SOCIETY OF FORENSIC TOXICOLOGISTS

ANNUAL MEETING
28 SEPTEMBER - 1 OCTOBER 1988
PHILADELPHIA, PA

ABSTRACTS OF PAPERS

1. Purification and Concentration of 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid in Urine using Solid Phase Extraction, R.C. PARRY (Supelco, Inc.) D.J. GISCH and G.D. WACHOB (Supelco, Inc.)

Marijuana abuse is detected in urine by identifying the presence of 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH). Many analytical procedures using gas chromatography (GC), high performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS) have been developed for determining the presence of THC-COOH in urine. However, the sensitivity and more importantly, the reliability of these methods are hindered by inadequacies of the techniques used in preparing urine samples for analysis. Current methods of sample preparation, using techniques such as thin layer chromatography, liquid-liquid extraction and other solid phase extraction techniques commonly suffer from low drug recovery, incomplete removal of interfering urine components, and/or long preparation times.

An alternative sample preparation procedure using a unique bonded phase for the solid phase extraction of THC-COOH from urine will be discussed in detail. This procedure provides absolute recovery of the metabolite in excess of 85% and relative recovery greater than 92% as determined by GC-MS. Initial urine concentrations of the metabolite ranged from 5ng/ml to 250 ng/ml. The versatility of the extraction procedure will also be discussed. Absolute recoveries in excess of 85% as determined by HPLC with UV detection and GC with flame ionization detection can also be obtained using this extraction procedure.

2. Matrix Modification of Serum by N,N-dimethylformamide (DMF) for Cannabinoid Analysis by GC/MS, KLINGER, R., BLUM, L. and RIEDERS, F (National Medical Services, Inc. Willow Grove, PA.)

Unacceptably low recovery rates (down to non-detectability) of deuterated THC, THCOH and THCOOH (internal standards) and/or excessive noise due to as yet undefined matrix variables are known to result in 3 to 8% failures of serum cannabinoid analyses by GC/MS. We have found that modifying the serum matrix by means of DMF renders tractable the extraction-refractory specimens, as well as markedly increasing and normalizing cannabinoid recovery for serum specimens in general. Thus, reliability (hardiness) of the method, as well as its LOD, LOQ, accuracy and precision are significantly improved.

Along with the method details, comparison of results by two standard methods without DMF matrix modification with those by the DMF method will be presented and its application to case issues will be discussed.

3. Delta-8-THC Fluoroligand: Synthesis and Properties of a Ligand for Fluoroimmunoassay of Delta-9-THC in Plasma and Blood. ANDREW P. MASON, Ph.D., and ARTHUR J. McBAY, Ph.D. (Office of the Chief Medical Examiner, Chapel Hill, NC 27599-7580).

The fluoroligand is a fluorescein-labelled δ^8-THC derivative that has a structure complimentary to that of a 5'-coupled THC immunogen. It displays optimal reactivity with antibodies produced using the immunogen, while it retains the fluorescence characteristics of fluorescein.

Fluorescein isothiocyanate (FITC) was converted to the thiocarbamate using 1,2-ethylenediamine. A peptide bond was then produced by attack of the primary amino group on the carbonyl in the activated N-hydroxysuccinimidoyl ester of 5'-carboxy-^{14}C-δ^8-THC. Yield of the TLC purified product was 39% based on the recovered ^{14}C activity.

The fluoroligand had fluorescence characteristics equivalent to those of FITC, (λ_{ex} 492 nm, λ_{em} 517 nm, ε_{492} = $7.8X10^4$ $M^{-1}cm^{-1}$ (0.1M $NaHCO_3$, pH 9.0)). It binds to rabbit anti-THC antiserum F-444-12 (prepared using a 5'-coupled δ^8-THC-BSA immunogen) with high affinity (K_D = $2.0X10^{-11}$ M). In contrast to the free state, the fluorescence of the bound fluoroligand was enhanced, with the degree of enhancement dependent on the affinity of binding (2X for F-444-12). Due to its structural similarity to the immunogen, the fluoroligand has a molar cross-reactive potency 10X greater than THC for antibody F-444-12.

This compound will be utilized as a non-isotopic alternative to isotopically labelled ligands in immunoassays for THC in blood and plasma.

4. Toxicological Findings in Several Fatal Overdoses Involving "Victory Sets", Frederick W. Fochtman, Ph.D. (Duquesne University and Clinical Pathology Facility), J. Douglas Bricker, Ph.D. (Duquesne University), and Nathan T. Scanzillo, Ph.D. (Clinical Pathology Institute).

Several drug deaths due to an overdose of "Victory Sets" over a two year period are reported. "Victory Sets" is a unique combination of of glutethimide, codeine, and diazepam. This report describes post-mortem findings and blood drug concentrations of several drug overdoses involving "Victory Sets." Most of the victims were males between the ages of 24 and 34.

Glutethimide concentrations were determined by HPLC and ranged between 5.0 ug/ml to 22.3 ug/ml. Codeine concentrations were determined by GC/MS and ranged between 0.384 ug/ml to 1.532 ug/ml. Total benzodiazepine concentrations were determined by HPLC and the values ranged between 0.12 ug/ml to 1.52 ug/ml. Comparisons of the data and conclusions are presented.

Chemical Detection of Recent Marijuana Use. Peggy Kelly (U.C. Berkeley) R.T.Jones (U.C.S.F.)

5. THC and its metabolites, THC-COOH and THC-COOH glucuronide, were quantitated in plasma and urine samples after frequent and infrequent marijuana users were infused with 5 mg of THC intravenously. Plasma THC concentrations were detectable 2 minutes post infused to 5 hours using solid phase extraction and GCMS. Plasma COOH-THC and COOH-THC glucuronide were detected 2 minutes post infusion to 12 days. The metabolites were at higher concentrations in the plasma and urine of frequent marijuana users when compared to infrequent marijuana users. A ratio of COOH-THC to COOH-THC glucuronide in plasma over 2, in either a frequent or infrequent marijuana user, occurred only during the 2 to 30 minute post-infusion interval, whereas all other ratios for the subsequent 12 days fell below 2. Additionally, a ratio of less than 1 for plasma total COOH-THC/THC occurred only between 2 minutes and 45 minutes post-infusion for frequent and infrequent marijuana users, with all subsequent ratios for the following 12 days being greater than 1.

6. Early Phase Dispositional Studies of Cocaine in the Rat. J. P. BONI*, A. POKLIS and R. V. BLANKE, (Department of Pathology, MCV/VCU, Richmond, Va., 23290).

The role of alterations in disposition after cocaine administration leading to acute toxicity is under current study. Naive Sprague-Dawley rats (225-275 gm) were surgically prepared with chronic indwelling abdominal aorta cannulae that were externalized from the dorsal neck region and sheathed in a flexible steel tether. Forty-eight hours after surgery, free-moving animals were administered cocaine HCl via tail vein injection. Arterial blood was immediately sampled from the cannula and aliquotted plasma was extracted using C-18 solid-phase cartridges. Quantitation of parent cocaine was performed using GC-MS in SIM mode with d_3-cocaine as internal standard. Time course profiles obtained were fitted to both 2 and 3 compartment body models (CBM) using NONLIN76. Diagnostic graphic and statistical tests showed significantly better fit of data for the 3CBM after 6.0 and 10.0 mg/kg doses ($p < 0.05$ and weight=1). Mean biologic half-lives of 1.45 and 1.32 hours respectively were not significantly different from each other as determined by t-test ($p < 0.05$) but were somewhat longer than previously reported findings of 0.42 hours for an 8mg/kg dose. Vd apparent (l/kg) of 1.93 and 1.53, Vdss (l/kg) of 17.18 and 15.79 and systemic clearance (l/kg•hr) of 13.97 and 15.94 were obtained for 6.0 and 10.0 mg/kg cocaine respectively This surgical model allowed for convenient sampling of arterial blood in free-moving animals in a reduced stress environment. In addition, by cannulating the abdominal aorta, compromise of blood flow to organs considered critical to acute toxicity is avoided. The ability to evaluate drug disposition reliably and repeatedly after administration in rodents allows the characterization of cocaine disposition especially during the early time course.

* 1987 Educational Research Assistantship Award recipient

7. Plasma Versus Bone Marrow Desipramine: A Comparative Study, Charles L. Winek, Ph.D. (Allegheny County Department of Laboratories), Wagdy W. Wahba, Ph.D. (Allegheny County Department of Laboratories).

Correlation between plasma and bone marrow tricyclic antidepressants has not been studied before. Three groups of rabbits were utilized; Group I (control group), Group II (10 mg desipramine per kg body weight) and Group III (20 mg/kg). Desipramine was administered to the animals once daily by mouth for 5 days. On the fifth day the animals were sacrificed and blood and bone marrow samples were collected and analyzed using a high performance liquid chromatographic (HPLC) method. Data showed that a correlation exists between bone marrow and blood desipramine. The bone marrow desipramine concentration increased as its blood levels increased. The average ratio of bone marrow to blood desipramine in both dosage groups was 37.2 ± 4.46 with a range of 30.99 to 44.82. This investigation is promising and shows that bone marrow could be used as an alternative tissue in the absence of a suitable blood sample.

8. Some Applications of the Photodiode-array Spectrophotometer Using Derivative Modes. B.P. Joynt, B.J. Perrigo (Royal Canadian Mounted Police, Central Forensic Laboratory, Ottawa, Canada).

A study of some applications of the photodiode-array detector including the use of 1st through 4th derivative modes. Of particular interest are potential applications to HPLC and differentiation of drug compounds of similar chemical structure.

9. Direct EMIT®-DAU Exclusion Screening Applied to NN-Dimethylformamide (DMF)-Modified Serum. BLUM, L., KLINGER, R., and RIEDERS, F. (National Medical Services, Inc. Willow Grove, PA)

The EMIT-DAU reagents with their well-established and widely published capabilities, are currently limited in their labelled applications to urine and are not directly applicable to serum. Not only is the latter a necessary analytical matrix for accurately establishing current case status, as opposed to the more historical value of urine testing, but frequently serum is available when urine is not. Extraction of analytes from serum by immiscible solvents, their evaporation and residue-reconstitution for EMIT® testing, adds substantial labor to screening, introduces the potential for adsorptive losses of analytes and may fail to recover co-reacting, water soluble conjugates and other metabolites. To overcome these limitations, we have developed a homogenous procedure involving serum matrix modification by DMF which yields a solution suitable for direct EMIT®-DAU analysis without requiring phase separation or concentration steps. The procedural details, method and validation for the benzodiazepine-, cannabinoid-, cocaine metabolite- and opiate-kits on the Syva® auto-carousel will be furnished; its applications to and limitations encountered with clinical-, "probable cause"-DUI- and postmortem- case material will be presented.

10. Screening of Blood and Urine for Methamphetamine Using the Abbott ADX® Fluorescense Polarization Immunoassay, Timothy A. Appel (California Department of Justice) and Norman A. Wade (California Department of Justice).

In recent years the proliferation of clandestine methamphetamine laboratories in the State of California has grown to astounding numbers (over three hundred laboratories seized in 1987 by our Bureau of Narcotics Enforcement). A vast majority of the solid dosage forms analyzed by our criminalistics laboratories throughout the state are positive for methamphetamine. Likewise, in our driving under the influence (DUI) cases a great deal (over 15%) are positive for methamphetamine, thus, the need for a rapid immunoassay screen for methamphetamine in whole blood and urine is of paramount importance to use and likewise to other forensic laboratories.

None of the radioimmunoassay or enzyme immunoassay kits commercially available are designed to detect methamphetamine in whole blood (70% of our submission are blood) Previously, we have tried extensive modifications of these kits with little if any success. However, recent commerical availability of fluorescent polarization immunoassay kits by Abbott Diagnostics led us to experimentation with these for detection of methamphetamine in whole blood and urine.

Data will be presented that shows after a methanolic extraction of blood or urine (as we currently perform for marijuana analysis) the extract may be used to quantitatively detect the presence of methamphetamine at 50 ng/ml. and was linear up to at least 1000 ng/ml. Cross reactivity with MDA and MDMA, but little or no cross reactivity with phenylpropanolamine, ephedrine, diphenhydramine, chlorpheniramine and other over-the-counter or endogenous amines. All positive screens to date have been confirmed by GC/MS as methamphetamine.

11. An Evaluation of the Abbott ADx Analyzer in a Clinical Toxicology Laboratory, RICKY P. BATEH (Consolidated Lab Services, 2549 Park Street, Jacksonville, FL 32204).

Over the past few years, there has been an increase in the use and abuse of drugs in society. In parallel to this increase in drug usage, several advances in technology have improved the test methods used to screen for drugs in biologic samples. While it is desirable to screen for as many drugs as possible in one procedure, most broad-spectrum screening methodologies are often time consuming and cumbersome.

Reported here is an evaluation of an automated method of screening for several drugs/classes of drugs in biologic samples. The Abbott ADx analyzer is an automated instrument utilizing the methods of fluorescence polarization immunoassay (FPIA) and the technology of radiative attenuation (REA). The FPIA assays available on the ADx include screens for amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine metabolite, opiates, and phencyclidine in urine as well as for acetaminophen, salicylates, and tricyclic antidepressants in serum. Currently, the REA assay available is the screen for ethanol in serum.

Results of the screens for these drugs in a variety of biologic samples will be discussed. The results obtained with the ADx will be compared to the results obtained with the SYVA EMIT procedure, the ROCHE ABUSCREEN procedure, and with gas chromatography/mass spectrometry.

The versatility of the ADx as an automated method of analysis will be discussed as it applies to its usage in a clinical toxicology laboratory.

12. Evaluation of ADx® for Determination of Urinary Cannabinoids, Cocaine Metabolite and Opiates. L.F. Rittenhouse, C. Cresalia, A.M. Cairns, D.S. Barberio, K.M. Monti and D.E. Moody, (Center for Human Toxicology., University. of Utah, Salt Lake City, UT 84112).

Automated ADx systems allow for simultaneous analysis of multiple drugs (flexible access) in urine by fluorescence polarization immunoassay (FPIA). We evaluated ADx for analysis of urinary cannabinoids (CAN), cocaine metabolite (BE) and opiates (OP). Precision was determined for 3 controls/assay run daily in replicates of 4, in either batch or flexible mode, for 10 days within a 14 day period of time. Precision results were:

	CAN			BE			OP		
	low	med	high	thres	low	high	thres	low	high
Expect(ng/ml)	35.0	50.0	120	300	500	3000	200	250	800
Found(ng/ml)	34.7	48.3	117	300	510	2940	193	233	763
W-R CV (%)	3.72	5.25	4.16	3.01	2.13	1.62	2.13	1.94	2.43
B-R CV (%)	4.32	5.58	4.50	3.76	3.70	2.68	3.41	2.67	4.14

Calibration curves were stable for a minimum of 21 days for all 3 assays. ADx assays were compared with TDx, RIA, and GC/MS for accuracy. Average percent recovery for all assays agreed within 20 %, and for most within 10 %, variation. Accuracy by clinical correlation was determined for 50 negative (GC/MS; CAN & BE only) and 50 positive (BE-48) samples. Correlation between methods was determined only for data pairs which were within the standard curves, with the following results:

	CAN(ADx vs)			BE(ADx vs)			OP(ADx vs)		
	TDx	RIA	GC/MS	TDx	RIA	GC/MS	TDx	RIA	GC/MS
# data pair	35	17	41	36	28	26	5	5	5
slope	1.01	0.77	0.37	0.99	0.92	0.96	1.02	1.72	0.55
r	0.99	0.85	0.86	1.00	0.82	0.92	1.00	0.82	0.92

The variation in slopes was consistent with the inherant antibody cross-reactivity differences between immunoassays (i.e. ADx/TDx vs RIA) and the specificity of GC/MS. No false-positives arose from any of the methods used. Based on the cutoffs employed (CAN: ADx, TDx & RIA, 25, GC/MS, 5ng/ml; BE: ADx, TDx & RIA, 300, GC/MS, 25 ng/ml; OP: ADx, TDx 200, RIA, 100, GC/MS, 20 ng/ml). All 50 CAN and OP positive samples were positive by all 4 methods. Two GC/MS positive BE samples, had one replicate negative by TDx, ADx, and RIA, and one sample was negative by RIA. The ADx system demonstrated excellent precision, and accuracy comparable to TDx and RIA.

13. The Federal Railroad Administration's Regulation on the Control o Alcohol and Drug Use in Railroad Operations. WALTER C. ROCKY Executive Assistant, (FRA Office of Safety).

Between 1975 and 1984, the FRA found that 48 train accident were caused by alcohol or drug using employees. These accident resulted in 37 fatalities, 80 injuries, and $34 million dollars i damages to railroad property. The FRA concluded that the use of alcohc and drugs by railroad operating employees was a significant nationa safety problem and that industry efforts to address it were inadequate.

In 1985, the FRA issued its final regulation on the control o alcohol and drug use on the railroads. This program prohibits railroa employees from possessing, using, or being under the influence o alcohol or drugs while on duty. The program also require post-accident toxicological testing after major accidents, improve reporting of alcohol or drug involvement in railroa accidents/incidents, preemployment drug screens, the identification c troubled employees and authorizes the railroads to conduct reasonabl cause breath and urine tests.

In 1987, the FRA found that 13 major accidents were caused o contributed to by alcohol or drug using employees. These accident resulted in 19 fatalities, 226 injuries, and over $17 million dollars i railroad property damages. The railroads reported performing 59 breath tests for alcohol, of which 28 or 4.7 percent were positive, an 5,439 urine tests of which 369 or 6.8 percent were positive. Durin 1987, the FRA concluded additional measures were necessary and issue a notice of proposed rulemaking on May 10, 1988, to require randon testing on the railroads.

14 Results of One Year of Drug Testing Under The Federal Railroad Administration's (FRA) Mandatory Post- Accident Testing Program. D. E. Moody, D. J. Crouch, R.P. Smith, C. Cresalia, D.G. Wilkins, and D. E. Rollins (Center for Human Toxicology, Univ. of Utah, SL C, Utah 84112)

Under federal law, railroad employees are tested for drugs following qualifying accidents. During the year beginning 4/1/87 specimens were collected for 183 qualifying events from 743 individuals, including 40 fatalities. Blood and urine, blood only, urine only, and tissue only specimens were provided for 714,17, 9, and 3 individuals respectively. Routinely, urine was screened for 8 drug classes and the blood for ethanol. Both blood and urine specimens from presumptive positives were confirmed and quantitated. Specimens confirmed ≥ cutoff in either blood or urine were positive. The results of testing were:

Screen			Confirmation			
Substance	Cutoff (ng/ml)	# Pos	Drug/ Metab's Tested for	Cutoff (ng/ml) Blood	Urine	# Pos.
Cannabinoids	20	61	THC-COOH	2	20	34
			THC	1	—	
Cocaine/Metab	300	9	Cocaine	50	50	8
			Benzoylecg.	50	150	
Opiates	300	8	Free Morphine	100	100	5
			Free Codeine	100	100	
Phencyclidine	25	1	Phencyclidine	25	25	0
Amphetamines	300	21	Amphetamine	100	100	1
			Methamphet.	100	100	
Barbiturates	200	1	Pento,Seco,Amo	200	200	1
			Phenobarbital	1000	1000	
Benzodiazepines	300	9	Diaz, Chlordiaz, Fluoraz & metab's	300	300	2
Methaqualone	750	1	Methaqualone	500	500	1
Alcohol	0.01(g%)	8	Ethanol (g %)	0.01	0.01	7

All presumptive and confirmed positive drug data will be presented. In specified cases, the list of drugs/metabolites was not restrictive, and could be extended by official authorization (e.g. the positive barbiturate was butalbital). Multiple drugs were present in 3 of the individuals, resulting in 48 (6.46 %) positive individuals for drugs, and 7 (0.94%) positive for alcohol.

15. PROBABILITY OF A TRUE POSITIVE AMPHETAMINE IMMUNO-ASSAY RESULT, Vina Spiehler, Howard Wilson, Said El Shami, Tom Sneath and Narish Jain, Diagnostic Products Corporation, Los Angeles, CA 90045 and National Toxicology Laboratories, Bakersfield, CA 93301

The probability that a positive amphetamine immunoassay result is a true positive can be calculated from experimental data using Bayes' theorem. This probability, called the predictive value of the assay, is useful in comparing screening tests, choosing cutoffs and deciding on the order and number of screening and confirmatory tests required. In this study the predictive value probabilities of four immunoassays were compared. Confirmation by SIM GC/MS using a n-propyl amphetamine internal standard was used as the gold standard. In the first experiment, using drug free urines and urines containing amphetamine or methamphetamine, the predictive value of a positive or negative result for all four immunoassays ranged from .94 to .97. In the second experiment ninety-two urines which contained clinical concentrations of sympathomimetic amines were assayed to determine the ability of the immunoassays to distinguish true positive amphetamines (amphetamine or methamphetamine confirmed by GC/MS) in a population of urine specimens containing phenylpropanolamine, ephedrine, pseudoephedrine, benzphetamine and chlorpheniramine. The prevalence of true amphetamine or methamphetamine was 50%. The predictive value of a positive single immunoassay screen run ranged from .48 to .95. The predictive value of a negative result ranged from .57 to .97. By using the assay with the highest predicitve value, the total number of initial screen positives can be decreased and the percent of presumptive positives confirmed can be increased, thus reducing the expense of confirmation.

16. Demographic Data and the Results of a Statistical Analysis of Paired Data Obtained at Arrest and Re-Interview of DUI-D Suspects (SOFT DUI-D Committee Report), EVERETT T. SOLOMONS*, JOHN HOLBROOK (Mercer University School of Pharmacy, Atlanta, Georgia), MARK PEVEY*, H. HORTON MCCURDY*, LISA CALLAHAN*, DONALD DICKS*, and CHRISTINE PAPADEA* (*GBI-Division of Forensic Sciences, Toxicology Section, Decatur, Georgia).

Twenty-five subjects suspected of DUI-Drugs were evaluated at arrest and approximately three days later after abstaining from alcohol and drugs. Demographics and statistical data resulting from the evaluations and including reaction time measurements and corresponding alcohol and drug analysis data will be presented.

17. PLENARY GUEST LECTURE: Indoles and Indians, Bo Holmstedt, Karolinska Institutet, S-104 01 Stockholm.

Description of chemical and toxicological field trips in the Amazon aboard the research vessel Alpha Helix with use of methods ranging from Drangendorff's reagent to gas chromatography/mass spectrometry. Discussion of indoles from dyes of antiquity to indigo substitutes to Goebel's 1841 isolation of harmine to the methylated indoles of South America. Survey of occurance, use, and mechanism of action of harmala alkaloids. Outline of parallels between the Pictet-Spengler reaction and possible condensations of aldehydes from tissues to form endogenous psychotoxic compounds.

18. Tissue Distribution of Cyanide in Two Fatal Overdose Cases, Charles L. Winek, Ph.D. (Allegheny County Department of Laboratories), Wagdy W. Wahba, Ph.D. (Allegheny County Department of Laboratories).

Two fatalities due to ingestion of cyanide are reported. Tissue distribution of cyanide was studied. Blood cyanide concentrations were 6.5 and 11.2 mg/L respectively. Other fluids and tissues analyzed included urine, eye fluid, bile, stomach contents, liver, kidney and brain. Total stomach contents were 188 mg and 8.58 mg respectively. The circumstances of the first case indicated suspected cyanide ingestion, while in the second case the possibility of cyanide overdose was not suspected at the offset. Routine screening for cyanide is easy to perform and should be carried out in all cases.

19. Determination of Phenelzine in Postmortem Blood
P.P. SINGER (Office of the Chief Medical Examiner, 4070 Bowness Road N.W., Calgary, Alberta T3B 3R7) and
G.R. JONES (Office of the Chief Medical Examiner, P.O. Box 2257, Edmonton, Alberta, Canada T5J 2P4).

A sensitive assay for phenelzine (eg. Nardil) in postmortem blood is required by forensic toxicologists because of the high potential for overdosage or serious drug-drug and drug-food interactions. However, the extremely low blood concentrations of phenelzine resulting from therapeutic doses, plus its chemical and thermal instability, have proved difficult barriers to analysis.

We have developed a GC/MS assay for phenelzine sensitive to at least 5 ng/ml in postmortem blood. Phenelzine is initially derivatized with pentafluoro-benzaldehyde (PFB) in situ (in blood), extracted with benzene, the extract evaporated and the residue further derivatized with trifluoroacetic anhydride. The resulting PFB-TFA derivative has excellent chromatographic properties and stability. Phenelzine-d_2 is used as the internal standard. Ions m/z 104 and m/z 106 are monitored in the EI SIM mode.

Preliminary data on the stability of phenelzine in postmortem blood and an application of the assay to one overdose case will be presented.

20. Chromatographic Separation of MDMA and MDA enantiomers, R.L. FITZGERALD (Medical College of Virgina), R.V. BLANKE (Medical College of Virgina), J.A. ROSECRANS (Medical College of Virgina) R.A. GLENNON (Medical College of Virgina) and A.POKLIS (Medical College of Virgina)

The methylenedioxy analogs of the amphetamines became popular when clandestine laboratories, attempting to bypass DEA regulations, sequentially altered the structure of 3,4-methylenedioxyamphetamine (MDA) first by forming the N-methyl (MDMA) then the N-ethyl (MDEA) homologs producing drugs with high abuse potential. Due to the street use of these compounds and lack of data on pharmacological and toxicological effects, studies by other groups were initiated, that demonstrated both enantiomers of MDA as well as S(+)-MDMA produced a long term depletion of serotonin in rats. Initial work done in this laboratory identified MDA as a major metabolite of MDMA and future work is directed at studying the kinetic relationship between the parent drug and metabolite. The purpose of this study was to develop a method that could be used to separate, identify, and quantitate the optical isomers of MDA and MDMA in small samples (200 ul) of blood for use in pharmacokinetic studies. Results of separating the enantiomers on a chiral (Pirkle) HPLC column will be compared with resolution as trifluoroacetyl-L-prolyl chloride (LTPC) diasteriomers. Extraction and derivatization schemes for both types of chromatography will be discussed and reasons for using LTPC instead of Pirkle chromatography will be presented. Both methods were used sucessfully to show that animals dosed with racemic MDMA preferentially formed the S(+) isomer of MDA.

21. Determination of Fentanyl in Whole Blood at Subnanogram Concentrations by Dual Capillary Column Gas Chromatography with Nitrogen Sensitive Detectors and Gas Chromatography/Mass Spectrometry, VICKIE WATTS (Mesa Police Department) Yale Caplan (State of Maryland, Office of the Chief Medical Examiner)

Two methods for the determination of fentanyl at subnanogram concentrations in whole blood have been developed and evaluated. The initial screening was by gas chromatography with nitrogen sensitive detection (GC-NPD) in a splitless injection onto two fused silica, 0.32 mm i.d. capillary columns (5% and 50% phenyl methyl silicone). Confirmation was by gas chromatography/mass spectrometry (GC-MS) using selected ion monitoring of a splitless injection onto a 0.1 mm i.d., 0.34 um 5% phenyl methyl silicone capillary column. The methods were studied at fentanyl concentrations over the range 0.05 ng to 5.0 ng/mL using 2 mL of blood. The detection limits were set at 0.10 ng/mL for GC-NPD and 0.05 ng/mL for GC-MS. The overall recovery of fentanyl was found to be greater than 75% over the range of 0.25 to 2.5 ng/mL. The within-run precision determined at fentanyl concentrations of 0.25 and 1.0 ng/mL showed coefficients of variation ranging from 8.7 to 14.8%. The between-run precision determined at concentrations of 0.4 and 0.8 ng/mL showed coefficients of variation ranging from 3.3 to 11.6%. The blood calibration curves in the range of 0.25 to 2.5 ng/mL monitored over a 3-month period showed a mean correlation coefficient of 0.99 for both the GC-NPD and GC-MS methods.

22. Tissue Distribution of Ketamine: Two Case Reports, SHERYL H. PEYTON, ANNE T. COUCH, and ROBERT O. BOST* (Southwestern Institute of Forensic Sciences, 5230 Medical Center Drive, Post Office Box 35728, Dallas, Texas 75235 (214)920-5960).

Two cases of death are reported; one involved an overdose of ketamine, the other involved a gunshot victim who was given ketamine during surgery. Distribution of ketamine throughout the body is reported for both cases. Concentration of ketamine in the various tissues and the presence or absence of norketamine may be helpful in distinguishing an alleged overdose case from other circumstances.

23. Analysis of Tissue Specimens for High Boiling (Mineral Oil) Hydrocarbons by IR and GC/MS, MORGAN,T. and RIEDERS,F. (National Medical Services, Inc., Willow Grove, PA)

Resolution of a toxic tort issue required identification and individualization of mineral oil residua in formalin fixed lung specimens which had originated from surgical resection. In the course of the analyses,we found that direct tests of the evaporated residua of sulfuric acid-washed, direct petroleum ether extracts by infrared spectrophotometry effectively detected and measured both light and heavy mineral oils, while TIC electron impact GC/MS allowed reasonably effective source-differentiation (individualization) between mineral oils from different sources.

Applying these methods to autopsy specimens, we have noted that similarly high boiling hydrocarbon fractions in substantial concentrations are still present as residua in aspiration pneumonitis deaths following ingestion of petroleum naphtha, kerosene or gasoline, even when death occurred so long after the incident that headspace gas chromatography no longer detected any of the usual hydrocarbons (with boiling points of up to approximately 180°C).

The relevant case details as well as the data from other ("control") autopsy tissues will be presented and the potential forensic toxicologic applicability of the method will be illustrated.

24. Determination of Synthetic Pyrethroid Insecticides in Biological Materials by GLC/ECD, Cao Xiu-peng, Gao Li-sheng, Institute of Forensic Science, Ministry of Public Security, People's Republic of China

Abstract: A method for simultaneously determining 4 synthetic pyrethroid insecticides: permethrin, cypermethrin, fenvalerate and deltamethrin in biological samples has been developed and the detailed procedures for extracting and purifying them from various tissues such as stomach, liver, blood, and urine etc. are described in this paper.

Analysis was performed by GLC/ECD and 1m X 1.75mm (i.d.) glass column packed with 10% DC-200 + 3% QF-1 (column I) or 2m X 1.75mm(i.d.) glass column packed with 5% OV-101 (column II).

Operating conditions: column teperature: 200°C for column I; 260°C for column II; injector teperature: 300°C; detector teperature: 350°C; nitrogen carrier gas 60-80ml/min.

Under the GLC conditions given above, the 4 insecticides were completely separated and determined in 30 minutes.

Detector responses were linear for the 4 synthetic pyrethroid examined in the range of 0.2-2ng. The detection limit was 0.2ng.

Measurement of unknown samples was based on peak area with a standard of appropriate concentration. The average recoveries for the 4 insecticides in fortified biological materials at the levels of 1ppm-2 ppm range from 70% to 90%. The coefficients of variation ranged from 0.03 to 0.08 (3%-8%).

Therefore this method can be used to detect them qualitatively and quantatively at the same time.

The method has been successfully used to detect synthetic pyrethroid insecticides in poisoning cases.

25. Death Due to Self Administered Fentanyl: A Case Report, ARVIND K. CHATURVEDI, Ph.D. and N.G.S. RAO, Ph.D. (Dept. of Toxicology, College of Pharmacy, North Dakota State University, Fargo, ND 58105)

Fentanyl, a synthetic opioid is an extensively used analgesic supplement for general anesthesia during surgery. It is considered superior to morphine since it has minimal hypotensive and hypertensive effects and short duration of action. These pharmacological effects also made it a choice drug of abuse for those with access. This case involves the death of a health care professional with a known history of drug abuse. A partly filled syringe with red colored liquid which was found by the side of the deceased was also submitted along with the tissue samples. The analysis was conducted using a GC with NP detector. The tissue concentrations of drugs found in the submitted samples are as follows:*

Sample	Fentanyl	Diazepam	Nordiazepam	Oxazepam
Fluid from syringe	2,800	ND**	ND	ND
Serum	17.7	Present	NA***	NA
Blood	27.5	206.0	35.8	3.8
Urine	92.7	123.6	128.0	22.8
Bile	58.2	492.7	147.2	4.7
Liver	77.5	271.4	164.7	9.5
Kidney	41.5	139.0	141.5	ND
Brain	30.2	155.7	21.5	9.3
Lung	83.4	72.8	199.0	6.8

*All drug concentrations are in mcg/L or kg except oxazepam levels which are in mg/L or kg.
**Not detected.
***Not analyzed.

26. **Cross Reactivity of Amphetamine Analogues With Radioimmunoassay Reagents**, John T. Cody, Ph.D. (Air Force Drug Testing Laboratory, Brooks AFB TX 78235-5000)

The use of amphetamines has long been evaluated by screening samples by radioimmunoassay (RIA). The cross reactivity of the reagents to various amphetamines and their analogues is of significance with regard to the detection of the illicit use of amphetamine analogues. A variety of compounds which are analogues of amphetamine are available on the illicit market and offer a challenge to the detection of these drugs. Since the establishment of the controlled substance analogue act of 1986, compounds which meet the criteria to be classified as analogues are considered schedule I controlled substances. The identification of these substances requires a system which is sensitive to the presence of the drug or its metabolites. Whether or not an RIA test procedure can detect the presence of the drug analogues depends on the extent of cross reactivity of the analogues with the reagent antibodies. Determination of the cross reactivity of amphetamine and a number of its analogues was studied to determine the ability of the RIA system to identify the presence of the compounds in urine. The study involved reagents from Roche Diagnostics and included the standard and high specificity test systems. The extent of cross reactivity of the analogues demonstrated the RIA system was very sensitive to MDA which showed activity greater than did D-amphetamine. MDMA and MDEA also showed measurable activity in the system. The reactivity of the high specificity reagents was substantially lower than the standard reagents for MDA.

27. APPLICATION of the ROCHE ABUSCREEN BENZODIAZEPINE ASSAY to POSTMORTEM URINE *SPECIMENS*, *C. LoDico, B. Levine, Ph.D. and Y. H.* Caplan, Ph.D. (University of Maryland, Department of Pathology, 111 Penn Street, Baltimore, MD 21201)

One hundred clinical urine specimens, 50 positive for benzodiazepines (BDPs) and 50 negative for BDPs were tested by RIA, EMIT, FPIA and a reference GC/MS assay. No false positives or false negatives resulted by any of the immunoassay techniques as compared to GC/MS. The limit of detection for BDPs was equivalent in reactivity to 100 ng oxazepam/mL.

The RIA Roche Abuscreen method was used to test 368 postmortem urine specimens for the presence of BDPs. Of the 33 testing positive, 31 were confirmed by GC/MS.

A specificity study revealed that diazepam, nordiazepam, oxazepam, temazepam and alprazolam reacted with the antibody while chlordiazepoxide, lorazepam and triazolam did not react with the antibody at a minimum concentration of 100 ng/mL of the drug.

We concluded that the Roche Abuscreen Benzodiazepine Assay compares favorably to EMIT and FPIA as a screening method for BDPs in urine specimens.

28. HYPOTHESIS: Prosecution of DUI (Alcohol) Based Upon Current Assumptions of Alcohol Pharmacokinetics is not scientifically and legally supportable;
ROBERT.K.SIMON (Washington Analytical Laboratory,Inc., Chantilly, VA), R.HUGH GRANGER (Washington Analytical Laboratory, Inc., Chantilly, VA).

This presentation will review the pharmacokinetic principles that have been interpreted in most jurisdictions as supportive of the legal use of the 2100:1 blood/breath ratio and the postabsorptive state in the prosecution of DUI Alcohol cases. The assumption used in medicolegal standard of practice is the absorption of alcohol occurs within 30 minutes of consumption during normal social drinking. This belief however, has been severely questioned in recent years by Dubowski, Simpson, and others and has been shown to be a misinterpretation of the actual data.

The authors will review the current status of the prosecutorial use of the assumptions on absorption, "provided" to the legal professional by experts. The original manuscript of Widmark will also be evaluated to determine if it actually supports the prosecutorial assumptions as currently used. In directing attention to the large population variability in alcohol pharmacokinetics as discussed by Dubowski, Simpson, et al., the authors will construct a model to evaluate the question of scientific and legal supportability of current breath alcohol evidence.

29. **Rapid Enzyme Immunoassay for Phencyclidine (PCP)**
J. TANSEY, J. FRAZER and H. BRANDWEIN,(Genetic Diagnostics Corporation, Great Neck, New York)

The rapid detection of phencyclidine (PCP) without labor intensive procedures or expensive, dedicated instruments becomes a more desirable goal as PCP abuse increases. We report here on the development of a 75 minute competitive ELISA for the qualitative detection of PCP and metabolites in human urine.
The test utilizes a 96-well microtiter plate coated with PCP linked to a carrier protein (bovine serum albumin) and a high affinity monoclonal antibody to PCP. After a 30 min incubation of test urine sample with the first antibody, an enzyme -labelled second antibody is added, and after an additional 30 min incubation and wash steps, the assay is visualized using 3,3,5,5'-tetramethylbenzidine as the enzyme substrate. After 15 min, the enzyme reaction is terminated by the addition of 1N HCl, and the absorbance is measured at 450nm. For qualitative positive/negative determination, test samples are compared to the positive standard which contains 25 ng/ml PCP in synthetic urine.
Using this competitive ELISA format and the Syva EMIT assay, we have analyzed 200 urine samples as shown below:

	Positive	Negative
SYVA EMIT	50	150
GDC	50	150

Cross reactivity studies with over 40 prescription drugs at concentrations of 50 ug/ml have revealed no interference with assay results. The assay can also detect PCP in blood and serum.
The ability to rapidly and specifically detect PCP and its metabolites using a competitive ELISA has been established and should be a valuable tool in identifying PCP abuse.

30. Comparative Sensitivity of the Abbott TDX and Other Immunoassay Techniques for Urine Cannabinoid Screening. DAVID J. WELLS (University of South Alabama Medical Center) MATTHEW T. BARNHILL, JR. (Alabama Dept. of Forensic Sciences) (Mobile, Alabama 36617)

Immunoassay testing is the preferred method of screening for the presence of cannabinoids in urine. Several methods are commercially available which are compatible with the NIDA recommended detection threshold of 100 ng per ml. These include the Roche Abuscreen RIA, Immunalysis RIA as well as the DPC double antibody cannabinoids RIA. Also, a fluorescence polarization based immunoassay for cannabinoid screening on the TDX (Abbott) has recently been introduced. The relative sensitivity of these techniques has been evaluated using urine specimens independently tested for the presence of cannabinoids by the Toxilab TLC technique and HPLC. The results indicate that, in general, the TDX is less sensitive than the prior established immunoassay procedures. When individual values are examined however, there is wide discrepancy of the results among all systems. Although disagreement of values determined by immunological methods has been reported at lower cut-off levels, this study indicates that positive screening values are method dependent even at the relatively high threshold of detection of 100 ng per ml. Further studies are in progress to determine if variation in cannabinoid metabolite cross-reactivity may be responsible for these observations.