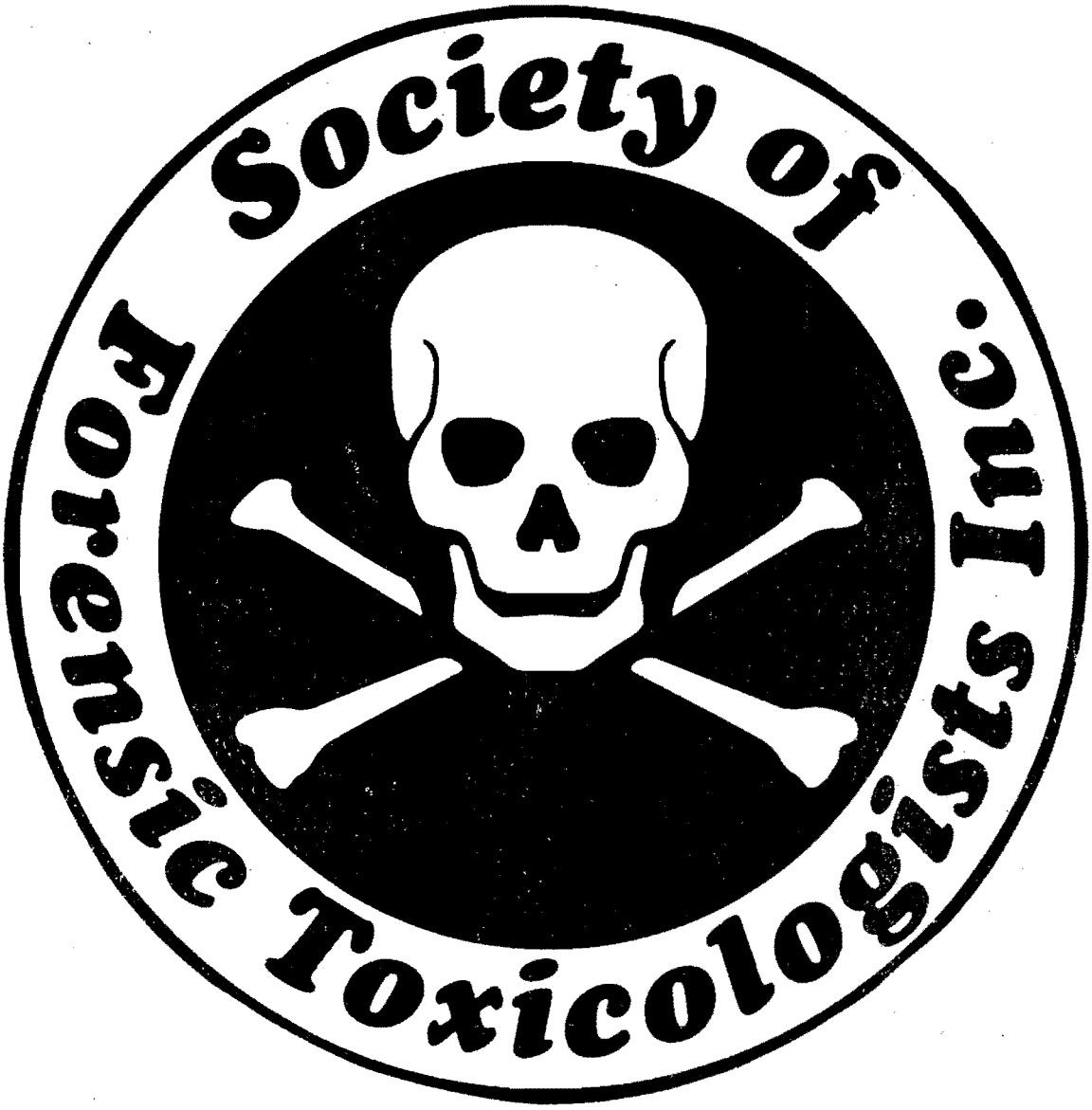

S.O.F.T. ANNUAL MEETING

OCTOBER 5 - 9, 1997

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SOCIETY OF FORENSIC TOXICOLOGISTS, INC.

Annual Meeting ABSTRACTS

October 5-9, 1997 Salt Lake City, Utah

Plasma Concentrations of Cocaine and Metabolites During Chronic Cocaine Administration to Humans. *Rebecca A. Jufer*[*1], *Sharon L. Walsh*[2] *and Edward J. Cone*[1]; [1]*Addiction Research Center, DIR, NIDA,* [2]*Dept. of Psychiatry, Johns Hopkins University School of Medicine, Baltimore, MD .*

Differences can exist in the distribution and kinetic pattern of a drug following single dose administration versus chronic dosing. We are currently developing a model to study chronic cocaine administration in humans. Cocaine abusing volunteers were administered oral cocaine in up to 16 sessions. In each session, volunteers received 5 equal doses of oral cocaine with 1 hour between doses. Across sessions, cocaine was administered in ascending doses with an initial dose of 100 mg (500 mg/day) up to 400 mg (2g/day), increasing by 25mg/dose/session (125 mg/session). Participation in the study was terminated if cardiovascular safety parameters were exceeded. Plasma was collected during and after dosing. Specimens were analyzed for cocaine and metabolites using solid phase extraction followed by gas chromatography-mass spectrometry. Preliminary results (n=4) indicated that chronic oral cocaine administration produced plasma concentrations of cocaine that were proportional to dose. In addition, a variety of cocaine metabolites were identified, including benzoylecgonine (BZE), ecgonine methyl ester (EME), norcocaine (NCOC), benzoylnorecgonine (BNE), *p*-hydroxycocaine (*p*-HOCOC), and *p*-hydroxybenzoylecgonine (*p*-HOBZE). Smaller amounts of *m*-HOCOC and *m*-HOBZE were also detected. Peak cocaine and metabolite concentrations (ng/mL) for each subject's maximum dose are summarized below.

Subject	Max Dose (mg)	C_{max} COC	C_{max} BZE	C_{max} EME	C_{max} NCOC	C_{max} BNE	C_{max} p-HOCOC	C_{max} p-HOBZE
A	400	1259	5389	3073	138	316	83	267
B	300	729	3825	2647	67	186	33	213
C	125	275	2242	380	27	69	19	109
D	400	1256	4421	2273	129	309	82	243
E	300	653	3700	1766	107	385	77	336
G	350	1899	4230	2719	168	302	114	325

These data indicated that chronic oral cocaine administration can be safely used to study the effects of repeated cocaine dosing in human subjects. However, there were substantive differences in the pattern of metabolism of cocaine compared to other routes of administration, such as intravenous and intranasal, including the appearance of several new metabolites previously undetected in plasma.

Key Words: Oral cocaine, chronic cocaine, metabolism, pharmacokinetics.

Fatty Acid Ethyl Esters in Long Evans Rats After Acute and Chronic Ingestion of Ethanol. *George S. Behonick*[*1], *Guy M. Vallaro*[2], *Jesse H. Bidanset*[3] *and Steven I. Baskin*[1]; [1]*US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010-5425;* [2]*Westchester Co. Dept. Labs and Research, Valhalla, NY 10595;* [3]*St. John's University, Jamaica, NY 11439.*

Fatty acid ethyl esters (FAEEs) are non-oxidative metabolites of ethanol. The objective of this research was to quantitate the concentrations of palmitic acid ethyl ester (PAEE) and stearic acid ethyl ester (SAEE) in the blood, liver, and brain of young adult (175-200 g), male Long Evans rats following the peroral administration of ethanol by gastric gavage (5g/kg, 25% w/v). The principal problem investigated was to determine the utility of quantitating FAEEs as biochemical indicators of consumption in acute and chronic paradigms. The acute study consisted of a single dose followed by timed blood collection (t = 2h) and euthanasia. The chronic study was conducted over a period of four weeks using a single, daily dose regimen. Timed blood collection, euthanasia, and necropsy were performed on the last day of the chronic study.

Mean blood alcohol concentrations (t = 2h) were 0.24 and 0.31 g% for the acute and chronic study respectively. After a single acute dose (H_2O control or ETOH treated) PAEE concentrations in blood, liver, and brain were: 98 or 1199 ng/mL; 630 or 5543 and 88 or 3295 ng/g respectively . The chronic study results were: 2093 ng/mL (blood), 6624 ng/g (liver), and 10,298 ng/g (brain). The SAEE data for both acute and chronic studies follow: blood (336 and 534 ng/mL), liver (4458 and 4845 ng/g), and brain (7416 and 5856 ng/g). FAEEs may be quantitated in tissues following ethanol consumption. Chronicity appears to be a factor in the non-oxidative biotransformation of ethanol to FAEEs. This may be relevant in postmortem toxicological evaluations in cases involving ethanol consumption. This study suggests measurement of FAEEs could provide a useful method for the assessment of acute and chronic alcohol intoxication.

The Incorporation of Phencyclidine Into Hair:A Comparison with Eumelanin and Pheomelanin Concentrations. *Matthew H. Slawson*, Diana G. Wilkins and Douglas E. Rollins. Center for Human Toxicology, Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, Utah 84112.*

Certain weak bases such as codeine, PCP, cocaine and nicotine are incorporated into hair with a preference for pigmented hair. This difference may be due to the presence of melanin in pigmented hair. Melanin can be divided into at least two distinct types. Eumelanin, a brown-black pigment consisting of quinone-like dihydroxyindole subunits and pheomelanin, a reddish-yellow pigment consisting of benzothiazine-derived subunits. The amount of each type of melanin dictates hair color. The relative amounts of each of these types of melanin may also influence the amount of drug incorporated into hair of different colors. To examine the relationship between PCP incorporation into hair and melanin concentration, rodents with a variety of hair pigmentation were studied. Sprague Dawley (SD; nonpigmented), Dark Agouti (DA; brown), Copenhagen (CP; white with a brown hood), Long Evans(LE; white with a black hood), $LBNF_1$ (deep brown) rats and Swiss-Webster (SW; nonpigmented), C57BL6 (black) and $C57BL6A^y/a$ (yellow) mice were administered PCP at 10 mg/kg/day for 5 days (n=5 for each strain). Fourteen (rats) or 35 (mice) days after the first dose, hair was collected and analyzed for PCP, eumelanin, and pheomelanin using published methods. PCP concentrations (mean±SEM) were 0.46±0.13, 12.25±1.24, 0.12±0.004, 9.16±2.8, 0.66±0.07, 21.2±1.4, 21.64±3.8, 0.48±0.36, 11.0±4.0 and 2.26±0.55 ng/mg for SD, DA, CPnonpigmented, CPpigmented, LEnonpigmented, LEpigmented, $LBNF_1$, SW, C57black and C57yellow hair, respectively. Eumelanin concentrations were 20.50±1.58, 19.43±0.40, 17.56±0.61, 27.26±2.52, 37.33±3.61 and 1.76± 0.02 µg/mg for DA, CPpigmented, LEpigmented, $LBNF_1$, C57black and C57yellow hair, respectively. Eumelanin was not detected in nonpigmented hair. Pheomelanin concentrations were 0.09±0.00, 0.20±0.03, 0.06±0.01 0.16±0.05 and 29.16±0.97 µg/mg for CPpigmented, $LBNF_1$, C57black and C57yellow hair, respectively. Pheomelanin was not detected in nonpigmented or LEpigmented hair. The results suggest that eumelanin concentration is a greater factor on the incorporation of PCP into hair than pheomelanin concentration. The concentration(s) of eumelanin and pheomelanin in hair may be important parameters to consider when interpreting drug concentrations in hair. This work was supported by NIDA grant DA07820.

Designing and Integrating Studies to Evaluate Drug Disposition in Hair. *Robert E. Joseph Jr.[*1], Edward J. Cone[1], Karin M. Hold[2], Diana G. Wilkins[2] and Douglas E. Rollins[2]; [1]Addiction Research Center, IRP, NIDA, NIH, Baltimore, MD.; [2]Center for Human Toxicology, University of Utah, Salt Lake City, UT.*

Drug disposition in hair was evaluated through a combination of *in vitro*, preclinical, and clinical research approaches. The results of *in vitro* studies indicated the presence of unique binding sites for cocaine and opioids in different hair types (i.e., dark and light colored hair). There was clear evidence of differences in binding due to hair color. Animal studies were performed to determine the relevance of *in vitro* findings. In preclinical studies, codeine and morphine incorporation was greater for black hair compared to brown hair and for brown hair compared to white hair. Clinical studies were implemented to evaluate how cocaine and opioids are deposited in hair. The clinical study design included collections of hair, sweat, sebum, plasma, and skin from males and females from different ethnic groups following controlled drug administrations. Drugs appeared in hair within 3 days and reached maximum concentrations in 1-2 weeks following drug administrations. The rapid elimination of cocaine and codeine in sweat and sebum within 1-2 days suggested that drugs in these biological secretions could have been deposited in hair collected within 3 days following drug administrations. However, incorporation of drugs into hair collected 1-2 weeks following drug administrations likely involved passive diffusion of drugs into developing hair cells in the follicle followed by emergence of drugs in growing hair. There was also preliminary evidence of a dose-concentration response relationship in hair similar to that observed for plasma. Overall, the deposition of drugs in hair appears to involve a combination of multiple pathways.

Measurment of Chloroquine in *Post-Mortem* Fluids by HPLC - DAD and ^{1}H - NMR. *Marc Deveaux[1]*, Valéry Hédouin[1], Nathalie Azaroual[2], Emmanuel Luneau[1], Michel Lhermitte[3] and Didier Gosset[1]; [1]Institut de Médecine Légale, 59000 Lille, France; [2]Laboratoire de RMN, Université de Lille 2, France; [3]Laboratoire de Biochimie, CHRU, 59037 Lille, France*

Chloroquine is a medical drug widely used for prophylaxis and treatment of malaria and various connective diseases. Suicidal overdosage is not so rare in France because this is sold without medical prescription and its use was emphasized a few years ago in a book called "Suicide: directions for use".

In two cases of intoxication by chloroquine, body fluids (blood, urine, bile and gastric content) were submitted to HPLC - Diode Array Detector and ^{1}H-NMR analysis. Chloroquine and its first active metabolite was detected and identified by comparing ^{1}H-NMR spectra with those of standards, using a TOCSY-1D sequence. Quantitation was achieved by relative integration of peaks areas, comparing to TSP-d_4.

Analysis were performed by HPLC-DAD with a modification of a previously published method [J Chromatogr. 574 (1992)305-312]: extraction by diethylether, C18 column, acetonitrile/ammonium sulfate (40/60) as a mobile phase. We confirmed the good linearity and sensitivity of this method.

In one case, body fluids concentrations were very high (femoral blood: 137 ng/mL; cardiac blood: 371 ng/mL; bile: 330 ng/mL, gastric content: 2052 ng/mL) and permit to conclude at a chloroquine self poisoning.

Key Words: chloroquine overdose, ^{1}H-NMR, HPLC-DAD

Clinical Findings and Drug Kinetics in Two Cases of Catastrophic Reactions to Cocaine. *Kari Blaho**[1], Barry Logan[3], Stephen Winbery[1], Lynda Park[1] and Stephen Geraci[2]; [1]Department of Emergency Medicine and Clinical Toxicology, and [2]Department of Medicine, University of Tennessee, Memphis, TN 38103; and [3]Washington State Toxicology Laboratory, Department of Laboratory Medicine, University of Washington, Seattle, WA 98134*

We report the clinical findings and cocaine and metabolite concentrations in 2 patients presenting with catastrophic reactions to cocaine. These patients were part of an ongoing study that included 120 patients acutely intoxicated with cocaine. Blood was drawn for cocaine and metabolite concentrations as soon as feasible after arrival and frozen immediately. Concentrations were determined by an extractive alkylation/GCMS procedure. Patient 1 was a 33 year old male with a history of IV cocaine abuse. On arrival his vital signs were: blood pressure 90/palpable, heart rate 188 bpm, respirations 28/min, temperature 108°F. Cocaine concentrations (mg/L) were 0.387, 0.207, 0.138, 0.086 ng/L at 30 min, 2hr, 4 hr, 10 hr after arrival, respectively. Ecgonine methylester, ecgonine, norcocaine, cocaethylene and benzoylecgonine concentrations were also measured. Despite aggressive resuscitation, the patient died after 14 hours. Patient 2 was a 22 year old male who presented after smoking crack cocaine. On arrival his vital signs were: blood pressure 250/140 mmHg, heart rate 176 bpm, respiratory rate 48/min, temperature 104.6°F. Cocaine concentrations (mg/L) were 0.266, 0.059, 0.052 at 2, 6, 11 hours, respectively. Ecgonine methylester, ecgonine, norcocaine, cocaethylene and benzoylecgonine concentrations were also measured. The patient was was discharged after 24 hours. Neither cocaine nor metabolite concentrations were predictive of the severity of clinical findings or outcome.

Key Words: cocaine

Does Alcohol Ingestion Increase Cocaine Toxicity? *Steven B. Karch**[1], Boyd G. Stephens[1] and Andrew Tseng[2]; [1]San Francisco Medical Examiner, 850 Bryant Street, San Francisco, CA, 94103; [2]University of Nevada, Dept of Mathematics, Las Vegas, NV*

Objectives: Cocaine (C) transesterification with ethanol (Etoh) produces cocaethylene (CE), which shares many actions with C, but has a longer half life. CE is thought to increase toxicity, and simultaneous consumption of Etoh and C leads to higher C blood concentrations. If these effects are clinically relevant, then in cocaine-related deaths, blood C should be lower in individuals who had also been consuming Etoh, than in those who had not. Experimental Design: 106 accidental, cocaine-related deaths, where C, benzoylecgonine (BE), and Etoh concentrations were known, and where no other drugs were detected were reviewed. Urine EMIT screening was followed by GC/MS confirmation. Nonparametric one-way analysis of variance was used to study the differences between Etoh+ and Etoh- groups.Results: Mean age was 40.2 years in Etoh- (n = 64) and 39.9 years in Etoh+ (n = 42). Mean Etoh was 0.13 (range 0.02-0.33) mg/dL. In Etoh- cases, the mean C was 1.04 mg/L, and BE was 2.09 mg/L. In Etoh+ case, the C concentration was 0.98 mg/L, and BE 1.46 mg/L. Neither the difference in C or BE concentrations were significant (p = .9305 and .2258 respectively). Conclusions: Since C and BE levels were not significantly different in the two groups, the presence of ethanol does not seem to enhance toxicity. However Etoh concentrations in these individuals were modest, and more CE is formed when ethanol concentrations are high. Increased toxicity from CE may only be apparent when larger quantities of alcohol have been consumed.

Key Words: Cocaine, Ethanol, Cocaethylen

Postmortem Forensic Toxicology of Trazodone in Man. *Kabrena E. Goeringer**[1] and Barry K. Logan[2]; [1]Department of the Air Force, HQ USAFA/DFC, 2355 Fairchild Drive Suite 2N225, USAF Academy, CO 80840 U.S.A; [2]Washington State Toxicology Laboratory, Department of Laboratory Medicine, University of Washington, 2203 Airport Way So. Suite 360, Seattle, WA 98134 U.S.A.*

Trazodone (Deseryl) is an atypical antidepressant with some serotonergic activity, although less than that of the classical SSRI's, such as fluoxetine or sertraline. Its combination of sedation without anticholinergic effects has made trazodone especially attractive in older, medically compromised patients or those who are otherwise sensitive to the anticholinergic actions of TCA's. Trazodone has been widely prescribed, occupying a large share of the US market for antidepressants. This paper reviews the literature on deaths involving trazodone, and describes drug concentrations in blood from fifteen deaths in which trazodone was detected, and discusses the possible role of the drug in each.

Basic drugs, including trazodone, were identified by gas chromatography/mass spectrometry, following an n-butyl chloride extraction. Analysis of peripheral blood from fifteen postmortem cases revealed trazodone concentrations ranging from 0.06-32.91 mg/L (the therapeutic range is 0.49-1.60mg/L). Postmortem distribution was studied in four of the cases, and the following

concentrations were found: 0.08-11.45 mg/L (central blood), 0.06-12.92 mg/L (bile), 0.42 mg/L (urine), 1.25-3.52 mg/kg (liver), 0.57-308.84 mg/kg (gastric contents), and 0.05-4.70 mg/L (vitreous). The average central:peripheral ratio in these cases was 1.5. Trazodone was typically present together with other drugs, and the potential for interaction with each is discussed. Over the one year period of this study there were no deaths ascribed solely to trazodone intoxication, although combined drug fatalities were frequent.

Key Words: Trazodone, GC/MS, Serotonin

Deaths Associated with Buprenorphine: Report of Fifteen Cases in France. *Marc Deveaux[1]*, Antoine Tracqui[2], Pascal Kintz[2], Bertrand Ludes[2]; Didier Gosset[1]; [1]Institut de Médecine Légale, 59000 Lille, France; [2]Institut de Médecine Légale, 67000, Strasbourg, France.*

Buprenorphine, a semisynthetic opioid derivative, was initially developed for treatment of pain under both injectable and sublingual forms. However, in 1996, the French Ministry of Health allowed prescription of buprenorphine by oral route for the treatment of heroin-dependant individuals, as an alternative to methadone substitution.

Unfortunately, many heroin addicts misused buprenorphine, i.e. increasing dramatically doses and using the intravenous route. Not less than 15 deaths occurred in one year. We present here the epidemiological data and the results of autopsies. Analysis of buprenorphine and active metabolite norbuprenorphine were performed by HPLC - MS with deuterated internal standard with a detection limit as low as 0.1 ng/mL in blood. Other toxicological analyses were performed by immunoassays and GC/MS. Concentrations of buprenorphine in blood range from 1.2 to 18 ng/mL, not far from the therapeutic range (0.5 to 8 ng/mL). Norbuprenorphine was not detected in all the cases. Buprenorphine was associated with benzodiazepines in all cases but one.

We conclude that, independently of the therapeutical efficacy of buprenorphine, the French way of medical prescription and delivery by a pharmacist of this drug in sublingual form is questionable and has to be supervised.

Key Words: buprenorphine, post-mortem blood, HPLC-MS

Buprenorphine and Norbuprenorphine in Human Hair After Multiple Doses: A Retrospective Study. *Angelique S. Valdez*[1], Diana G. Wilkins[1], Atsuhiro Mizuno[1], John D. Laycock[1], Edward J. Cone[2] and Douglas E. Rollins[1]; [1]The Center For Human Toxicology, Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, UT 84112 USA; [2]Addiction Research Center, NIDA, NIH, Baltimore, MD 21224 USA.*

The analysis of hair has been proposed as a tool for monitoring drug treatment compliance. This study was performed to determine if buprenorphine (BPR) and norbuprenorphine (NBPR) could be detected in human hair up to one year after drug administration. Subjects with dark hair (six males, six females) received 8 mg sublingual BPR for up to 180 days. Single hair collections were made once after BPR treatment and stored at -20°C until analysis. Hair was aligned root to tip and segmented in 3-cm sections. It was assumed that the mean hair growth rate was 1 cm/month. Deuterated internal standards were added to hair segments (2-20 mg of hair) and digested overnight at room temperature with 1N NaOH. Specimens were extracted with a liquid-liquid procedure and analyzed by LC/MS/MS. The limits of quantitation for BPR and NBPR with 10 mg of hair were 3 pg/mg and 5 pg/mg respectively. BPR and NBPR concentrations were highest for all subjects in hair segments corresponding to the subject's period of drug treatment. NBPR was present in higher concentrations than the parent compound. NBPR concentrations in hair segments ranged from 4 pg/mg to 1518 pg/mg. BPR ranged from 3 pg/mg to 124 pg/mg. However, in 4 of 12 subjects BPR or NBPR was detected in hair segments that did not correspond to the period of drug treatment suggesting that drug movement had occurred in hair possibly by diffusion in sweat and by other mechanisms. Further prospective and retrospective research is required to evaluate whether hair can serve as a historical record of drug use. This study was supported by NIDA grant #DA09096.

Key Words: Buprenorphine, Hair, Segmentation

A Mouse Model for Studying Drug Incorporation Into Human Hair. *Pamela R. Nagasawa*[1], Gerald G. Krueger[2], Matthew H. Slawson[1], Diana G. Wilkins[1] and Douglas E. Rollins[1]; [1]Center for Human Toxicology and [2]Division of Dermatology, University of Utah, Salt Lake City, UT, 84112.*

In studies of the incorporation of drugs into hair, the typical models utilized are animals or humans. We have developed a unique model in which human hair is grown on nude, athymic mice. This model offers the advantage of retrieving human hair samples from an animal that can be dosed with known concentrations of drugs and/or metabolites.

Human scalp tissue is prepared and cut into 1 cm^2 pieces. The tissue is then grafted subcutaneously on the lateral thoracic area of the mouse using sterile surgical techniques. After a period of 2.5 to 3 months the graft is surgically exposed and left to heal for 4 to 6 weeks. By this time mice with viable grafts (1.5 to 2 cm in diameter) have 2 to 4 cm of hair growth. These mice can then be used for experimental protocols.

Histological studies were performed on a graft taken from a fully developed model. These results showed significant structural similarities to normal human hair. The rate of hair growth on grafts was monitored to evaluate the variability among grafts and identify a mean growth rate. An area was selected on each graft and hair was cut to 1 mm. Ninety-one days later the mean ± SEM hair length was 15.50 ± 0.76 mm. This calculates to a mean ± SEM growth rate of 1.69 ± 0.24 mm/week; slightly slower than

normal human scalp hair. This model has been used to study the incorporation of phenobarbital, codeine and PCP into human hair. Results from these studies demonstrate that the grafted athymic mouse can be a successful model, used in addition to other animal and human models, for studying the incorporation of drugs into human hair. Supported by NIDA Grant No. DA07820.

Key Words: Graft, Hair, Growth

Nicotine Studies of Hair from Passive Smoke Contamination, Smokers, and Non-Smoke Ingested Drug Along with a Color Bias Investigation. *K.L. Egli and K. S. Kalasinsky; Office of the Armed Forces Medical Examiner, Division of Forensic Toxicology, Armed Forces Institute of Pathology, Washington, DC 20306-6000.*

Environmental contamination and wash procedures for hair has dominated much of the literature on drug hair analysis. We have investigated these issues using drug-free hair that we exposed to cigarette smoke, hair from smokers and hair from rats that had been infused with nicotine. We also investigated the affinity of the drug for the colored versus non-colored hair. Grayed hair from smokers as well as black and white hairs from hooded rats were used for the study.

Drug-free hair was contaminated with "passive smoke" from a cigarette in a closed environment chamber. Analysis of the hair from the "passive smoke" sample showed the hair to have significant amounts of nicotine bound to the hair that was not removed through wash techniques. Approximately 85 ng/mg of nicotine was in the unwashed hair and 15 ng/mg in the washed hair.

The smoker's hair was washed and analyzed and found to contain about 75 ng/mg in the darker hair and 45 ng/mg in the lighter from the grayed hair smoker's sample.

The hooded rats who had been infused with 5 mg of nicotine per day for 14 days through an osmotic pump were found to have about 15ng/mg of nicotine in the dark hair and 0.25 ng/mg in the white hair.

Key Words: Nicotine, hair analysis, contamination

Implementation of the State of Florida Hair Analysis Proficiency Testing Program. *Jeri D. Ropero-Miller*[1], Bruce A. Goldberger[1], Roger Vance[2] and Pascal Kintz[3]; [1]University of Florida College of Medicine, Gainesville, FL 32610-0275; [2]Agency for Health Care Administration, Tallahassee, FL 32308; [3]Institut de Medecine Legale, Strasbourg, France 67000.*

Recent changes in the State of Florida Drug-Free Workplace Act (Chapter 96-289) now permit the use of hair analysis. In an effort to regulate laboratories seeking licensure, the State of Florida Agency for Health Care Administration (AHCA) has recently implemented a Proficiency Testing program. The Forensic Toxicology Laboratory at the University of Florida College of Medicine has been chosen to provide the proficiency samples and oversee the laboratory aspects of the licensure program.

Drug-free hair was obtained from laboratory personnel. Drug-positive hair was obtained from known drug users, or if authentic samples were not available, drug-free hair was fortified with drug analyte. Drug-positive hair contained one or more of the following analytes: amphetamine, methamphetamine, cocaine, benzoylecgonine, cocaethylene, THC-acid, morphine, codeine, 6-acetylmorphine, and phencyclidine. All hair samples were reduced to a powdered form to ensure homogeneity and verified by GC/MS.

Initial licensure requires satisfactory performance in 3 testing events. Each testing event consisted of 5 hair samples. Following licensure, the laboratory must continue to participate in the Proficiency Testing Program.

An in-depth description of the State of Florida Drug-Free Workplace Act, including its requirements regarding hair analysis, will be provided.

Key Words: Proficiency Testing, Drugs of Abuse, Hair Analysis

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Epimerization Studies of LSD Using ^{1}H Nuclear Magnetic Resonance (NMR) Spectroscopy. *Salvatore J. Salamone*, Zhuyin Li, Alan J. McNally, Stephen S. Vitone and Robert S. Wu. Roche Diagnostic Systems, Inc., 1080 U.S. Highway 202, Somerville, NJ 08876-3771 USA.*

A study was conducted to determine the conditions needed to achieve equilibrium concentration for the epimerization of d-lysergic acid diethylamide (LSD) to iso-LSD. The reaction was followed by integration of the C-9 resonance of LSD and iso-LSD by proton nuclear magnetic resonance (NMR). The C-9 resonance of LSD and iso-LSD appear as singlets at 6.35 ppm and 6.27 ppm respectively. Starting with pure LSD, the conversion to iso-LSD is attained at temperatures above 37°C and pHs above 7.0. The LSD/iso-LSD ratio of 9:1 is achieved after one week at 45°C or two weeks at 37°C. Starting with iso-LSD, the conversion to LSD requires more vigorous conditions. The 9:1 LSD/iso-LSD ratio is attained only after six weeks at a temperature of 45°C and a pH of 9.7. At lower pHs, the reaction proceeds more slowly. The 9:1 LSD/iso-LSD ratio is achieved whether the starting material is LSD or iso-LSD and therefore represents an equilibrium concentration (K=9). In addition, the more vigorous conditions needed to achieve equilibrium of iso-LSD to LSD demonstrates the difficulty in extraction of the epimerizable proton of iso-LSD. This study is the first to quantitate the epimerization of LSD by NMR techniques and establishes the conditions needed to induce epimerization in solution.

Key Words: LSD, iso-LSD, ^{1}H-NMRR

Detection of iso-LSD in Urine After Sodium Ethoxide Isomerization to LSD. *Buddha D. Paul*, Division of Forensic Toxicology, Office of the Armed Forces Medical Examiner, Armed Forces Institute of Pathology, 1413 Research Blvd., Rockville, MD 20850, U.S.A.*

Iso-LSD is present in many illicit preparations of LSD and some of it is excreted unchanged in urine following ingestion. It is unstable in solution and approximately 33% was converted to LSD during storage at 2-6^{0}C over a period of 10 months. Iso-LSD was extracted from urine and converted to a C-8 carbanion by sodium ethoxide in ethanol. Protonation of the carbanion by water selectively produced LSD. The conversion was almost quantitative (98%). Best result was found by heating iso-LSD in 0.5M ethanolic sodium ethoxide at 50^{0}C for 10 min. The product was purified by solid-phase fractionation and acid-base separation techniques. Trimethylsilyl derivative of LSD was then detected by a GC-MS method. Identification was based on monitoring three ions and comparing retention time and ion ratios with that of a standard. The overall recovery of extraction was approximately 69%. Quantitation of iso-LSD was linear over the concentration range 50-2000 pg/mL. Inter-run variation was within ±10%. Iso-LSD was detected in many urine specimens when the LSD concentration was below the limit of detection of the procedure (50 pg/mL). Because iso-LSD is a byproduct of illicit preparation of LSD, presence of iso-LSD in urine is an indication of LSD use.

Key Words : Iso-LSD, Isomerization, GC-MS

Applications of Liquid Chromatographic / Mass Spectrometry (LC/MS) in Detection of LSD in Human Urine Matrices. *Gregory K. Poch*, Domingo Hallare, Marilyn Manglicmot , David R. Lesser, and Gary C. Vias; Navy Drug Screening Laboratory, San Diego, CA.*

Traditionally, GC/MS has been used as the confirmation method for LSD. The current methodology requires a large sample volume, a lengthy 8-hour extraction /derivatization, and a detection limit of 200 pg/mL. A urine assay for LSD that requires a sample volume of 5 mL, no derivatization, and a detection limit of (100 pg/mL has been developed utilizing LC/MS. The internal standard is the isomeric Lysergic acid methyl, propyl amide (LAMPA). The procedure currently uses a liquid-liquid and SPE combination extraction for specimen clean up. Preliminary evidence suggests that the extraction time can be shortened by as much as 6 hours, by a combination SPE clean up. The resulting fluorescence and TIC chromatograms show no interfering compounds. The HPLC conditions result in a retention time of 11.9 and 12.4 (monitored by fluorescence detection) for LSD and LAMPA, respectively, and the three ions monitored (by the Finnigan LCQ) are 324, 251, and 223 for both compounds. Detection levels at ~100 pg/mL have been observed in both the SIM and MS-MS mode for the LCQ.

Key Words: LSD, urine, LC/MS

Immunoaffinity Purification of LSD Prior to Confirmation by GC/MS. *Paul Morrill. Roark Gallowav, Jeffrey Shindelman,. David Davoudzadeh,. Neal Bellet* and William Coty; Beohringer Mannheim Corporation, Concord, CA.*

In order to simplify GC/MS confirmation of LSD, we developed a sample preparation method based on immunoaffinity chromatography. A highly-specific monoclonal antibody to LSD was attached to cyanogen bromide-activated Sepharose 4B. Urine samples containing LSD were incubated with affinity resin, the resin was washed and bound drug eluted in a small volume of methanol. Greater than 95% recovery of LSD added to drug-free urine was obtained; recovery could be monitored by addition of lysergic acid methyl-propylamide (LAMPA) or deuterated LSD as internal standards. Recovery of the weakly cross-reactive LSD metabolite, 2-oxo, 3-hydroxy LSD was 65%. Affinity resin extracts were derivatized for GC/MS analysis with N, O-bis(trimethylsilyl)trifluoro- acetamide (BSTFA) and subjected to gas chromatography on a Model DB5-MS column (15 m x 0.2 mm (id) 33 micron film, J & W, Folsom, CA). Immunoaffinity extracts gave dramatically reduced background signal as compared to conventional solid-phase extraction. An LOD of 50 pg/mL and an LOQ of 61 pg/mL were obtained using a Hewlett Packard model 5971 mass spectrometer with electron ionization. The immunoaffinity resin also appears to extract metabolites of LSD. Urine samples testing positive for LSD by immunoassay and GC/MS were extracted by the immunoaffinity method and further by reverse-phase HPLC. Testing of the resulting fractions by immunoassay (CEDIA® method, Boehringer Mannheim Corp.) showed an immunoreactive peak eluting earlier than LSD, and separate from the peak resulting from injection of 2-oxo, 3-hydroxy LSD. Studies to characterize the metabolite further by mass spectrometry are going.

Key Words: LSD, immunoaffinity purification

Effects of Adulterants & Interferents on CEDIA DAU Assays. *Gregory F. Grinstead*; Marshfield Laboratories, 1000 N. Oak Ave., Marshfield, WI 54449.*

Objectives: 1) Determine effects of commonly used adulterants on CEDIA DAU assays. 2) Recommend a strategy for detecting adulterated specimens.

Methods: Two types of specimens were used: Negative Urine and Urine spiked with the "NIDA 5" drugs at 50-100% above the DHHS cutoff levels. To each specimen type we added one of the following: Bleach, Glutaraldehyde, Visine, Vinegar, Ammonia, NaOH, Sodium Chloride, Liquid Soap, Tolmetin, or a buffer solution at pH 2, 3, 4, 5, 9, or 10. We assayed treated and untreated specimens for the NIDA 5 drug classes using CEDIA DAU reagents on a Hitachi 717 analyzer.

Results: Glutaraldehyde and bleach produced very depressed delta absorbances (about 200 units less than a typical negative) and effectively knocked out positives for all drugs. Ammonia and strong alkaline pH depressed responses to all drugs, but cocaine was by far the most sensitive. Moderate alkaline pH (9-10) had no effect on opiates, but depressed results for all other drugs, especially cocaine and THC. Vinegar, moderately acidic pH (2.0 -5.0), NaCl, and Visine selectively knocked out THC, but had little effect on other drugs. Liquid soap enhanced the response to THC, and had no effect on other drugs. Tolmetin had no effect on any drug.

Conclusion: The lab can detect many adulterants affecting CEDIA DAU assays by depressed delta absorbances, odor, pH, specific gravity, and full scan GC-MS for glutaraldehyde.

Key Words: Adulterants, CEDIA DAU, Tolmetin

Integration of Multiple Spreadsheets for Efficient Quantification and Reporting. *A. Louisa Martin,* Kelly M. Wood and Matthew T. Barnhill, Jr.; Alabama Department of Forensic Sciences, P.O. Box 7925, Mobile, AL 36670.*

Lotus 1,2,3 for Windows is used to integrate calibration data, drug data and case data into a report suitable for use in a forensic toxicology setting. The integration of multiple spreadsheets allows the discreet calibration of any number of drugs using a customized spreadsheet template, the storage of these calibrations within named ranges and the importation of relevant information into a customized report form quickly and easily through the use of custom macro buttons that appear on-screen.

Calibration data are recorded on a calibration spreadsheet where the regression analysis is performed, average retention times and relative retention times are calculated and therapeutic, toxic and lethal ranges recorded. Specific information on each drug is recorded in an inventory spreadsheet from which tables are derived for a listing of drugs by RRT and by filename. Reports are created on a separate spreadsheet by entering case information and importing the appropriate calibration file. Use of macro buttons makes the report generating process fast and efficient.

Quantification is performed and analyte information included in the report automatically, resulting in reports that are clear, concise, easy to interpret and contain the appropriate information pertinent to the case being investigated..

Key Words: Spreadsheet, customized reports, calibration and quantification

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A PC-Based Computer Program to Handle Records of DHHS-Mandated Specimens. *William N. Bennett[1], Daniel D. Bretl[1], Judy K. Musich[1] and Anthony Wu*[2]; [1]Bayshore Clinical Labs, Brown Deer, WI 53223; [2]A. Wu & Associates, Inc, Brookfield, WI 53005.*

The PC-based program described here has passed National Laboratory Certification Program inspections to meet Department of Health and Human Services (DHHS) guidelines. The program maintains a data base which is forensically sound and password protected. The system is stand-alone and is impervious to outside entry via networking or phone lines. Its features include: entry of demographics, result entry, search capabilities, help menus, formatting for telecommunication, uploading of results from Hitachi 717 autoanalyzer, calculation and printing of quarterly statistical reports in DHHS format, highly specified safeguards to ensure proper result reporting, production of daily worksheets, encrypted audit trail of all record edits, and hardcopy data lists to aid in certification.

Another feature is that reporting of all results requires initiation by a Certifying Scientist.

Key Words: Forensic, Computer, Program

Non- Halogenated Elution Solvents for Mixed Mode Solid Phase Extraction Sample Preparation of Urinary Benzoylecgonine, Opiates, Phencyclidine, and Amphetamines. *Catherine L. Schilling*[1] and Leon R. Glass[1,2]; [1]Pathology and Laboratory Medicine Service, 113-B; [2]Research Department of Veteran's Affairs Medical Center, 10000 Brecksville Rd., Brecksville, OH 44141 (USA).*

The chlorinated series of methane has been used for sample preparation with liquid/liquid and solid phase extraction (SPE) due to its extraction efficiency, selectivity, and ease of solvent removal. The toxicity of this solvent family has resulted in the classification of carbon tetrachloride, chloroform, and most recently dichloromethane (DCM), as carcinogens. In an effort to eliminate daily exposure of laboratory personnel to halogenated solvents during C_8/benzenesulfonyl SPE sample preparation, non-halogenated solvent mixtures (NHS) were investigated.

Columns from three suppliers were used in the study. Recoveries at SAMSHA cutoff concentrations were determined by GC-MS SIM quantitation using external standardization. The selectivity of the solvents was assessed qualitatively by comparison of chromatograms collected in scan acquisition mode.

NHS that produced recoveries equivalent to DCM/isopropanol/NH_4OH (78:20:2) on all SPE columns, and absolute recoveries greater than 80% for all analytes on the fully optimized columns, were: amphetamines, opiates, & phencyclidine - ethyl acetate/isopropanol/NH_4OH (84:12:4), and benzoylecgonine - ethyl acetate/methanol/NH_4OH (68:28:4).

Key Words: Solid-Phase Extraction, Halogenated solvents, Sample preparation

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Very Unusual Ethanol Distribution in a Fatality. *Peter P. Singer* and Graham R. Jones; Office of the Chief Medical Examiner, 7007 - 116 Street, Edmonton, Alberta, Canada T6H 5R8.*

A 48 year old man with an extensive history of alcoholism was found dead at home, lying face down on a carpet. The distribution of ethanol was very unusual (concentrations in mg/100 mL or mg/100 g): femoral blood 257 and 273 (two samples); heart blood 643, vitreous humor 763, urine 84, bile 616, liver 250, gastric 4660 (2470 mg/53 g). In addition, this man had ingested isopropanol, and according to the history, may also have ingested acetone in the form of nail polish remover. The distribution of both isopropanol and acetone was as expected, approximately in proportion to the aqueous content of the respective tissues. At autopsy and on histologic examination there was evidence of gastric aspiration. The lungs were heavy and congested, the heart was normal and there were marked fatty changes in the liver, but no cirrhosis. It is proposed that agonal or postmortem aspiration of the ethanol-rich vomitus and postmortem fermentation could account for the apparently elevated concentrations of ethanol in heart blood and bile. The elevated vitreous ethanol could be explained if ethanol diffused across the eye in the agonal phase or postmortem, from gastric aspirate in the carpet. The relatively low urinary ethanol concentration would be consistent with a recent binge drinking episode which allowed only a limited time period for excretion into an already partially full, but relatively ethanol-free bladder.

Key Words: Ethanol, Unusual distribution, Diffusion

Fatalities Associated with Moclobemide and Serotonin Reuptake Inhibitors. *Peter P. Singer* and Graham R. Jones; Office of the Chief Medical Examiner, 7007 - 116 Street, Edmonton, Alberta, Canada T6H 5R8.*

Moclobemide is a newer, reversible inhibitor of monoamine-A oxidase (a RIMA). It is available in Europe and Canada as "Manerix" and is undergoing clinical trials in the USA. Because of its reversibility and site specificity it has much lower toxicity than the older monoamine oxidase inhibitors (MAOIs), and single drug overdose is relatively benign; no fatalities have been reported. MAO-A sites are responsible for the metabolism of serotonin. After moclobemide overdose, however, the MAO reversibility is lost, and, if a serotonin reuptake inhibitor (SRI) is co-ingested, serotonin accumulates in the neural gap. This combined effect leads to greatly enhanced serotogenic neurotransmission and development of potentially life-threatening "serotonin syndrome". We present four Medical Examiner's fatalities involving moclobemide overdose. All four cases additionally involved varying concentrations of SRIs. Cases 1, 2 and 3 had postmortem blood moclobemide concentrations of 18.5, 9.4 and 13.0 mg/L and in liver 28.5, 39.1 and 25.0 mg/kg, respectively. Blood concentrations of moclobemide after therapeutic use are typically less than 2.5 mg/L. The SRIs occurring with the moclobemide were paroxetine (cases 1 & 2), fluvoxamine (case 2), fluoxetine (case 3) and sertraline (cases 2 & 3). Case 4 involved a women who was treated in hospital. The moclobemide concentration in admission serum was 23.0 mg/L and was 5.65 mg/L in postmortem blood; venlafaxine was the co-ingested SRI. Case 1 has been described in some detail elsewhere.[1]

1 "An uncommon fatality due to moclobemide and paroxetine" J. Anal. Tox. Oct 1997. P.P. Singer and G.R. Jones

Key Words: Moclobemide, Serotonin reuptake inhibition, Serotonin syndrome

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Distribution of Paroxetine in Two Postmortem Cases. *Tanya M. Vermeulen; Orange County Sheriff-Coroner, 320 N. Flower, Santa Ana, CA 92703.*

Paroxetine is a selective serotonin reuptake inhibitor, one of a new class of antidepressants used in the treatment of obsessive-compulsive disorder, panic disorder, and depression. Paroxetine potentiates serotonergic activity through the selective inhibition of the reuptake of serotonin in the central nervous system. There are relatively few reported overdoses in the literature, and of those, none were fatal.

We report two coroner's cases in which paroxetine was directly associated with the cause of death. In case #1, paroxetine was the only drug detected. No drugs were found at the scene and the autopsy findings were unremarkable. The concentrations of paroxetine were distributed in the tissues as follows: heart blood, 4.0 mg/L, liver, 110 mg/kg, and 102 mg total in the gastric contents. In case #2, paroxetine, fluoxetine, norfluoxetine and ethanol were detected. This case was a known suicide in which the decedent herself admitted taking pills and alcohol. The hospital blood sample drawn at admission was analyzed and found to contain 0.25% ethanol and no paroxetine. Fluoxetine and norfluoxetine were present in low levels. At death 10 hours later, the ethanol concentration in postmortem blood was 0.06% and paroxetine, fluoxetine and norfluoxetine were distributed as follows:

Specimen	Paroxetine	Fluoxetine	Norfluoxetine
Postmortem blood	3.7 mg/L	0.86 mg/L	0.65 mg/L
Peripheral blood	2.9 mg/L	0.68 mg/L	0.62 mg/L
Liver	113 mg/kg	28 mg/kg	17 mg/kg

The paroxetine levels in these 2 cases will be compared to paroxetine levels seen in Orange County Coroner's cases for 1995 and 1996.

Key Words: SSRIs, Paroxetine, Postmortem levels

Acetylcodeine Abuse – A Case Report. *Andrew L. Falzon, Marilyn Hall*, Robert M. Brissie, and C. Andrew Robinson; Department of Pathology, Divisions of Forensic Pathology and Laboratory Medicine, University of Alabama at Birmingham, Birmingham, AL.*

We present a case report of a 24 year old black female who was found dead in her bed the morning after she had spent the night partying with friends. A full autopsy performed the day of her demise revealed the presence of severe pulmonary congestion and edema as well as mild coronary atherosclerosis. No anatomical cause of death was identified at autopsy. Toxicological evaluation of blood revealed the presence of promethazine metabolite, diazepam, nordiazepam and acetaminophen. None of the aftermentioned drugs were present at a fatal level. Examination of urine revealed the presence of benzoylegconine, promethazine, promethazine metabolite, acetaminophen and codeine. Analysis of the gastric contents (total volume=250 mL) revealed the presence of diazepam and acetaminophen, in addition to acetylcodeine. The presence of acetylcodeine within the stomach and the relative ease with which acetylcodeine can be produced in the laboratory, suggest that this compound may represent a non-controlled chemical modification of codeine which is being pushed as a street drug. Review of the literature reveals the (ab)use of acetylcodeine in Southeast Asian populations in traditional folk medicine. However, to date, no reports were found suggesting the use of acetylcodeine as a street drug.

Key Words: acetylcodeine, toxicology, acetylation

Quantitation of Psychoactive Cannabinoids in Urine Samples from Driving Under the Influence Arrests. *Lionel P. Raymon*, Richard G. Maclure, Philip H. Dickson, Bernard W. Steele and H. Chip Walls; U. of Miami, Sch. of Med., Forensic Tox. Lab., Miami FL 33177.*

While 9-tetrahydrocannabinol (THC) and 11-hydroxy-9-tetrahydrocannabinol (11-OH-THC) are psychoactive, the inactive urinary metabolite 11-nor-9-carboxy-9-tetrahydrocannabinol (THC-COOH) is accepted as forensic evidence of recent marijuana abuse. This study adapted a procedure to quantitate THC and 11-OH-THC in urine samples (Kemp et al., 1995). Extensive validation and data from 6 months of specimen collections at the time of DUI arrests are presented. THC-COOH was determined in parallel.

Hydrolysis of the glucuronide conjugates was followed by hexane: ethyl acetate extraction under alkaline conditions. Recoveries at 50 and 5 ng/ml for 11-OH-THC and THC were above 90%. Analytes were derivatized with BSTFA prior to GC/MS analysis. Data was acquired in SIM mode. LODs were below 1 ng/ml and LOQ were 0.5 and 2.0 ng/ml for THC and 11-OH-THC, respectively. Linearity was observed from 0.5 to 500 ng/ml. CV between assays were 15 and 9% for THC and 11-OH-THC. CV within assays ranged from 0.5 to 3% for THC and 1.6 to 7.5% for 11-OH-THC. Precision at 50 ng/ml was 47±7 and 55±5 ng/ml for THC and 11-OH-THC, respectively. Analysis of 186 samples revealed that 11-OH-THC is readily present when THC-COOH is confirmed in the samples. 11-OH-THC and THC-COOH ranged from 3 to 2268 ng/ml (mean=156 ng/ml) and from 6 to 1853 ng/ml (mean=225 ng/ml), respectively. THC was not always present with THC-COOH, ranging from 2 to 942 ng/ml (mean=57 ng/ml). Correlations between the levels of psychoactive cannabinoids and the degree of impairment at the time of arrest and determination of urinary kinetic parameters of THC and 11-OH-THC under controlled conditions are warranted.

Key Words: Psychoactive cannabinoids-Single Ion Monitoring-DUI arrests

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Potentiation of the Vasoconstrictive Effect of Serotonin, Norepinephrine, and Tyramine by Cocaine on the Isolated Human Umbilical Artery. *Tessa L. Long[1]*, Kenneth E. Ferslew[1], Peter J. Rice[1], Uchenna C. Nwosu[2] and Frederick R. Jelovsek[2]; [1]Depts. of Pharmacology and [2]OB/GYN, Quillen College of Medicine, East Tenn. St. Univ., Johnson City, TN, 37614.*

Perinatal complications associated with cocaine (CO) abuse have generally been attributed to its inhibition of presynaptic reuptake of catecholamines resulting in vasoconstriction. Our objective was to evaluate the ability of CO to enhance the vasoconstrictive effects of serotonin (5HT) and norepinephrine (NE) in the human umbilical artery. Rings of umbilical arteries (4 mm) collected from normal pregnancies and perfused under physiological conditions in isolated tissue baths for isometric recording. Cumulative concentrations, (10^{-8} to 10^{-5}M) of NE and 5HT were added in the presence and absence of CO (10^{-5}M) to produce paired tissue comparisons. The mean vasoconstrictive effect of 5HT was increased 13% at 10^{-8}M, 16% at 10^{-6}M, and 17% at 10^{-5}M by cocaine. Cocaine potentiated the mean vasoconstrictive effect of NE 2.25-fold at 10^{-7}M, 2.50-fold at 10^{-6}M, and 3.71-fold at 10^{-5}M. The mean effective concentration of NE producing 50% of the maximum response (EC-50) was significantly decreased ($p=0.03$) in the presence of CO from the EC-50 without CO. Because methoxamine, a potent alpha-1 agonist, produced no vasoconstrictive effect over the dosage range of 10^{-8} to 10^{-5}M in the presence or absence of CO, NE may not be acting via alpha-1 receptors in this tissue. Doses of TYR
(10^{-4} and 10^{-5}M) were added to tissue segments in the presence and absence of CO (10^{-5}M) to assess the presynaptic activity of CO. The mean vasoconstrictive response to TYR was increased by 28% in the presence of CO, significance ($p=0.05$) at 10^{-4}M. We hypothesize that this vasoconstriction was due to a direct postsynaptic effect of TYR on the tissue. These data indicate that enhanced catecholamine activity in the umbilical artery is an important component of perinatal CO toxicity.

Key Words: Cocaine, Human Umbilical Arteries, Vasoconstriction

Plasma and Urine Concentrations of Flunitrazepam by Immunoassay and GC/MS After a Single Oral Dose. *Salvatore J. Salamone*[1], *Joseph J. Passarelli*[1], *Kathryn S. Schwenzer*[1], *Rudolf Brenneisen*[2], *Mahmoud A. ElSohly*[3] *and Shixia Feng*[3]; [1]*Roche Diagnostic Systems, Somerville, NJ, USA;* [2]*University of Bern, Bern, Switzerland;* [3]*ElSohly Laboratories, Oxford, MS, USA.*

A clinical study was conducted to assess the ability to detect flunitrazepam (FNP) in plasma and urine samples by immunoassay and gas chromatography-mass spectrometry (GC/MS). The clinical study consisted of four individuals (2 male, 2 female) who had taken a 2 mg dose of FNP. Plasma was collected over a 48-hour period and urine was collected over a 72-hour period. The plasma samples were analyzed by an OnLine Serum Based Assay for Benzodiazepines which had a 3 ng/mL limit of detection (LOD) and GC/MS which had a 1 ng/mL LOD. The urine samples were evaluated with the OnLine Assay for Benzodiazepines (using β-glucuronidase treatment) which had a 27 ng/mL LOD and by GC/MS which had a 1 ng/mL LOD. The immunoassay results on plasma samples showed that peak concentrations appeared between seven and eight hours for three individuals and one hour for the fourth individual. The maximum levels of cross reactive FNP ranged from 5 to 13 ng/mL and the time of last detection of drug ranged from six to eight hours for three individuals and one hour for the fourth individual. The immunoassay results on the urine samples showed peak concentrations between 12 and 35 hours with levels of cross reactive FNP ranging from between 66 and 163 ng/mL. All urine profiles displayed a biphasic nature and the time to the last positive sample ranged from 12 hours to 36 hours. GC/MS analysis of the plasma samples for FNP, nor-FNP, 7-amino-FNP and 7-amino-nor-FNP showed that only 7-amino-FNP was detectable. The maximum concentration of 7-amino-FNP ranged from 5 to 17 ng/mL in the four individuals and appeared at four to six hours for three individuals and at 24 hours for the fourth individual.

Key Words: Flunitrazepam, Immunoassay, GC/MS

Methylephedrine Concentrations in Blood and Urine Specimens. *Gary W. Kunsman*, Robert Jones, Barry Levine and Michael L. Smith; Forensic Toxicology Laboratory, Division of Forensic Toxicology, OAFME, AFIP1413 Research Blvd., Rockville, MD 20850-3125.*

Methylephedrine is a sympathomimetic amine that appears in many over-the-counter cough and cold medications throughout the world. In Japan, abuse of methylephedrine containing medications has been reported. Although methylephedrine is not available in the United States, it was identified in 15 cases received by the Forensic Toxicology Laboratory, Division of Forensic Toxicology, Office of the Armed Forces Medical Examiner, Armed Forces Institute of Pathology over a 2 year period; 12 of the 15 cases were collected from patients or decedents located within the confines of the Continental United States. Methylephedrine was identified in each case by gas chromatography/nitrogen phosphorus detection following an alkaline extraction and subsequently confirmed using full scan electron impact mass spectrometry. Quantitation of underivatized methylephedrine was performed using the same technique. Blood methylephedrine concentrations ranged from < 0.05 – 0.28 (n=14) and the mean methylephedrine concentration in urine was 1.6 mg/L (range: 0.15 – 6.8, n=11 [excluding case # 6]). A literature search revealed little information pertaining to the interpretation of methylephedrine concentrations in the blood. Six (6) of the 15 cases presented here were positive for methylephedrine in the blood, 3 of which were postmortem cases and 3 were non-fatal aircraft mishaps. There is no evidence in any of these cases that methylephedrine was present at toxic concentrations, therefore, it appears from the cases reviewed in this study that blood methylephedrine concentrations less than 0.3 mg/L are not associated with significant toxicity.

Key Words: methylephedrine, blood interpretation, sympathomimetics

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Determination of D,L Methamphetamine Isomers by Chiral Derivative/Chiral Column GC/MS. *Donald W. Jehn*, Gerald E. Kananen, Paula J. Underwood and Edwin K. Armitage; Forensic Toxicology Drug Testing Laboratory, Fort Meade, MD 20755.*

Department of Defense (DoD) reporting for methamphetamine requires the determination of the distribution of D and L isomers to preclude incarceration for legitimate inhaler use. In compliance with this requirement, our laboratory validated a procedure for the determination of D and L isomer distribution in samples containing methamphetamine. Over-the-counter products contain the L-isomer of methamphetamine. Abuse of methamphetamine normally involves use of D-methamphetamine or a racemic mixture of methamphetamine consisting of equivalent portions of the D-isomer and L-isomer. This method uses the DoD established 20% D-isomer value as the cutoff.

The procedure uses a combination of liquid-liquid extraction incorporating a chiral deriva- tizing reagent (S-trifluoroacetyl prolyl chloride) and chiral chromatographic column (Supelco's Gamma Dex 120, 30 m, 0.25 mm id, 0.25 um film thickness) for subsequent GC/MS analysis of the analytes using selected ion monitoring. The D, L isomer method is loaded onto a Chemstation that is linked to HP's 6890 GC and 5973 MSD. Data reduction was accomplished using a custom macro. The GC/MS run calculation accounts for D-isomer impurity in the chiral derivatizing agent. Limit of detection (LOD) was determined as 2% D-isomer; Limit of quantitation (LOQ) as 10% D-isomer. Within-run cutoff precision was established with a mean of 19.33, SD 0.08, CV 0.41%, and N 11. Linearity was established over a range of 10-100% D isomer ($r^2 = 0.9998$). Run-to-run precision (mean ± SD, CV, N) was established for LOD 2.62 ± 0.50, 18.90%, 16; LOQ 10.25 ± 0.61, 5.92%, 23; cutoff 19.13 ± 0.34, 1.80%, 31; positive control 47.22 ± 0.29, 0.61%, 21; and nonextracted standard 19.73 ± 0.75, 3.79%, 12. The assay was certified by an external agency through participation in their proficiency certification program.

Key Words: Methamphetamine Chiral Analysis

The Determination of Amphetamine, Methamphetamine and other Phenethylamines in Blood and Urine by a Dual Derivatization Technique. *Randal E. Clouette*, Jeffrey P. Brendler, Gary H. Wimbish and James C. Garriott; Harrison Laboratories, Inc. 9930 W. Hwy 80, Midland, TX 79706.*

The analysis of amphetamine in biological fluids is complicated by the fact that hydroxyphenelthylamines interfere with the analysis of amphetamine and methamphetamine by producing a "methamphetamine artifact". The solution to the problem has included reduction of injections port temperature, derivivative changes and pretreatment of samples with periodate to oxidize the hydroxyphenethylamines prior to extraction. The issue is further complicated by the fact that amphetamine can be produced from methamphetamine in the presence of periodate at an alkaline pH. Even with the delicate balancing act of time, temperature, pH and periodate concentration most laboratories employ the periodate oxidation step.

This method employs a dual derivatization technique that first silylates the hydroxyl group and primary or secondary amines with MSHFBA (N-Methyl-N-trimethlsilylheptafluorobutyramide). Then a second derivatizing agent, MBHFBA [N-Methyl-bis-heptafluorobutyramide] is added which replaces the silyl group on the amine fuctions with the heptafluorobutyrol group. Thus, the hydroxyphenelthylamines will have the silyl group on the hydroxyl moiety and the heptafluorbutyramide on the amino moiety while amphetamine and methamphetamine will have only the heptafluorobutyramide group. The byproducts of the derivatizating agents are not acidic and the derivitization can be performed directly in the autosampler vials without the need for wash steps or damage to columns. The silyl group on the hydroxyl group adds thermal stability to ephedrine, pseudoephedrine, and phenylpropanolamine preventing formation of the "methamphetamine artifacts". This dual derivatization technique allows chromatographic resolution of amphetamine, phentermine, methamphetamine, ephedrine, pseudoephedrine, and phenylpropanolamine as discrete entities thereby improving forensic quality for the analysis of amphetamines

Key Words: Amphetamine, Methamphetamine, Ephedrine

Labetalol Interference with Methamphetamine Analysis: Assessment and Elimination. *Donna Hensley* and John T. Cody; Clinical Investigations, Wilford Hall Medical Center, Lackland AFB TX 78236-5319.*

Administration of labetalol, a commonly used anti-hypertensive medication, has been shown to pose a potential threat to the proper analysis of methamphetamine in biological samples (Gilbert et al., JAT, 19(2):84-6, 1995). 1-Methyl-3-phenylpropylamine (MPP), a metabolite of labetalol, differs from amphetamine by a single methylene group making it isomeric with methamphetamine (MA). Under some GC conditions, the compound has been shown to co-elute with MA, posing a serious problem when using non-specific GC detectors such as FID or NPD. Mass spectra of MPP and MA differ thus eliminating the possibility of a false positive report, but both share common ions. Therefore, proper identification of MA is dependent upon ensuring MPP does not interfere.

Samples containing MA (50 - 10,000 ng/mL), MPP (50 - 15,000 ng/mL) and MA-D_{11} as internal standard were derivatized with HFBA, PFPA, or TFAA then analyzed by GC/MS. Three different GC conditions were evaluated: (#1) 80 (1 min)-180°C @20/min; (#2) 50-200°C @30/min to 260°C @15/min; (#3) 150-270°C @8/min.

Interference was seen with each of the conditions depending on the concentrations of the compounds, temperature programs and derivatives used. Interference manifested itself most commonly as lack of resolution (valley > 10%) of the m/z 118 peaks of MA and MPP. Condition #1 was able to confirm the presence of MA up to 2,500 ng/mL with as much as 15,000 ng/mL of MPP (a level consistent with administration of labetalol). Other GC parameters and derivatives suffered greater degrees of interference. These results demonstrate the proper GC/MS identification of MA in the presence of MPP requires care in selecting conditions to avoid false negatives.

Key Words: Interference, GC/MS, Methamphetamine, Labetalol

Fen-Phen: A Major Cause of False Positive Results in CEDIADAU-Amphetamine Assays. *Gregory F. Grinstead*, Mary R. Dommer and Diane M. Beyer; Marshfield Laboratories, 1000 N. Oak Ave., Marshfield, WI 54449.*

Objectives: 1) Assess frequency of false positive CEDIA DAU Amphetamine results caused by Fenfluramine-Phentermine (Fen-Phen). 2) Easily detect Fen-Phen by GC-MS.

Methods: Fenfluramine at 5000 ng/mL or phentermine at about 50,000 ng/mL give a positive CEDIA DAU Amphetamine result. We used PFAA derivatives and selected ions 190, 118, 91 for Amphetamine confirmation by GC-MS. PFP-Phentermine (prominent 91 ion and lesser 118 ion) and PFP-norfenfluramine (prominent 190 ion and lesser 118 ion) coelute with retention time about .15 minutes after amphetamine. A peak with prominent 190, 118, and 91 ions eluting .15 minutes after amphetamine indicates use of fen-phen.

Results: From Jan. through May, 1997, Marshfield Labs had 133 positive CEDIA DAU Amphetamine results in workplace drug testing specimens. Of those, 33 (25%) were confirmed positive by GC-MS for amphetamine and/or methamphetamine, 33 (25%) contained fen-phen and not amph/methamph, and 67 (50%) had neither fen-phen or amph/methamph. Of 70 GC-MS confirmatory batches for Amphetamine, 5 batches (7% of total) included only fen-phen samples.

Conclusion: Fen-phen is a major cause of false positive initial test results for labs using the CEDIA-DAU Amphetamine assay.

Key Words: Amphetamine, Fen-Phen, CEDIA DAU

Multi Component Immunoassay Screen for Amphetamine Drugs of Abuse in a Department of Defense Drug Testing Laboratory. *Daniel C Nichols, Paula J Underwood, Matthew J Schofield*, Gerald E Kananen and Edwin K Armitage; Forensic Toxicology Drug Testing Laboratory (FTDTL), Fort Meade, MD 20755.*

An effective immunoassay amphetamine screen for drugs of abuse must deal with interfering amines (phenylpropanolamine, phentermine, fenfluramine and ephedrines). To decrease costs, migrate to an environmentally friendlier immunoassay and decrease turn-around-time, the Department of Defense recently moved from radioimmunoassay (RIA) to kinetic interaction of microparticles in a solution (KIMS) assay. The FTDTL at Fort Meade has extensive experience with both assays. The percentage of presumptive positives (greater than 500 ng/mL of base compound) identified by KIMS (On-Line Amphetamine Kits, Roche, Somerville, NJ) requiring confirmation by GC-MS was increased five fold of the specimens requiring confirmation with RIA (Abuscreen Methamphetamine Kits, Roche, Somerville, NJ). While phenylpropanolamine and ephedrine were significant interfering substances in the KIMS assay, phentermine and fenfluramine did not interfere. At levels up to 1.0 mg/mL, the addition of sodium periodate (0.4 M) to the microparticle reagent (40:1000) oxidizes ephedrine to eliminate its interfering potential. Emit®II (Monoclonal Amphetamine/Methamphetamine Assay, Behring Diagnostics Inc, Cupertino, CA) was also evaluated. Phentermine, fenfluramine, and ephedrine presented significant Emit®II interference, while phenylpropanolamine did not significantly interfere. Although the KIMS assay could be modified to include sodium periodate to eliminate ephedrine, the Emit® assay did not allow this modification. The following procedures reduce the percentage of presumptive positives as follows: (a) KIMS plus periodate (70 %); (b) Emit®II (74 %); (a) and (b) in combination (87 %). Based on this data a decision was made to use KIMS, Emit®II and KIMS with periodate in combination to reduce the number of presumptive positives.

Key Words: Drugs of Abuse Screen, Amphetamine, other Interfering Amines

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Enhanced Sensitivity for the CEDIA dau Benzodiazepine Screening Assay. *Robert C. Meatherall*[1] and Albert D. Fraser[2]; [1]Biochemistry Laboratory, St. Boniface General Hospital, Winnipeg, Canada, R2H 2A6; [2]Toxicology Laboratory, Queen Elizabeth II Health Sciences Centre, Halifax, Canada, B3H 2Y9.*

The CEDIA dau Benzodiazepine (Microgenics) screening assay has been reformulated to provide increased sensitivity toward urinary benzodiazepine metabolites. Hydrolysis of benzodiazepine conjugates using E. coli b-glucuronidase has been incorporated into the kit as an on line procedure. The antibody now has increased cross-reactivity toward benzodiazepines which are excreted in urine in low metabolite concentrations.

Four subjects each ingested a single 1 mg lorazepam tablet. Urine samples were collected at 2, 5, 8, 11, 14, 24, 26, 29, 32, 35, 38 and 48 hours. Using a 200 ng/mL nitrazepam cut-off, positive results were obtained for the 4 individuals in samples collected at 5-24, 5-35, 2-35 and 2-32 hours.

There was little difference in assay response when aliquots of the same urine sample were hydrolysed on line for 5 minutes at 37°C using 40,000 U of E. Coli b-glucuronidase per mL of reaction mixture or off line for 2 hours at 56°C using 5,000 U of Helix pomatia b-glucuronidase per mL of reaction mixture. Without hydrolysis, all urine samples tested negative.

By comparison, the EMIT II (Syva) kit performed poorly. Only samples collected at 8-24,14, 5-32 and 14 hours were positive. A 100 ng/mL oxazepam cut-off was used.

The cross-reactivities of lorazepam in the CEDIA method ranged from 108% - 178% for lorazepam concentrations between 50 and 2,500 ng/mL. For the EMIT II screening method, the cross-reactivities ranged 0% - 31% over the same lorazepam concentrations.

Key Words: benzodiazepines, drugs of abuse, immunoassay

Assessment of the Status DS, a New Rapid Screening Test Kit for the Analysis of Drugs in Urine. *Maoxin Wu , Jerry Meena, and Michael Lehrer *; Dept. of Toxicology, Long Island Jewish Medical Center, Campus for the Albert Einstein College of Medicine, New Hyde Park, New York 11042.*

This study was designed to assess a new rapid drug screening test kit called Status DS which is manufactured and distributed by Orion Diagnostica, Inc. Urine drugs tested and their cutoffs include cannabinoids (50 ng/mL), opiates (300 ng/mL), cocaine metabolite (300 ng/mL), amphetamines (1000 ng/mL), phencyclidine (25 ng/mL), barbiturates (300 ng/mL), and benzodiazepines (300 ng/mL). The operational principle of the self contained test cartridge is based on a one step solid phase immunoassay utilizing highly specific monoclonal/polyclonal antibodies. Drug-BSA conjugates are immobilized at specific locations on the test membrane. Antibody dye conjugates are present on a pad near the sample well. Three drops of urine is placed into the sample well. Urine migrates by capillary action and drugs present compete for antibody binding with drug-BSA conjugates along the membrane. After 10 minutes the results are interpreted as follows: the appearance of a reddish-purple control line and a line for each specific drug indicates a negative result; the appearance of only a reddish-purple control line and no visible line next to a specific drug indicates a positive result for that drug.

The study assessed the sensitivity and specificity of the Status DS and compared it to that of Syva's EMIT and Abbott's FPIA assays. Specimens that gave result inconsistencies (among the 3 types of assays) were followed-up by performing quantitative GC/MS analysis. A total of 165 patient urine specimens were assayed: with about 40% of these specimens being drug positive for one or more of the drug classes. The results of the testing with the Status DS compared with Syva's EMIT DAU are listed below:

EMIT DAU

	THC		OPI		COC		AMP		BAR		BZO		PCP	
STATUS	+	-	+	-	+	-	+	-	+	-	+	-	+	-
DS +	10	2	9	1	10	0	10	7	10	4	10	6	5	2
-	0	98	1	99	0	100	0	93	0	96	0	94	0	98

The results of this study demonstrate excellent sensitivity (90% for opiates and 100% for all other drugs tested) and specificity (between 93-100%) when compared to the EMIT and FPIA methodologies. The specificity and sensitivity combined with the speed and breadth of drug classes being screened make the Status DS a very useful product for the hospital emergency room setting.

Key Words: Status DS, Rapid Drug Screening

Evaluation of Three Rapid Immunoassay Devices for Screening of DHHS Five Drugs in Urine. *Stuart C. Bogema*; Forensic Testing, Inc., Box 196, Clifton, VA 20124.*

This study evaluated the ability of three non-instrumented, on-site drug testing devices to screen for the presence of marijuana metabolites, cocaine metabolite, amphetamines, morphine/codeine and phencyclidine in urine. The study consisted of three parts : 1) ability of devices to identify drug/metabolite in reference calibrators above and below the DHHS cutoffs for initial screening, 2) ability of the devices to identify drug/metabolites in urine specimens from drug rehabilitation patients, and 3) the effect of potential interferents to mask the presence of marijuana and cocaine metabolites in patient specimens. The devices evaluated were the American Bio Medica Corp. (Ancramdale, NY) Rapid Screen (ABM), the Princeton BioMeditech Corp. (Monmouth Jct, NJ) AcuSign DOA 5/AMP (PBM), and the Roche Diagnostic Systems, Inc. (Somerville, NJ) OnTrak TesTcup-5 (RDS). Patient specimens were initially screened by the RDS OnLine immunoassay. The reference calibrators for precision around the DHHS cutoffs were made at 50%, 75%, 125% and 150% of the cutoff. The percent negative screen results for each device at 50% and 150% of the DHHS cutoffs was:

	AMP		COC		MOR		PCP		THC	
Device	50%	150%	50%	150%	50%	150%	50%	150%	50%	150%
AMB	90%	80%	100%	100%	90%	20%	60%	10%	70%	10%
PBM	70%	0%	100%	0%	100%	0%	90%	20%	90%	30%
RDS	100%	2.5%	100%	2.5%	100%	0%	100%	2.5%	100%	7.5%

Patient specimens that either screened negative for all drugs or positive for a drug with OnLine were tested with each device. (N)=number of specimens, FN=false negative, FP=false positive.

	AMP(136)		COC(234)		MOR(220)		PCP(120)		THC(215)	
Device	FN	FP	FN	FP	FN	FP	FN	FP	FN	FP
ABM	0%	8%	21%	0%	8%	4%	33%	6%	18%	12%
PBM	8%	0%	1%	0%	0%	0%	15%	0%	7%	0%
RDS	0%	0%	0%	0%	0%	0%	0%	1%	2%	0%

Potential interferents were used in excessive amounts. Dry bleach, salt, liquid Drano, Klear and Visine had little or no effect on PBM and RDS, but more on ABM. Liquid detergent and glutaraldehyde had some effect on all devices but did not change all positives to negative. THC Free caused all devices not to perform properly, resulting in invalid tests.

Key Words: Rapid Immunoassay Devices, On-Site Drug Testing, Adulterants

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GC/MS/MS and GC/FTIR Methods for the Determination of Fentanyl in Biological Matrices of Opiate Abusers. *J. Sklerov, T.Z. Bosy and K.S. Kalasinsky; Office of the Armed Forces Medical Examiner, Division of Forensic Toxicology, Armed Forces Institute of Pathology, Washington DC.*

Fentanyl is a synthetic analgesic with "opiate-like" properties which lends itself to abuse. Many different derivatives of fentanyl are sold as designer drugs on the illicit drug market. It is difficult to titer the dose as the derivatives have potencies that ranges from 500-7,000 times that of morphine. Many overdose cases result. Detection of fentanyl is complicated by the low doses, rapid metabolism and myriad of possible analogues that may be abused.

Ion trap GC/MS/MS offers enhanced detectivity over other analytical techniques because of the ability to eliminate background and matrix interferences. Cryogenic deposition GC/FTIR is useful when mass spec fragmentation patterns are indistinguishable; IR data increase the confidence of compound identification.

Blood, brain and hair from a number of fentanyl overdose cases were obtained for the study. The ion trap MS/MS method isolates the 245 parent ion of fentanyl which is then subjected to resonant dissociation. A mass-selective rf scan is used to eject and detect the product ions 146, 158, 189, & 202. FT-IR spectra are collected at 8 cm^{-1} resolution by cryogenic deposition of GC eluant on a ZnSe window held at -150^{0}C.

Therapeutic ranges of fentanyl are 1-3 ng/ml in blood and 0.1-0.5 ng/10mg in hair. Reported ranges for fentanyl overdoses are 2-100 ng/ml in blood and 10-30 ng/mg in brain. Detection limits of fentanyl for the various techniques are: immunoassay, 0.5 ng/ml; GC/MS, 2.0 ng/ml; GC/MS/MS, 0.1 ng/ml; GC/FTIR, 10 ng/m l.

Factors Affecting Enzymatic Hydrolysis, Mixed Mode Solid Phase Extraction, and GC-MS Analysis of Urinary Nordiazepam, Oxazepam, Temazepam, and alpha-Hydroxyalprazolam. *Leon R. Glass[1,2*], Stephen T. Ingalls[1,3] and Charles L. Hoppel[1,3]; [1]Research, [2]Pathology and Laboratory Medicine Service (113B), Department of Veterans Affairs Medical Center, 10000 Brecksville Rd., Brecksville, OH 44141; [3]Departments of Pharmacology and Medicine, Case Western Reserve University, Cleveland OH, 44106.*

Determination of four urinary benzodiazepines was investigated during sample pretreatment, C_8/benzenesulfonyl copolymer solid phase extraction (SPE), derivatization, and chromatographic stages of analysis. Pooled patient urine was used for hydrolysis studies. Drug-free urine was fortified with individual benzodiazepine standard materials to approximately 200 ng/ml for all other studies. Variables were isolated and investigated using external standardization for recovery calculation.

No single set of conditions was optimal for recovery of all four benzodiazepines. Oxazepam and temazepam were thermally labile during beta-glucuronidase hydrolysis. Tributyldimethylsilyl (TBDMS) derivatization improved GC peak shapes in relation to those observed with trimethylsilyl (TMS) derivatives on 95% silicon/5% phenyl capillary columns.

The composition of the aqueous wash, organic wash, and eluent used in SPE was the principal determinant of recovery efficiencies. Hydrolyzed urine was applied to prepared SPE columns. Interfering substances were eluted from the columns with 3 mL of type I water, 2 mL of 100 mM phoshpate buffer (pH 6.0) / MeCN (75:25 v:v), and 2 mL of n-hexane / EtOAc (90:10 v:v). The drugs were eluted from the columns with 3 mL of EtOAc. These SPE conditions afforded overall recoveries of alpha-hydroxyalprazolam, nordiazepam, oxazepam, and temazepam of at least 80%.

Key Words: Benzodiazepines, Solid Phase Extraction, GC-MS

Heroin and Scopolamine Combination Makes an Appearance in the Midwest. *Laureen J. Marinetti-Sheff*[1], Phyllis J. Good[1] and Kirk C. Mills[2]; [1]Michigan State Police Crime Laboratory, 714 South Harrison, East Lansing, MI 48823; [2]Detroit Receiving Hospital, Detroit, MI 48201.*

Nine subjects were admitted to the emergency room at Detroit Receiving Hospital exhibiting symptoms consistent with opiate toxicity. This occurred over a two day time span in August of 1996 in Detroit, Michigan.

After administration of a narcotic antagonist, six of the subjects continued to display adverse drug effects diagnosed as either anti-colinergic toxicity or CNS stimulant activity. An emergency room physician with a speciality in toxicology, recognized the anticholinergic toxicity and opiate combination as a phenomenon that had occurred on the East coast. Supportive treatment was given and all of the subjects recovered. Eight urine specimens were analyzed by the Michigan State Police Crime Laboratory - Toxicology Subunit. The analysis consisted of immunoassay screening with confirmation by gas chromatography/mass spectrometry in full scan EI+ mode. All of the urine specimens which confirmed for opiates (morphine and codeine) also contained scopolamine among other drugs. Both positive and negative results for the eight urine specimens are presented along with clinical presentation and user perceptions of the drug(s) ingested. The suspected heroin was purchased from the same trafficker although the subjects were not "using" together nor did they know one another.

Key Words: Heroin, Scopolamine, Toxicity

Self-intoxication of Morphine Obtained from an Infusion Pump - A Case Report. *Susan B. Gock*[1], Steven H. Wong[1,2], K. Alan Stormo[1,2] and Jeffrey M. Jentzen[1,2]; , Milwaukee County Medical Examiners' Office; and [2]Department of Pathology, Medical College of Wisconsin, Milwaukee, WI 53223.*

A 36 year old white male, who had a history of opiate abuse, was found unresponsive with white foam around his mouth by his wife, and pronounced dead shortly after. Approximately 6 hours before, his wife was awaken by his labored breathing. He had a history of back pain, and was treated with epidural morphine delivered to the spinal cord via a catheter of an infusion pump, set at 10 mg/24hours. This treatment was necessitated by his dependence on oral opiate medications. Due to crimping of the pump catheter, replacement was performed 4 days before. The capacity of the pump reservoir was 18 mL for delivery of 30 days of morphine totaling about 300 mg. Toxicological findings included: urine positive for amitriptyline, nortriptyline, opiates, hydrocodone metabolite, ibuprofen, acetaminophen, caffeine, and nicotine and metabolite. Drug concentrations were: blood - amitriptyline, 260 µg/L., nortriptyline, 160 µg/L., unconjugated morphine, 460 µg/L., total morphine, 624 µg/L., vitreous humor - unconjugated morphine, 34 µg/L, and total morphine, 80 µg/L., and CSF, unconjugated morphine, 99 µg/L. Residual pump content, collected by a syringe, accounted for only 22 mg instead of the expected 260 mg of morphine. The high blood morphine concentrations did not correlate with the infusion dose to the spinal cord. The symptoms were consistent with opiate overdose. The type of death was determined to be fatal morphine intoxication, and manner of death - accidental. This case is intended to alert the pathologists and toxicologists to the abuse potential of the newer analgesic delivery systems.

Key Words: Morphine Self-Intoxication, Abuse Potential Of Analgesic Infusion Pump, Morphine Vitreous Humor And CSF Concentrations

Release of Molecular Weight Silicones and Platinum from Silicone Breast Implants. *Ernest D. Lykissa*, Subbarao V. Kala, Jennifer B. Hurley and Russell M. Lebovitz; Department of Pathology, Baylor College of Medicine, Houston, TX 77030.*

We have conducted a series of studies addressing the chemical composition of silicone gels from breast implants as well as the diffusion of low molecular weight silicones (LM-silicones) and heavy metals from intact implants into surrounding media. LM-silicones in both implants and surrounding media were detected and quantitated using gas chromatography coupled with atomic emission (GC/AED) as well as mass spectrometric (GC/MS) detectors,.

Platinum, a catalyst used in the preparation of silicone gels, was detected and quantitated using inductive argon-coupled plasma/mass spectrometry (ICP/MS). Our results indicate that low molecular weight silicones contribute approximately 1-2% to the total gel mass and consist predominantly of cyclic and linear polydimethylsiloxanes ranging from 3 to 20 Siloxane [$(CH_3)_2$-Si-O] units (molecular weight 200-1500). Platinum can be detected in implant gels at levels of approximately 700 µg/kg by ICP/MS.

We studied the rates at which LM-silicones and platinum leak through the intact implant outer shell into the surrounding media under a variety of conditions. Leakage of silicones was greatest when the surrounding medium was lipid-rich, and up to 10 mg/day of LM-silicones was observed to diffuse into a lipid-rich medium per 250 g implant at 37°C. This rate of leakage was maintained over a 7-day experimental period. Similarly, platinum was also observed to leak through intact implants into lipid-containing media at rates of approximately 20-25 µg/day/250 g implant at 37°C. The rates at which both LM-silicones and platinum have been observed to leak from intact implants could lead to significant accumulation within lipid-rich tissues and should be investigated more fully *in vivo*.

Key Words: Siloxanes, Breast Implant, Platinum

Detection of Doxepin and Desmethyldoxepin in Hair During and Following Drug Therapy. *Adam Negrusz*[1], Christine M. Moore[2] and Jennifer L. Perry[1]; [1]Department of Pharmaceutics and Pharmacodynamics, College of Pharmacy, University of Illinois at Chicago, 833 S. Wood St., Chicago, IL 60612; [2]U.S. Drug Testing Laboratories, 1700 S. Mount Prospect Rd., Des Plaines, IL 60018.*

It is estimated that the number of suicides caused by depression in the U.S. varies from 26,000-75,000 and is the tenth highest cause of death for all ages. Doxepin (DOX) is widely used for the treatment of depression and has strong sedative effects. Its major metabolite, desmethyldoxepin (DDOX) also contributes to the pharmacological effect. Five hair samples (100 mg each) were collected between July of 1996 and March 1997 from the scalp of a 36 year old female taking 25 mg of DOX daily. Samples were washed, dried and pulverized. Internal standard, D_3DOX (4 ng/mg) was added to aliquots of hair (50 mg) and the samples were incubated in 0.1 M HCl for 18-24 hours. The drugs were removed from the hair using solid phase extraction and the final elution of the drugs was achieved using methylene chloride:isopropanol:ammonium hydroxide (78:20:2, v/v/v). Extracts were evaporated to dryness, reconstituted in acetonitrile (40 µL) and derivatized using BSTFA with 1% TMCS (40 µL). A Hewlett Packard GC-MS system comprising a 6890 GC and a 5973 MSD detector operated in SIM mode was used for analysis. For DOX *m/z* 58, for D_3DOX *m/z* 61, and for DDOX *m/z* 116 ions were monitored. Standard curves for DOX and DDOX prepared in negative hair, were linear over the range 0.25-20 ng/mg with correlation coefficients of 0.984 and 0.985, respectively. Intra-day variability was determined using three replicates of controls (2 and 15 ng/mg) prepared in negative hair and analyzed on a single day. Inter-day variability was determined over a one month period on 8 separate days. All precision and accuracy values were within acceptable limits. The highest concentration of DOX and DDOX was observed in hair samples collected one month after DOX therapy was terminated.

Key Words: Hair Analysis, Tricyclic Antidepressants, GC-MS

Lack of Dose-Concentration in Hair Relationship in a Controlled Heroin Maintenance Program, *Rudolf Brenneisen*[1], Pascal Kintz[2], Petra Bundeli[1] and Bertrand Ludes[2]; [1]Institute of Pharmacy, University of Bern, Baltzerstrasse 5, CH-3012 Bern, Switzerland; [2] Institut de Médecine Légale, 11 rue Humann, F-67000 Strasbourg, France.*

Hair specimens were collected in the vertex area from 20 subjects participating in the Swiss heroin maintenance program. Heroin hydrochloride was intravenously self-administered 2 or 3 times per day, with daily doses ranging from 30 to 800 mg. In all cases, a 4-cm segment from the proximal zone (root) was analyzed. This corresponds to about 100 days of hair growth. During that period, the total heroin administered ranged from 14100 to 71540 mg. All special features of hair such as coloring, bleaching etc. were noted. Each sample was twice washed with dichloromethane and, after drying, cut into small pieces of about 1 mm. A 30-35 mg aliquot was incubated overnight at 45°C in 1 mL methanol in presence of 200 ng of heroin-d_9, 6-acetylmorphine-d_3, and morphine-d_3. The methanolic extract was then evaporated to dryness and the residue derivatized by silylation. Drugs were analyzed by GC/MS. Limits of quantitation were set to 0.1 ng/mg. Concentrations ranged from 0 to 4.5, 0.4 to 10.1, and 0.7 to 5.2 ng/mg for heroin, 6-acetylmorphine, and morphine, respectively. In only 5 samples, 6-acetylmorphine was not the major analyte present in hair. Heroin dominated in 3 specimens, while morphine, probably due to hydrolysis, was the major opiate in 2 specimens. No correlation between the doses of administered heroin and the concentrations of total opiates in hair was observed (correlation factor was 0.346). Unless more is known about the factors that may influence the incorporation of drugs into hair, and the way to reduce the observed variability, these results suggest that the idea of using quantitative drug measurements in hair to determine the amount of heroin used will remain inapplicable.

Evaluation of 15 Non-Instrumented Drug Testing Devices. *Robert E. Willette*[1] and Leo J. Kadehjian.[2]; [1]Duo Research Inc. 2419 East Fifth Avenue, Denver, CO 80206 U.S.A.; [2]765 Chimalus Drive, Palo Alto, CA 94306 U.S.A.*

Renewed interest in conducting drug tests on-site has prompted a proliferation of "hand held" or non-instrumented drug test (NIDT) devices on the market. The study reported here was conducted in an effort to evaluate the performance characteristics of as many available devices as possible for use in criminal justice and workplace drug testing programs.

Fifteen commercial NIDT devices were tested with a reference instrumented system, a Syva ETS using Emit d.a.u. reagents. The devices included multiple and single test formats and dipstick and "plate" designs.

Ninety samples were selected for each of the five HHS-authorized drug classes from specimens received at the contract laboratory serving the U.S. Federal Probation and Pretrial Services. In order to challenge the devices on their accuracy around the cutoff, based on the initial laboratory screening results, 800 samples were selected to provide about 15 giving negative responses, 60 between minus 25% and plus 25% of the cutoff, and 15 greater than 25% above the cutoff for each drug class. Ten known control samples were also tested. All samples were analyzed by GC/MS, and the results evaluated using criteria established by the U.S. Courts and HHS, using conventional calculations: e.g., sensitivity, specificity, and positive and negative predictive values. Significant variations were found between the various devices across each drug class and between drug classes. Although false positive and false negative values ranged from 88% and 87%, resp., to 0%, several devices produced results comparable to the instrumented on-site reference method.

Data comparing device performance and operational characteristics will be presented. (Supported by the Administrative Office of the U.S. Courts, Contract No. USCA 67001)

Key Words: Non-Instrumented Drug Test Devices, On-site Drug Testing, GC/MS

The Effect of Consumption of Dihydroepiandrosterone (DHEA) on Testosterone: Epitestosterone Ratios in Human Urine. *Karla A. Moore*, Thomas Z. Bosy and Alphonse Poklis, Department of Pathology, Medical College of Virginia, Richmond, Virginia 23298-0165*

Use of selected anabolic, androgenic steroids is illegal in the military. For synthetic anabolic androgenic steroids, the identification of the parent steroid and metabolites in urine is evidence that abuse has taken place. For substances that are produced naturally, like testosterone, the mere presence of the substance in the urine obviously cannot constitute proof of an offense. In recognition of this situation, the International Olympic Committee (IOC) and the Department of Defense have worded their rules to state that testosterone administration is banned and that the testosterone (T): epitestosterone (E) ratio may not exceed 6. Exogenous testosterone administration results in an increase in the T:E ratio because exogenous testosterone is not incorporated into the manufacture of epitestosterone.

The purpose of this investigation was to determine the effect of the consumption of 50 mg/day dihydroepiandrosterone (DHEA), a readily available, legal, over-the-counter nutritional supplement, on the normal T:E ratio in human urine. Steroid abusers are claiming that DHEA is responsible for their abnormal T:E ratios rather than illegal steroid consumption. Baseline DHEA and T:E ratios were determined for six (6) male volunteers. Subsequently, each volunteer took one 50-mg DHEA tablet each morning for 30 consecutive mornings. Each day, a pre-dose and 3-hour post-dose urine sample was collected. DHEA, T:E and T:DHEA ratios were determined using a previously established gas chromatography/mass spectrometry procedure. This dose of DHEA taken for 30 days appears to have no significant effect on either of these ratios. Moreover, administration of this dosage for this period of time does not result in an elevation of the T:E ratio to levels which would be consistent with exogenous testosterone abuse as defined by the IOC.

Key Words: Dihydroepiandrosterone, Anabolic Steroids, Steroid Abuse

11-Hydroxy-Δ^9-THC and 8β,11-di-hydroxy-Δ^9-THC and Their Glucuronides as Major Metabolites of Δ^9-THC in Meconium: GC/MS Analysis. *Mahmoud A. ElSohly* and Shixia Feng; ElSohly Laboratories, Incorporated (ELI), 5 Industrial Park Drive, Oxford, MS 38655 USA.*

GC/MS analysis of meconium specimens screening positive for cannabinoids by the EMIT 20 Assay showed a low confirmation rate for 11-nor-Δ^9-THC-9-COOH (THC-COOH). A study was designed to investigate the possible contribution of other Δ^9-THC metabolites to the overall response of the EIA, including glucuronides. Delta-9-THC-glucuronide was synthesized in order to develop the most efficient procedure for hydrolysis of glucuronides in meconium. Procedures were developed for the extraction and GC/MS analysis of Δ^9-THC, 11-OH-Δ^9-THC, 8α- and 8β-OH-D^9-THC, 8β,11-diOH-Δ^9-THC, and THC-COOH, after enzymatic hydrolysis of meconium extracts. The data concluded that (a) enzymatic hydrolysis of meconium extracts is necessary for efficient recovery of Δ^9-THC metabolites; (b) Δ^9-THC and its 8-OH metabolite(s) are basically absent in meconium specimens; (c) 11-OH-Δ^9-THC, and 8β,11-diOH-Δ^9-THC contribute significantly to the immunoassay response of meconium extracts. Analysis of several meconium specimens which screened positive for cannabinoids but failed to confirm for THC-COOH showed significant

amounts of 11-OH-Δ^9-THC and 8β,11-diOH-Δ^9-THC. Therefore, GC/MS confirmation of cannabinoids in meconium should include analysis for these two metabolites in addition to the THC-COOH.

Supported in part by National Institute on Drug Abuse SBIR Contract No. N43DA-6-7057

Key Words: Meconium, GC/MS Analysis, Δ^9-THC-Metabolites in Meconium

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An Analysis of LSD in Urine Under Varios Storage Conditions. *Alan J. McNally*, Zhuyin Li, Ian A. Pilcher, Haiying Wang and Salvatore J. Salamone; Roche Diagnostic Systems, Inc., 1080 U.S. Highway 202, Somerville, NJ 08876-3771.*

Few formal studies have been conducted on the stability of LSD in urine under various storage conditions. A controlled study was conducted to determine the stability of LSD in pooled urine samples at 0.5 ng/mL and 500 ng/mL of LSD. The urine samples were followed over time at various temperatures, in different types of storage containers, at various exposures to different wavelengths of light, and at varying pHs. LSD concentrations were measured quantitatively by the Abuscreen RIA and by HPLC using a fluorescence detection method. Good correlation of data was observed between the immunoassay binding and the fluorescent integrity of the LSD molecule. Thermostability studies were conducted in the dark, in amber glass, and polyethylene containers demonstrated no significant loss at 25°C for four weeks. At higher temperatures there was a 30% loss at 37°C and up to a 40% loss at 45°C for four weeks. These thermostability results were the same for each of the container materials tested. Three container types, amber glass, polyethylene, and quartz, were used as storage containers for testing the stability of LSD during light exposure. Various light conditions used and tested at various time points at 25°C were: 15 watts of fluorescence light at 15 cm or 200 cm distance, 254 nm UV light with 345 watts, and 365 nm light with 345 watts. The urine containing LSD in the amber glass showed no loss under any light conditions. The polyethylene container after one month revealed a 20% loss when placed 200 cm from the fluorescence source (normal lab conditions) and displayed a 60% loss when placed 15 cm from the fluorescence source. In addition, when using the polyethylene container at a distance of 15 cm from a 254 nm strong UV light source, no loss was observed after eight hours. When this polyethylene container was placed 15 cm from a 365 nm strong light source for eight hours, a 50% loss was observed. In the quartz container, losses of 28% and 50% were observed using strong light sources of 254 nm and 365 nm respectively, at a 15 cm distance for eight hours.

Key Words: LSD, urine, stability

A Fatal Drug Interaction Between Clozapine and Fluoxetine. *Kenneth E. Ferslew[1]*, Andrea N. Hagardorn[1], Gretel C. Harlan[2] and William F. McCormick[2]; [1]Section of Toxicology, Department of Pharmacology, [2]Department of Forensic Pathology, James H. Quillen College of Medicine, East Tennessee State University, Johnson City, TN 37614.*

A case is presented of a fatal drug interaction caused by ingestion of clozapine (Clozaril) and fluoxetine (Prozac). Clozapine is a tricyclic dibenzodiazepine derivative used as an "atypical antipsychotic" in the treatment of severe paranoid schizophrenia. Fluoxetine is a selective serotonin reuptake inhibitor used for the treatment of major depression. Clinical studies have proven that concomitant administration of fluoxetine and clozapine produces increased plasma concentrations of clozapine and enhances clozapine's pharmacological effects due to suspected inhibition of clozapine metabolism by fluoxetine. Blood, gastric and urine specimens were analyzed for fluoxetine by GC/MS and for clozapine by GLC. Clozapine concentrations were: plasma, 4.9 μg/ml; gastric contents, 265 mg; and urine, 51.5 μg/ml. Fluoxetine concentrations were: blood, 0.7 μg/ml; gastric contents, 3.7 mg; and urine 1.6 μg/ml. Analysis of the biological specimens for other drugs revealed the presence of ethanol (blood, 35 mg/dl; vitreous, 56 mg/dl; and urine 153 mg/dl) and caffeine (present in all specimens). The combination of these drugs produced lethal concentrations of clozapine and high therapeutic to toxic concentrations of fluoxetine. The deceased had pulmonary edema, visceral vascular congestion, paralytic ileus, gastroenteritis and eosinophilia. These conditions are associated with clozapine toxicity. The combined central nervous system, respiratory and cardiovascular depression of these drugs was sufficient to cause death. The death was determined to be a clozapine overdose due to a fatal drug interaction.

Key Words: Clozapine, Fluoxetine, Drug Interaction

Drug Reviews in Progress at the Drug Enforcement Administration. *Kira D. Hutchinson; Drug and Chemical Evaluation Section, Office of Diversion Control, Drug Enforcement Administration, Washington, DC 20537.*

The staff of the Drug and Chemical Evaluation Section of the Drug Enforcement Administration (DEA) is composed of scientists that among many things, evaluate drugs for control under the Controlled Substances Act (CSA). This poster presentation will explain the process of controlling a drug under the Controlled Substances Act and those drugs which are currently under review at the Drug and Chemical Evaluation Section of DEA.

A substance can be controlled under the CSA by several different venues, all of which are specific legal procedures that are based on scientific data. The most common route for controlling or decontrolling a substances involves a petition review process in which the abuse potential of the substance is evaluated on the basis of 8 factors. This evaluation, referred to as an eight factor analysis is transmitted to the Department of Health and Human Services (DHHS) by the DEA. The DHHS in turn, performs an independent analysis of the abuse potential of the substance and returns a scientific and medical evaluation as well as a scheduling recommendation to the DEA. The DEA then considers the DHHS scientific and medical evaluation and all other relevant data. If

the DHHS has recommended control of the substance under the CSA then DEA determines the appropriate schedule for that substance. All scheduling actions are published in the federal register as proposed rules, subject to comment and requests for a hearing by interested parties. The following drugs are among those currently under evaluation at the DEA: butorphanol, carisoprodol, fenfluramine, flunitrazepam, GHB, and ketamine.

Key Words: DEA, CSA, Drug Scheduling

Nitrite Adulteration of Workplace Urine Drug Testing Specimens: Concentrations of Nitrite in Urine, and Distinction Between Natural Sources and Adulteration. *Francis M. Urry*, Gabor Komaromy-Hiller, Brian Staley, David K. Crockett, Mark Kushnir, Gordon Nelson, and Richard E. Struempler; ARUP Laboratories, Salt Lake City, UT, 84108.*

The commercial adulterant Klear contains nitrite ion. Nitrite compromises the confirmation of some drugs, notably carboxy-THC. A reported bisulfite step overcomes some nitrite adulteration, but not all. Since nitrite is present in normal urine, it is necessary to provide evidence that the amount of nitrite in a workplace urine specimen could not arise by normal means in order to defend a report of nitrite adulteration. Our objectives were to identify all sources of nitrite in urine and their concentrations, and to determine if nitrite adulteration can be supported by a quantitative result. The literature was reviewed for all nitrite sources, and their concentrations are reported. Separately, nitrite was measured by a spectrophotometric method in the following: specimens nitrite positive by test strip, <15 ug/mL; specimens culture positive for nitrate reducing microorganisms, <36 ug/mL; specimens from patients on medications which might metabolize to nitrite, <6 ug/mL; and drug test specimens which appeared to be adulterated with nitrite, >1,900 ug/mL. The literature and the nitrite measurements indicate a substantial difference between concentrations from natural sources and adulteration. Nitrite quantitation can provide valid evidence of adulteration with a nitrite-containing substance.

Key Words: Adulteration, workplace, nitrite

Potency of Cannabis in New Zealand from 1976 to 1996. *Helen A Poulsen* and Graeme J Sutherland; Institute of Environmental Science ~ Research Limited Wellington Science Centre, P O Box 30-547, Lower Hutt, New Zealand.*

Cannabis plant and cannabis oil are the most frequently abused illegal drugs in New Zealand The origin of cannabis plant and its products available to the user has changed dramatically over the last 20 years. In recent years there have been frequent media claims that cannabis is much stronger now than 20 years ago.

The potency of cannabis plant and its products found in New Zealand over the period of 20 years has been studied. The first part of the study includes imported cannabis oil, cannabis resin and cannabis plant seized by police. The latter part of the study includes little imported material as cannabis plant was grown in New Zealand, cannabis oil was manufactured locally and imported cannabis resin became rare.

Cannabis plant for the study was extracted with isopropyl alcohol in a manner similar to that used by local cannabis oil manufacturers. The cannabis oil produced and the oil and resin seized by the Police were taken up in ethanol and analyzed by capillary gas chromatography.

The average potency of cannabis plant available to the user has not increased over the 20 year period with leaf remaining at around 1% THC(tetahydrocannabinol) and the female flowering heads at around 3.5%. The average potency of cannabis oil has dropped from 45% in 1985 to 13% in 1995.

Key Words: Cannabis Plant, Cannabis Products, Potency

Toxicity of Breast Implant Cyclic Siloxanes on Rat-1 Fibroblasts and MCF-7 Human Breast Carcinoma Cells. *Subarao V. Kala*, Ernest D. Lykissa, Gene Isabel and Russel M. Lebovitz; Department of Pathology, Baylor College of Medicine, Houston, TX 77030.*

We have recently demonstrated that intact silicone breast implants release a relatively constant stream of low molecular weight silicones (LM silicones) plus small amounts of platinum into surrounding media. To evaluate possible effects of this effluent on cells and tissues, we exposed rodent and human cell lines to different concentrations of a distillate prepared from implant gels that consists of LM-silicones plus platinum, and assayed toxicity both microscopically and by monitoring release of lactate dehydrogenase (LDH). Both Rat-1 rodent fibroblasts and MCF-7 human breast carcinoma cells showed substantial cell membrane damage as assayed by LDH release within 4 hours of exposure to silicone concentrations of 10 mM or greater. Longer exposure periods (24 hr) of these cells to cyclic siloxanes resulted in the release of LDH even at lower concentrations (1 mM). At higher silicone concentrations, greater than 95% of cells were killed within 24 hours when assayed microscopically. Significant amounts of LM-silicones were detected in cell membrane fraction, suggesting that LM-silicones may induce cell injury and death primarily by direct damage to membranes. LDH release correlated most closely with the amount of octamethylcyclotetrasiloxane (D4) bound to the cell membrane, suggesting that D4 and to a lesser extent, D5-D7 are responsible for cell membrane damage. Interestingly, hexamethyl-cyclotrisiloxane (D3), the most water soluble of the LMsilicones, does not bind appreciably to cell membranes. We conclude that LM-silicones, and particularly the cyclic LM-silicones D4-D8, can cause direct cell injury and therefore pose a potential threat to implant recipients that should be investigated in greater detail.

Key Words: Siloxanes, Breast Implant, LDH, Toxicity

CE-TOFMS for the Analysis of Drugs of Abuse. *Milton L. Lee,[1]* Iulia M. Lazar,[1] Kenneth D. Onuska[1] and Gary Naisbitt[2]; [1]Department of Chemistry and Biochemistry, Brigham Young University, Provo, UT 84602, USA; [2]Utah State Crime Laboratory, Salt Lake City, UT 84114, USA and [2]Utah State Crime Laboratory, Salt Lake City, UT 84114, USA.*

Capillary electrophoresis (CE) which rapidly grew in the 1980s has become a mature and well established separation technique. The fast development of CE (and its related techniques) is due to some of its characteristics such as: broad applicability (ionic/non-ionic. and low/high molecular weight compounds), high separation efficiency in short analysis time, very good mass limits of detection, and relatively low cost and simplicity. Rapid progress has also been made in interfacing CE to mass spectrometry (MS).

We have recently developed an electrospray time-of-flight mass spectrometer (ESI-TOFMS) for use as a detector for fast and efficient liquid phase separations. The main features of the instrument are speed and sensitivity. Low attomole detection limits have been achieved with continuous infusion experiments, and the acquisition rate can be as high as 10,000 spectra per second.

The present report describes briefly the instrumental set-up and the analysis of some mixtures of illicit drugs through CE-TOFMS and two-dimensional isotachophoresis (ITP)-TOFMS. Seized drug samples were analyzed either through continuous infusion or CE-TOFMS experiments. Detection in the low femtomole region was obtained using CE-TOFMS

Acknowledgment. This work was funded through a Grant/Cooperative agreement sponsored by the National Exposure Research Laboratory-Characterization Research Division (NERL-CRD), U.S. Environmental Protection Agency, Las Vegas, NV.

Key Words: Capillary Electrophoresis, Time-of-Flight Mass Spectrometry, Drugs of Abuse

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Automated Solid-Phase Extraction and GC/MS Quantitation of Therapeutic and Fatal Levels of Basic Drugs in Post-mortem Whole Blood and Liver. *Moria A. Lymburn, Diana C Kappatos, Stuart J Dickson and Helen A Poulsen*; Institute of Environmental Science & Research Limited, Wellington Science Centre, P O Box 30-547, Lower Hutt, New Zealand.*

The extraction of basic drugs has been developed using the Zymark Rapid Trace automated solid phase extraction system for both post-mortem liver and whole blood samples; with the use of deuterated internal standards for quantitation using capillary GC/MS

The solid phase extraction and clean up procedure entails the use of Varian Bondelut Certify SPE cartridges (mixed-mode bonded phase siliça). Clean up of both enzymatically digested liver and whole blood extracts is achieved on the SPE with solvent concentration prior to GC/MS.

Use of the automated SPE system has increased through put of samples, reduced solvent/reagent expense while still giving excellent reproducibility. Intra-day and inter-day variations, linearity, recovery levels, coefficient of variations, limits of detection and limits of quantitation are evaluated for a wide variety of common basic drugs. The cleanliness of both the digested liver and whole blood extracts from the SPE are suitable for both capillary GC/MS and capillary GC with Nitrogen-Phosphorus-Detection

This method of extraction is also excellent for basic drugs that are difficult to extract by traditional means. eg. paroxetine from liver extracts.

Key Words: Solid Phase Extraction, Post-mortem Blood and Liver

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Determination of Buprenorphine and Naloxone in Human Plasma by LC/MS/MS. *Alan C. Spanbauer*, John D. Laycock, Dennis J. Crouch, David E. Moody and Rodger L. Foltz; Center for Human Toxicology, University of Utah, Salt Lake City, UT.*

Buprenorphine exhibits partial μ-agonist properties and k-antagonist properties. It is a potent synthetic opioid that has received attention recently in the treatment of opioid dependence. A combination of buprenorphine with the opiate antagonist, naloxone, is suggested to preclude parental abuse while retaining sublingual efficacy. The low plasma concentrations of buprenorphine and naloxone require a sensitive and selective technique for their determination in biological samples.

Plasma samples (1 mL) are fortified with internal standards of buprenorphine d_4 and naltrexone d_3 at 5 ng/mL. A liquid-liquid extraction is performed by raising the plasma pH to 9.5 by addition of 1 N NaOH and mixing with 4 mL of 4:1 butyl chloride:acetonitrile. After centrifugation, the organic layer is separated and evaporated under air. The residue is reconstituted in LC mobile phase for injection into the LC/MS/MS. A Zorbax RX-C18 narrow-bore (2.1 x 150 mm, 5 μm particle size) LC column and gradient elution are employed. The binary gradient consists of H_2O-0.1% formic acid and acetonitrile-0.1% formic acid. The method uses a Finnigan TSQ7000 tandem mass spectrometer with electrospray ionization (ESI). The tube lens and capillary voltages are optimized for the $[M+H]^+$ ions of buprenorphine and naloxone. The MS/MS transitions monitored are m/z 472 to 400 for buprenorphine d_4, m/z 468 to 396 for buprenorphine, m/z 328 to 310 for naloxone, and m/z 345 to 327 for naltrexone d_3.

The method has been validated in plasma with an LOQ of 0.1 ng/mL for both analytes. Both the buprenorphine and naloxone curves are weighted quadratic curves from 0.1 to 10 ng/mL. Quality control samples at 0.25, 1.0, and 5.0 ng/mL were employed for precision and accuracy validation runs. Calculated concentrations for the LOQ and all QC's were within 15% of the target value and % cv's are all less than 15%. All precision and accuracy, recovery, selectivity, and stability data will be described in detail.

(Supported in part by NIDA Contract No. NO1DA-1-9205)

Key Words: Buprenorphine, Naloxone, LC/MS/MS, Plasma

Determination of Risperidone and 9-Hydroxy-Risperidone in Human Plasma by LC/MS/MS. *Wei Huang*, John D. Laycock , Rodger L. Foltz, and David E. Moody; Center for Human Toxicology, University of Utah, Salt Lake City, UT.*

Risperidone, a benzisoxazole derivative, is a novel antipsychotic agent used for the treatment of schizophrenia. Risperidone and its major metabolite, 9-hydroxy-resperidone, have been analyzed by RIA and by HPLC with electrochemical or UV/VIS detection. We have developed an LC/MS/MS method with improved sensitivity, selectivity and dynamic range.

A structural analog of resperidone, methyl risperidone (5 ng/mL), is added as the internal standard to 1 mL of human plasma. After adjustment of the plasma pH to 10.5 by addition of saturated sodium carbonate, the plasma is extracted into 4 mL of pentane:methylene chloride (3:1). The organic phase is collected, evaporated to dryness, and the residue is reconstituted in water with 1% formic acid. For LC/MS/MS analysis a Metachem Inertsel HPLC column (2.1 x 150 mm, 5 µ particle size) is connected to a Finnigan TSQ7000 tandem mass spectrometer via the Finnigan API interface. Both electrospray (ESI) and atmospheric pressure chemical ionization (APCI) produce predominantly MH^+ ions for the two analytes and the internal standard. Ions detected by selected reaction monitoring (SRM) correspond to the following transitions: m/z 411 to 191 for resperidone, m/z 427 to 207 for 9-hydroxyresperidone , and m/z 421 to 201 for the methyl risperidone.

ESI provided better sensitivity with a signal-to-noise ratio > 10:1 at 25 pg/mL. APCI, however, provided a larger dynamic range (0.1 to 100 ng/mL) and better precision and accuracy. Precision and accuracy for the APCI method are presented below as % Target ± %CV.

	Target	Intra-Assay		Inter-Assay	
Sample	(ng/mL)	Risperidone	9-OH-Ris.	Risperidone	9-OH-Ris.
LOQ	0.10	94 ± 13.6	97 ± 9.5	99 ± 5.5	109 ± 12.0
QC1	0.25	102 ± 7.3	85 ± 3.1	97 ± 5.8	92 ± 7.2
QC2	2.50	94 ± 3.8	94 ± 3.5	93 ± 6.2	93 ± 8.4
QC3	15.0	97 ± 7.0	98 ± 5.6	97 ± 0.7	99 ± 1.6

(Supported in part by NIDA Contract No. NO1DA-1-9205)

Keywords: Risperidone, 9-Hydroxy-Risperidone, LC/MS/MS, Plasma

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Review of Postmortem Toxicology in Twenty Deaths Involving Sertraline. *Kabrena E. Goeringer*[1] and Barry K. Logan[2]; [1]Department of the Air Force, HQ USAFA/DFC, 2355 Fairchild Drive Suite 2N225, USAF Academy, CO 80840 U.S.A;, [2]Washington State Toxicology Laboratory, Department of Laboratory Medicine, University of Washington, 2203 Airport Way So. Suite 360, Seattle, WA 98134 U.S.A.*

Sertraline (Zoloft) is a selective serotonin reuptake inhibitor (SSRI) whose major metabolite, N-desmethyl sertraline (NDS) is also selective for inhibition of serotonin reuptake. SSRIs are generally associated with fewer side effects than the classical tricyclic antidepressants. However, use of SSRIs has been linked to a new constellation of side effects, including serotonin syndrome, and the co-ingestion of drugs with serotonergic properties must be carefully evaluated when interpreting blood sertraline concentrations. Furthermore, because of the common isoenzymes of cytochrome P450 involved in the metabolism of these drugs and other common therapeutic drugs, they can interact significantly with each other, altering half lives, leading to accumulation. These factors and others were evaluated in the investigation of twenty deaths occuring over a one year period where sertraline was detected.

Basic drugs, including sertraline and NDS, were identified by gas chromatography/mass spectrometry, following an n-butyl chloride extraction. Analysis of blood samples from twenty postmortem cases revealed concentrations ranging from 0.09-2.52 mg/L of sertraline (therapeutic concentrations of sertraline range from 0.03 to 0.19 mg/L) and 0.08-4.05 mg/L of NDS. Postmortem tissue distribution studied in one case revealed the following concentrations of sertraline and NDS, respectively: 1.79 and 1.95 mg/L (central blood), 2.52 and 4.05 mg/L (peripheral blood), 3.08 and 3.95 mg/L (bile), 5.65 and 9.21 mg/L (urine), 42.14 and 59.69 mg/kg (liver), 8.72 and 19.91 mg/kg (gastric contents), and 0.89 and 1.46mg/L (vitreous). Sertraline was typically present together with other drugs, including antidepressants, alcohol and drugs of abuse. The potential for interaction with each is discussed. Over the one year period of this study there were no deaths ascribed solely to sertraline intoxication.

Key Words: Sertraline, GC/MS, Postmortem, SSRI

Is *N*-OH-MDA a Possible Metabolite in MDMA Intoxications? *D. de Boer,* L.P. Tan and R.A.A. Maes; Netherlands Institute for Drugs and Doping Research, Sorbonnelaan 16, 3584 CA Utecht, The Netherlands.*

Using a chiral derivatization with *N*-heptafluorobutyryl-(*S*)-propyl chloride combined with a consecutive reaction with *N*-methyl-*N*-trimethylsilyltrifluoroacetamide resulting in *N*-(hepta-fluorobutyryl-(*S*)-propyl)-*O*-trimethylsilyl derivatives, we identified the enantiomers of several chiral metabolites in 3 urine samples obtained from MDMA intoxications. Detection was carried out with positive chemical ionization ion trap mass spectrometry. Besides MDMA, the major chiral metabolites were 3,4-methylenedioxyamphetamine (MDA), 4-hydroxy-3-methoxymethamphetamine and 4-hydroxy-3-methoxyamphetamine. In addition to these substances, *N*-hydroxy-3,4-methylenedioxyamphetamine (*N*-OH-MDA) was identified in 2 out of 3 samples.

Because *N*-OH-MDA itself is sometimes the main pharmacologically active compound in 'Ectasy' street samples, it cannot be excluded that the presence of *N*-OH-MDA is caused by the combined use of MDMA and *N*-OH-MDA. However, the low frequency of the presence of *N*-OH-MDA in 'Ectasy' street samples and the observation that *N*-OH-MDA was found in two out of three urine

samples from totally different and unrelated intoxications suggests that the observed *N*-OH-MDA is a metabolite rather than a coadministered compound. Moreover, the indication that *N*-OH-MDA is of metabolic origin in these two cases was confirmed as the 'metabolic' enantiomeric ratios of *N*-OH-MDA were in the same range as those of MDA, the assumed precursor of *N*-OH-MDA. The significance of this observation is that *N*-OH-MDA may be converted in the body into a nitroso compound which is known to be relatively hepatotoxic.

Key Words: MDMA, Ectasy, N-OH-MDA, Metabolite

Simultaneous GC/MS Assay for Cocaine, Codeine, 6-Acetylmorphine and Metabolites in Human Biological Specimens. *William D. Darwin*, Jonathan Oyler and Edward J. Cone; Addiction Research Center, IRP, NIDA, NIH, Baltimore, MD.*

Cocaine is extensively metabolized in man to benzoylecgonine, ecgonine methyl ester, nor-metabolites and several hydroxylated metabolites. Cocaethylene and its metabolites are produced if alcohol is consumed with cocaine. Codeine and heroin (via 6-acetylmorphine) are metabolized to morphine and related metabolites. A combined assay for cocaine, opiates and their metabolites has been difficult in the past due to inefficient isolation techniques. A simultaneous assay has been developed in our lab for 19 cocaine and cocaine metabolites/internal standards and 9 opiates and opiate metabolites/internal standards. This assay has been employed with a variety of human biological specimens including blood, saliva, sebum, hair and skin. Samples of the fluid or digest are treated with internal standards buffered with sodium acetate (pH 4.0, 2.0 M). However, the initial extraction step may vary with different matrices. The solution is extracted with a 200 mg/10 mL CLEAN-THRU® DAU solid phase extraction column. The extract is concentrated, derivatized with BSTFA (with 1% TCMS) and analyzed by GC/MS (HP-1 column; 12 M x 0.2 mm ID x 0.33 μm DF) in the SIM mode. Correlation coefficients of the calibration curves were _0.98. Responses were linear across a concentration range of 0.5-500 ng of drug/mL (or mg) in zero-control biological specimen with the lower limit of sensitivity 0.1-2.0 ng/mL (or mg) depending on the drug and type of specimen. The intra-assay and inter-assay coefficients of variation for components were between 2.4-15.4%. This assay is currently in use in pharmacokinetic studies of cocaine, opiates and their metabolites in a variety of alternative biological matrices.

Key Words: GC/MS Assay, Biological Matrices, Cocaine and Opiates

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Detection of Methadone, LAAM and Their Metabolites by Methadone Immunoassays. *Matthew L. Cheever,* Gerardo A. Armendariz and David E. Moody; Center for Human Toxicology, University of Utah, Salt Lake City, UT.*

l-alpha-Acetylmethadol (LAAM) is a recently approved substitute for methadone in maintenance programs for treatment of opioid addiction. LAAM and methadone are metabolized by N-demethylation. LAAM is also hydrolyzed and methadone is also oxidized to methadol (M). Methadone can then be further metabolized by N-demethylation. Due to structural similarity, LAAM and its metabolites could potentially cross react with methadone immunoassays. To test this hypothesis, drug-free urine was fortified with LAAM, norLAAM, dinorLAAM, M, norM, dinorM, methadone, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), or 2-ethyl-5-methyl-3,3-diphenylpyrroline (EMDP). Twelve concentrations per analyte were prepared and ranged from 30 to 100,000 ng/mL. These urine samples were then analyzed using six different immunoassays. The immunoassays included two enzyme immunoassays (Behring Diagnostics, EIA-b; Diagnostic Reagents, Inc., EIA-d), one fluorescent polarization immunoassay (Abbott Diagnostics, FPIA); two enzyme-linked immunosorbant assays (Diagnostix, Ltd., ELISA-d; STC Technologies, Inc., ELISA-s) and one radioimmunoassay (Diagnostic Products Corp., RIA). All samples were analyzed in duplicate using the manufacturer's recommended procedures. The methadone samples prepared in-house were used as uniform calibrators for all assays. The percent cross-reactivities are summarized below.

Assay	LAAM	norL	dinorL	M	norM	dinorM	EDDP	EMDP
EIA-b	0.2	0.1	0.1	1.9	0.2	0.1	0.1	0.0
EIA-d	100.8	98.8	2.0	97.8	71.8	2.2	7.6	0.3
FPIA	14.0	1.2	0.2	37.7	1.4	0.2	0.0	0.0
ELISA-d	318.3	4.9	0.9	70.3	2.4	0.7	0.0	0.0
ELISA-s	75.3	3.6	0.2	16.2	5.0	0.2	0.0	0.0
RIA	249.5	7.3	1.4	15.0	3.8	0.6	0.0	0.0

These findings suggest that LAAM use could result in positive immunoassay results for many of the commercially available methadone kits. (We gratefully acknowledge vendor donation of all reagents).

Key Words: Methadone immunoassay, LAAM, LAAM Metabolites, Methadone Metabolites

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Cocaine Disposition in Saliva After Intravenous, Intranasal and Smoked Administration. *Edward J. Cone*, Jonathan Oyler and William D. Darwin; Addiction Research Center, IRP, NIDA, NIH, Baltimore, MD.*

Drug testing with alternate matrices such as saliva offers a number of distinct advantages over urine testing including ease of collection and the possibility of broader pharmacologic interpretation. We studied the disposition of cocaine and related analytes in

saliva following different routes of administration. Cocaine (COC), benzoylecgonine (BZE), ecgonine methyl ester (EME) and anhydroecgonine methyl ester (AEME) were measured in six healthy male subjects by GC/MS following intravenous (IV), intranasal (IN) and smoked (SM) COC administration. COC was present in saliva immediately following all routes of administration and saliva/plasma (S/P) ratios were generally >1. There was evidence of moderate to extreme contamination of saliva by COC immediately following IN and SM COC, but contamination of saliva cleared rapidly; saliva appeared to be free of contamination 2 h after dosing and demonstrated comparable cocaine S/P ratios to IV administration. BZE and EME concentrations were consistently low and were only comparable to COC at times when COC concentrations had declined below 100 ng/mL. AEME was detectable in saliva following SM drug, but was quickly cleared. Terminal half-life estimates for COC by the IN and SM routes were significantly shorter in saliva compared to plasma. Half-life estimates following the IV route tended to be lower for saliva than plasma, but the differences were not significant. Duration of pharmacologic effects was generally equal to or shorter than detection times of COC in plasma and saliva. Overall, the study demonstrated the usefulness of saliva as a test matrix for the detection and measurement of COC following different routes of administration.

Key Words: Cocaine, Saliva, Routes of Administration

Urine and Sweat Monitoring of Illicit Cocaine Use. *Marilyn A. Huestis*, Kenzie L. Preston, Conrad J. Wong, and Edward J. Cone; Division of Intramural Research, NIDA, NIH, Baltimore, MD 21224.*

A new technique, sweat testing, offers the advantage of monitoring drug use over an extended period of time, up to one week or longer. Sweat patch drug analysis was compared to urine drug testing for the evaluation of cocaine use in methadone maintenance patients in an outpatient setting. The results of urine drug tests (collected on Mondays [M], Wednesdays [W] and Fridays [F] and analyzed by EMIT, 300 ng/mL cutoff) from 44 human subjects (N = 1065 specimens) were compared to results of 355 sweat patches that were applied each Tuesday [T] and worn for 7 days. All sweat patches were analyzed for cocaine by an ELISA immunoassay (cutoff 10 ng/mL). A subset (N = 591) were confirmed by GC/MS (cutoffs = 5 ng/mL for cocaine, benzoylecgonine, and ecgonine methyl ester). It was first necessary to compare the windows of detection of cocaine use for urine and sweat monitoring. Results of four different groups of urine specimens were compared with each T sweat patch result: 1) 3 specimens collected the following W, F, & M; 2) 4 specimens collected the preceding M, and the following W, F, & M; 3) 4 specimens collected the following W, F, M, & W; and 4) 5 specimens collected the preceding M and the following W, F, M, W. If any of the urine specimens was positive, drug use was assumed. If all urine specimens were negative, drug abstinence was assumed. Agreement between the paired sweat and urine results for cocaine in the four groups were as follows: 1) 77.7%, 2) 78.9%, 3) 78.3% and 4) 79.4%. The initial ELISA sweat test appeared to be a more sensitive method for detecting cocaine use than urine testing across all four comparison groups. The accuracy, sensitivity and specificity of sweat ELISA cocaine results as compared to sweat GC/MS results were 93.5%, 90.5%, and 93.1%. Cocaine was detected in 99% of GC/MS positive sweat patches with a mean GC/MS concentration of 989.7 ng/mL. In contrast, the mean benzoylecgonine and ecgonine methyl ester concentrations were 133.5 and 115.9 ng/mL. Additional controlled drug administration studies are needed to differentiate whether the improved detection of cocaine use by sweat analysis is due to greater sensitivity of sweat testing over urine analysis, to environmental contamination of the sweat patch, analytical error or other factors.

Key Words: sweat patch, drug testing, sweat testing

Ante/Post Mortem Kinetics of Cocaine in Juvenile Swine. *Barry K. Logan[1]*, Kari Blaho[2], Gene Schwilke[1] and Timothy Mandrell[3]; [1] Washington State Toxicology Laboratory, Department of Laboratory Medicine, University of Washington, Seattle WA 98134; [2] Department of Emergency Medicine, and [3] Department of Comparative Medicine, University of Tennessee, Memphis TN 38103.*

These experiments were devised to examine the *in vivo* and post mortem distribution of cocaine between the arterial and venous circulation in the pig, and to assess changes in drug and metabolite concentration occurring surrounding death. Chronic indwelling vascular access ports (internal carotid and external jugular) were implanted in four juvenile swine as part of a chronic cocaine study. Cocaine (6mg/Kg) was administered via the venous port, blood pressure and heart rate were measured via the arterial port. Blood samples were drawn from the arterial port and an additional venous catheter placed in the mammary vein. The animals were euthanized 30 to 60 minutes after administration. Additional post mortem arterial and venous blood samples were collected at 120, 240, and 360 minutes post administration. In selected cases, heart blood, vitreous humor and spinal fluid samples were also collected at intervals post mortem. All samples were analyzed using an extractive alkylation/GCMS procedure, to permit the analysis of cocaine, and its three major metabolites.

The arterio/venous ratio *in vivo* exceeded one for all metabolites, up to thirty minutes, while the ratio for the parent drug was less than one. During the immediate postmortem interval, there was a consistent rise in the arterial and venous blood concentrations of up to 60% for cocaine and its major porcine metabolite ecgonine methyl ester, with the largest increases occurring in the venous compartment. Similarly, concentrations of drug and all metabolites in vitreous and CSF increased over the postmortem interval. Changes in heart blood concentrations were less predictable. This work illustrates that arterio venous differences exist in the immediate post administration period *in vivo*, and further that tissue release or postmortem redistribution of cocaine, benzoylecgonine, ecgonine methyl ester and ecgonine does indeed occur in a time dependent fashion in the immediate postmortem interval.

The Detection of Acetylcodeine and 6-Monoacetylmorphine in Opiate Positive Urines. *Carol L. O'Neal* and Alphonse Poklis; Department of Pathology, Box 980165, Medical College of Virginia/Virginia Commonwealth University, Richmond, VA 23298-0165.*

Acetylcodeine (AC), an impurity of heroin, has been suggested as a biomarker for the use of illicit heroin in addition to 6-monoacetylmorphine (6MAM), but there have been no reports in the literature on the detection of AC in biological fluids. One hundred criminal justice urine specimens that had been confirmed positive for morphine at concentrations >5000 ng/ml by GC/MS were analyzed for AC, 6MAM, codeine, norcodeine and morphine. The GC/MS analysis was performed by solid phase extraction and derivatization with propionic anhydride. Total codeine and morphine concentrations were determined by acid hydrolysis prior to the above procedure. AC was detected in 37 samples in concentrations ranging from 2 to 290 ng/ml with a median of 11 ng/ml. 6MAM was also present in these samples at concentrations ranging from 49 to 12,600 ng/ml with a median of 740 ng/ml. Of the 63 specimens negative for AC, 36 were positive for 6MAM at concentrations ranging from 12 to 4600 ng/ml (median of 124 ng/ml). When detected, the AC concentrations were an average of 2.2% (0.25 to 10.2%) of the 6MAM concentrations. There was a positive relationship between AC concentrations and 6MAM concentrations (r=0.878). Although it is indicative of illicit heroin use, AC would not make a suitable biomarker in place of 6MAM due to the low concentration in urine compared to that of 6MAM. 6MAM was found to be present in twice as many specimens at much higher concentrations.

Key Words: Acetylcodeine, 6-Monoacetylmorphine, Heroin

Pharmacokinetic-Pharmacodynamic Relationships Following Acute Marijuana and Cocaine Administration. *Stephen J. Heishman*[1] and Dennis J. Crouch[2]; [1]Addiction Research Center, IRP, NIDA, Baltimore, MD; [2]Center for Human Toxicology, University of Utah, Salt Lake City, UT.*

The complex relationships between plasma drug concentration and various pharmacological effects are not well characterized for most drugs of abuse. We have undertaken a series of clinical studies to explore these relationships, with an emphasis on determining plasma concentration profiles during periods of drug-induced behavioral alterations. On six separate sessions, 18 healthy research volunteers smoked two marijuana cigarettes containing 0, 1.75, or 3.55% THC or insufflated cocaine hydrochloride (4, 48, or 96 mg/70 kg). Δ^9-Tetrahydrocannabinol (THC), 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THCCOOH), cocaine (COC), benzoylecgonine (BZE), and ecgonine methylester (EME) were measured using GC-MS, and a battery of physiological, subjective, and performance measures were assessed before and up to 260 min after drug administration. THC peaked immediately after smoking at levels of 52 and 76 ng/ml for low and high doses, respectively. Plasma area-under-the-curve ($AUC_{0-\infty}$) for THC was 1606 and 2652, and terminal half-life (T 1/2) was 71 and 74 min for 1.75 and 3.55% THC doses, respectively. Marijuana produced dose-related increases in heart rate and ratings of drug strength and impaired performance on two field sobriety tests (FST), One Leg Stand and Finger to Nose. THC plasma levels were 15-28 ng/ml at the time of behavioral impairment. COC concentrations peaked at 45 min postdrug and were 81 and 201 ng/ml for low and high doses, respectively. $AUC_{0-\infty}$ for COC was 19,060 and 43,065, and T 1/2 was 108 and 96 min for the 48 and 96 mg doses, respectively. COC increased heart rate, blood pressure, and ratings of drug strength. In contrast to marijuana, COC produced a trend toward improved performance on the FST when plasma COC levels were 75 and 181 ng/ml for low and high doses, respectively.

Key Words: Marijuana, Cocaine, Pharmacokinetic-pharmacodynamic Analyses

Chiral Analytical Toxicology and Pharmacology of Fluoxetine and Norfluoxetine. *Lily Yee[1,3], Steven H. Wong*[1,2,3,4], Victor A. Skrinska[3], Harold H. Harsch[2], James C. Neicheril[1], Rebecca D. Cannon[1,4] and Laura E. Manis[1]; [1]Depts. Pathology., and [2]Psych. & Behav. Med., Med. Coll. of Wisconsin; [3]Dept. Health Sci., Univ. Wisconsin/Milwaukee; [4]Milwaukee County Medical Examiners' Office, Milwaukee, WI 53226.*

Fluoxetine(F) is a potent serotonin reuptake inhibitor and is demethylated to norfluoxetine(NF). Both are racemic. S-Fluoxetine(SF) and S-norfluoxetine(SNF) are more potent serotonin re-uptake inhibitors than R-fluoxetine(RF) and R-norfluoxetine(RNF). The purposes of this study were to establish: firstly, the chiral selectivity using LC columns., secondly, a solid phase extraction protocol., and thirdly, a limited biodistribution study in rabbits. Chiral analyses were systematically investigated using three bonded HPLC silica columns - acetylated beta cyclodextrin(CD), vancomycin(V) and teicoplanin(T). Using methanol or ethanol/aqueous triethylamine, chiral separations were achieved with differing elution orders and capacity factors: for CD - SNF, 6., SF, 7., RNF, 8., and RF 9., and for V - SNF, 5., RNF, 6., SF, 7., and RF, 9. However, achiral analysis was achieved by T. After performing the Varian's protocol for solid phase extraction for F and NF, the extracts were analyzed by using either CD or V columns. Precision studies of the CD assay showed that day-to-day means of 82 µg/L and CVs of 7.0 to 10.5%, and detection limit of 10 µg/L. In order to investigate the chiral pharmacology, biodistribution study was performed by administering i.v. 2 mg/kg of F to five rabbits. Blood samples were collected between 0 and 24 hrs. After euthanizing the rabbits, collected samples included: urine, liver, heart, kidneys, brain and vitreous humor(VH). CD assays showed:

Plasma	Urine	VH

		(n=5)				(n=4)	(n=-5)
	0h	0.75 h	2 h	6 h	24 h	24 h	24 h
RF(μg/L)	ND	32	21	17	ND	8	ND
SF(μg/L)	ND	37	21	13	ND	76	ND
RNF(μg/L)	ND	ND	12	32	29	34	ND
SNF(μg/L)	ND	ND	ND	ND	ND	51	ND

In conclusion, chiral analysis may be performed by either CD or V columns - potentially useful for patient monitoring, and chiral discrimination was evident in the metabolism of enantiomeric F and NF in rabbits.

Key Words: Fluoxetine, Chiral Pharmacology, Enantiomeric/Chiral Hplc Analysis

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Increased Incidence of PHEN-FEN Associated Complaints in an Emergency Department. *Stephen L. Winbery*, Robert Smith and Kari E. Blaho; Department of Emergency Medicine and Clinical Toxicology, UT Medical Group, Memphis, TN 38103.*

There has been renewed interest over pharmacological weight control since the recent availability of the combination of a CNS stimulant phentermine and serotonergic antagonist fenfluramine (PHEN-FEN). The toxicology of these compounds is largely suspected and not proven. In our Emergency Medicine and Toxicology Department we have seen 26 patients with complaints related to or associated with PHEN-FEN prescribed for weight loss. During the same period of time last year (1996) we had no complaints related to PHEN-FEN as there were very restrictive regulations reducing the use of these medications in the state of Tennessee. However, neighboring Arkansas had liberal prescribing laws. The overall incidence is probably grossly under estimated due to many patients do not admit to use the first 3-4 visits. Patient age ranged from 15 to 60 years. Complaints included headaches, palpitations, chest pain, shortness of breath, memory loss, sleep disturbance. Clinical findings included tachycardia, near syncope, memory problems, hypertension, pleural effusion and cardiac arrhythmias. There were three critically ill patients admitted to the hospital; two with myocardial infarctions and one patient died with a dissecting aortic aneurysm. All had been prescribed PHEN-FEN in accordance with current regulations. There were two overdose patients, both with CNS depression and lethargy as their primary manifestation. With the increased use and prescription for the drugs, the clinical pharmacology and toxicology of these drugs should be emphasized.

Key Words: Phen-Fen, CNS Stimulant, Phentermine

Simultaneous Quantitation of Cocaine, Opiates and Metabolites in Human Hair by GC/MS. *Karin M. Höld*[1], Diana G. Wilkins[1] Mike S. Hill[1], Robert E. Joseph Jr.[2], Edward J. Cone[2] and Douglas E. Rollins[1]; [1]Center for Human Toxicology, University of Utah, Salt Lake City, UT 84112; [2]Addiction Research Center, National Institute on Drug Abuse, Baltimore, MD 21224.*

A sensitive method was developed for the combined extraction of cocaine, cocaethylene (CE), benzoylecgonine (BE), ecgonine methyl ester (EME), norcocaine, 6-acetylmorphine (6-MAM), codeine, norcodeine, morphine, and normorphine from human head hair using an enzyme-based digestion technique. Deuterium labeled internal standards were added to 20 mg of hair samples that were digested overnight in 6 mL of Protease VIII/DTT/Tris-buffer pH 6.5 at 22^0C in a shaking water bath. Calibration standards and conversion controls containing known concentrations of drugs dried onto drug-free hair were also prepared and digested. The pH of the digests was adjusted to 5.5 with 0.1 N HCl. The digest solutions were extracted with a solid phase extraction procedure using Bond-Elut Certify^{TM} columns. The extract residues were evaporated at < 40^0C and reconstituted in 20 μL ethyl acetate and derivatized with 30 μL of Nmethyl-N-trimethylsilylheptafluorobutyramide and trimethylsilylimidazole (1000:40,v/v) for 20 min at 80^0C. Then 5 μL of N-methyl-bis-heptafluorobutyramide were added and the mixture was heated for 25 min at 80^0C. Analyses were performed by positive ion chemical ionization GC/MS on a Finnigan-MAT^{TM} mass spectrometer with methane/ammonia as reagent gas, helium as a carrier gas and a DB-1 (30M-0.32 mm i.d.-1.0 μm film thickness) capillary column. Two injections were performed on each extract to optimize sensitivity for all analytes. The assay was capable of quantitating 200 pg/mg of all compounds and was linear to 50 ng/mg. The method was used to analyze human hair samples obtained from cocaine and heroin users. Cocaine, BE, and EME were detectable in all samples, whereas norcocaine, CE, codeine, 6-MAM, and morphine were detected in only some samples. Norcodeine or normorphine were not detected. The assay is currently being used to analyze samples from a study investigating the mechanisms of drug disposition in hair. (Supported by NIDA grant DA09096).

Key Words: Cocaine, Opiates, Hair analysis

The Preferential Incorporation Of Cocaine And Its Metabolites Into Pigmented Hair: A Dose Response Study. *Deanna L. Hubbard*, Diana G. Wilkins and Douglas E. Rollins; Center for Human Toxicology, Department of Pharmacology and Toxicology, University of Utah, SLC, UT 84112.*

Drugs of abuse that are weak bases are preferentially incorporated into pigmented hair. The purpose of this study was to evaluate the incorporation of cocaine (COC) and its metabolites, benzoylecgonine (BE), ecgonine methyl ester (EME) and norcocaine (NCOC) into pigmented and nonpigmented hair of Long Evans (LE) rats. Cocaine was administered to male LE rats at 3 doses (5,

10 and 20 mg/kg; n=8 in each group) once daily for five days by i.p. injection. Fourteen days after the initial injection, the new hair growth was collected and analyzed for COC, BE, EME and NCOC. Deuterated internal standards were added to 20 mg aliquots of hair, which were then digested enzymatically and the analytes isolated by a solid phase extraction protocol optimized for the simultaneous recovery of all analytes. Concentrations of COC, BE, EME and NCOC were determined by gas chromatography/positive chemical ionization mass spectrometry. Mean concentrations (ng/mg) ± S.E.M. of each analyte in pigmented hair at the 5 mg/kg dose were: COC, 8.76 ±0.73; BE, 0.22 ±0.02; EME, 0.82 ±0.02; and NCOC, 0.39 ±0.02. At the 10 mg/kg dose, COC was 14.46 ±0.65; BE was 0.32 ±0.02; EME was 1.60 ±0.14; and NCOC was 0.60 ±0.03. Mean concentrations at the 20 mg/kg dose were: COC, 21.94 ±1.03; BE, 0.39 ±0.03; EME, 2.20 ±0.17; and NCOC, 2.27 ±0.17. The analytes were virtually undetectable in nonpigmented hair. The plasma pharmacokinetic profile of each analyte was also determined and used to normalize the hair concentration to the plasma concentration. Our data suggest that COC, EME, and NCOC (but not BE) are distributed into pigmented hair in a dose-related manner and that none of the analytes demonstrate a dose-related incorporation into nonpigmented hair. This study was supported by NIDA grant DA 07820.

Key Words: Cocaine, Pigmented Hair, Dose Response

Drug Testing with Alternative Matrices I. Pharmacological Effects and Cocaine Disposition in Plasma. *Ann Basham*, Diana Lafko, Janeen Nichels, Jonathan M. Oyler, Chideka Ohuoha, Deborah Price, Robert E. Joseph Jr, and Edward J. Cone; IRP, NIDA, NIH, Baltimore, MD.*

A clinical study was designed primarily to evaluate the disposition of cocaine and codeine in alternative biological matrices. Behavioral and physiological data were also collected for comparison to plasma drug concentrations. This report describes the pharmacological effects and disposition of COC in plasma following subcutaneous (SQ) COC administrations. Subjects were administered low doses (70 mg/70 kg) and high doses (150 mg/70 kg) of COC hydrochloride. Pharmacological measures (heart rate, pupil diameter, subject "High" and "Liking") were obtained simultaneously with blood specimens. Plasma was analyzed by GC-MS for parent drug and metabolites. Plasma COC concentrations typically peaked at 30 min following drug administrations. The mean peak plasma COC concentration (Cmax ± S.E.M.) for 5 subjects after the high dose (706 ng/mL ± 133) was significantly greater ($p < 0.05$) than following the low dose (363 ng/mL ± 34). Mean AUC was also significantly greater ($p < 0.05$) after the high dose (132,590 ng-min/mL ± 19,280) compared to the low dose (79,977 ng-min/mL ± 18,264). Increases in heart rate compared to predrug measures for individual subjects ranged from 24-64 bpm after the low dose and from 29-56 bpm after the high dose. The magnitude of physiological and behavioral effects was not significantly different ($p > 0.05$) after the low versus the high dose. Peak heart rate, pupil diameter, "Liking" and "High" measures consistently occurred prior to peak plasma COC concentrations resulting in a clockwise hysteresis effect. The observed hysteresis effects suggested the development of acute tolerance which may have limited the magnitude of pharmacological effects at peak plasma COC concentrations.

Key Words: Plasma, Cocaine, Effects

Analysis of Fenfluramine and Norfenfluramine in Blood. *David M. Andrenyak*; Center for Human Toxicology, University of Utah, Salt Lake City, Utah 84112.*

Fenfluramine is a sympathomimetic drug that is used as an appetite suppressant Fenfluramine is often prescribed in combination with phentermine. Fenfluramine is metabolized by N-dealkylation to norfenfluramine which is active. A method that has been used to analyze methamphetamine and other sympathomimetic drugs has been adapted to analyze fenfluramine and norfenfluramine in blood samples. This method involved liquid-liquid extraction at basic pH with butyl chloride:chloroform (4:1) and derivatization with trifluoroacetic anhydride (TFAA). The extracts were analyzed by gas chromatography - positive ion mass spectrometry using methane / ammonia as reagent gases. The protonated ammonia adducts of the molecular ions for the derivatized fenfluramine (m/z 345) and norfenfluramine (m/z 317) were monitored. A DB-1 column was used for chromatographic separation. Amphetamine -d_5 and methamphetamine-d_8 (meth-d_8) were evaluated as internal standards with meth-d_8 giving the best precision and accuracy. The analysis was linear from 10 ng/mL to 1000 ng/mL. Blank blood samples spiked at 40 ng/mL, 100 ng/mL, and 400 ng/mL were used to evaluate precision and accuracy. Using meth-d_8 as the internal standard, the intrassay CVs ranged from 5.2% to 14.6% for norfenfluramine and 6.3 to 10.7% for fenfluramine. The interassay CVs ranged from 4.2% to 10.8% for norfenfluramine and 4.9% to 8.2% for fenfluramine. The interassay accuracy ranged from 99.6% to 102.7% of the target for norfenfluramine and 92.6% to 100.9% for fenfluramine. This method has also been used to analyze fenfluramine and norfenfluramine in plasma samples for a research study.

Key Words: Fenfluramine, Gas Chromatography Mass Spectrometry, Positive Ion Mass Spectrometry

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Rapid Gas Chromatographic Procedure for the Determination of Topiramate in Serum. *Carl E. Wolf, C. Richard Crooks* and Alphonse Poklis; Department of Pathology, Medical College of Virginia, Virginia Commonwealth University, PO Box 980165, Richmond, VA. 23298-0165.*

A rapid gas-liquid chromatographic (GLC) method for the routine determination in serum of the new anticonvulsant drug topiramate (Topamax®) [TOP] is described. The method involves extracting 0.50 mL of sample with pH 9.5 saturated borate buffer

and ethyl acetate. Microliter aliquots of the extract are injected into a 10 m x 0.53 mm ID x 0.5 µm, 100% methyl silicone megabore capillary column connected to an NPD. The column temperature was initially at 170 °C for 0.1 min., then programmed at 10 °C/min. to 240 °C, then 20 °C/min. to 280 °C for 0.5 min. Under the conditions of the assay, the retention times of TOP and mepivicaine internal standard were 3.5 and 4.0 min., respectively. Quantitation was performed with peak height ratios of TOP to the internal standard. Calibration curves were linear from 5 to 500 mg/L TOP. The assay had an LOQ of 5.0 mg/L. The within-run precision of the method yielded CVs of: 3.9% at 10 mg/L (n=10) and 3.1% at 100 mg/L (n=10). The overall between-run precision calculated by three determinations on a single day for four days yielded CVs of 7.3 % at 23 mg/L (n=12) and 7.8 % at 85 mg/L (n=12). Common anticonvulsant and basic/neutral extractable drugs were found not to interfere with the assay. At present, no correlation has been demonstrated between trough plasma TOP concentrations and clinical efficacy (1). However, in a recent study of TOP in refractory partial epilepsy, 5 of 6 seizure-free patients had TOP concentrations exceeding 15 mg/L (2). To date, TOP values observed in our laboratory in serums from patients receiving adjunctive treatment for seizure disorders ranged from 5.0 to 35 mg/L.

1. Topamax: Prescribing information. McNeil Pharmaceutical, Springhouse, PA. 1996.
2. Personal communication, OXIS International, Portland, OR. 1997.

Key Words: Topiramate (TOP), Gas-Liquid Chromatography (GLC), Anticonvulsant

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Tricyclic Antidepressant Concentrations in Overdose Patients Presenting to an Inner City Emergency Department. *Stephen Winbery*[*1], *Barry Logan*[2], *and Kari Blaho*[1]; [1]*Department of Emergency Medicine and Clinical Toxicology, UT Medical Group, Memphis, TN 38103;* [2]*Washington State Toxicology Laboratory, Department of Laboratory Medicine, University of Washington, Seattle, WA 98134.*

We report the clinical findings, outcomes and drug concentrations in 14 patients presenting to an inner city emergency department (ED) with a history of tricyclic antidepressant (TCA) overdose. Blood for TCA concentrations was drawn as soon as feasible after arrival. Drug concentrations were determined by GCMS following extraction with n-butylchloride. Six patients ingested amitryptyline, 3 ingested imipramine, 4 ingested desipramine and 1 ingested doxepin. Of the 14 patients, 12 were obtunded on initial presentation. Six patients required hospital, the remaining patients were discharged from the ED. Urine drug screens in 5 of the 14 patients indicated recent use of cocaine, 8 patients ingested additional CNS depressants. Amitriptyline concentrations ranged from 0-293 ng/ml, doxepin concentrations ranged from 0-229 ng/ml, desipramine concentrations were 0 and imipramine concentrations ranged from 0-902 ng/ml. No patient died or had a poor clinical outcome. TCA concentrations are not predictive of the clinical course and outcome especially in the setting of multiple drug overdose. There appears to be an association between cocaine abuse, clinical depression and suicide gesture by self ingestion of CNS depressants. This report would suggest that post mortem TCA concentrations may be difficult to interpret without significant clinical history.

Key Words: Tricyclic antidepressants, overdose, postmortem

Dose-Proportional Distribution of Drugs for The Treatment of Substance Abuse into Rat Hair. *Diana Wilkins*[*], *Pamela Nagasawa, Angelique Valdez and Douglas Rollins; Center for Human Toxicology, Department of Pharmacology and Toxicology, University of Utah, SLC, UT 84112.*

l-α-Acetylmethadol (LAAM), buprenorphine (BPR) and methadone (MD) are drugs that may be used for the treatment of substance abuse. The purpose of this study was to study the relationship between dose, plasma concentration, hair concentration and hair pigmentation for these compounds and their major metabolites. Male Long-Evans rats received either LAAM (1 mg/kg and 3 mg/kg; n=6), BPR (1 mg/kg and 3 mg/kg; n=5), or MD (4 and 8 mg/kg; n=5) by i.p. injection daily for five days. Fourteen days after beginning drug administration, newly grown hair was collected and analyzed for either: LAAM and two metabolites (norLAAM and dinorLAAM), MD and two metabolites (EDDP and EMDP), or BPR and norBPR. The plasma time courses (AUC) for LAAM, MD and BPR were also determined after a single administration of each drug at the specified doses.

There was an approximate dose-proportional increase in measured hair concentration of parent drug in both pigmented and non-pigmented hair. At the highest dose, the mean hair concentrations for LAAM (3 mg/kg), MD (8 mg/kg) and BPR (3 mg/kg) in pigmented hair were 1.57 (±0.23), 16.73 (±3.46), and 1.88 (±0.22) ng/mg, respectively. At the lower dose, the mean hair concentrations for LAAM (1 mg/kg), MD (4 mg/kg) and BPR (1 mg/kg) and in pigmented hair were 0.66 (±0.08), 5.27 (±0.46), and 0.67 (±0.07) ng/mg, respectively. The concentrations of LAAM, MD and BPR in non-pigmented hair were significantly less than that measured in pigmented hair at either the high or low dose. The metabolites norLAAM and EDDP were detected at lower concentrations than either LAAM or MD in pigmented hair. However, the dinorLAAM metabolite concentrations in pigmented hair were significantly greater than the parent drug after either LAAM dose. The data from this study suggests that LAAM, MD, BPR and metabolites are distributed into hair in a dose-proportional manner with a preference for pigmented hair. This study was supported by U.S. Public Health Service Grant DA09096.

Key Words: LAAM, Buprenorphine, Hair

Drug Testing with Alternative Matrices II.Stratum Corneum. *Jonathan M. Oyler*[*], *Robert E. Joseph Jr., Abraham Wtsadik and Edward.J. Cone; Addiction Research Center, IRP, NIDA, NIH, Baltimore, MD.*

This study evaluated the disposition of cocaine and codeine in stratum corneum. Five, africoid male subjects with a recent history of cocaine and heroin use resided on a closed residential ward. Following a three week wash out period, 75 mg cocaine hydrochloride/70 kg (subcutaneous) and 60 mg codeine sulfate/70 kg (oral) doses were administered on alternating days for six days. The same dosing schedule was repeated during week eight with 150 mg cocaine hydrochloride/70 kg and 120 mg codeine sulfate/70 kg. Stratum corneum (ca.13 mg) was collected once a week by scraping alternating regions of the back with a scapel. Thawed specimens were suspended in 0.02 M phosphate buffer (pH 2.5) followed by solid phase extraction followed by GC/MS analysis for cocaine, codeine and eighteen analytes. The primary analytes in stratum corneum were cocaine and codeine. Drug concentrations peaked within 24 hours of the last dose with peak concentration ranges of 0-4.8 ng cocaine and 0-5.6 ng codeine/mg stratum corneum. Cocaine and codeine concentrations subsequently decreased in the ensuing two weeks and were near detection limits (0.1 ng/mg) 3 weeks following drug administration. No dose-response relationship was apparent for either drug in stratum corneum. Benzoylecgonine (0.2-2.0 ng/mg) and ecgonine methyl ester (0.1-0.8 ng/mg) were the major cocaine metabolites detected with a time course similar to that of cocaine. No codeine metabolites were detected. Mechanisms of drug deposition in stratum corneum could be complex. Sources of drug could have been blood, sweat, sebum and/or hair. Regardless of the mechanism, further study is needed to explore the possible role of stratum corneum as an alternative matrix for drug testing.

Key Words: Skin, Cocaine, Codeine.

Drug Testing with Alternative Matrices III. Sebum. *Abraham T. Wtsadik*, Jonathan Oyler, Robert E. Joseph, Jr. and Edward J. Cone; Addiction Research Center, IRP, NIDA, NIH, Baltimore, MD.*

Sebum is comprised primarily of waxy fatty acid esters synthesized in cells of sebaceous glands in skin. Sebum is secreted into the canal of hair follicles and channeled onto the skin surface in most body regions. This report describes the time course for secretion of these drugs in sebum. Sebutape™ patches were applied periodically to the foreheads of 5 Africoid males following oral administrations of codeine sulfate (60 and 120 mg/70 kg) and subcutaneous administrations of cocaine hydrochloride (75 and 150 mg/70 kg). Sebutape is a microporous, adhesive patch designed for collection of sebum. In this study, Sebutape patches were weighed prior to application and again after removal to determine the amount of sebum collected. Drugs were extracted from Sebutape with hexane followed by solid phase extraction and GC/MS analysis for parent drugs and metabolites. Sebum contained mainly cocaine and codeine and metabolites represented less than 10 % of parent drug concentrations. Peak cocaine concentrations (Mean ± SEM) occurred within 4 hr following low doses (21.1 ng/mg ± 18.1) and high doses (6.3 ng/mg ± 2.1). Peak codeine concentrations (Mean ± SEM) also occurred within 4 hr following low doses (0.6 ng/mg ± 0.5) and high doses (1.7 ng/mg ± 1.4). Drugs were not detected in sebum collected 1-2 days following drug administrations. The mechanism(s) for drug deposition in sebum may involve the following: diffusion of drug from blood into sebum just prior to secretion; and/or transfer of drug from sweat and skin to sebum on the skin surface. Similarly, transfer of drug from sebum to other biological matrices (i.e. hair and sweat) may also occur.

Key Words: Sebum, Cocaine, Codeine

A Comparison of Poisoning Deaths in Medicolegal Autopsies of Lausanne and Those of China. *Z.G. Liao[1]*, B. Horisberger[2], F. J. Huang[1], K. Michaud[2], C. Brandt Casadevall[2], T. Krompecher[2] and P. Mangin[2]; [1]Faculty of Forensic Medicine, West China University of Medical Sciences, Chengdu 610041, China; [2]Institute of Forensic Medicine, University of Lausanne, Rue du Bugnon 21, CH 1005, Lausanne, Switzerland.*

For the purpose of analyzing the poisons, the route of administration and the nature of poisoning deaths in medicolegal autopsies, 161 poisoning autopsies were strictly chosen and retrospectively studied from 523 medicolegal autopsies at the Institute of Forensic Medicine in Lausanne during the past 3 years (1992-1994). The statistical results are compared with the statistical data of large groups surveyed from China.

The results showed that the commonest poisons used in Lausanne are narcotics (50.93%), whereas the most popular poisons are agrochemical pesticides (52.70% to 87.69%) in China, even higher than this rate in the countryside. As for the route of administration of poisons, the highest rate in Lausanne is injection (59.63%), so that most modes of death are overdose (65.84%). However, the route of administration is mostly oral (78.84-94.48) as most people used pesticides as a suicidal dose (71.13-86.90%) in China. All of the proportions but overdose about the nature of death arc much higher in China than in Lausanne.

The authors consider that the statistical differences are contributed to the various social and cultural backgrounds in Switzerland and in China. On the other hand, this variate reflects, in some degree, the economical development of a country.

Key Words: medicolegal autopsy, poisoning death, statistical comparison

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Additional Testing of DHHS Specimens Reported Positive to the MRO. *Roger Rutter*[1], Elizabeth S. Keith[1] and William B. Keith[2]; [1]Laboratory Corporation of America, Memphis, TN; [2]University of Mississippi, University, MS.*

Final review of drug testing results by a Medical Review Officer (MRO) is required for urine specimens regulated by US Department of Health and Human Services (DHHS). Additional testing for specimens reported positive by the laboratory may be

requested by the MRO on the original bottle or by the donor through the MRO on the split specimen. This study determined the frequency and types of additional drug tests requested. DHHS regulated specimens (N = 341,482) received at LabCorp-Memphis from November 1, 1996 to April 30, 1997 were screened with Roche Abuscreen Online reagents. 3.86% (N=13,184) screened positive and 2.97% (N=10,132) were confirmed as positive by GC/MS and reported as such to the MRO. Of these specimens, 277 specimens were sent to other SAMHSA certified laboratories for 'retesting' under Federal guidelines. Additionally, 137 requests were received for D/L isomer analysis, and 42 requests were received for 6-MAM.

The specimens which screened positive, the number that confirmed as positive, and the number of retests are as follows: cannabinoids - 5737, 5597, 159; cocaine metabolites - 2550, 2396, 115; opiates - 2866, 1266 (codeine 872; morphine 907), 7; amphetamines - 1907, 826 (amphetamine 614; methamphetamine 659), 39; phencyclidine - 124, 81, 2.

Percentages of positives by 'Reason for Test' were also summarized as follows: PreEmployment - 3.4%; Random - 2.4%; For Cause - 14.6%; Post Accident - 4.1%, Return to Duty - 3.9%; Follow Up - 5.7% and Other 2.8%.

Key Words: Drug, Retest, MRO

Adulterating Effects of Various Salts on Urine Drug of Abuse Testing. *William E. Wingert* and David G. Perini; Laboratory Corporation of America, Raritan, NJ 08869.*

Adulteration during the collection process may present a significant problem in urine drug testing. In some cases this adulteration may not be detected by commonly measured parameters such as creatinine, specific gravity, pH or temperature. A recent report indicates that the addition of potassium nitrite is effective in preventing the confirmation of carboxy-THC by GC/MS. In this study, the effect of other commercially available salts on initial screening results was investigated.

Eighteen salts were added individually to 10 mg/ml to a commercially purchased control which was positive for ten drugs of abuse (amphetamines, barbiturates, benzodiazepines, THC, propoxyphene, cocaine, methaqualone, opiates, PCP, methadone) at approximately twenty percent above cutoff. These samples were then tested on Olympus 5131 using OnLine reagents. Eight salts produced at least a twenty percent decrease. These included: sodium periodate (THC, opiate), potassium dichromate (THC, piate), potassium manganate (barbiturates, THC, opiate),ferric chloride (benzodiazepines, THC, methaqualone, methadone), manganese chloride (THC, benzodiazepines, opiate, PCP, methadone), sodium peroxide (cocaine), chromium trioxide (THC, opiate), sodium persulfate (THC, opiate).

The possibility of adulteration was illustrated by this study. THC and piate initial screening tests were the most often influenced by the potential adulterants tested. More studies are needed to identify the mechanisms by which the adulterants act and to develop antidotes.

Quantitation of Cocaine in Human Hair: The Effect of Centrifugation of Hair Digests. *Karin M. Höld*, Deanna L. Hubbard, Diana G. Wilkins and Douglas E. Rollins; Center for Human Toxicology, University of Utah, Salt Lake City, UT 84112.*

Hair pigmentation is a critical factor in interpreting the concentration of certain compounds and their metabolites incorporated into hair. Melanin is responsible for the pigmentation and the color of hair. The melanin content of human hair samples differs over a wide range. Once deposited into hair, drug remains detectable for a period of months to years. However, if drug disposition is influenced by those properties of hair attributed to a particular ethnic group, e.g. hair color, then certain ethnic groups may test positive more frequently than other ethnic groups. In this study, the effect of melanin removal by centrifugation of hair digests on cocaine concentrations was investigated. Two sets of hair samples from five cocaine users were analyzed for cocaine and metabolites. Ten mL of 0.5 M Tris buffer, pH 6.4, to which is added 60 mg DTT, 200 mg SDS and 200 U Proteinase K was used as the digestion solution. Two mL of this solution was added to 20 mg of hair and incubated at 37 ^{0}C in a shaking water bath (90 oscillations/min) overnight. The samples were removed from the water bath and mixed. One set was centrifuged at 2000 rpm and divided into supernatant and melanin pellet. The other set was not centrifuged. Internal standards were added to all tubes. The samples were further extracted, derivatized and analyzed by GC/MS. A mean of 8.8% (SD 7.0%) of the total cocaine concentration was left behind in the pellet. These data demonstrate that centrifugation after protein digestion and analysis does not significantly change the amount of cocaine measured as compared to the non-centrifuged controls.

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Key Words: Melanin, Hair color, Cocaine
