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ABSTRACTS

These abstracts are included in the December, 1993, issue of ***ToxTalk*** as a courtesy to S.O.F.T. members

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MEASUREMENT AND INTERPRETATION OF HEROIN, 6-ACETYLMORPHINE AND MORPHINE CONCENTRATIONS IN BIOLOGICAL TISSUES OBTAINED FROM HEROIN USERS AND HEROIN-RELATED DEATHS. Bruce A. Goldberger* and Yale H. Caplan, National Center for Forensic Science, Baltimore, MD 21227 and Edward J. Cone, Addiction Research Center, NIDA, Baltimore, MD 21224.

Understanding the role of heroin in heroin-related deaths was facilitated by the development of an assay for heroin and its metabolites in biological specimens. Recognizing heroin's susceptibility to rapid chemical and metabolic hydrolysis, procedures were developed for the efficient recovery of heroin, 6-acetylmorphine (AM) and morphine from tissues utilizing liquid-liquid and solid-phase extraction. Aprotic solvents, mild elution solvents and an enzyme inhibitor were employed to ensure maximum analyte stability. Extracts were analyzed by gas chromatography/mass spectrometry. Specimens were collected and analyzed from 21 cases of death due to acute narcotic intoxication. The mode of death in these cases was categorized as rapid, delayed or undetermined. Compared to delayed deaths, rapid deaths were characterized by the following trends: higher mean concentrations of AM, free morphine and total morphine in blood; higher ratio of free to total morphine concentration in blood; lower mean concentrations of AM and morphine in urine; greater likelihood to detect AM in blood; and lesser likelihood to detect heroin in urine. In a detailed study of two heroin overdose cases, heroin was present only in urine specimens. Concentrations of AM in cerebrospinal fluid, spleen and brain were substantially higher than in blood, liver, lung and kidney. All specimens were positive for morphine.

Hair analysis was used to corroborate prior heroin exposure. Hair specimens, collected from 20 heroin users and two heroin-related deaths, contained AM and morphine. Heroin was found in 7 of 20 heroin user specimens. Generally, the AM concentration in hair was higher than that of heroin or morphine.

The identification of heroin and AM in biological tissues effectively established heroin use in cases of acute narcotic intoxication. These studies demonstrated that measurement of heroin and its metabolites provides useful information in the differential diagnosis of heroin-related deaths.

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DISPOSITION OF HEROIN AND METABOLITES IN BLOOD FROM SUBJECTS WHO SMOKED HEROIN

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Smoking has been the traditional route of self-administration for drugs such as nicotine, marijuana and phencyclidine. In recent years, there has been an increase in the abuse of other drugs, such as cocaine and heroin, by this route. Technical difficulties associated with delivering precise doses of smoked drugs have limited research of this route. We modified a method for smoked cocaine base delivery for the delivery of smoked heroin to human subjects. The system utilized a nichrome wire coil heating element for drug volatilization. Drug was applied in solution to the coil and dried. The coil was placed in a smoking chamber connected to a differential pressure transducer and pneumotachograph sensitive to changes in air flow. Inhalation produced an activation of the power supply which volatilized the drug. The entire dose was inhaled as a single puff. Studies showed that approximately 89% of the parent drug was delivered to the subject by the device.

Two subjects completed ten experimental sessions during which they received a total of four intravenous, one oral and four smoked doses of heroin in an ascending dose design. One of the smoked doses was randomly repeated. Physiological, behavioral, subjective and pharmacokinetic parameters were measured. Blood samples were analyzed by solid phase extraction-gas chromatography/mass spectrometry for heroin, 6-acetyl morphine (6-AM) and morphine (M).

Heroin, 6-AM and M were detected in blood one minute after heroin administration by the smoking route. Time to peak heroin concentrations varied between 2-5 minutes after smoking, compared with 2 minutes after intravenous administration. A peak heroin concentration of 108 ng/mL was reached after smoking 10.5 mg of heroin base compared to a peak of 315 ng/mL following IV administration of 6 mg of heroin HCl. Within 15 min, concentrations had declined to 2.0 ng/mL following smoking and 3.1 ng/mL following IV administration. Following smoking, a peak 6-AM concentration of 55 ng/mL was achieved at 2 minutes. Concentrations declined to 1.7 ng/mL by 30 minutes, compared to a peak of 126.7 ng/mL at 2 min and none detected at 30 min by the IV route. M concentrations peaked at 11.9 ng/mL following smoking and 105.0 ng/mL following IV administration.

These data indicate that smoking heroin produced blood concentrations of heroin, 6-AM and M in a pattern similar to that by IV administration; however, the bioavailability appeared to be substantially reduced by this route of administration.

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POSTMORTEM CLOMIPRAMINE CONCENTRATIONS: THERAPEUTIC OR TOXIC LEVELS?

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This study was undertaken to compare postmortem blood and liver clomipramine concentrations, and to provide an interpretation of their toxicological significance.

The analysis of clomipramine was by HPLC/dual ultraviolet wavelength detection. The detector was operated at 220 and 254 nm. Blood or liver homogenates were made basic and extracted with hexane/butanol (95%/5%). The organic layer was then extracted with 100 uL of 0.2% phosphoric acid. 30 uL was injected into the chromatographic system. The mobile phase consisted of 40% acetonitrile in 0.1M sodium dihydrogen phosphate buffer containing 2.5% diethylamine, pH 8.0. Total flow rate was 2.0 mL/min, and the run time was 40 min. The isocratic conditions were capable of separating, identifying and quantifying 20 antidepressants and selected metabolites. Clomipramine eluted at about 32 min with a relative retention time (RRT) to the internal standard (cianopramine) of 3.60. The main metabolite, desmethyl clomipramine, was detected at about 10 min with a RRT of 1.13, but this was not quantified.

Ten cases containing clomipramine were identified by routine toxicological screening. Clomipramine was then quantified with the above method and found to range from 0.21 to 4.9 mg/L in blood, and from 7.0 to 320 mg/kg in liver. Two cases of clomipramine toxicity were clearly differentiated by the analysis of liver homogenates. The liver concentrations were 10 - 30 times greater than the other cases where overdose was not suspected. One case involving a natural death, where mixed cardiac blood was collected in place of femoral blood, showed the highest blood level (4.9 mg/mL), but a therapeutic liver concentration (13 mg/kg).

It is concluded that femoral blood concentrations up to 0.9 mg/L, and liver levels up to about 20 mg/kg may constitute the therapeutic range for clomipramine. The analysis of peripheral blood, together with liver, reduces interpretation problems arising from postmortem redistribution and diffusion from sites of high concentrations.

4

Fentanyl Related Deaths in New Jersey

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Twelve cases of fentanyl related deaths were reported in New Jersey in early February 1991, when fentanyl was being sold as heroin under the name of "Tango and Cash" and "Goodfellas". Victims were all males (10) blacks, (1) white and (1) hispanic with ages that range from 23 to 39.

Fentanyl concentrations in the postmortem body fluids and tissues were determined by the GC/MS method utilizing the deuterium-labeled fentanyl as the internal standard. Fentanyl concentrations ranged from 0.014 - 0.064 mg/L in blood, 0.036 - 0.293 mg/kg in brain, 0.003 - 0.290 mg/L in urine, 0.008 - 0.0220 mg/L in bile, 0.031 - 0.377 mg/kg in liver, 0.032 - 0.0408 mg/kg in kidney, 0.047 - 0.422 mg/kg in spleen, 0.002 - 0.015 mg/L in vitreous humor and 0.046 - 3.899 mg/kg in stomach contents. Fentanyl was also detected in the corresponding formalin-fixed tissues: brain, liver, kidney and spleen.

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THE FORENSIC INVESTIGATIONS OF "DEATH IN THE CAMPGROUNDS", J. Bidanset*, H. Rind and C.J. Abraham, Inter-City Testing & Consulting Corproration, Inc., 167 Willis Avenue, Mineola, NY, 11501.

During the last three years, our office has investigated three separate instances of deaths which have occurred while families were tent camping. In each instance, a propane heater produced sufficient carbon monoxide to cause the death of the tent occupants. Were those deaths the result of a defect in tent design, a heater malfunction or an expected and predictable result of using a propane heater in a confined space? our studies included a permeability measurement of the fabrics used in the floor, walls and roofing of the tents. The effect of moisture in the fabric dramatically altered the permeability of the material. When camping in freezing temperatures was reconstructed using a low temperature chamber, frozen moisture in the tent fabric produced a nearly impermeable barrier with virtually no gaseous exchange possible. Wind velocity (turbulence at the tent surface) markedly increased gas exchanges through the fabric. Because one of the incidents occurred in a high altitude campground (>6,000 feet) the tent and heater were tested at different altitudes. At higher altitudes, oxygen depletion occurred more quickly, with accompanying rapid buildup of carbon dioxide and carbon monoxide. When oxygen concentrations approach 16%, the buildup of carbon dioxide and carbon monoxide accelerate markedly. In some of the instances, drug substances had been used by the tent occupants prior to their death. The relatively minor role of these drugs will be discussed.

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METABOLIC TESTING OF SUDDEN INFANT DEATH SYNDROME CASES: EVIDENCE FOR FUMARIC ACIDEMIA AS A NEW CAUSE OF SIDS. *William Shaw, Ph.D., Ellen Kassen, (Children's Mercy Hosp., Kansas City, MO) and John Overman, M.D. (Jackson County Medical Examiner's Office, Truman Med. Ctr., Kansas City, MO)

The Medical Examiner's Office in large metropolitan areas can play a key role in the elucidation of the metabolic causes of Sudden Infant Death Syndrome (SIDS). Blood and urine samples from 30 cases of SIDS were collected at the Jackson County Medical Examiner's Office over a period of one year. Serum samples were tested for sodium, potassium, chloride, uric acid, glucose, cholesterol, bilirubin, urea,calcium, phosphorus, total protein, albumin, triglycerides, magnesium, bicarbonate, several enzymes, amino acids, and organic acids. Organic acids were tested on urine when available. Postmortem changes in electrolytes,glucose, and serum enzymes were so large that this information was not able to be interpreted.

Urine organic acids appear to be the single most useful laboratory test for evaluation of SIDS deaths and even 0.1 ml of urine may be useful in determining cause of death. We found that fumaric acidemia, a genetic deficiency of the Krebs Cycle enzyme fumarase appears to be a new metabolic cause of SIDS and we have found that fumaric acid episodes, possibly indicating that fumarase deficiency may be a significant risk factor for apnea episodes and SIDS. Organic acid testing of the urine by GC/MS is an extremely powerful tool for testing metabolic diseases since it can detect abnormal metabolites from about one hundred different diseases including those involving abnormalities in carbohydrate, fatty acid, purine, pyrimidine, and amino acid metabolism.

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Review and Recommendations: Ethylene Glycol Analysis, A. Poklis, Dept Pathology, Medical College of Virginia, Richmond, VA. 23298-0597

Ethylene Glycol (EG) intoxication, although infrequent, is often life-threatening. The rapid determination of EG by Clinical laboratories is important for the diagnosis and treatment of intoxication. Postmortem analysis by Forensic laboratories in conjunction with autopsy findings is necessary to accurately establish EG poisoning as the cause of death. An extensive review of the literature reveals that gas chromatography (GC) is the most popular method for EG analysis. Analysis of EG is usually performed on polar, polyethylene glycol, columns while Boronic acid derivatives of EG are analyzed on intermediate polarity substituted polysiloxane columns. 2,3-Butanediol and 1,2-propanediol, novel metabolites in chronic alcoholics have been reported as possible interferences with GC methods. The apparent misidentification of an organic acid as EG in serum from an infant with methylmalonic acidemia (Shoemaker, et al, J. Pediat 120, 417, 1992) raises questions concerning EG identification by GC. Although this case involved extremely unusual circumstances, toxicologists may wish to consider the following recommendations: 1)Clinical laboratories should expand interference studies to include organic acids and other metabolites associated with inborn errors of metabolism or chronic alcoholism 2)Forensic laboratories, particularly in death of infants, should perform qualitative analysis of derivatized EG by GC/MS.

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FATAL METHANOL POISONING: METHANOL, FORMIC ACID CONCENTRATIONS AND THE COURSE OF METABOLIC ACIDOSIS

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Reports on the formation of formic acid and acidosis following methanol poisoning have been published before, but rarely for the same case. We report on a fatal methanol overdose which was monitored extensively as the patient deteriorated. A large number of antemortem specimens allowed for the determination of the methanol, ethanol, formic acid and acetic acid levels with the corresponding values for the acidosis, osmolar gap and anion gap.

Methanol and ethanol were assayed by head-space gas chromatography (GC) using 1-propanol as internal standard.[1] Formic and acetic acid were esterified, *in situ*, with methanol and sulfuric acid catalysis. The resulting methyl esters were analyzed by automatic head-space GC, using di-isopropylether as the internal standard.[2]

The patients admission blood pH was 6.75. The first antemortem blood specimens show methanol 3.1 g/l, formic acid 1.7 g/l, anion gap 41 mmol/l and an osmolality of 462 mmol/kg. Levels of formic acid this high are normally fatal and despite specific therapy: bicarbonate to correct acidosis, ethanol to competitively inhibit alcohol dehydrogenase, and hemodialysis to accelerate elimination, the patient died the next day. The postmortem levels were:

	methanol	formate	ethanol	acetate
blood	0.4 g/l	0.0 g/l	0.2 g/l	0.2 g/l
vitreous	0.5 g/l	0.8 g/l	0.1 g/l	0.8 g/l

The kinetics of the methanol/ethanol elimination and the formic/acetic acid formation and their relationship to acidosis will be presented.

1. D.S.Christomre, R.C.Kelly and L.A.Doshier. Improved recovery and stability of ethanol in automated head-space analysis. J.Forensic Sci. 29:1038-1044,1984.
2. P.P.Singer and G.R.Jones. Formate analysis in cases of methanol poisoning by automated head-space gas chromatography. Can.Soc.Forensic Sci.J. 18:250,1986.

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MARIJUANA TEA: TOXICOLOGICAL FACT OR FICTION?

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In two recent cases, a military member was charged with a Uniform Code of Military Justice offense based on the Positive finding of THC-Acid in a urine sample. In each case, we were asked to comment on the member's claim that he had consumed a marijuana tea beverage a short time before the urine collection, which purportedly explained the presence of THC-Acid in the urine sample. In the absence of useful literature data, we performed controlled experiments to determine whether or not the defendants' claims could be supported during expert testimony.

In one case, four different methods of brewing were attempted - hot and cold infusion, percolation and decoction - followed by GC-FID analyses of the resulting marijuana beverage solutions. In this experiment, it was demonstrated that all of the hot methods of brewing resulted in recovery of the hallucinogen THC in approximately 8% yield from the botanical material.

In the second case, reconstruction of the purported circumstances of unknowing ingestion of marijuana tea was attempted using boiling water containing crumbled marijuana leaf and tea bags. After 30-minutes of brewing the mixture was cooled, and TLC and GC-MS testing of the beverage identified THC. In addition, one male volunteer ingested 250-mL of the beverage, and urine samples were collected at timed intervals after ingestion. EMIT and GC-MS testing of these urine samples demonstrated that consumption of the marijuana tea beverage led to Positive urinalysis findings for THC-Acid.

These experiments demonstrate that it is quite possible to brew a marijuana tea which, when ingested, will lead to confirmed Positive THC-Acid urinalysis findings. Therefore, due caution must be exercised in the interpretation of similar circumstances when profered to explain such findings during administrative or judicial actions.

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FORMATION OF BENZOYLECGONINE FOLLOWING THE ADMINISTRATION OF COCAETHYLENE IN DOGS: A DOSE RESPONSE STUDY

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Following an IV dose-response study to evaluate the pharmacokinetics and cardiotoxic effects of cocaine and metabolites, and cocaine in combination with ethanol in a dog model, a second dose-response study was undertaken to evaluate the cardiotoxic effects of cocaethylene and any metabolic products.

Specimens were extracted using a SPE technique. Residues were derivatized with MSTFA and quantitatively analyzed via SIM utilizing a MSD for Cocaine (COC), Benzoylecgonine (BZE) and CocaEthylene (CE).

Data from the studies is as follows:

	t_{max} (min)	Cp_{max} (ng/mL)
· COC(7.5 mg/kg):n=5		
COC		5790±2761
BZE	132±27	859± 196
· COC(7.5 mg/kg) + ETOH(1 g/kg): n=4		
COC		6178±2451
BZE	188±37	882± 283
CE	83±10	77± 58
· CE(7.5 mg/kg): n=4		
CE		8623±7145
BZE	270±35	730± 196
· CE(3.75 mg/kg):n=4		
CE		2253± 684
BZE	285±58	371± 55

Utilizing this technique postmortem specimens can also be analyzed and cocaine related deaths may potentially be more easily interpreted.

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A CASE STUDY OF ETHANOL, COCAINE AND HEROIN USE,

J. Bidanset*, R. Dettling, C. Salerno, J. Segelbacher, W. Redner, and F. Zugibe, St. John's University, Jamaica, NY and Rockland County Office of the Medical Examiner, Pamona, NY.

This paper provides detailed analytical results on autopsy specimens in comparison to paraphernalia from the scene. A 38 year old white male collapsed at home and was subsequently pronounced dead at the scene. A history revealed smoking crack/snorting cocaine on the night preceding death. No specific timing could be obtained between drug use and death. In addition to the routine autopsy specimens (blood, bile urine, liver and brain), nasal swabs, a foil packet, and paraphernalia, including four straight glass tubes (used as pipes) and a straw were submitted to the toxicology laboratory for analysis. The following Table provides the tissue distribution :

	BLOOD	URINE	BRAIN
Ethanol	0.18%	0.25%	0.18%
Cocaine	<0.1 mg/L	1.0 mg/L	
Benzoyl Ecgonine	1.6 mg/L	16.7 mg/L	
Morphine	0.9 mg/L	3.5 mg/L	

Based upon the above toxicological data, the cause of death was determined to be "Multiple Drug Synergism". Analysis of the evidence revealed the presence of cocaine on the aluminum foil packet and the four glass pipes in amounts greater than 30 mg. Heroin was found in the straw and the nasal swabs in amounts greater than 1 mg. No needle punctures were observed at autopsy. Based upon the analysis of the evidence, one could conclude that the cocaine was smoked and the heroin was snorted.

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BREATH AND ROOM AIR CONCENTRATIONS OF COCAINE AFTER SMOKING CRACK

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Many drugs of abuse are self-administered by the smoking route. During "crack" smoking, the pipe is heated rapidly resulting in vaporization of the cocaine "rock." A single inhalation delivers the majority of drug as a bolus of vapor to the pulmonary system where it is quickly absorbed.

We studied the release of residual cocaine into the atmosphere from "side-stream" smoke during "crack" cocaine smoking and from expired breath. On separate occasions, four subjects smoked 25 and 50 mg of cocaine base. Room air samples were collected prior to smoking, immediately after, and at 5 and 15 min after smoking. Air samples were collected approximately 0.4 m from the smoker by vacuum withdrawal through SPE extraction cartridges (1-L/min). The SPE cartridges were eluted and the eluates were analyzed by GC/MS. Immediately after "crack" inhalation, three subjects collected breath samples in a 2-L Tedlar® bag. The bag was sealed immediately. Breath cocaine was determined by analyzing bag air by GC/MS. Also, residual cocaine in the bag was removed with solvent and measured. Total combined cocaine in bag air and residue was used for estimation of %dose recovery.

Analysis of room air in the vicinity of "crack" smokers indicated that room air cocaine increased from background to approximately 300 ng/L of air within a few minutes of smoking. Concentrations remained elevated through 15 min. Recovery of cocaine in breath (bag air & residue) ranged from 0.7-2.5% of the administered dose. These data demonstrate that relatively minor environmental exposure to cocaine occurs when drug escapes into room air during smoking and from breath exhalation after "crack" smoking.

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PRELIMINARY RESULTS INDICATE THAT PASSIVE INHALATION OF COCAINE VAPOR FAILS TO PRODUCE POSITIVE URINE RESULTS AT DHHS CUTOFF CONCENTRATIONS

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Cocaine is released into the atmosphere when "crack" cocaine is smoked. Individuals in the immediate vicinity could absorb cocaine from environmental air, resulting in passive exposure. The amount of cocaine absorbed would be highly dependent upon numerous factors including amount of cocaine vaporized, length of exposure, ventilation conditions, and individual pharmacologic factors such as rate of metabolism and excretion. Several claims have been made that positive urinalysis results occurred due to passive exposure to "crack" smoke.

We investigated whether passive exposure to cocaine vapor would lead to positive urine results at concentrations above the DHHS cutoff for cocaine metabolite. Drug-free subjects who provided informed consent and had a recent history of cocaine use were exposed to the vapor from 100 and 200 mg of cocaine base for one hr in an unventilated 7 X 8 X 8 ft room. All urine samples were collected after exposure. For comparison, the same subjects were administered, at separate times, 1 mg of cocaine HCl by the intravenous (IV) route. In addition, urine samples were collected from medical staff who assisted in a research protocol involving "crack" smoking by volunteer subjects. Samples were analyzed by EMIT®II Cocaine Metabolite assay and by GC/MS for cocaine, benzoylecgonine (BE) and ecgonine methyl ester.

All urine samples collected after drug-exposure consistently tested negative for cocaine and metabolites by EMIT II assay. By GC/MS, some samples collected after exposure to 100 and 200 mg of vaporized cocaine contained traces of cocaine (0-20 ng/mL). BE concentrations peaked at approximately 125 ng/mL in 4-8 hrs. Similar concentrations were obtained when subjects received 1 mg of cocaine IV. Staff urine samples contained BE in concentrations below 10 ng/mL.

These preliminary data suggest that although DHHS cutoff concentrations were not achieved through passive exposure, absorption of cocaine can occur readily from environmental air resulting in excretion of detectable amounts of cocaine and metabolite in urine.

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USE OF A SKIN PATCH TO MONITOR COCAINE ABUSE

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This study investigated a method of monitoring cocaine use. A collection device ("patch") was placed on a subject's (S's) torso, biceps, or back and worn for up to 12 days. The patch consists of an adsorption pad and an outer layer of polyurethane/adhesive, which allows the water content of sweat to evaporate while acting as a barrier to the outside environment. Non-volatile components from the skin are retained and concentrated on the adsorption pad. After a pad is removed, it is extracted in 2.5 ml of 0.2 M pH 5.0 acetate buffer with 0.1% Triton-X and analyzed by RIA (Diagnostic Products Corp.) or GC/MS.

In an initial phase of the study, 18 non-cocaine using volunteers wore 14 patches for 2 to 12 days. The mean cocaine content of the pads (N=194) by RIA analysis was 0.1 ng/mL. No pad contained >4 ng/mL.

In a second study phase, patches were applied to 16 volunteers who met cocaine-use criteria. After signing an informed consent, each S participated in two treatment periods, separated by a week washout, and took 30 and 125 mg cocaine HCl intranasally. Ss were assigned to counterbalanced treatment order by random procedures. With the exception of one of the authors (M.B.), Ss and SCRI and CTI staff were blind to treatment order and specimen sequence and code.

Under both treatment conditions, a patch was removed and urine specimen obtained at 1-hr post-dose intervals for six hours. The remaining patches were removed during the next seven days. GC/MS analysis identified parent cocaine as the dominant cocaine-related component in the pad contents, which reflected a clearcut dose-response relationship. Analysis of some pads revealed >15 ng/mL cocaine within a few hours of dosing, while others did not become positive for 24 hrs. After the 30 mg dose, the mean pad content was 30 ng/ml on Day 1 and remained above 27 ng/ml through Day 7. Following the 125 mg dose, the corresponding findings were 98 ng/mL on Day 1 and 145 ng/mL through 7 days.

With an arbitrary selection of 15 ng/mL cocaine by RIA as the positive/negative decision point, 70% of all patches from both doses were positive from Day 1 through Day 7. A Day 1 95% positive rate for urine specimen (>300 ng/ml BE by RIA) dropped to zero by Day 4. The patch technology appears to offer a sensitive and reliable method of monitoring for cocaine abuse.

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ANALYSIS OF 3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA) AND ITS METABOLITES IN HUMAN PLASMA AND URINE BY HPLC-DAD, GC/MS AND ABUSCREEN-ONLINE®

H.-J. Helmlin*, K. Bracher, S.J. Salamone and R. Brenneisen

Since 3,4-methylenedioxymethamphetamine (MDMA) is legally used in Switzerland as an adjunct in psychotherapy it was the aim of this study to establish and validate the analytical methodology for monitoring MDMA and its metabolites in body fluids and to investigate the pharmacokinetic behavior of MDMA in man under controlled conditions.

After administration of 1.5 mg/kg MDMA to patients, body fluids (plasma and urine) were collected over a period of 9 and 22 h, respectively. The solid-phase extracts of the plasma and urine samples (before and after enzymatic hydrolyzation) were analyzed by HPLC with photodiode array detection (DAD) and GC/MS (HFBA derivatives). In addition, some urine specimens were measured by the Abuscreen-ONLINE® immunoassay for amphetamines on a COBAS MIRA Plus automated analyzer.

Maximum plasma levels of MDMA (about 300 ng/ml) were reached after about 140 min. >17 µg/ml MDMA were measured in 22 h-urine specimens. The main urinary metabolites of MDMA are 4-hydroxy-3-methoxymethamphetamine (HMMA) and 3,4-dihydroxy-methamphetamine (HHMA), both eliminated mainly as conjugates, and 3,4-methylenedioxyamphetamine (MDA). Minor metabolites are 4-hydroxy-3-methoxyamphetamine (HMA) and 3,4-dihydroxyamphetamine (HHA).

The cross-reactivity of the Abuscreen-ONLINE immunoassay varied between 0% (HMMA, HMA) and 35 % (MDA). An increase of the cross-reactivity was observed in the mixture of MDMA and MDA, e.g. to 100% when adding 1500 ng/ml MDA to 500 ng/ml MDMA. For example, at a cut-off of 1000 ng/ml d-amphetamine 1.5 h- and 3.5 h-urine samples gave negative results, whereas 10 h- and 22 h-urine samples were positive.

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URINE CREATININE LEVELS IN RANDOM AND DAILY URINE COLLECTIONS IN A CONTROLLED CLINICAL STUDY

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Random urine samples are routinely used for urine drug testing. Individuals may attempt to lower drug levels below the mandated HHS cutoffs by imbibing large quantities of liquid. Urine creatinine analyses monitor possible adulteration of the sample due to dilution. However, the within and between subject variation in urine creatinine levels has been poorly characterized. The variation in urine creatinine levels of six healthy male subjects, and the relationship of time of collection to urine creatinine level were studied. Each urine void was collected over a three week period for six subjects participating in a controlled clinical study at the Addiction Research Center, NIDA. Creatinine analyses were performed on 895 specimens on a Hitachi 704 analyzer utilizing Boehringer Mannheim reagents. The mean ± SD urine creatinine level across subjects was 127.3 ± 44.0 mg/dL (CV=34.6%), with a range of urine creatinine from 6-360 mg/dL. Individual subject's mean urine creatinine levels were as follows: 118.8 ± 48.3 (CV=40.7%); 155.7 ± 39.0 (CV=25.0%); 123.6 ± 47.8 (CV=38.7%); 101.3 ± 61.2 (CV=60.5%); 68.8 ± 48.6 (CV=70.7%); and 195.9 ± 57.1 (CV=29.1%). Twenty-six urines (2.9%) had a creatinine level below 20 mg/dL and would be considered dilute according to current policy. Four of six subjects produced at least one dilute urine, with 17 of 26 produced by a single subject. Mean urine creatinine concentrations across time were determined from 2400 to 0600, 0600 to 1200, 1200 to 1800, and 1800 to 2400 h. The mean creatinine concentrations varied by less than 10% across these time periods. Urine 24 h creatinine values for each subject over a 21 day period ranged from 431.7 to 2359.9 mg/day, with a mean across subjects of 1556.3 ± 506.8 mg/day. Normalization of drug concentrations to urine creatinine levels in serial urine samples have been shown to be useful for determining drug reexposure in a drug treatment setting. However, the inherent variation in random urine creatinine levels within and between subjects will increase the variability of the normalization of drug levels to urine creatinine concentration.

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EVALUATION OF TOLMETIN AND OTHER NONSTEROIDAL ANTI-INFLAMMATORY DRUGS FOR INTERFERENCE WITH EMIT® AND TDx® ASSAYS FOR DRUGS OF ABUSE

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Nonsteroidal anti-inflammatory drugs (NSAIDs) are frequently prescribed for the relief of pain and inflammation. Some NSAIDs have been found to interfere with immunoassays for drugs of abuse. Recently, a number of urine samples collected from employees tested under DHHS guidelines were determined to be "unsuitable for analysis" by forensic urine drug testing (FUDT) laboratories. It is possible that the presence of NSAIDs in these samples was the cause of the immunoassay failure. This study systematically evaluated the effects of the following fourteen commonly prescribed NSAIDs on EMIT® and TDx® assays for drugs of abuse: fenoprofen; ketoprofen; ibuprofen; naproxen; indomethacin; sulindac; tolmetin; diclofenac; meclofenamate; mefenamic acid; piroxicam; flurbiprofen; acetaminophen; and aspirin. NSAID urine standards were prepared by adding the maximum recommended daily dose of each NSAID to 1-L of drug-free urine. In addition, urine samples were collected from an arthritic patient who ingested tolmetin. Tolmetin was the only NSAID that interfered with EMIT assays; it produced extremely high absorbance readings resulting in non-linear detector responses and instrument alarms. The sample was unacceptable for analysis by this technique. Similar results were obtained with specimens collected after ingestion of tolmetin. In contrast, TDx analyses of NSAID urine standards produced acceptable negative results for all assays; but, fenoprofen, ketoprofen, indomethacin, tolmetin, and flurbiprofen produced higher than background readings in the TDx benzodiazepine assay. Samples that contained NSAIDs combined with drugs of abuse produced depressed responses in all EMIT assays. GC/MS analysis of selected samples containing tolmetin and drugs of abuse was successful. These results indicate that EMIT analysis of urine samples containing tolmetin may result in a report of "unacceptable specimen," but these samples may be successfully analyzed by TDx and GC/MS assays.

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A COMPARISON STUDY BETWEEN RADIOIMMUNOASSAY AND TWO ENZYME IMMUNOASSAYS ON UNTREATED WHOLE BLOOD AND URINE SAMPLES

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The Department of Justice Toxicology Laboratory has used radioimmunoassay (RIA) for the screening of drugs of abuse in biological specimens since the early 1980's. Confirmation rates for this method have exceeded 95% by GC/MS. In recent years, however, environmental concerns and spiraling cost increases for low-level radioactive waste disposal have fostered research into alternative methods for detecting drugs of abuse.

Enzyme immunoassay (EMIT®) procedures for the analysis of drugs of abuse in whole blood have been published, but required manipulation of the sample and were therefore rejected. Fluorescence Polarization Immunoassay (ADx/TDx®) procedures were summarily dismissed as time consuming and prohibitively expensive.

Two immunoassay screening procedures, Sigma immonoassay (SIA®) and Milenia®; investigated by the Toxicology Laboratory utilize microtiter plates, permitting drug analysis in whole blood without deproteinizing. Each case screened by one of the new procedure was also screened by the current RIA. Only discrepancies between RIA and the new procedures were confirmed by GC/MS and GC/MC/MS.

Seventy-one (71) cases were screened by SIA®, and 142 by Milenia® for free Morphine 10ng/ml, d-Methamphetamine 100ng/ml, Benzoylecgonine 300ng/ml, Phencyclidine 10ng/ml, and 11-Nor-9-Carboxy-Delta-9-Tetrahydrocannabinol 30ng/mL. Results were as follows:

Sigma Immunoassay	1 False positive Methamphetamine
	4 False negative Benzoylecgonine
Milenia®	1 False positive Phencyclidine
	6 False negative Methamphetamine
Radioimmunoassay	3 False positive Benzoylecgonine
	1 False positive Methamphetamine

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EVALUATION OF THE TRIAGE™ PANEL FOR DRUGS OF ABUSE TEST FOR THE SIMULTANEOUS DETECTION OF MULTIPLE DRUGS IN URINE

J. F. Bruni*[1], T. Koch[2], S. H. Wong[3], J. A. Callas[4], and A. H. Wu[5]
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The Triage™ Panel for Drugs of Abuse is a 10 minute, solid phase, monoclonal antibody based, competitive immunoassay that simultaneously tests a urine specimen for the presence of multiple drugs of abuse. Approximately 100 positive specimens from each drug class (phencyclidine, barbiturates, benzodiazepines, opiates, amphetamines, cannabinoids methadone, and cocaine metabolites) were selected to be positive using Emit immunoassays, and confirmed to be positive using GC/MS. The Triage test is normally configured to contain the 5 drugs recommended by HHS plus benzodiazepines and barbiturates. An additional version of this test contains the seven drugs previously mentioned plus methadone. Another study was performed to assess the performance of the Triage test when the threshold concentration is lowered to 50 ng/mL for cannabinoids. 100 negative specimens were evaluated for each drug class.

	Triage™/SYVA Sensitivity(%)	Triage™/SYVA Specificity(%)	Triage-SYVA Agreement (%)
PCP	100/100*	100/100	100
Benzodiazepines	98/92	94/63	88
Cocaine Metabolites	100/100	100/100	100
Amphetamines	97/100	97/85	90
THC (cannabinoids) 100 ng/mL	93/100	100/100	97
THC 50 ng/mL	100/100	100/100	100
Opiates	100/100	97/97	100
Barbiturates	97/100	100/100	98
Methadone	100/100	100/98	99

* By design, Emit should be 100% sensitive, positive specimens were selected to be Emit positive. Therefore, the Emit test should only sacrifice performance in the specificity.

The performance of the internal quality control zones and the effects of various adulterating substances will be presented, in addition to the ability of the Triage test to detect a broad class of benzodiazepine metabolites as compared to other screening tests. The clinical performance, speed, accuracy and precision of the Triage™ device make it a rapid alternative or supplement to instrument based screening tests.

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EFFECT OF HYDROLYSIS PRETREATMENT ON URINE BENZODIAZEPINE SCREENING USING EMIT II® AND TDX® Robert Meatherall, PhD, Clinical Biochemistry, St. Boniface General Hospital, Winnipeg, MB, Canada, R2H 2A6

Urine specimens were collected from individuals who were prescribed oral doses of one of the benzodiazepines: diazepam, oxazepam, temazepam, lorazepam, alprazolam, flurazepam, and chlordiazepoxide. An aliquot was enzymatically hydrolyzed to cleave the glucuronide and sulfate conjugates. Both the hydrolyzed and unhydrolyzed urine were subjected to the EMIT II and TD_X screening immunoassays for benzodiazepines and to GC/MS confirmation.

GC/MS analysis showed greater than 95% of the urine benzodiazepine metabolites are conjugated. Flurazepam metabolites are detected equally well on either hydrolyzed or unhydrolyzed urine. An increased response is observed after hydrolysis for all other benzodiazepines. Hydrolysis is required to adequately detect oxazepam, temazepam and lorazepam. The manufacturers' 200 ng/mL cut-off must be lowered to 100 ng/mL to detect lorazepam following therapeutic doses. EMIT II is a more sensitive benzodiazepine screening test than the TD_X.

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IMMUNOASSAY DETECTION OF BENZODIAZEPINES AND BENZODIAZEPINE METABOLITES IN BLOOD

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The ability of commercial benzodiazepine immunoassays to detect 24 benzodiazepines and 16 benzodiazepine metabolites in blood samples was investigated. Abuscreen's and Diagnostic Products Corperation's serum radioimmunoassays; the EMIT TOX serum enzyme immunoassay; and X-systems Serum fluorescence polarization immunoassay were evaluated. Drug-free human blood was fortified with the different compounds at concentrations of 7, 30 and 70 ng/mL. Blood samples were extracted with butyl chloride (7:1), reconstituted with buffer to one-half the original volume, and assayed per manufacturer's instructions, with nordiazepam fortified blood used as the calibrator in all instances. The following were detected by all four assays at concentrations of 70 ng/mL or lower: alprazolam, α-hydroxyalprazolam, norclobazepam, diazepam, estazolam, flunitrazepam, norflunitrazepam, midazolam, 4-hydroxymidazolam nitrazepam, oxazepam, prazepam, 3-hydroxyprazepam, temazepam, and triazolam. Haloxazepam, loprazolam, oxazolam, and the reduced and/or N-acetylated metabolites of nitro-benzodiazepines (7-amino-clonazepam, 7-acetamidoclonazepam, 3-hydroxy-7-acetamido-clonazepam, 7-aminoflunitrazepam, 7-aminonitrazepam and 7-acet-amidonitrazepam) were not detected by any of the four assays at concentrations up to 70 ng/mL. A number of benzodiazepines or their metabolites were detectable by some but not all of the assays. The detectablitiy of benzodiazepines depends upon their structure and the immunoassay being employed. Metabolism alters the cross-reactivity, for those benzodiazepines flowing through nordiazepam and oxazepam it is generally improved, for others, immunoreactivity can be greatly diminished.

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EVALUATION OF THREE SCREENING TESTS FOR THE DETECTION OF BENZODIAZEPINE METABOLITES IN URINE, CONFIRMATION USING GAS CHROMATOGRAPHY - MASS SPECTROSCOPY, J.F. Bruni[1], T. Koch[2], R. Raglin[2], J. Buechler[1] and S. Kirk[1] ([1]Biosite Diagnostics Incorporated, San Diego, CA 92121 and [2]Maryland Medical Laboratories, Baltimore MD 21227)

The benzodiazepines are a class of hypnotic sedatives that are among the most frequently abused prescribed drugs. The benzodiazepines undergo minor to extensive biotransformation followed by conjugation to glucuronic acid prior to excretion by the kidney. Very little of the ingested compound is excreted unchanged in the urine. Three commercially available screening tests were evaluated to detect the presence of benzodiazepines in approximately 300 urine specimens; Triage™ Panel for Drugs of Abuse, Biosite Diagnostics, San Diego, CA, Emit®, SYVA Co., Palo Alto, CA and the TDx®, Abbott Diagnostics, North Chicago, IL. The Triage benzodiazepine test, unlike the Emit and TDx tests, was standardized to the glucuronide conjugates of the benzodiazepine metabolites. All methods were performed according to the recommendations described in the package insert of the respective kit. The screening and confirmation cut-off was 300 ng/mL. All specimens producing a positive screening result were confirmed using Gas Chromatography/Mass Spectrometry using mass selective detection to simultaneously identify and quantify the following major metabolites of the most commonly prescribed benzodiazepines: desalkylflurazepam, nordiazepam, oxazepam, temazepam, lorazepam, alpha-hydroxy-ethylflurazepam, alpha-hydroxy-alprazolam, and alpha-hydroxy-triazolam. The results compared to GC/MS are presented below:

	Triage™/SYVA/TDx Sensitivity(%)	Triage™/SYVA/TDx Specificity(%)	Triage™/SYVA-TDx Agreement (%)
Benzodiazepines	98/92/76	94/63/97	88/84

Statistical analysis of the clinical sensitivity and specificity of these screening tests provided the following results:

Sensitivity	Triage > Emit > TDx	($p < 0.05$)
Specificity	Triage = TDx > Emit	($p < 0.05$)

These results demonstrate that the Triage benzodiazepine test is a sensitive and specific screening test for benzodiazepine metabolites in urine. A discussion of the standardization and specific differences in the test systems will be presented.

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A COMPARISON OF TWO GC/MS METHODS FOR CONFIRMATION OF NORDIAZEPAM AND OXAZEPAM.

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Benzodiazepine analysis is a growing area in workplace testing. The NRC, CAP, and governmental agencies such as those in the states of New York and Florida require an ability to test and quantify benzodiazepines. However, in the case of benzodiazepines different GC/MS methodologies may yield quantitative levels of oxazepam and/or nordiazepam that are vastly different from each other. This has the potential to create gross inconsistencies in results and hence to pose significant problems in result interpretation.

Negative urine was spiked with nordiazepam, oxazepam, or both compounds. The spiked samples were analyzed by two different GC/MS methods. The initial method was based on acid hydrolysis to yield 2-amino-5-chlorobenzophenone (ACB). Hydrolysis was performed at 100°C to ensure complete ACB formation. The second method employed enzyme hydrolysis and trimethylsilyl derivatization with BSTFA and 1% TMCS. This method yielded two different derivatives for oxazepam and nordiazepam. A comparison of recoveries for ACB and the sum of TMS-oxazepam and TMS-nordiazepam demonstrated agreement for both GC/MS methods.

Human urine samples that tested positive for benzodiazepines by immunoassay were analyzed by both GC/MS methods. The mean ACB concentration was 1,143 ng/mL. The sum of the mean TMS-oxazepam levels (689 ng/mL) and TMS-nordiazepam levels (387 ng/mL) was 1,076 ng/mL. The correlation coefficient of a linear plot of the two methods was 0.980.

In summary, our study indicates that calibrators should consist of both nordiazepam and oxazepam, and that enzyme hydrolysis with trimethylsilyl derivatization is a preferred methodology.

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GC/MS CONFIRMATION OF URINARY BENZODIAZEPINE METABOLITES

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A GC/MS method is described for the confirmation of benzodiazepines in urine. The targeted drugs/metabolites are diazepam, nordiazepam, temazepam, oxazepam, lorazepam, α-hydroxyalprazolam, α-hydroxytriazolam, 2-hydroxyethylflurazepam, N-desalkyl-3-hydroxyflurazepam and N-desalkylflurazepam. They are the metabolic end product following the ingestion of the most commonly prescribed benzodiazepines.

The conjugates of these urinary metabolites are hydrolyzed with β-glucuronidase (Helix Pomatia). The urine is made alkaline and extracted with an organic solvent. The solvent is evaporated and the residue subjected to sequential derivitization. The secondary amine on the lactam ring is propylated then the free hydroxyl groups are propionylated to form esters.

The derivatives are separated on a merhyl silicone capillary column. Full scan mass spectra were acquired with a Finnigan MAT ITS40 ion trap mass spectrometer. Quantitation is based on ion ratios of the analyte to one of four internal standards - oxazepam D5, lorazepam D4, α-hydroxyalprazolam D5 and α-hydroxytriazolam D4.

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"QUANTITATIVE ANALYSIS OF CODEINE AND ITS MAJOR METABOLITES IN HUMAN HAIR BY POSITIVE CHEMICAL IONIZATION MASS SPECTROMETRY: A CLINICAL APPLICATION". D. Rollins, *D. Wilkins, J. Seaman, G. Krueger and R. Foltz. Center for Human Toxicology, Dept. of Pharmacology and Toxicology and Div. of Derm., Univ. of Utah, Salt Lake City, UT, 84108.

Pharmacokinetic and epidemiologic studies of the disposition of drugs of abuse into human hair requires sensitive analytical methods. A highly sensitive and specific method has been developed for the quantitative analysis of codeine and its major metabolites in human hair. Hair samples were collected from four male volunteers by plucking or cutting from the back of the head after a single 120 mg oral dose of codeine. At least two mg of hair and 100 ng of deuterated codeine, morphine and normorphine were digested overnight in flat-bottom glass vials containing 500 µl of 1M NaOH at 37°C. Eleven calibration standards containing known concentrations of drugs dried onto human hair were also prepared and digested. Digest solutions were extracted using a modified solid-phase procedure with Bond-Elute Certify® bonded silica extraction columns. The final eluate containing drug was evaporated to dryness, derivatized with TFAA, evaporated and reconstituted in 50 µl of ethyl acetate. Derivatized extracts were analyzed by gas chromatography/mass spectrometry on an ITS40™ mass spectrometer. Chromatographic separation was achieved with helium carrier gas on a DB5MS-15m-.25µ capillary column. Positive chemical ionization mode was utilized with acetone as reagent gas to enhance sensitivity and specificity. Regression analysis of the extracted standards indicated a linear range of 0.1 ng/mg to 75 ng/mg for codeine and morphine (0.998 to 1.000). The assay is capable of detecting as little as 10 pg of codeine and/or morphine on column. Intra-assay precision ranged from 8-22%. Accuracy was verified with control hair specimens of known concentration.

Hair samples were plucked from the scalp for 21 days and then cut at the scalp for eight weeks. The maximum mean measured concentration of codeine was 2.9 ng/mg in the proximal 1cm of hair (containing the bulb) at 12 hours following administration of codeine. Codeine was detected in hair for at least 8 weeks following the single dose. No norcodeine, normorphine or morphine detected in hair samples at this single dose. Hydrolysis of samples with ß-glucuronidase prior to extraction yielded no evidence of the presence of codeine or morphine glucuronide conjugates. The method is currently being used to quantitate codeine and its major metabolites in further dose-response disposition studies in human subjects. Supported by NIDA Grant DA07820.

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HAIR CONTAMINATION FROM ENVIRONMENTAL COCAINE
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Drugs are often released into the environment as vapors, powders, and aqueous solutions. It is important in hair testing for drugs of abuse to be able to discriminate active drug use from environmental exposure. We deliberately contaminated hair with cocaine and studied its characteristics to determine if use of specific markers or wash methods would allow distinction between active use and passive exposure.

We found that "cut" hair can be readily contaminated with either cocaine vapor or aqueous cocaine solutions. In the cocaine vapor experiments, cocaine and anhydroecgonine methyl ester were absorbed in a linear manner over time. Significant contamination occurred after 15 min of cocaine vapor exposure; however, the amount of cocaine adsorption appeared to be capacity-limited and reached a plateau with ca. 25 mg of vaporized cocaine in room air (7 X 8 X 8 ft room dimensions). There was near quantitative removal of cocaine with a brief methanol wash, but residual cocaine remained detectable in hair. After aqueous contamination with cocaine HCl (0.01-0.1 mg/mL), hair was highly contaminated. Less cocaine was found in the wash fraction than in the extract fraction indicating that aqueous cocaine contamination differs substantially from contamination by cocaine vapor.

Head hair of two subjects (A & B), who had no previous exposure to cocaine, was contaminated with cocaine vapor (100 mg) in the passive exposure room. Cocaine extract concentrations in hair immediately after exposure were 5.6 ng/mg and 8.6 ng/mg, respectively. After 1 and 8 days, with regular shower and shampoo, cocaine concentrations fell to 1.2 and 0.6 ng/mg for Subject A and 0.7 and 0.5 ng/mg for Subject B, respectively.

We conclude that hair can be contaminated from environmental cocaine and may be difficult to distinguish from active drug use.

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HAIR DETERMINATIONS BY INFRARED MICROSCOPY

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Infrared microscopy has been shown to be a unique method of analysis for drugs of abuse determinations in hair, with the ability to analyze only the central core or medulla region of sectioned hair. By spectrally mapping infrared functional groups related to the various drugs, a three dimensional image of the drug location can be obtained from cross-sectional and laterally cut hairs. This technique eliminates the question of externally contaminated hair by analyzing only that portion of the hair which is formed from within the root where ingested material would transport.

Compositional differences at various lengths of a hair from a single root have been found and determinations of the relative concentrations of the various components at different lengths can be obtained. This technique utilizes curve fitting routines and principle component regression of the hair in question. Compositional differences can also be obtained across the hair defining the outer and inner core material. Variations in overall composition have been found in hair with different color and type.

Prepared hair used as standards can be mapped for effective concentrations across the hair. Wash kinetics can also be monitored and removal of the drug at various layers of the hair can be determined.

Visual differences in the hair can be seen by the infrared microscope in the viewing mode before the hair is sectioned. These hairs can then be tediously separated and sent on to analysis by the established GC/MS technique. Differences found by infrared microscopy can be compared to the overall homogeneous analysis of GC/MS and critical differences in the hair sampling can be determined.

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OPTIMIZATION OF DRUG ANALYSIS USING GAS CHROMATOGRAPHY AND THERMODYNAMIC RETENTION INDICES
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Method development in gas chromatography involves making choices for many different parameters. Many combinations of column dimensions and instrument conditions may be tested in order to find the combination that provides the optimum separation for a set of analytes. In order to make the best choices, the analyst should be knowledgeable about the variables that effect separation efficiency and retention time. Too often the process of method development is a time consuming process that involves empirically varying column length, inside diameter, stationary phase film thickness, oven parameters, carrier gas velocity and many other parameters. This often results in a hit or miss approach to method development that may result in a method that is acceptable but not optimized for maximum resolution in the shortest amount of time.

Thermodynamic retention indices are related to the equilibrium solubility of an analyte in the stationary (liquid) phase and the mobile (gas) phase at specific temperatures. These thermodynamic characteristics can be used to predict the retention time and peak width of a compound under a given set of chromatographic conditions. By combining a computer program designed to model retention time with a database of compounds, separations involving a large number of compounds can predicted.

This paper will describe the basis for this computer model and demonstrate examples of drug separations using the model to predict the optimum set of chromatographic conditions. Data will be shown comparing predicted and actual separations.

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DRUGS OF ABUSE TESTING IN EUROPE: QUO VADIS

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Drug abuse has increased and spread rapidly in Europe in recent years and, consequently, there is much debate on whether and how to implement drugs of abuse testing (DAT) programs, both at the political and at the scientific level. An additional factor that needs to be considered is the abolishment of the internal borders between the 12 member states that form the European Community (EC).

To get an adequate overview, various surveys have been held lately and discussions between national representatives and experts are taking place at the EC level.

This presentation will evaluate the present status of DAT in the EC. Though legislation or regulations on DAT are virtually non-existent in most member states, a lot of testing is already being done. On the other hand, the drugs of interest, the analytical strategies used, and the interpretation of the results show considerable differences as compared to the situation in the US. Specific problems will be highlighted and some projections into the future will be made.

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OSMOLAL GAP, ENZYMATIC ETHANOL AND INHALANT ABUSE WITH ETHYL CHLORIDE

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Clinical toxicology laboratories make use of delta osmolality (the difference between measured and calculated osmolality) and enzymatic ethanol assay to determine the need to measure volatiles by GC. Osmolality is measured by freezing point depression. Calculated osmolality is determined by the formula [1.86 (sodium) + glucose/18 + urea nitrogen/1.8] / 0.93. The contribution due to ethyl alcohol is calculated by multiplying the delta osmolality by 4.6 (weight of ethanol per dL, to give ethanol in mg/dL). Conditions causing an increase in delta osmolality include alcohols, ketones, mannitol, elevated proteins, lipemia and inhalants.

We report here the use of delta osmolality, ethanol assay and the finding of ethyl chloride in two cases. Ethyl chloride is a vapor coolant used for topical application to control pain associated with minor surgical procedures. Ethyl chloride is available at local "head shops" as VCR head cleaner. Inhalation may produce narcotic and general anaesthetic effects. Ethyl chloride sensitizes the myocardium to epinephrine which can lead to ventricular fibrillation.

The first case was a 22 year old college student discovered by his parents. He was taken to the hospital where he was declared DOA. An antemortem serum sample revealed the following: glucose 92 mg/dL, Na 142 meq/L, BUN 18 mg/dL, measured osmolality 321, ethanol by ADH < 5 mg/dL. Delta osmolality was 25 mOsm/kg. Volatiles by GC showed ethyl chloride to be 20 mg/dL.

Case two was a passenger in a traffic fatality, no serum was available, vitreous humor results were as follows: Na 129 meq/L, glucose 60 mg/dL, ethyl alcohol 90, osmolality 300, calculated osmolality was 261, delta osmolality was 39. Increase in osmolality due to ethanol was 21 mOsm/kg. In this case, ethyl chloride was 20 mg/dL.

These cases demonstrate the usefulness of obtaining an osmolality measurement and the finding of an unusual volatile, ethyl chloride, in the blood of inhalant abusers.

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APPLICATION OF A PALMTOP TYPE COMPUTER TO FORENSIC TOXICOLOGY

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The extraordinary database program of a new palmtop type computer has been extended to applications in forensic toxicology. Although the computer can literally fit into a pocket, it is the equivalent of an IBM-XT, but faster because all programs are built into ROM. Through a connectivity pack, it can run almost any DOS program that a desktop computer can run. The computer comes equipped with 1 megabyte of RAM memory and has up to 20 MB of mass storage capability in its PCMCIA slot.

One database application has been constructed to contain the 6 most prominent GC/MS ions of over 1200 hundred drugs as well as the Base Ion and M.W. for each drug. Depending upon the complexity of the search parameters, the entire database of 1260 drugs can be searched in less than 10 seconds. Complex search parameters involving searches of multiple GC/MS ions in random order will, of course, take longer; but can be usually accomplished in less than 60 seconds. Another database was designed to contain over 150 of the most common drugs and stores information on therapeutic and lethal levels, relative retention times, base ion, M.W., metabolites, trade names, etc. A search of this database, depending upon the search parameters, often takes as little as 3 seconds.

Also, built into the computer is a full-blown version of Lotus 1-2-3, a statistics package capable of plotting regression lines with ease, DOS 5.0 and a host of other remarkable features.

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DEVELOPMENT OF CEDIA® ASSAYS FOR DRUGS OF ABUSE IN URINE: Rueyming Loor*, Neal Bellet, Greg Marr, Terry Morton, Harkanwal Singh, Heidi Scholz, Noah Schuyler, David Davouzadeh, Mary Crenshaw, Jeff Shindelman, and Pyare Khanna (Microgenics Corporation, Concord, CA 94520). Dave Moorman, Gerry Singler, and Michael Hansen (Boehringer Mannheim Corporation, Indianapolis, IN 46250).

We have developed seven homogeneous enzyme immunoassays, listed on the table below, for detection of drugs of abuse in urine using the CEDIA technology. Assays have been applied to the BM/Hitachi 717 and other Analyzers. In these assays, the enzyme ß-galactosidase has been genetically engineered into two inactive parts: Enzyme Acceptor (EA) and Enzyme Donor (ED), which can spontaneously reassociate to form active enzyme. Each drug derivative is covalently attached to the ED molecule so that enzyme reassociation is not affected, however, binding of the antibody to the ED-drug conjugate inhibits the reassociation with EA. The assays are performed on the Hitachi 717 as follows: Sample (3-12 µL) is pipetted into a reaction cuvette, followed by 130 µL of Reagent 1 containing EA and antibody, mixed and incubated for 5 min at 37°C. Then, 130 µL of Reagent 2 containing ED-drug conjugate and substrate CPRG is added, and the incubation is continued for 4 min at 37°C. Drug present in the sample binds to antibody in the first reagent, thus preventing binding of ED-drug conjugate to the antibody. The unbound conjugate is thus available for reassociation with EA, resulting in formation of active ß-galactosidase. The amount of enzyme formed is proportional to the drug concentration in the sample which is determined by the rate of CPRG hydrolysis measured at 570 nm. The concentration of drug in the sample can be determined by (1) a qualitative result positive or negative (above or below the cut off level), or (2) a semi-quantitative measurement by point-to-point interpretation using 4 calibrators.

Using these CEDIA drugs of abuse assays, the following results are obtained:

Assays (cut-off, ng/ml)	Assay range (ng/ml)	ΔA (mA) (0 to cut-off)	Dose precision at cut-off: %CV	LOD ng/ml
Opiates (300)	0 - 2,000	150	2.6	13.5
Cocaine met (150, 300)	0 - 10,000	100	4.0	14.7
Cannabinoids (25, 50, 100)	0-50,100,150	210, 240, 220	0.8, 1.0, 0.9	1.8, 4.2
Phencyclidine (25)	0 - 150	190	1.5	4.6
Meth/Am (500, 1000)	0 - 3,000	90	4.0	27
Barbiturates (200, 300)	0 - 3,000	118, 165	3.6, 3.8	10.0
Benzodiazepines (200, 300)	0 - 3,000	108, 146	3.2, 2.2	13.4

We have also developed several assays listed on the table with multiple cut-off levels to meet the requirement of different customers. The method comparison of CEDIA assays against GC/MS revealed 97%-100% assay sensitivity and specificity. In comparison with other commercial assays, the CEDIA assays are more precise at the cut-off level and better zero to cut-off separation due to a greater signal to noise ratio. In summary, the seven CEDIA drugs of abuse assays provide a rapid, convenient and effective method for screening drugs in urine.

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USE OF EMPORE™ EXTRACTION DISKS FOR PREPARATIVE PURIFICATION AND CONCENTRATION OF IMIQUIMOD AND METABOLITES FROM LARGE VOLUMES OF URINE. R.L. McQuinn, J.H. Machacek, A.M. Draper, P.E. Myhre, G.L. Carlson and D.A. Wells[1]. 3M Pharmaceuticals, Drug Metabolism Department and [1]3M New Products Department, St. Paul, MN 55144

Empore™ Extraction Disks were investigated for processing large volumes of urine (up to 100 mL) from human, rabbit and rat for chromatographic and mass spectral analyses of the investigational compound imiquimod and its metabolites. Solid-phase extraction (SPE) by means of small disposable columns is a commonly used technique to concentrate and purify drugs and their metabolites from biological matrices prior to analysis. However, the sample volume throughput in most SPE columns is slow due to a narrow diameter (5 to 13 mm); capacity can be insufficient for concentrating several metabolites present in low concentrations; and resulting eluants sometimes need further workup before GC/MS analysis. Empore™ Extraction Disks overcome the deficiencies of SPE columns for this purpose. The disks used in this study were 47 mm diameter, 0.5 mm thick, and contained either C-18, mixed mode C-8/cation exchange or poly(styrene divinylbenzene) within a stable, inert matrix of fibrillated polytetrafluoroethylene. Single aliquots of urine (up to 100 mL) were extracted rapidly (5 min for human urine using filter aid beads) and with nearly 100% recovery. Solvent eluants were analyzed directly by GC/MS and radiomonitored HPLC and TLC. A low level of contamination from endogenous urine substances was noted with an improved signal-to-noise ratio for the analyte of interest. Extraction of fecal homogenates also yielded excellent recoveries and clean chromatograms. Mixed mode disks allowed for selective elution of basic and acidic metabolites. Empore™ disks are useful in toxicology applications to purify and concentrate drugs and metabolites from large volumes of urine prior to GC/MS and HPLC analyses.

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CORROBORATION STUDY - A COMPARISON OF DRE OPINIONS TO TOXICOLOGY EVALUATIONS OF IMPAIRED DRIVERS IN MINNESOTA

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The purpose of this study was to compare the opinions of Drug Recognition Experts (DRE) to toxicological evaluations of urine specimens from impaired drivers.

There were 71 cases, at this writing, in which DRE opinions were compared with the results obtained from urine samples.

The urine samples were analyzed by our laboratory. Ninety-seven percent of DRE samples submitted to this laboratory were urine. The number of blood samples submitted was insignificant to warrant inclusion in this study. Urine samples were screened using SYVA EMIT® for cannabinoids, cocaine, opiates, amphetamines, phencyclidine, barbiturates, and benzodiazepines. Positive urine samples were confirmed by gas chromatography-mass spectrometry procedures using selected ion monitoring with deuterated analogues as internal standards.

At this writing, the overall corroboration rate for the presence of at least one predicted category was 84.5 percent. The corroboration rates for individual drug categories were: Cannabis, 91.8 percent; Central Nervous System Depressants, 64.3 percent; Central Nervous System Stimulants, 66.7 percent; and Narcotic Analgesics, 54.6 percent.

The DRE program, if followed properly, appears to be a useful screening tool for predicting drug categories.

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SENSITIVITY AND SPECIFICITY COMPARISONS OF THE ROCHE ON-LINE AND SYVA EMIT 2000 AMPHETAMINE ASSAYS. **Michael Sheehan* and Ray Cordery, Southwest Washington Medical Center, Vancouver, Washington**

Abuscreen On-line (Roche) and EMIT 2000 (Syva) amphetamine assays were compared on a Cobas FARA II analyzer. Both assays were performed according to the manufacturers' directions for qualitative analyses with 1000 ng/ml cutoffs set for each assay. Spiked urine specimens containing 800, 1000, and 1500 ng/ml of d-amphetamine were (-), (+), and (+), respectively, by the On-line assay and (-), (-), and (+), respectively by the EMIT 2000 assay. Of the 15 urine specimens (emergency room patients) confirmed (+) for amphetamine/methamphetamine by GC/MS and/or TLC, all specimens gave (+) results by both assays. By serially diluting each of these specimens and reassaying them by both assays until a (-) result was obtained, we found that the EMIT 2000 assay gave (-) results at lesser dilutions than the On-line assay by roughly a factor of 2.5. Specificity experiments of a similar nature are currently in progress using urine specimens that gave (+) screen results by either assay but were negative for amphetamines by GC/MS. Data to date indicates both methods to be comparably specific.

In conclusion, we found the On-line assay to be more sensitive at detecting amphetamine abuse than the EMIT 2000 assay.

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EMIT® THC 50/100 ng ASSAY, A DUAL CUT-OFF, LIQUID, READY-TO-USE ASSAY FOR THE DETECTION OF CANNABINOIDS IN URINE ON THE OLYMPUS AU5000 ANALYZERS. **J. Visor*, L. Anne, E. Berger, J. Blanc, M Henson, M. Hu, S. Hussain, and C. Messenger (Syva Company, San Jose, CA 95161)**

We have developed an improved Emit® assay for the detection of cannabinoids in urine. The Emit THC 50/100 ng assay utilizes either a 50 ng/mL or 100 ng/mL 11-nor-Δ^9-THC-9-carboxylic acid (Δ^9-THC-COOH) cut-off. This homogeneous immunoassay is provided in a stable, liquid two-reagent format, with liquid ready-to-use calibrators that contain only the L isomer of Δ^9-THC-COOH. Reagents and calibrators have been formulated for increased stability and precision on the Olympus AU5000 series analyzers. Adsorption of Δ^9-THC-COOH from the calibrators has been minimized to the degree that special handling instructions to prevent adsorption are no longer required.

One thousand five hundred ninety-nine (1,599) patient samples were analyzed on the Olympus AU5121 analyzer by the Emit THC 50/100 ng Assay and the corresponding Emit® II Cannabinoid 50 ng or 100 ng Assay. Using the 100 ng/mL cutoff, the Emit THC 50/100 ng assay and the Emit II Cannabinoid 100 ng assay showed >99% agreement. Using the 50 ng/mL cutoff, the Emit THC 50/100 ng assay and the Emit II Cannabinoid 50 ng assay showed >99% agreement. More than 99% of the samples that were positive by both Emit assays were confirmed as positive for Δ^9-THC-COOH by GC/MS. Within-run rate precision at the 50 ng/mL and 100 ng/mL cut-off calibrators (N=24) yielded coefficients of variation of 1.0 and 0.9%. The reagents typically provide at least 5 days of calibration stability on the AU5121 analyzer. Samples containing Δ^9-THC-COOH at ±20% of the 50 and 100 ng/mL cut-off calibrators were accurately assessed with greater than 95% confidence.

The Emit THC 50/100 ng assay offers dual cut-offs at 50 or 100 ng/mL Δ^9-THC-COOH. Liquid reagents and calibrators are stable, ready-to-use, and give accurate and precise results on Olympus AU5000 series analyzers.

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SPECIFICITY OF THE BIOSITE TRIAGE™ URINE DRUGS OF ABUSE TESTING DEVICE FOR BENZODIAZEPINES.

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A new point of care urine drugs of abuse testing device, the BIOSITE "TRIAGE™" was evaluated for specificity for benzodiazepines (BNZ). TRIAGE™ utilizes a unique, competitive, visual immunoassay methodology to simultaneously detect seven classes of drugs of abuse. The technology utilized permits the use of multiple drug specific antibodies within each drug class. For BNZ, the monoclonal antibodies in TRIAGE™ are specific for the glucuronide conjugates of oxazepam and temazepam not the parent compounds. These two factors may produce conflicting test results when TRIAGE™ is compared to conventional immunoassay techniques. Apparent TRIAGE™ benzodiazepine false positive results may occur when GC/MS confirmation utilizes oxazepam as the analyte used to confirm BNZ presence. We evaluated the specificity of the BNZ component of TRIAGE™ by comparing TRIAGE™ to Syva EMIT™ results of urines screened in our laboratory. Both negative and positive urines are confirmed by GC/MS. The following drugs were monitored during confirmation; oxazepam, nordiazepam, desalkyl flurazepam, hydroxy ethyl flurazepam, lorazepam, temazepam, alpha hydroxy alprazolam and alpha hydroxy triazolam. Initial screening results (n=150) found an 85% agreement between TRIAGE™ and EMIT™. GC/MS data will be presented.

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COMPREHENSIVE DRUG ANALYSIS BY TANDEM MASS SPECTROMETRY IN A FORENSIC TOXICOLOGY LABORATORY

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Tandem mass spectrometry (GC/MS/MS) has been shown to be an effective tool in the analysis of forensic samples for various types of drugs. The California Department of Justice Toxicology Laboratory has developed methods which utilize this technology for the analysis of morphine and codeine. In order to more effectively utilize available resources, this laboratory has now extended the use of GC/MS/MS to the analysis of cocaine and its metabolites ecgonine methyl ester and benzoylecgonine. In addition, we are now able to detect the heroin metabolite, 6-monoacetylmorphine. This paper will summarize the extraction schemes and instrumental parameters utilized to detect the above drugs in blood samples.

The extraction of the above drugs from blood samples all follow the same basic scheme. One milliliter of blood with deuterated internal standard added undergoes a brief clean-up step followed by an extraction at pH 8.5. The opiate compounds are extracted using ethyl acetate while the cocaine compounds use a methylene chloride/isopropanol solvent system. All samples are derivatized with trimethylchlorosilane-BSTFA. Previous methods used by this laboratory utilized pentafluoropropyl derivatives. The present derivative was chosen because it is less caustic to instrumental hardware with no loss of detection sensitivity.

The derivatized drugs are detected by monitoring parent to daughter ion transitions that are specific for the analyte of interest. Initial ionization of the GC eluate in the mass spectrometer source is by either electron impact (opiate compounds) or positive chemical ionization (cocaine and related compounds). The ions produced in the source undergo an argon induced dissociation to produce daughter ions. Transitions for each drug are chosen by using the molecular ion (i.e. 304 for cocaine) as the parent ion and determining which daughter ion is produced in the highest abundance (i.e. 182 amu for cocaine). The deuterated internal standards are detected by monitoring simultaneous and analagous transitions (307 to 185 amu for D3-cocaine). The specific ion transitions for each drug will be detailed.

Limits of detection for each compound will be outlined. In general, opiates can be detected to a level of 1 ng/ml while cocaine and its related metabolites are detected to 5 ng/ml. Quantitation curves for all the compounds are linear with excellent correlation values.

The use of tandem mass spectrometry in this laboratory has improved many facets of laboratory operation. The increased sensitivity and selectivity of this technology allows the toxicologist to use small sample volumes, decrease sample preparation time and increase sample throughput while increasing sensitivity. This laboratory screens over 5,000 samples annually. Therefore, there is a premium on efficiency utilization of available resources. Tandem mass spectrometry provides an effective tool for this purpose.

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DETECTION OF URINE SPECIMENS ADULTERATED WITH URINAID

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UrinAid is a commercial product that is sold for the purpose of defeating urine drug tests. The major component of UrinAid has been identified as glutaraldehyde, a highly toxic compound which reacts with proteinaceous material. UrinAid can be effective in preventing the detection of drugs in urine by the EMIT® test, presumably because the glutaraldehyde reacts with the enzyme and prevents a change in absorbance. As a result, urine specimens containing UrinAid give an abnormally negative test result, which often is the first indication of the presence of the adulterant. UrinAid is not effective in defeating other immunoassays, such as the Abuscreen® RIA and Online®, and can even cause an apparent false positive result when these tests are performed on UrinAid adulterated specimens.

GC/MS confirmation of drugs in urine containing UrinAid is often difficult due to the tendency of glutaraldehyde to inhibit efficient extraction of the drug and its internal standard. We have developed a simple GC/MS test for glutaraldehyde in urine consisting of extraction with methylene chloride and injection of a small fraction of the extract into the GC/MS using a 15 m x 0.2 mm i.d. methylsilicone capillary column which is held at 50 °C for 1.5 min, and then temperature programmed from 50 to 110 °C at 15 °/min. Under these conditions glutaraldehyde elutes at approximately 2.5 min and gives a characteristic mass spectrum consisting of abundant ions at m/z 44 (100% RA), 57, 72 and 82. With chemical ionization glutaraldehyde gives an intense protonated molecule ion at m/z 101.

Because of the increasing use of UrinAid, we recommend that laboratories institute procedures for identifying urine specimens containing this adulterant.

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DETECTION OF A METABOLITE OF HALOPERIDOL IN URINE BY LIQUID CHROMATOGRAPHY.

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Haloperidol is commonly administered to psychiatric patients but is rarely detected in toxicological screens. We evaluated the use of REMEDi HS™, a multi-column drug profiling system, for detection of haloperidol in urine.

Sera and urines were collected from seven hospitalized psychiatric patients who were receiving therapeutic doses of haloperidol. Serum haloperidol levels were measured by a reference laboratory, using a gas chromatographic method. The urines were analyzed without prior extraction or hydrolysis, using the automated LC system.

The serum levels ranged from 2 ng/ml (subtherapeutic) to 22 ng/ml (above upper limit of therapeutic range) with a mean value of 8.3 ng/ml. All of the urines contained a peak with an LC retention of 1.9 minutes and maximum absorbance of 252 nm. The spectrum of this peak was similar but not identical to the spectrum of haloperidol. No peak was seen for the parent drug at its expected retention time (about 5.5 minutes) in any sample; this was consistent with literature reports. The spectra observed for all urines were satisfactory for matching, except for the urine collected with the 2 ng/ml serum.

We conclude that therapeutic levels of haloperidol produced a metabolite which was consistently observed using REMEDi HS. Studies are in progress which will establish the structure of this metabolite.

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EFFECT OF SALICYLATE ON DETECTION OF URINARY BENZOYLECGONINE BY THE BIOSITE TRIAGE™ IMMUNOASSAY. Mark W. Linder* and Roland Valdes Jr. Department of Pathology, University of Louisville, Louisville KY 40292.

Our laboratory has recently demonstrated that urinary salicylates negatively interfere with the Syva EMIT II d.a.u. assays. This negative interference effectively increases the concentration of benzoylecgonine (BE), a metabolite of cocaine, required to produce a positive result in the EMIT assay.

This study evaluates the Triage™ (Tr) Drugs of Abuse immunoassay system for detection of BE in urine samples shown to have negative interference in the EMIT cocaine assay. Benzoylecgonine was added into urine containing salicylate at 0, 2.8, 84 and 123 mg/dL. As indicated in the Table, in samples with salicylate concentrations of 84 and 123 mg/dL, the change in absorbance rate determined by the EMIT assay was less than that determined for the cutoff, even at concentrations of BE of 375 ng/mL (25% above the cutoff). Further, in the sample with 123 mg/dL salicylate, the rate obtained at 560 ng/mL BE is only slightly above the cutoff.

Benzoylecgonine Assay

[BE]	Salicylate Concentration							
ng/mL	0 mg/dL		2.8 mg/dL		84 mg/dL		123 mg/dL	
	Emit	Tr	Emit	Tr	Emit	Tr	Emit	Tr
0	-2 -	-	-1 -	-	-9 -	-	-8 -	-
300	52 -	+	50 -	+	*39* -	+	*29* -	+
375	59 +	+	60 +	+	*49* -	+	*47* -	+
560	76 +	+	80 +	+	67 +	+	*56* -	+
750	93 +	+	96 +	+	83 +	+	81 +	+

The delta absorbance rate (mAU/min) of the cutoff calibrator (300 ng/mL BE) was 52.

In contrast, the sensitivity of the Triage™ assay for detection of BE was not effected by concentrations of salicylate in urine up to 123 mg/dL.

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EVALUATION OF THE UTILITY OF SERUM AND AQUEOUS HUMOR SAMPLES IN THE TRIAGE® IMMUNOASSAY DRUG SCREENING DEVICE. Guy Purnell*, Lucy Thomas, Office of the Medical Examiner, Philadelphia, PA and Tully J. Speaker, Temple University School of Pharmacy, Philadelphia, PA.

Aliquots from a series of approximately two hundred sets of samples of human blood serum, urine, and aqueous humor were screened for the presence of several classes of drugs and metabolites using the Triage® immunoassay device. The test results obtained with serum and aqueous humor were compared with those from urine, the matrix for which the device was developed, to assess the utility of the alternative matrices in the device. In addition aliquots from the same sample sets were analyzed using the standard protocols of the toxicology laboratory of the office of the medical examiner (OME). All positive drug findings were subjected to analytical confirmation by gas chromatography-mass spectrometry (GC-MS). Results from Triage® screening and from OME protocols were kept blinded until the outcome of both series of analyses were completed for each sample set and then results from Triage® screening were compared with outcomes from the standard protocols.

Aqueous humor and serum samples, including samples from partially hemolyzed blood, were found to give results consistent with those from corresponding urine samples when screened with the Triage® device. Positive screens with the device were confirmed by GC-MS. The modest sample size (140 µL), rapid response and easily recognized signal of the Triage® device recommend it to routine screening for several drug classes. The range of sample matrices which may be usefully screened with this device may be extended to include blood serum and aqueous humor.

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SCREENING FOR DRUGS OF ABUSE ON THE CHEM I ANALYZER USING SYVA EMIT II REAGENTS

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The Miles CHEM I analyzer was evaluated for use in screening drugs of abuse in urine for federally mandated testing using the SYVA EMIT II reagents. The EMIT II reagents were evaluated for accuracy, precision, patient correlation, linearity around the cut-off, and carry-over.

EMIT II reagents (100 mL test kits) were reconstituted with 72 mL deionized water, except cannabinoids, which were reconstituted with 52 mL deionized water. Reagents cassettes were prepared by using 10 mL of reagent and 125 uL of wetting reagent.

Accuracy was evaluated using by controls fortified at 20% above and below the cut-off concentration for each analyte for five days (N=10). The results indicated that the CHEM I could satisfactorily distinguish positive from negative results at these concentrations. Precision studies were performed by assaying replicates of the EMIT II calibrators. Coefficients of variation for both in-run and between-run precision studies were less than 5% for all analytes tested.

Over 1500 patients were assayed using both the SYVA d.a.u. and EMIT II reagents. Few discrepancies were found except in the case of a limited number of specimens at or near each assay's cut-off. There was a 50% decrease in amphetamine positives due to sympathomimetics other than amphetamine or methamphetamine using the EMIT II methodology.

Linearity around the cut-off was evaluated by assaying 10 replicates each of the cut-off calibrator and controls fortified at 20% above and below the cut-off concentration for each analyte over several days. The average correlation coefficients obtained (>0.98 for each analyte) indicated good linearity around the cut-off.

The effect of carry-over was studied by assaying fortified samples containing high drug concentrations followed by five negative controls. No appreciable carry-over (>20% of the negative to cut-off delta absorbance) was observed for any assay except for PCP at concentrations exceeding 1,000 ng/mL.

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CLINICAL COMPARISON OF THE BIOSITE TRIAGE PANEL FOR DRUGS OF ABUSE WITH THE ABBOTT ADX EMERGENCY TOXICOLOGY DRUG PANEL. *J. Coppola, D. Kraft, D. Hohnadel, T.K.Mayer and J. D'Souza, Clinical Labs. The Genesee Hospital, Rochester, NY 14607

The Biosite Triage™ method tests urine samples for drugs giving qualitative results. The emergency toxicology drug panel consisted of amphetamines, benzodiazephines, barbiturates, cannabinoids, cocaine metabolites, opiates and phencyclidine. The Triage was compared with Abbott's AD_x Semi-Quantitative method for the same drug panel. We found that the Triage agreed well with the AD_x and had an advantage in time saving, reducing both our STAT turn around time and the number of FTE's needed to run the assay.

The Triage is a competitive immunoassay procedure that employs monoclonal antibodies which are specific for the metabolites of the tested drugs. The Triage panel takes about fifteen minutes to complete and has a visual end point appearing as a colored bar on the test strip. The cutoff values for each drug are based on recommendations made by NIDA (National Institute on Drugs of Abuse).

Seventy drug levels were tested from frozen proficiency samples by Triage and AD_x and all were found in agreement except one sample for cannabinoids which was positive by the AD_x but negative by Triage. The disagreement was attributed to differences in cutoff levels. The sample read 50 ng/ml on the AD_x (cutoff = 25 ng/ml) and negative on the Triage (cutoff = 100 ng/ml).

Seventy-seven frozen urine samples were tested by both methods and all were found to be in agreement except one sample for benzodiazephines which read positive by Triage and negative by AD_x. The difference was attributed to the fact that AD_x measures the parent compound (present in the urine at approximately 25%) and Triage measures the metabolites (present in urine at approximately 75%).

Three levels of commercial quality control material were tested consisting of one negative panel, one positive panel with cutoff values close to the stated Triage cutoffs and one higher positive panel. Both the AD_x and Triage agreed with manufacturer stated results for all three levels of control.

In conclusion, we found the Triage to be a good alternative method in our lab for emergency toxicology screening which will produce greater productivity because of savings in instrument maintenance, turn around time and FTE's.

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WORKFLOW & COST ANALYSIS, SYVA EMIT d.a.u. vs. ROCHE ONLINE; WHICH ASSAY SYSTEM DELIVERS THE GREATER VALUE? Craig Lehmann, Ph.D., CC(NRCC)* and Alan Leiken, Ph.D., SUNY at Stony Brook, Stony Brook, NY 11794

A comparative analysis was performed to evaluate the labor required to prepare Syva and Roche reagents for drug abuse testing (DAT). Additionally, the time and cost of calibration requirements of the two methods was compared. The study was conducted in a laboratory which has contract pricing with both manufacturers. The laboratory is affiliated with a non-profit corporation which provides human services to persons experiencing mental illness, emotional disorder or substance abuse problems. The overall cost differences using the two methods was then determined. Assays under study included amphetamines, barbiturates, benzodiazepines, cocaine metabolite, opiates, PCP, and THC.

The lab processes approximately 19,000 reportable patient tests for DAT per year. Syva Emit d.a.u. and Roche ONLINE reagents were used to process the work on two respective COBAS MIRA systems, by the same technologist with appropriate experience and training. Time and motion studies were performed to determine labor time associated with daily calibration and daily reagent preparation. Any additional costs associated with differences in calibration such as calibrators, reagents and cuvettes were also determined based on the prices as established with respective manufacturers. Differences in reagent waste were also identified. Finally, differences in annual operating costs were computed.

Annual expenditures using Syva Emit d.a.u. amounted to $30,865 as compared to annual expenditures of $14,730 when Roche ONLINE were used. The difference was largely attributed to the additional calibration cost with Syva and the large amount of waste when Syva was used for low volume tests. In such situations, between calibrators and controls, three tests were needed to produce one patient result. Additional labor costs associated with the increased frequency of calibration with Syva amounted to $2,193 per year. One hour of labor time is spent each evening, 5 days per week preparing reagents for the following day. Using an average salary for a medical technologist of $15 per hour yields an average additional labor cost of $3,900.

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A MEXILETINE INTOXICATION

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A case is presented of a 26 year old white male with a history of depression and previous suicide attempts. No anatomic cause of death was ascertained at autopsy. The blood was negative for ethanol or other volatiles. Comprehensive drug testing of the blood and urine identified mexiletine, a class 1B antiarrhythmic drug. This drug had been prescribed to the decedent's roommate. The presence of mexiletine was confirmed by full scan electron impact gas chromatography/ mass spectrometry. Drug quantitations as performed by gas chromatography were (in mg/L or mg/kg): heart blood-38; subclavian blood-14; urine-370; liver-190; kidney-170; vitreous humor-17; bile-440. The normal therapeutic range of mexiletine in plasma is 0.75-2.0 mg/L. The medical examiner ruled that the cause of death in this case was mexiletine intoxication and the manner of death was suicide.

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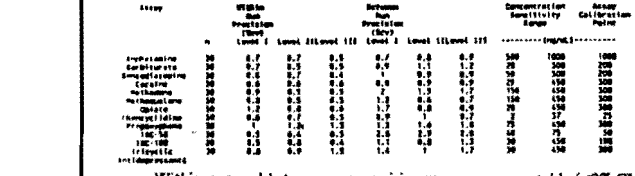
EVALUATION OF TWELVE SYVA EMIT II DRUG ASSAYS ON THE ROCHE COBAS MIRA S ANALYZER: CONCENTRATION SENSITIVITY, PRECISION, CALIBRATION STABILITY AND METHOD COMPARISON

R. Lyle Christensen* (Toxicology Laboratory, Department of Pharmacology and Toxicology, Indiana University Medical School, Indianapolis, IN 46202) and Chris B. Zurface (Syva Company, 3403 Yerba Buena Road, P.O.Box 49013, San Jose, CA)

Twelve Syva Emit II assays were evaluated for application as forensic drug screening tests using a Cobas Mira S analyzer. The test kits were prepared by the procedures outlined in the Syva application bulletin for the instrument. The data below resulted from studies performed as follows: precision, using the three level ConDOA control material (Diagnostic Product Corp.) in daily independent replicates of five repeated over a five day period; concentration sensitivity, using analytically diluted Syva kit calibrators. Calibration stability was monitored using Condoa level II control material.



Within run and between run precision were very acceptable (<2% CV in most cases). Most of the assays showed acceptable sensitivity to concentration changes by approaching or entering their respective therapeutic ranges of the drugs. All of the assays exhibited calibration stability of at least 14 days (defined by the level II control remaining above the calibration concentration point).

These data illustrate that the Syva Emit II assays can be used for the routine evaluation of urine in forensic drug related cases by being precise, stable, easy to use, relatively cost effective assays. Furthermore, the presented concentration sensitivities, which are well below the SAMSHA (NIDA) cutoffs, should allow these assays to effectively address toxic situations as well as drug involvement in the impaired subject where lower drug concentrations can be involved.

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DETERMINATION OF Δ^9 - TETRAHYDROCANNABINOL (THC) AND ITS METABOLITE 11-NOR- Δ^9 - THC - 9 - CARBOXYLIC ACID (THCA) IN WHOLE BLOOD

Bethany Telepchak, Gerald Long* and Christine Moore*

A GC/MS method has been developed for the analysis of parent THC and its metabolite in whole blood. Due to the very low levels of THC and THCA present, very sensitive extraction and analytical procedures are required. Determination of both compounds in a single sample, to date, has involved tedious, exhaustive, time consuming liquid-liquid extractions resulting in poor recoveries and matrix interfering substances.

The method presented uses a Clean Screen® bonded phase extraction column that provides a simple, purified extract containing both compounds. A whole blood sample is first precipitated, then acidified and extracted on a copolymeric bonded-phase extraction column. The effect of pH and solvent strength on the retention and recovery of both compounds is demonstrated. Samples were spiked at the 1-10 ng/ml range and relative standard deviations within a run were assayed to be less than 10%. Absolute recovery was determined to be greater than 80% for both compounds. The derivatizing reagent, BSTFA with 1% TMCS was used to enhance the chromatography and separation on GC/MS using electron impact detection in a selected ion monitoring mode for data acquisition.

Case samples which tested positive for cannabinoids were analyzed using the bonded-phase extraction method and accurately quantitated by GC/MS. The bonded phase extraction procedure required less extraction time and reduced solvent use as compared to liquid-liquid methods. Cleanliness and recovery were increased due to the selective copolymeric functionality of the Clean Screen® bonded-phase extraction column

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CHARACTERIZATION OF ONE MECHANISM OF NEGATIVE INTERFERENCE IN THE GC/MS ANALYSIS OF DRUGS OF ABUSE IN URINE. D. Ostheimer (Anderson)*, M. Cremese, E. Forte, A. Wu (Clin Chem Lab, Hartford Hospital, Hartford, CT, 06115),D. Hill (Microchem Lab, Univ of CT, Storrs, CT 06269).

Negative interference due to the presence of unwanted drugs in the targeted GC/MS analysis of drugs of abuse in urine can theoretically occur in the extraction, derivatization, gas chromatographic or mass spectrometric steps of the analysis. Recently, we reported on the negative interference of fluconazole (FLUC) in the GC/MS confirmation analysis of benzoylecgonine (BE) following derivatization to trimethylsilyl (TMS) analogs in both the full scan and SIM modes. Four patients on FLUC had positive results for BE by the Syva EMIT and Abbott FPIA immunoassays at approximately 0.400 mg/L but negative results (< 0.150 mg/L) for BE by GC/MS analysis. FLUC-TMS coeluted with BE-TMS using our chromatographic conditions. For a 0.500 mg/L sample of BE, the recovered concentration was below the threshold concentration when the ratio of FLUC to BE was 40 in the SIM mode and 60 in the full scan mode. Experiments were conducted to determine at which point in the analytical process the interference occurred and whether the interference was specific to FLUC or if it was a general problem of coelution.

Methanolic solutions of BE were prepared at an equivalent urine concentration of 0.500 mg/L, and were spiked with varying concentrations of FLUC ranging from 0 to 50 mg/L. They were derivatized with BSTFA with 1% TMCS. The GC/MS analysis was performed on a HP 5890/5970B GC/MSD using a HP Ultra-1 methyl silicone gum column in the full scan and SIM mode. The intensity of the BE-TMS chromatographic peak decreased with increasing concentrations of FLUC in both modes ruling out the extraction step as the source of interference. The above experiment was repeated except the solutions BE and FLUC were combined after derivatization with BSTFA. The same decrease in BE was observed indicating that derivatization was not the cause of interference. A direct probe analysis was conducted on solutions of BE and FLUC without derivatization using a HP 5988 MS. The same interference appeared to occur ruling out the GC as the source of interference. Two other coeluting drug pairs, propoxyphene/propoxyphene-d7 and methaqualone/methaqualone-d4 in methanol were tested without derivatization. The deuterated analogs were kept at a constant concentration of 0.050 mg/L for propoxyphene-d7 and 0.500 mg/L for methaqualone-d4. They were spiked with increasing concentrations of propoxyphene and methaqualone from 0 to 50 mg/L. A similar concentration dependent decrease in ionization occurred with these compounds as with FLUC/BE. These results suggest that the source of the problem likely occurs in the ionization chamber of the MSD. The negative interference appears to be of a general nature and not unique to FLUC and BE.

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A COMPARISON OF DRUG INDUCED SIGNS AND SYMPTOMS BETWEEN D.R.E. (DRUG RECOGNITION EXPERT-EXAMINATION) AND THE MEDICAL LITERATURE.

Chester Flaxmayer of Forensic Alcohol Science & Technology(FAST)

The purpose of this paper is to present the reported drug induced signs and symptoms from the NHTSA D.R.E. program and compare them to the spectrum of reported drug induced signs and symptoms published in the relevant medical literature, as well as listing the underlying mechanisms causing the effects, if known, with references.

The signs and symptoms will include the presence or absence of bronchorrhea, bruxism, diaphoresis, emesis, erythema, extrapyramidal reactions, lacrimation, nystagmus, ocular convergence, tremors with variations, vasoconstriction, vasodilation, as well as drug induced changes in blood pressure (systolic & diastolic with variations), body temperature, bowel sounds, capillary refill, conjunctiva, coordination, gait, muscle tone, perception, pulse rate (tachycardia & bradycardia with variations), pupil size (mydriasis, miosis), pupillary reaction to light, reflexes, respiration, salivation, sclerae, and ventricular rhythms.

Drug related medical literature generally provides information concerning direct and indirect drug actions. This includes which nerve receptors are affected, central and peripheral nervous system effects, and the necessary information to allow a differential diagnosis to be reached. Familiarity with this literature can aid toxicologists in their understanding of the DRE program and ease their preparation for court testimony.

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SELECTING APPROPRIATE INTERNAL STANDARDS FOR GAS CHROMATOGRAPHY/MASS SPECTROMETRY ANALYSIS OF DRUGS OF ABUSE. Ray H. Liu*, Wayne D. Bensley, Graduate Program in Forensic Science, University of Alabama at Birmingham, AL 35294; Edward J. Cone, Addiction Research Center, National Institute on Drug Abuse, Baltimore, MD 21224; and Shiv D. Kumar, Radian Corporation, Austin, TX 78720

Drug and metabolite analogs that are labeled with three or more deuterium atoms (isotopic analog) at appropriate positions are considered to be the most effective internal standards for the quantitative determination of drugs of abuse and their metabolites (drug/metabolite) in biological fluids and tissues by selective ion monitoring (SIM) gas chromatography/mass spectrometry (GC/MS). Before a specific isotopic analog can be adopted as an internal standard in a GC/MS assay, the mass spectrum of the isotopic analog must be evaluated along with that from the parent drug/metabolite. There should be an adequate number of sufficiently high mass ions that can be attributed to each of the pair (typically three for the drug/metabolite and two for the isotopic analog); and these ions should be sufficiently free of interference by contributions from the other component of the pair. Interferences may be caused by the presence of an isotopic impurity in the isotopic analog (extrinsic factor) or may be due to the ion fragmentation characteristics of the compound (intrinsic factor). The extrinsic factor may be corrected by the manufacturer using different manufacturing or purification procedures, while the intrinsic factor may be partially or wholly corrected by adapting different chemical derivatization (sample preparation stage) or ionization (GC/MS assay stage) procedures.

With the intention to systematically evaluate the suitability of currently available isotopic analogs (commercial and research sources) as internal standards, we have compiled full-scan mass spectra of approximately 50 drugs/metabolites along with approximately 80 isotopic analogs. Approximately 100 mass spectra of chemical derivatives of these drugs/metabolites and their isotopic analogs have also been compiled and compared. Exemplary data are presented to illustrate the selection of appropriate ions for the development of a SIM assay, the identification of the interference source (extrinsic or intrinsic), and the assessment of the extent of possible (extrinsic or intrinsic) interference. Compilation and evaluation of a mass spectral data base of deuterated drugs/metabolites (along with their parent compounds) should aid future efforts in developing GC/MS assays for drugs of abuse.

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URINARY DISTRIBUTION OF COCAINE METABOLITES RESULTING FROM ETHANOL-MEDIATED METABOLISM IN NON-FATAL EXPOSURES

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It has become increasingly apparent that ethanol-mediated cocaine metabolism plays a role in the toxicity associated with coabuse of these drugs. Cocaethylene, which is formed via the ethyl transesterification of cocaine in the presence of ethanol, has been implicated in cocaine related deaths. The objective of this paper is to present data collected from 40 clinical urine drug screen specimens which were screened positive for cocaine by EMIT and ethanol by GLC.

A GC/MS (SIM) procedure was developed to quantitate cocaine, benzoylecgonine, ecgonine methyl ester, cocaethylene, and norcocaine. Cocaine and the above metabolites were quantitated in the positive urines. The procedure had an LOQ of 25 ng/ml for each compound.

The results showed ethanol concentrations ranging from 13 mg/dL to 467 mg/dL in urines positive for both cocaine and cocaethylene. Cocaethylene concentrations ranged from 49 ng/ml to 5,220 ng/ml. Cocaine concentrations ranged from 64 ng/ml to 27,250 ng/ml. Benzoylecgonine concentrations ranged from 638 ng/ml to 227,470 ng/ml. Ecgonine methyl ester concentrations ranged from 238 ng/ml to 64,500 ng/ml.

Urines that contained cocaethylene also contained significant quantities of cocaine, whereas those negative for cocaethylene contained little or no cocaine.

Also presented with the above data, are graphs showing correlations between cocaine and the metabolite concentrations.

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ANALYSIS OF MECONIUM SPECIMENS FOR DRUGS OF ABUSE: I EVALUATION OF EMIT AND TDx IMMUNOASSAYS AS SCREENING PROCEDURES. Mahmoud A. ElSohly, ElSohly Laboratories, Incorporated, 5 Industrial Park Drive, Oxford, MS 38655.

The analysis of meconium specimens for metabolites of THC, cocaine, and morphine was used as an indication of prenatal exposure to these drugs. Most of the methods reported in the literature rely on radioimmunoassays. In this presentation both the EMIT and TDx immunoassays were evaluated, and a procedure was developed and validated for the screening of meconium extracts for cannabinoids, cocaine, opiates, amphetamines, and phencyclidine. The data showed that meconium has to be extracted and the extract has to be cleaned up prior to analysis. Levels as low as 20 ng/g THC-COOH, 75 ng/g benzoylecgonine, 100 ng/g morphine, 200 ng/g amphetamine, and 10 ng/g phencyclidine could be detected in meconium using the EMIT/ETS system. Much higher levels were detectable with the TDx system. The procedure was applied to screen 118 meconium specimens. GC/MS analysis confirmed the presence of 11-nor-Δ^9-THC-9-COOH in 3 out of the 10 specimens screened positive for cannabinoids, the presence of benzoylecgonine and/or cocaine in 16 out of 23 specimens screened positive for cocaine, and the presence of morphine and/or codeine in 3 out of 5 specimens screened positive for opiates. The presence of amphetamine and/or methamphetamine was not confirmed in 2 specimens screened positive for amphetamines. There was no PCP positive specimens. The high rate of unconfirmed positives for cannabinoids suggests the possibility of a different THC metabolite than the 11-carboxy derivative as the major metabolite in meconium. Work is in progress to identify this metabolite.

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META-HYDROXYBENZOYLECGONINE: AN IMPORTANT CONTRIBUTOR TO THE IMMUNOREACTIVITY IN ASSAYS FOR BENZOYLECGONINE IN MECONIUM

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Meconium has been reported to be a better specimen than maternal or neonatal urine for detecting fetal exposure to cocaine. In a study comparing various immunoassays with GC/MS for analyzing meconium, several discrepancies among the assays were noted. Thin layer chromatography of methanol extracts from meconium samples revealed an immunoreactive spot more polar than benzoylecgonine. GC/MS analysis of the eluted spot yielded a fragmentation pattern indicative of an aryl-hydroxylated benzoylecgonine. Standards of ortho-, meta-, and para-hydroxybenzoylecgonines were synthesized, and m-hydroxybenzoylecgonine yielded the same retention time and ion ratios as the TLC immuno-reactive spot. Furthermore, m-hydroxybenzoylecgonine proved to be immunoreactive. Ten meconium samples immunoreactive for benzoylecgonine were analyzed by GC/MS with and without B-glucuronidase (Type IX) pretreatment. Free m-hydroxybenzoylecgonine comprised 59 to 94% of the total m-hydroxybenzoylecgonine, and total m-hydroxybenzoylecgonine ranged from 0.2 to 6.3 times as high as the benzoylecgonine. Therefore, m-hydroxybenzoylecgonine appears to be a quantitatively important cocaine metabolite in meconium that is responsible for a significant portion of the difference between immunoassay readings as benzoylecgonine and actual benzoylecgonine concentrations, measured by GC/MS, in meconium extracts.

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IDENTIFICATION OF DRUG ANALYTES EXCRETED IN SWEAT AFTER COCAINE AND HEROIN ADMINISTRATION

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Sweat composition and volume varies widely from individual to individual and collection methods have been less than satisfactory. Consequently, the study of the excretion of drugs of abuse in sweat has been highly limited. We investigated the usefulness of a new sweat patch device (Sudormed Sweat Patch, Sudormed, Inc.) designed to collect non-volatile drugs of abuse from human skin. The device is applied like a Band-Aid® to the skin. Substances with the volatility of water or greater leave the device through a membrane barrier. Non-volatile substances are concentrated on an absorption pad inside the patch. Subjects can wear the patch for periods up to several weeks, followed by removal, storage and analysis of the contents of the absorption pad.

Volunteer subjects who participated in these studies provided informed consent and were drug-free. Patches were applied to the stomach and back area 24 hrs prior to drug administration. Control patches were removed prior to administration of single doses of cocaine (25 mg, iv) and heroin (20 mg, iv). Drug patches were removed at 24 and 48 hrs. Deuterated internal standards were added to each patch, the patch was extracted with buffer by vigorous shaking, drugs were isolated by solid phase extraction and the eluate was derivatized and analyzed by GC/MS operated in full scan and selective ion monitoring modes. Stability studies of heroin, 6-acetylmorphine and cocaine added to patches indicated that all analytes were stable for a minimum of 48 hrs.

Following heroin administration, sweat extracts contained heroin, 6-acetylmorphine and morphine in varying proportions. Heroin and 6-acetylmorphine often predominated over morphine content. Following cocaine administration, sweat extracts consisted primarily of cocaine, followed by ecgonine methyl ester and benzoylecgonine.

These data indicate that lipophilic compounds are excreted in sweat in greater amounts than are polar metabolites. Further, there appears to be potential for use of sweat analysis for source differentiation of opiates and for use in monitoring human drug exposure.

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KINETIC STUDIES OF COCAINE EXCRETION IN SWEAT

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Initial studies in this laboratory have shown that parent cocaine is the primary analyte excreted in sweat; however, there is little information on the relationship of drug concentration in sweat to time of appearance and disappearance and to dose.

We investigated the time course of appearance of cocaine and metabolites in sweat and the relationship of dose to amount of excreted drug. Sweat patch devices (Sudormed Sweat Patch, Sudormed Inc.) were used for sweat collection. To determine the minimum amount of cocaine that was detectable in the patch, drug-free volunteer subjects were administered single graded doses of intravenous (IV) cocaine HCl on a daily basis in ascending order (0, 1, 2.5, 5, 10, 25 mg). Sweat patches were applied prior to drug administration and removed in 24 hr timed intervals. The time course of appearance of cocaine in sweat was evaluated by application and removal of patches at different intervals after administration of single doses of cocaine by the IV, smoked (SM) and intranasal (IN) routes. Patches were stored frozen until time of analysis. Deuterated internal standards were added to each patch; the patch was extracted with buffer by vigorous shaking; drugs were isolated by solid-phase extraction; and the eluate was derivatized and analyzed by GC/MS.

The minimum dose of cocaine that could be detected in the sweat patches was 5-10 mg administered by the IV route. Although there was substantial inter-subject variability, concentration in sweat appeared to be dose-related. Cocaine was detected in sweat as early as 2 hr after drug administration, but the majority was excreted in the 2-24 hr period. Intra-subject variability was evaluated by analysis of duplicate patches. Mean peak cocaine concentrations (ng/patch) of duplicate sweat patches from 2 subjects were as follows: IV, 38.8, 12.8; SM, 51.2, 8.7; IN, 35.4, 34.6. Further excretion was observed during the 24-48 hr period. Results indicated that cocaine concentrations in duplicate patches were highly correlated ($r = 0.93$).

Overall, these data suggest that cocaine is excreted in sweat in detectable amounts over a period of 2-48 hrs after drug. Use of the sweat patch in a treatment or surveillance setting could provide a means of monitoring cocaine exposure.

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COCAINE IS EXCRETED IN SEMEN AFTER USE

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The use of cocaine by pregnant women has become a serious health issue of major concern because of cocaine's effects on the developing fetus. In contrast, cocaine's effects on the fetus and the neonate arising from the father's drug use have attracted less attention. Recently, it was discovered that cocaine binds tightly to human spermatozoa when added *in vitro*. However, there is no information on *in vivo* excretion of cocaine and its metabolites in semen after drug use.

In the present study, we describe the detection of cocaine and its metabolites in semen of human subjects following administration of cocaine by the intravenous (IV), intranasal (IN) and smoking (SM) routes. Single doses of cocaine were administered to drug-free volunteers, who gave informed consent and had a history of cocaine use, as follows: 25 mg, IV; 32 mg, IN; 42 mg, SM. Semen samples were collected prior to drug administration, approximately 1-hr after drug administration, and 24-hr later. Blood samples were collected before, and periodically after cocaine. Samples were analyzed by solid phase extraction coupled with GC/MS assay. For five subjects, the mean semen:plasma concentration ratio of cocaine and benzoylecgonine (BE) at 1-hr were as follows: cocaine, 0.70 (IV), 0.56 (IN), 0.91 (SM); and BE, 0.67 (IV), 0.57 (IN), 0.59 (SM). Pre-drug samples were negative. Samples collected 24 hrs after drug administration were negative for cocaine, but contained traces of BE (<20 ng/g).

This study demonstrated that cocaine and metabolites are excreted in semen immediately after use in a pharmacokinetic pattern similar to that observed in blood. The discovery of cocaine and BE in semen after cocaine administration could be important in understanding the causes of abnormal development in offspring of cocaine users.

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BENZTROPINE IDENTIFICATION AND QUANTITATION IN A SUICIDAL OVERDOSE CASE.

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In a human fatality involving suspected overdose with an anticholinergic agent, benztropine, gas chromatography/ mass spectrometry analysis was used for drug detection/ quantitation and for investigation of drug metabolism. Chromatography of a hexane extract of serum or urine was performed on a DB-5 capillary gas chromatography column with an open split interface to an ion trap mass spectrometer programmed for full spectrum data acquisition. Electron impact ionization fragments at m/z 82 (base peak), 96, 105, 124, 140, 165, 167 and 201 were used in drug detection and a structural identification of the fragments is proposed. Chemical ionization was used as further confirmation of molecular weight. Benztropine-d3 was synthesized and used as internal standard in determining the concentration of benztropine in blood (183 µg/L) and urine (7,124 µg/L) from the decident. Drug levels were interpreted in relationship to the case findings, the limited published data and an evaluation of therapeutic drug levels in a group of psychiatric patients. Potential metabolic conversion to n-desmethylbenztropine was investigated by organic synthesis of the n-desmethylated derivative and gas chromat ography/mass spectrometry analysis. Fragment ions at m/z 68 (base peak), 82, 105, 110, 126, 165, 167 and 187 are consistent with the proposed fragmentation of the parent drug and the extent of bioconversion is determined. In conclusion, gas chromatography with a combination of electron impact and chemical ionization mass spectrometry provide a useful forensic methodology for toxicological evaluation of potential overdose with benztropine.

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QUININE ELIMINATION AFTER INGESTION OF TONIC WATER

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Biological specimens from eight fatal aviation accidents out of 775 fatal aviation accidents analyzed in 1991 and 1992 were found to contain quinine. In one case the investigators wanted to identify the source of quinine found in the pilot. It was suggested that the quinine may have come from the consumption of tonic water. Since no recent use of quinine or tonic water could be found, the investigators asked how long quinine could be detected in a urine specimen. A limited research project was undertaken to establish the approximate length of time quinine could be detected in urine and blood. Each of two male subjects was given a 16 oz bottle of tonic water which contained 35 mg of quinine. Quinine was detected using standard laboratory TLC and HPLC methods. One subject reached a maximum urine level of 3.92 µg/mL of quinine 9 hours after drinking tonic water. In one subject a maximum concentration of 0.291 µg/mL of quinine in blood was detected between 2 and 3 hours after ingestion of one bottle of tonic water. After 24 hours the blood quinine level had dropped to 0.184 µg/mL. The experiment was terminated at 8 days, at which point the urine still demonstrated quinine, albeit at a very low level of 0.058 µg/mL.

Quinine is used for muscle cramps, malaria, and as an additive in tonic water. Since adverse affects have been identified at plasma concentrations between 10-15 µg/mL, no performance effects would be expected from the maximum concentrations of quinine found (0.291 µg/mL) after the ingestion of one 16 oz bottle of tonic water. However, the possibility of pronged detection of quinine should (a) serve as a warning against using this as a sign of recent drinking and (b) alert the investigators to inquire about disorders or conditions which might impair performance but for which quinine Rx was terminated days before the accident.

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THE ISOLATION AND IDENTIFICATION OF COMPLEX VOLATILES IN POSTMORTEM TISSUES

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Fatal poisoning through the deliberate or accidental ingestion of commercial products presents a unique problem for the coroner's toxicologist. Conventional analytical techniques for drugs or poisons in biological materials generally do not apply to cases of consumer product poisoning, where the analyte tends to be of a complex chemical nature. When the poisoning involves products containing petroleum hydrocarbons (turpentine) or other mixtures of volatile compounds, an alternative procedure is required.

A method is presented for the recovery of complex mixtures of volatiles from biological fluids and whole tissues. The procedure is based on the passive diffusion of volatile substances onto commercially prepared activated charcoal strips (Pro=Tek®), carbon disulfide elution and capillary GC analysis. It is a commonly used method for the recovery and identification of arson accelerants from fire debris.

Two fatalities due to the ingestion of pine oil based commercial products are reported. The sensitivity of this method is demonstrated by the recovery of pine oil in brain tissue of one subject who had undergone a 24 hour therapeutic intervention prior to death. In the second subject pine oil was recovered from blood and gastric contents following a fatal intentional ingestion and IV injection of Real Pine ® disinfectant.

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A LEAD POISONING SCREENING PROJECT.

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The Centers For Disease Control and Prevention has recently recommended that all children between the ages of 6 months and 6 years be screened for lead poisoning by blood lead determinations. Since, blood lead determinations are seldom requested for Utah Children, it is unclear whether routine screening is necessary in this state. The purposes of this study were to determine whether children in the Salt Lake City area are at risk for Lead Poisoning, to identify risk factors for lead poisoning, and to determine whether blood lead concentrations correlate with serum iron concentrations in this population.

Informed consent was obtained prior to collecting blood by fingerstick or venipuncture for blood lead and serum iron determinations. Samples were collected from children ages 6 months through 5 years at area Women, Infants and Children's clinics, day care centers and from private pediatricians. A questionnaire was completed for each subject to gather demographic, and socioeconomic information. Age of housing information was determined from county records. Blood lead concentrations were determined using an Atomic Absorption Spectrophotometer with a graphite furnace. Standard measures of central tendency were used to describe the continuous variables. Regression analysis was used to test the relationship between the continuous variables. Analysis of variance was used to test the relationship between lead and the categorical variables.

A total of 317 subjects were screened for lead poisoning. The mean blood lead concentration was 4.6 mcg/dl with a range of 0.6-20.5 mcg/dl. There was no relationship between lead and iron lead and the categorical variables such as age, race, sex or age of house.

In the population studied, no significant risk factors for lead poisoning were identified. Although the mean blood lead concentration was low in this sample, further data is needed before we can definitively say routine screening is not necessary in Utah.

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DRUG FINDINGS IN DECOMPOSED TISSUES OF A MULTIPLE DEATH INVESTIGATION.

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The discovery of a common drug in the decomposed organs of all seven bodies was a key element of the prosecution's case against a woman who ran a boardinghouse on F Street in Sacramento, California. Due to the lack of studies in the literature involving decomposed tissues relating to concentration of drugs found, the effects of the decomposition process on drugs, the state of hydration of the tissues, and the fact that blood was not available for comparative evaluation all seven deaths were ruled "undetermined" in cause. Other facts concerning the case will be discussed.

The first body was unearthed November 11th 1988. The estimated date of how long the remains had been buried varies from four months to two and one half years according to dates of last sightings. Both liver and brain tissues were available in five of the seven cases. In one case the victim's head was not found and in another the organs were mummified and the liver was unidentifiable. The brains and livers varied in states of decomposition and brain weights varied from 100 to 600 grams and the livers from 160 to 1790 grams.

Facts in the case led the coroner to believe that flurazepam was involved in several of the deaths. The coroner's toxicologist knew that due to the nature of the tissue and lack of specialized instrumentation, he would seek help. The instrumentation needed for the analysis was available at the California Department of Justice. A specific request was made to look for traces of flurazepam. Homogenates of the tissues from each victim were supplied by Sacramento County coroner's office to the California Department of Justice, Toxicology laboratory(DOJ).

DOJ utilizes a 3 step screen and confirm scheme, routinely involving RIA, GC, GC/MS. Benzodiazepines were identified in all tissues by RIA and flurazepam or its metabolites, N-1-hydroxyethylflurazepam, and N-1-desalkylflurazepam were confirmed in all brain and liver tissue homogenates from the seven victims. Electron capture gas chromatography (EC/GC) and gas chromatography-electron capture negative chemical ionization Mass Spectrometry (GC/EC/NCIMS) were used in the confirmation and quantitation. Calf liver and brain tissue were utilized in preparation of the quality assurance material. Deca-deuterated flurazepam was used as the internal standard during a selective ion monitoring quantitation.

Other drugs found in the samples include amitriptyline, nortriptyline, carbamazepine, codeine, diazepam, diphenhydramine, doxylamine, haloperidol, and phenytoin. Quantitative results will be presented for all drugs and metabolites in a graphical presentation.

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CONCENTRATIONS OF BUTRIPTYLINE IN BIOLOGICAL FLUIDS FOLLOWING A FATAL OVERDOSE.

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A fatality resulting from the ingestion of butriptyline is reported with the methodology of analysis and blood, urine, vitreous levels of the parent butriptyline and major metabolite n-desmethyl norbutriptyline.

A 16 year old white female was found dead. 7 empty bubble blister packs and two unmarked orange, biconvex, film coated tablets found near the body matched the mother's prescription, obtained during a visit to Mexico, for butriptyline.

Acidic/neutral and basic drug extractions were screened with HP 5890 GC/FID and GC/NPD dual column J&W DB-5 and DB-17 and confirmed by Finnigan GC/MS/IT J&W DB-5MS.

The following concentrations of butriptyline and norbutriptyline expressed as mg/l were found: Blood: 14.9/3.6, Vitreous: .5/.4, Urine: 3/3. In addition salicylate, acetaminophen, dextromethorphan, doxylamine and pheniramine were detected.

Since, at the time of this report, no other fatality with butriptyline could be located in the literature, the values were compared with those of other tricyclics. An autopsy was not performed so we were unable to provide tissue distribution levels. In consideration of the circumstances surrounding the death and the toxicological findings, the cause of death had been certified as butriptyline toxicity and the manner of death was ruled as a suicide.

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THE SIMULTANEOUS ANALYSIS OF TEN BENZODIAZEPINE DRUGS AND METABOLITES BY GAS CHROMATROGRAPHY, INCLUDING REPRESENTITIVES OF THE "NEW GENERATION" OF DRUGS.

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Ten drugs and metabolites of the benzodiazepine class were simultaneously extracted and analyzed. The ten drugs and metabolites were subdivided into two groups: (1) the "new generation" drugs: Alprazolam, Clonazepam and Triazolam; and (2) the "old generation" drugs: Chlordiazepoxide, Deealkylflurazepam, Diazepam, Lorazepam, Nordiazepam, Oxazepam, and Temazepam. The "new generation" (1) are typically prescribed in low doses, so lower levels of detection, as low as 10 ng/mL, must be possible in order to confidently assess their presence. Dynamic ranges were found to be 10 - 500 ng/mL and 50 - 2000 ng/mL for (1) and (2), respectively. The drugs were extracted from whole blood, serum, plasma or urine using Varian Bond Elute Certify or Worldwide Clean Screen solid phase extraction columns. The extractions were then derivitized using MTBSTFA and analyzed by gas chromatography with an electron capture detector. Enzymatic treatment using β-Glucoronidase enhanced the extraction of benzodiazepines from urine samples. The average between-run precision was 16% and the average within-run precision was 6%.

The data presented illustrates the utilization of a combination of solid phase extraction and -TBSTFA derivatization to produce a quantitative assay capable of confirming the presence of ten benzodiazepines. The dynamic ranges of this procedure permits the evaluation of low dosed benzodiazepines throughout their respective therapeutic ranges. This procedure may be utilized to evaluate forensic cases involving either physical impairment or questionable overdoses.