Society of Forensic Toxicologists, Inc.

P.O. Box #5543, Mesa AZ 85211-5543 Telephone/ FAX: 602-839-9106

ToxTalk

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EDITOR: Joseph R. Monforte, PhD DABFT **EDITORIAL BOARD**: H. Chip Walls, BS, Jim Wigmore, BSc, Carl Selavka, PhD
PUBLISHER: Patricia Mohn-Monforte

FROM THE EDITOR'S DESK . . . . .

Joseph R. Monforte, Ph.D., DABFT

The SOFT 25th Annual Meeting was one to remember. An outstanding program was organized by Yale Caplan and his meeting committee. The scientific sessions were informative, and the social activities were enjoyed by all. We all owe Yale and his dedicated meeting committee a great deal of thanks for allowing us to benefit from the time and effort which they devoted to the meeting.

For those of you who were unable to attend, the abstracts of the meeting are enclosed in this issue of **ToxTalk**. Ed Cone, again, graciously prepared these for distribution to the membership.

Ted Siek has provided for this issue of ToxTalk an informative article on the application of the standard addition technique to post mortem toxicology. Some useful points to consider when applying this technique are summarized.

Happy Holidays to All!!!

IN THIS ISSUE

ToxTalk is mailed quarterly (bulk mail) to members of the *Society of Forensic Toxicologists, Inc.* It is each member's responsibility to report changes of address to the SOFT mailing address (above). Non-members may now receive **ToxTalk** for $15 per calendar year. Make your check payable to SOFT, and mail it to the **ToxTalk** Editor.

All members and others are encouraged to contribute to **ToxTalk**. Mail material to :
Joseph R. Monforte, Ph.D., DABFT, **ToxTalk** Editor, 846 Smoki Drive (H.P.), Prescott, AZ 86301
Phone/FAX: 520-717-0617 *(after 11 a.m. E.S.T.)*

DEADLINES: Feb. 1, May 1, Aug. 1, and Nov. 1. **NEXT DEADLINE:** ***February 1, 1996***

SOFT is a supporting organization of the American Board of Forensic Toxicology

PRESIDENT'S MESSAGE . . . Vina Spiehler, Ph.D., DABFT

What is Good Laboratory Practice in *Post Mortem* Forensic Toxicology?

In this time of government downsizing and privatization, the United States is in danger of losing the sil infrastructure of our criminal justice and public health - medical examiner autopsies and forensic toxicology laboratory investigation of death. Because of budget cuts combined with increasing workload, many laboratories have retreated to doing minimal or less than minimal cases. Many cases are given a limited immunoassay screen and no further work is done until the case goes to trial. This leads to wild and unfounded speculation in court, loss of perspective on the national drug problem and ignorance. Ignorance is incompatible with democratic government by the people.

The SOFT Forensic Toxicology Laboratory Guidelines are recognized internationally as a standard of practice in forensic toxicology, but how many state, county or city governments in the United States feel compelled to provide services at the level of good laboratory practice recognized in our Guidelines? One way to bring these standards into practice is the planned laboratory accreditation program of the joint SOFT-AAFS committee. Laboratory directors can use accreditation as a stick to prod government agencies to fully fund and support the forensic laboratory. In an interview which appeared on the front page of the Orange County (California) edition of the Los Angeles Times on October 29, 1995, OCSO Forensic Science Services Director Frank Fitzpatrick declared that the cuts necessitated by the County of Orange bankruptcy in the staff and operating budget of the Orange County Sheriff's crime lab have endangered the laboratory's accreditation. Mr. Fitzpatrick cited as a result of the cuts an increasing backlog of cases, unsolved crimes and the court system's expectation that the laboratory may be a source of delay rather than a source of information and knowledge.

All SOFT members can support the goal of maintaining adequate levels of excellence in forensic toxicology services by incorporating the SOFT Guidelines recommendations into their Standard Operating Procedures documentation, by testifying in court to the SOFT Guidelines as the standard of practice, by conducting self-inspections of their own laboratories using the SOFT-AAFS Committee checklists and by volunteering as laboratory inspectors in the coming program.

I would like to suggest three areas in which SOFT should lead in establishing good laboratory practice for forensic toxicology: full body distribution determinations in drug overdose cases including analysis of stomach contents, brain, liver, kidney, vitreous and bile, in addition to heart and peripheral blood drug and drug metabolite concentrations; comprehensive toxicology screens in undetermined deaths; and collaboration or confirmation of blood alcohol concentrations in addition to confirmation of drug screens.

SOFT champions quality assurance, quality control and good laboratory practices in forensic toxicology. One of the objectives of SOFT in the SOFT ByLaws is "to promote and assist in (1) the continued development of the field of forens toxicology and (2) bringing about adequate availability of forensic toxicology services to units of government, organizatic and persons in need thereof." My grandmother, Pearl Boyington-Hunt Thompson, a pioneer woman of the old west, used to say, "Anything worth doing is worth doing well." In my opinion, quality assurance in forensic toxicology involves not only quality control of the analytical procedures but also performing sufficient tests on the appropriate specimens to sustain or rule out the identity and source of the drug taken, the amount taken, and the time taken, chronic or acute use and contribution to death. This can only be done by applying comprehensive screens, analyzing tissue and stomach contents in addition to blood and urine and by collaboration or confirmation of screen results. Anything less is not good laboratory practice.

What do SOFT members think? What are the obstacles to good laboratory practice in your region? Do you think that these are goals which are important to interpretation of toxicology results? Do you think that we can achieve them in the traditional government laboratory? If not, how can we practice excellence in forensic toxicology? Should the accreditation of forensic toxicology laboratories require that the laboratory do confirmation? Of alcohol? Body distribution? Comprehensive screens? If you have an opinion please write a letter to the editor and send it to ToxTalk to start a dialog amongst SOFT members on how to meet the challenges of diminishing resources which face forensic toxicologists today. I am sure that the ToxTalk Editor, Dr. Joseph Monforte, would publish any contributions on these topics from interested persons.

Editor's note: The laboratory guidelines referred to above are the AAFS/SOFT Forensic Toxicology Laboratory Guidelines, copies of which are available for $25 U.S. and $35 non-U.S. through the SOFT office (602-839-9106). Also, the laboratory accreditation program will be designed and implemented by the American Board of Forensic Toxicology. More information on this laboratory certification program will be published when it is available. Furthermore, any comments regarding this article are encouraged and would be considered for publication.

CHANGE OF ADDRESS?
NEED A MEMBERSHIP APPLICATION OR OTHER S.O.F.T. INFORMATION?

All requests for database changes, materials, or any information relative to SOFT (except **ToxTalk**), should directed to **P.O. Box 5543, Mesa, AZ 85211-5543, or phone/fax 602-839-9106**. Sending any mail to the obsolete, temporatry Peoria, AZ, address or to Prescott, AZ, will cause a definite delay in fulfilling your request. **ToxTalk** materials only should be sent to the Prescott, AZ, address on the front page of **ToxTalk**.

MEMBERSHIP ACCEPTS NOMINATING COMMITTEE SLATE FOR 1996 S.O.F.T. LEADERSHIP

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	Joseph R. Monforte, Ph.D., DABFT (ToxTalk editor)

1996 S.O.F.T. DUES NOTICES HAVE BEEN MAILED DIRECTLY TO MEMBERS

A Closer Look at S.O.F.T.:

TOXTALK

Editor: Joseph R. Monforte, Ph.D., DABFT; Editorial Board: H. Chip Walls, B.S., Jim Wigmore, B.Sc., Carl Selavka, Ph.D.; Publisher: Patricia Mohn-Monforte

ToxTalk is the official quarterly newsletter of the Society of Forensic Toxicologists. Members and non-members are encouraged to submit items of interest to the membership. The Health and Safety Committee is a good example of a committee utilizing **ToxTalk** to dissimenate information. Hopefully, more committee chairs will use the publication to inform SOFT members of specific committee activities. Information on annual meetings and special events can also be found in the newsletter. SOFT is fortunate to have regular contributors, such as Chip Walls, Jim Wigmore, and Carl Selavka and his group from NMS on whom I have been able to rely for material for years.

How does one submit material? It's easy. Send a hard copy and disc (we use Microsoft Works/Word 2.0), if possible, to:

Joseph R. Monforte, ToxTalk Editor, 846 Smoki Dr. (HP), Prescott, AZ 86301-7347

You may also FAX to 520-717-0617 if someone is here to turn on the fax. Always mail a hard copy to make sure your material is received. If your material is more than 1 page, it should be submitted "print ready" - 10 point, no wasted space, 1/2" margin on the bottom, 1/4 " top and side margins. Look at other issues and follow the obvious perameters.

Around the deadline date, Pat puts all the materials received into the computer and does a draft layout, indicating the number of pages necessary, what materials are still needed, etc. An initial proof is done at this time. Also, and only if the production schedule allows, people who still have not submitted promised or needed materials are contacted. Layout adjustments are then made, often numerous times. On the cut-off date determined by the editor, a final draft is prepared for final proofing. Then the print copy is produced which is taken to a local printer. Depending on the printer's schedule, ToxTalk can be printed in 3-7 days. After picking up **ToxTalk**, the pages are collated and inserts are included. These are then placed into envelopes and labels are added. We then sort and bag per post office requirements for bulk mail. Then we "go to town" and take ToxTalk to the Prescott post office. Non-U.S. copies, which are separated, are also taken to the post office to determine appropriate postal fees according to the new (and not very logical) international rates.

I am considering publishing "articles I'd like to receive for the next issue" in the next **ToxTalk** as a reminder to specific members to submit appropropriate materials that should be included in **ToxTalk** and to encourage more members to participate. So send material - a short case note, a general item of interest, information on your committee . . . We'd like to get something from YOU.

1996 SOFT ANNUAL MEETING

DENVER, COLORADO — OCTOBER 14-18

Mark your calendars now! Set aside those travel dollars!

Plans for SOFT 1996 are well underway. An excellent meeting was planned and carried out in Baltimore - we aim to do the same for 1996. In this issue fo **ToxTalk**, you will find a flyer with some preliminary information that should be helpful in your planning and the initial call for papers. The forms needed to submit an abstract are included - remember the deadline has been moved up one month to June 1, 1996. All pre-registration information will be in the March issue of **ToxTalk**.

We are looking forward to having you all come to colorful Colorado. Plan for a scientifically stimulating meeting. Dress code for the meeting will match the casual Colorado lifestyle. Thanks to all of you who have so willingly offered to help.

Co-hosts:	Laurel Farrell (303-691-4727) and Bob Zettl (303-691-4738)
Scientific Program:	Amanda Jenkins and Bruce Goldberger
Workshops:	Dan Isenschmid and Dennis Crouch
Exhibitors:	Lisa O'Dell

Proposed workshop topics:

Capillary Gas Chromatography
Drugs & Driving
Fundamentals of Medical Examiner Toxicology
Inhalants
Internet
New Concepts in Forensic Urine Drug Testing
Principals of Drug Metabolism

Hotel: Denver Marriott Tech Center 1-800-228-9290 or 303-779-1100
Special reduced room rate: $77 including tax (same as current federal government rate)

Shuttle service from Denver International Airport: $15 one way

S.O.F.T. 1995 PRESENTATION WINNERS

Submitted by Amanda J. Jenkins

At the highly successful annual meeting in Baltimore, prizes were donated by vendors to honor the best poster and platform papers presented during the meeting. A group of reviewers evaluated all presentations according to three criteria: overall presentation quality; scientific validity; and interest to forensic toxicologists. The winners were recognized by the membership during the Honors and Awards section of the 25th Anniversary Commemorative Program. Congratulations to the winners listed below and to all those who contributed to the scientific program.

Platform Presentation (2):

The Analytical and Phramacological Characterization of a-Benzyl-N-methylphenethylamine, An Impurity of Illicit Methampheatmine Synthesis. Karla Moore, Alphonse Poklis, William H. Soine, Joseph F. Bozelleca, Joseph J. Saady, and James C. Valentour, Medical College of Virginia and Office of the Chief Medical Examiner, Richmond, VA.

Determination of Colchicine in Human Biofluids by HPLC/ISP-MS. Antoine Tracqui, Pascal Kintz and Patrice Mangin, Institute of Legal Medicine, Strasbourg, France.

Poster Presentation (2):

Improved Chromatographic Separation of Opiates as TMS Derivatives by Formation of the Oxime-TMS Derivatives of Hydrocodone. Randal Clouette and Gary H. Wimbish, Laboratory Specialists, Inc., Belle Chasse, LA.

Profile of Drug Use in California: A Composite of Vehicle and Health and Safety Code Drug Findings. William H Phillips, Jr., California Department of Justice, Sacramento, CA.

Technical Notes: BLUTALKOHOLKONZENTRATION No. 11

Submitted by: J.G. Wigmore, B.Sc., Toxicology Section, Centre of Forensic Sciences, Toronto, Ontario, Canada

THE COURSE OF THE BLOOD ALCOHOL CURVE AFTER CONSUMPTION OF LARGE AMOUNTS OF ALCOHOL P Zink and G Reinhardt, **Blutalkohol** 21:422-442, 1984.

German title: Der Verlauf der Blutalkoholkurve bei grossen Trinkmengen

There are many studies on the BAC curve in subjects after drinking moderate amounts of alcohol (0.8-1.2 g/kg), however, there are very few studies on subjects drinking large amounts of alcohol under controlled conditions. In this study 12 male subjects (ages 25 to 39) consumed between 3.0 and 5.7 g/kg alcohol over a period of 4 to 10 hours. The subjects consumed the beverage of their choice (beer, whiskey, brandy, or stomach bitters) 'ad libitum'. Blood samples were taken frequently (on average 28 times) during the course of the experiment from a heparin lock in the cubital vein. The BACs were determined by GC and ADH methods.

The peak BACs varied between 0.200 g/100mL and 0.400 g/100mL (mean 0.280 g/100mL). The peak BACs were up to 50% lower than expected, and the apparent Widmark factor f was on average 1.08 rather than the expected 0.7. The rate of alcohol elimination in these subjects was between 0.012 and0.024 g/100mL/h (mean 0.017 g/100mL/h). During the experiment two subjects showed severe intoxication.

Five of the subjects in another series of tests consumed a much lower amount of alcohol (between 1.1 and 1.3 g/kg) within 1.5 and 2.3 hours. The peak BACs were between 0.095 and 0.170 g/100mL. The rate of elimination of alcohol at this lower dose was between 0.017 and 0.029 g/100mL/h (mean 0.022 g/100mL/h). The average Widmark r factor was 0.77.

The authors conclude that there are individuals whose BACs do not increase to toxic concentrations after drinking dangerously large amounts of alcohol.

Case Notes: MASSIVE CYANIDE OVERDOSE

Submitted by: Jeanne M. Beno, Ph.D., Monroe County Medical Examiner's Office, Rochester, NY 14623

A 51 y.o. caucasian male was found dead on the living room floor of his locked apartment. Nothing in the home appeared out of place and there was no evidence supporting the recent ingestion of any food, beverage, drugs or chemicals. Lividity was purple in coloration and there was a moderate degree of cervicofacial cyanosis. His wife related that he was under some stress since being laid off from work 4 mo. prior but did not relate any significant depression or suicidal ideation. Autopsy showed slight to moderate coronary artery disease and marked pulmonary and visceral congestion.

Routine post-mortem toxicology screening on blood, urine, liver and vitreous humor was significant only for the presence of therapeutic levels of alprazolam. On routine observation of the gastric contents it was noted that there was 400 mL of reddish brown fluid with a pH of 10.7. Four of 5 laboratory personnel who smelled the gastric contents indicated that it smelled of ammonia. In discussing the case with Dr. William Anderson of the OCME in North Carolina, he suggested that we test for cyanide as , in his experience, cyanide in gastric contents smelled like ammonia. Cyanide was isolated from samples using Conway diffusion cells and analyzed colorimetrically with the following results:

Heart blood	920 mg/L	Brain	15.5 mg/kg
Vitreous humor	211 mg/L	Urine	1.5 mg/L
Gastric contents	35 g as CN; 87.5 g as KCN		

Upon reinvestigation of the scene, and empty, 100g bottle of KCN was found hidden in a closet.

This case is remarkable for several reasons. First, this is the highest blood level of cyanide we have ever seen, either in our own laboratory or reported in the literature. Given the massive amount of cyanide in the stomach, blood levels may be due, in part, to continued diffusion of cyanide out of the stomach and into the blood post mortem. Secondly, one should not overestimate the speed with which cyanide can kill. Despite ingesting close to 100 g of KCN, the decedent managed to hide all evidence of chemical ingestion and collapse in his living room in what appeared to be a natural death. Aqueous solutions of KCN are alkaline (a 0.1N solution of KCN has a pH of 11.0) and are less rapidly fatal than an acidified solution in which HCN is generated. Cherry pink lividity cannot be relied upon to signal a cyanide fatality either. Routine determination of gastric pH and gastric odor can be instrumental in determining a cause of death. Of the 4 laboratory personnel who could smell ammonia the gastric contents of this case, none has the genetic ability to smell the "bitter almond" cyanide odor. Our technician who could not smell ammonia is a "cyanide sniffer" and could smell cyanide in the sample. Finally, this case pointed out a gross deficiency in our office Chemical Hazard Plan. Neither the autopsy rooms nor the laboratory has a cyanide antidote kit! Had the gastric contents been acidified in this case, it would have posed a significant hazard to all personnel involved.

Case Notes: BUTANE, 2-METHYLPROPANE AND PROPANE

Submitted by: Dean F. Fritch, Ph.D., DABFT, DABCC, National Medical Services, Inc., 2300 Stratford Ave., Willow Grove, PA 19090

Over the past several years, our laboratory has been receiving increasing numbers of requests to screen for the low-molecular weight aliphathic hydrocarbon gases. However, there is little information in the literature correlating concentration of these gases with effects.

In the next few paragraphs, I am going to summarize some of the sources of exposure, the results of our determinations, and some of the effects of the above-mentioned gases.

Sources of exposure: Propane and 2-methylpropane (isobutane) have largely replaced fluorocarbons as low-pressure aerosol propellants. Propane and butane are used in bottled gas (propane in the northern states and butane in the southern states). Butane is also found in cigarette lighters. Although there are other sources of exposure, these are the most common.

Results of blood screens for butane, propane and isobutane (1993-1995):

	No. Positive	Range (ppm)
Butane	16	2 - 60
Propane	11	5 - 85
Isobutane	4	11 - 69
Butane and Propane	2	
Butane and Isobutane	2	
None Detected	217	2 - 5

Population characteristics of positive results:

Gender		Age	
Male	21	<20 years	22
Female	4	>20 years	4
Unknown	8	Unknown	7

Effects of exposure: Clinically, volatile substance abuse has a rapid onset of intoxication followed by rapid recovery. Euphoria, disinhibition, hallucinations, tinnitus, ataxia, confusion, nausea and vomiting may be present.[1]

In high concentrations, these compounds exert a mild narcotic effect, but also act as simple asphyxiants. Arrhythmias leading to cardiac arrest are the cause of most deaths, but anoxia, respiratory depression, and vagal stimulation leading to cardiac arrest may contribute.[1] Seizures, hypotension and indirect causes, such as trauma, also contribute to deaths from butane, isobutane and propane abuse.[2] One report indicated that the brain may yield the highest concentrations of propane, followed by liver, lung, blood and kidney.[3]

It has been estimated that 3 to 10% of of young people have experimented with volatile substance abuse with a ratio of males to females of 3:1.[4] Our data support the male to female ratio (5:1) and the fact that most abusers are young (85% were less than 20 years old).

References

1. Flanagan R.J., et al. *An introduction to the clinical toxicology of volatile substances.* **Drug Safety** 5(5):359-383, 1990.
2. Siegel E., Wason S. *Sudden death caused by inhalation of butane and propane.* **N Engl J Med** 323(23):1638, 1990.
3. Haq M.Z., Hameli A.Z. *A death involving asphyxiation rom propane inhalation.* **J For Sci 23**(1):25-28, 1980.
4. Billington A.C. *Volatile substance abuse.* **Hum Toxicol** 8(4):323-5, 1989.

CALL FOR CASE NOTES

Your case note should be about 1/2 page in length, no more than a full page. Material or a disk (using Microsoft Works/Word 2.0) may be mailed to: **Joseph R. Monforte, Ph.D., DABFT, ToxTalk Editor**
846 Smoke Dr. (H.P.), Prescott, AZ 86301
- or - **Telephone/FAX: 520-717-0617** *(after 11:30 E.S.T.)*

Other items of interest to SOFT members are also welcome. **Next deadline: FEB. 1, 1996**

Jones AD, Dunlap MR, Gospe S Jr. **Stable-isotope dilution GC-MS for determination of toluene in submilliliter volumes of whole blood** Journal of Analytical Toxicology 18(5):251-4 1994

Aggarwal SK, Orth RG, Wendling J, Kinter M, Herold DA **Isotope dilution gas chromatography/mass spectrometry for cadmium determination in urine** Journal of Analytical Toxicology 17(1):5-10 1993

Ellerbe P, Long T, Welch MJ **The determination of amphetamine and methamphetamine in a lyophilized human urine reference material** Journal of Analytical Toxicology 17(3):165-70 1993

Wu AH, Onigbinde TA, Wong SS, Johnson KG **Identification of methamphetamines and over-the-counter sympathometic amines by full-scan GC-ion trap MS with electron impact and chemical ionization** Journal of Analytical Toxicology 16(2):137-41 1992

Hughes RO, Bronner WE, Smith ML **Detection of amphetamine and methamphetamine in urine by gas chromatography/mass spectrometry following derivatization with (-)-menthyl chloroformate** Journal of Analytical Toxicology 15(5):256-9 1991

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Musshoff F, Daldrup T, Bonte W **Gas chromatographic-mass spectrometric screening procedure for the identification of formaldehyde-derived tetrahydro-beta-carbolines in human urine** Journal of Chromatography 614(1):1-6 1993

van Rhijn JA, Traag WA, van de Spreng PF, Tuinstra LG **Simultaneous determination of planar chlorobiphenyls and polychlorinated dibenzo-p-dioxins and -furans in Dutch milk using isotope dilution and gas chromatography-high-resolution mass spectrometry** Journal of Chromatography 630(1-2):297-306 1993

Lundh T, Akesson B **Gas chromatographic determination of primary and secondary low-molecular-mass aliphatic amines in urine using derivatization with isobutyl chloroformate** Journal of Chromatography 617(2):191-6 1993

Desai DM, Gal J **Reversed-phase high-performance liquid chromatographic separation of the stereoisomers of labetalol via derivatization with chiral and non-chiral isothiocyanate reagents** Journal of Chromatography 579(1):165-71 1992

Bagnati R, Paleologo Oriundi M, Russo V, Danese M, Berti F, Fanelli R **Determination of zeranol and beta-zearalanol in calf urine by immunoaffinity extraction and gas chromatography-mass spectrometry after repeated administration of zeranol** Journal of Chromatography 564(2):493-502 1991

Franceschini A, Duthel JM, Vallon JJ **[Specific detection of urinary sympathomimetic amines for control of anti-doping by gas chromatography-mass spectroscopy]. [French]** Journal of Chromatography 541(1-2):109-20 1991

IMPLEMENTING STANDARD ADDITION TECHNIQUE IN FORENSIC TOXICOLOGY

Submitted by: Theodore J. Siek, Ph. D., DABFT, Analytic Bio-Chemistries, Feasterville, PA

If standard addition technique is commonly used in laboratories regularly involved in forensic toxicology, the key players have been reluctant to promote or even admit to this technique. No one questions that conceptually and in reality, standard addition is matrix-matched calibration. This technical note outlines how and when to apply standard addition technique to quantify and validate analytical determinations.

1. Since I don't know what Cx (concentration to be determined), how do I know how much analyte to add? If in a given forensic case , one does not have a clue as to the concentration of analyte, a solution is to do an uncalibrated run with an internal standard to obtain an approximate concentration, then do a standard addition run.
2. How many standard additions will give reliable, accurrate results? If an estimate is made of the analyte concentration prior to standard addition, then 1X and 3X the estimate (X is the estimated concentration) will yield an acceptable linear regression line for accurate results. Two points plus the no addition specimen plot will yield the analyte concentration on the X intercept (FIG. 1). More points can be included (1X, 2X, 4X, 8X) if ample speimen is available.
3. How do you set up standard additions? Treat all aliquots the same except for added analyte. Whatever volume of solvent or water is added should be the same for all aliquots including the unspiked aliquot.
4. What about standard dilution? You cannot remove analyte from the specimen without altering it, and diluting the specimen with blank identical specimen is not possible. Diluting with water may serve the purpose of bringing the signal into the linear range of the detection system. The dilution point cannot be plotted to establish the test line since you do not know where to plot the X point (concentration is not known in advance).
5. What specimens are amenable to standard addition? Any homogeneous specimen of which you have at least 3X as much as you need to do one test. Blood, bile, urine, other fluid, or homgenized tissue is appropriate.
6. How do you know you will be in the linear range? For solid phase and solvent extrations, partition constants are not concentration dependent, so the key is to recover sufficient analyte to be within the linear range of the detection system, whatever that might be (UV, FID, NPD detector). The initial run with sufficient internal standard added to be within the linear range of the detector (no calibration) will show if <u>any</u> analyte is detectable. If not, calibaration by any technique is not required.

The rationale for using standard addition technique for postmortem specimens is that no two postmortem bloods are identical, particularly aged specimens. A "blank" from prior subjects may significantly interfere with analyte or internal standard. If after standard addition, a significant signal increase does not occur, you may not be measuring analyte, but some interference. Standard addition provides validation of the determination in those very critical cases which might be challenged as to reliability of results obtained.

There are times when standard addition is not wise. If you are doing multiple specimens of the same type, then external calibrators are appropriate, since the number of calibrators for standard

additions would grow rapidly with number of test specimens. Standard addition when recoveries approach 100% offers no advantage. Non-linear test lines are more difficult to adapt to standard addition (1). If the specimens to be tested are all bloods, for example, standard addition to one of the bloods can establish a calibration for all specimens. In FIG. 1, line A is the standard addition line and line B a calibration for all specimens (runs parallel to B with same slope but zero intercept). Determining concentrations near the detection limit is inaccurate by normal calibration and standard addition.

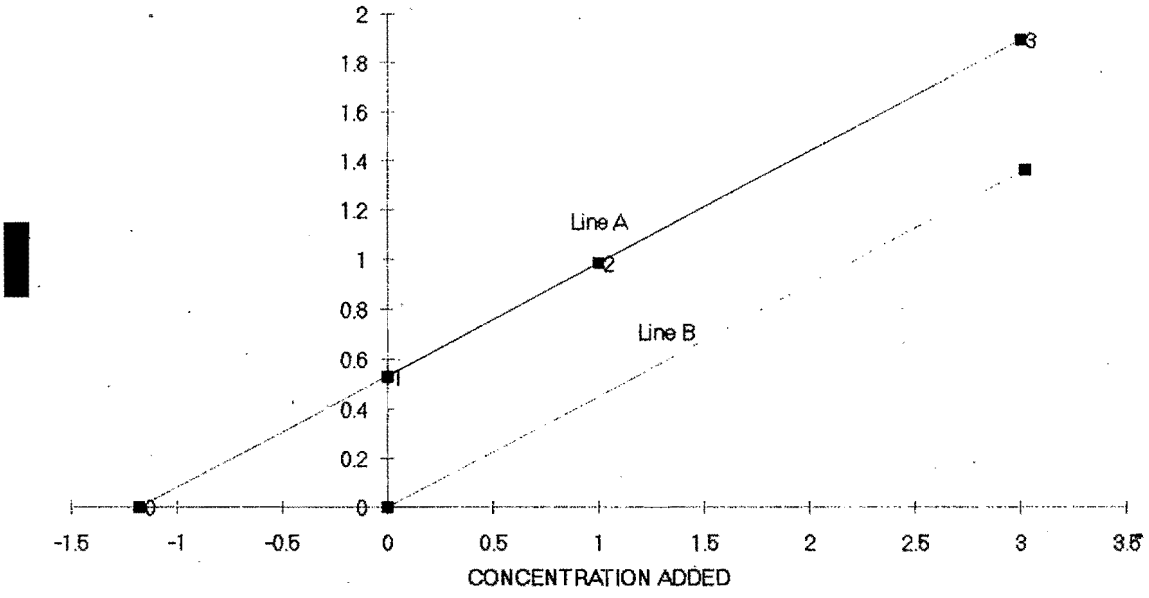

Figure 1. Line A is the standard addition test line. Points 1, 2, and 3 are unspiked, 1X, and 3X spiked concentrations, respectively. The intercept on the X axis at -1.1 gives the concentration of the test specimen., which is 1.1. Line B is the transposed test line for normal calibration.

If a preliminary analytical run is made without external calibrators or without standard addition, an external standard can beadded just before the final determination step to monitor recovery of internal standard. Recovering 80% or more of added internal standard indicates that the uncalibrated run gives a reasonable extimate of the analyte concentration. Standard addition accuracy diminishes if recoveries are poor, as is the case for normal calibration.

The techniques described here are for the purpose of saving time and simultaneously documenting analyte concentration by the best matrix-matching that can be done. Standard addition can be applied to GC, HPLC, TLC, and spectrophotometric assays which give linear test lines. Many immunoassays are non-linear in response, but can be done by variations of standard addition (author, unpublished).

1. T. J. Siek, and W. A. Dunn, "Quantitation by Standard Addition and Use of Three Instrumental Techniques," J. Forensic Sciences, Vol. 38, No. 3, 1993, pp. 713-720.
2. R. E. Van Atta and R. L. Vanatta, "An Experiment Employing Standard Addition," J. Chem. Ed., Vol 57, No. 3, 1980, pp. 230-231.

FROM THE HEALTH AND SAFETY COMMITTEE

Members: Daniel Isenschmid (Chair), John Cody, Laurel Farrell and Elizabeth Marker

OCCUPATIONAL EXPOSURE

Submitted by, John T. Cody, Ph.D.

Many different hazards face toxicologists as they do their everyday work. The very nature of the business (the analysis of toxic substances) brings the toxicologist into contact with hazardous substances. Many of the hazards are found in virtually all analytical laboratories, whether they be clinical, forensic, environmental, etc.

One difference between forensic and other laboratories is the potential exposure to illicit drugs. These can be the drug itself in either the solid or liquid form or in the standard materials used every day in the analysis of drugs. The hazard of exposure from the material itself is more evident in that the material is visible and contact can usually be envisioned by the individuals potentially exposed to the drugs. To some extent, the hazard of working in such an environment depends on the nature of the employment situation. Studies which evaluated the potential exposure and absorption of drugs in a laboratory setting have shown that the amount of drug absorbed is typically low, and, for most drugs, poses no real danger from the drug itself. If, however, the individual is part of a random drug testing program, even the low-level exposures found in the laboratory can cause positive urine drug testing results. These can also be at levels below which an individual would feel any effects from the drug, but would, nonetheless, test positive for the presence of the drug (or the metabolite).

Remember, if you work in a laboratory where the drug materials are handled, you must be aware of when and where they are used. If the bench at which you are about to work was just used for sampling powered cocaine and not properly cleaned, for example, you may be exposed to the drug without actually working with it. Another potential area of exposure to the drug is with the standard materials themselves, although the amounts of the drug used in the preparation of standard materials is relatively small.

Also, remember that the solvents used in the preparation of these standard materials are typically very effective in acting as a vehicle to transport compounds, such as drugs, across what would otherwise be barriers, like your skin. The solution to these problems, however, is found in standard laboratory personal protective equipment, such as gloves, a lab coat, working in a hood, and cleaning up your work area. Washing your hands after working in the lab before eating or drinking, no eating or drinking in the lab, etc. are all it takes.

The references listed below are related to this topic, but the list is not intended to be exhaustive. The references do, however, list a comprehensive bibliography of studies in this area.

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5. R Aaron and P Lewis. *Cocaine residues on money.* **Crime Lab Digest** 14:18 (1987).
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FUTURE ARTICLES: Chemical Spills (Isenschmid).

ABFT NEWS

ALTERNATIVE SPECIALIST EXAM: In the past, the ABFT offered the examination for Forensic Toxicology Specialist through the NRCC. This exam is offered once a year. The ABFT Board has approved an alternative to this examination which is to offer it at the annual SOFT and AAFS meetings. This provides more opportunities to write the examination and coincides with the current examination schedule for Diplomate (Ph.D.) applicant examinations.

Forensic toxocologists interested in certification by the American Board of Forensic Toxicology should contact:

ABFT Administrative Office, P.O. Box 669, Colorado Springs, CO 80901-0669
Telephone: 719-636-1100

BIBLIOGRAPHY: CHEMICAL HAZARDS

mitted by: Daniel Isenschmid, Ph.D.

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FREE SAFETY NEWS MAGAZINE #2: Occupational Health and Safety is an excellent magazine that includes many feature articles on health and safety. Some recent articles have included HIV/AIDS in the Workplace, Protected Workers Breathe Easier, How to Develop a Company Safety Manual, Truckers Prepare for Alcohol "Stupidity Test" and Friction Underfoot. This journal is free to "qualified" subscribers. You may contact the publisher at: Stevens Publishing, Occupational Health and Safety, Reader Service Management Department, P.O. Box 2573, Waco, Texas 76702. You may also reach the publishers by telephone (817) 662-1134 or fax (817) 662-7075.

Please feel free to submit comments or suggestions to: Daniel Isenschmid, Ph.D., Wayne County Medical Examiners Office, 1300 E. Warren, Detroit, MI 48207

S.O.F.T. MEMBERS TO RECEIVE A.A.F.S. AWARDS

The American Academy of Forensic Sciences recently announced the following recipients of the 1996 Toxicology Section awards.

Joseph R. Monforte - Rolla Harger Award - outstanding contributions to the profession

Amanda J. Jenkins - Irving Sunshine Award - contributions to forensic toxicology by a young scientist

Dennis Crouch - Raymond Abernethy Award - outstanding practioner of forensic toxicology

Congratulations to all! The awards will be formally presented at the 1996 AAFS annual meeting in Nashville.

ELMER GORDON OPEN FORUM
AN OPPORTUNITY FOR INFORMAL DIALOGUE

From The Arizona Republic 12/3/95: France has lowered to .05 per cent the blood alcohol concentration level at which it is illegal to drive. Until September, the limit had been .07 per cent. The new level conforms with a recommendation by European Transport Safety Council already in effect in Belgium, Norway, Finland, Portugal and the Netherlands.

CAREER OPPORTUNITIES

Positions available are listed for the consideration of SOFT members. There is no fee for this service. The information will be repeated in the next issue only if the information is confirmed by the person who submitted it.

Ph.D. experienced in development of assays for steroids using GC and LC separations and MS detectors, possible administrative and supervisory responsibility. Non-tenure track researcher, UCLA School of Medicine sport drug testing lab. CV and 3 reference letters to R. L. Hilderbrand, Ph.D., OAL, UCLA Pharmacology Dept., Los Angeles, CA 90095-1735.

Postdoctoral Fellow in Forensic Toxicology Ph.D. in analytical chemistry, biochemistry, pharmacology, toxicology or closely-related science, experience in bioanalytical tox methods and appreciation for Forensic tox casework desirable. Strong communication skills imperative. Send CV to National Medical Services, Inc., Attn: Dr. Dean Fritch. 2300 Stratford Ave., Willow Grove, Pa 19090.

PROFESSIONAL CALENDAR

California Association of Toxicologists (CAT) quarterly meetings and workshops. For information contact Vickie Watts at 602-644-2077, FAX 602-644-2478. **2/3/96** San Diego, CA, **5/4/96** Sacramento, CA, **8/3/96** San Diego, CA, **11/9/96** San Francisco, CA.

American Academy of Forensic Sciences (AAFS) annual meeting: Contact Brenda Papke, 719-636-1100. Future AAFS meetings: **2/19-24/96** Nashville; **2/17-22/97** New York City.

2nd IACP DRE Drugs, Alcohol and Impaired Driving Training Conference: May 12-14, Aspen, CO. Contact Chuck Peltier, IACP 703-836-6767 x224; Tom Page, LAPD, 213-485-8565; or Paul Helzer, Colorado Office of Transportation Safety, 303-757-9462.

Analytical and Molecular Biological Techniques in Environmental Toxicology and Forensic Sciences: September 11-12, San Juan, Puerto Rico. Sponsored by Puerto Rico Chemists Assoc and the American Registry of Pathology, $200. Contact Dr. Jose Centeno, AFIP, 14th & Alaska Ave. NW, Washington, DC 20306-6000. Ph 202-782-2839, Fax 202-782-9215

SOFT Annual Meeting: Oct. 14-18, 1996, Denver, Colorado. Co-hosts: Laurel J. Farrell and J. Robert Zettl, CDPHE - Division of Laboratories, P O Box 17123, Denver, CO 30217. Phone: 303-691-4727/4738. Fax: 303-393-7881.

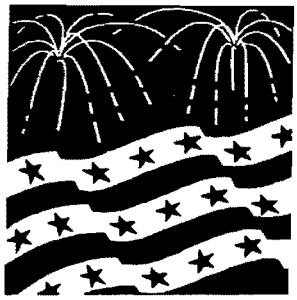
REMINDER - S.O.F.T. CONTACT INFORMATION:

VOICE MAIL & FAX 602-839-9106

MAILING ADDRESS P.O. Box 5543, Mesa, AZ 85211-5543

Submit your items for ToxTalk to:
Dr. Joseph Monforte, 846 Smoki Dr (HP), Prescott, AZ 86301

(ToxTalk print date: 12/14/95)



Baltimore 1995

25th Anniversary Meeting

October 9-14, 1995

ABSTRACTS

Special thanks to Dr. Edward Cone and his colleagues for providing the following abstracts from the 1995 S.O.F.T. meeting for inclusion in this issue of ToxTalk.

Joseph R. Monforte, Ph.D., DABFT
ToxTalk Editor

ToxTalk Volume 19, No. 4 (December 1995)

Meeting Host:
Yale H. Caplan, Ph.D.
3411 Philips Drive
Baltimore, MD 21208
phone: (410) 536-1700 fax: (410) 536-1617

1996 Annual Meeting

of the

Society of Forensic Toxicologists, Inc.

October 14 - 19

Denver, Colorado

Hotel: Denver Marriott Tech Center 1-800-228-9290

For information, check ToxTalk or contact 1996 SOFT Meeting Hosts:

Laurel J. Farrell or J. Robert Zettl
CDPHE - Division of Laboratories
P.O. Box 17123
Denver, CO 80217

FAX: 303-393-7881 Phone: 303-691-4727 or 303-691-4738

Other inquiries regarding S.O.F.T. should be addressed to:

Society of Forensic Toxicologists, Inc.
P.O. Box 5543
Mesa, AZ 85211-5543

FAX/Voice Mail: 602-839-9106

Detection Times of Marijuana Metabolites in Urine by Immunoassay and GC/MS

Marilyn A. Huestis[1], John M. Mitchell[2], and Edward J. Cone[1], ARC, NIDA, NIH, P.O. Box 5180, Baltimore, MD 21224 and [2]Navy Drug Screening Laboratory, H2033, Naval Air Station, Jacksonville, FL 32212*

It is assumed that marijuana use may be detected for extended periods after smoking. Detection times of cannabinoids in urine by GC/MS and eight cannabinoid immunoassays were determined in a controlled clinical study of marijuana smoking. The EMIT® d.a.u.™ 100, EMIT II 100, EMIT® d.a.u.™ 50, and EMIT II 50 from Syva, Co., San Jose, CA; Abuscreen® Online™ and Abuscreen RIA from Roche Diagnostic Systems, Branchburg, NJ; DRI™ from Diagnostic Reagents, Mountain View, CA; and AD_X® from Abbott Diagnostics, Abbott Park, IL were evaluated. Mean detection times were calculated from the times of the last positive immunoassay results equal to or greater than the specified cutoff. Mean ± SD detection times after smoking a 1.75% or 3.55% THC cigarette were 18.7 ± 6.7 h (range 0 to 48.5 h) and 45.7 ± 8.8 h (range 30.8 to 56.9 h), respectively. GC/MS detection times at 15 ng/mL were substantially longer, 33.7 ± 22.6 h (range 8 to 68.5 h) and 88.6 ± 23.2 (range 57 to 122.3 h). Detection times of cannabinoid metabolites in urine are shorter than commonly thought and are decreasing as the specificity of immunoassays increase. Knowledge of the sensitivity and specificity of immunoassays is essential for their proper use. Significant differences exist between the available immunoassay products and affect the efficiency of detection of drug use. These results indicate that recent reductions in cannabinoid cutoffs by military and Federally-mandated programs will increase detection times and improve sensitivity, as expected.

Relationship of Three Blood and Urine Cannabinoids and PerformanceAfter Smoking Marijuana

Barbara R. Manno[1,4], Joseph E. Manno[2,4], Imad K. Abukhalaf[2,4], Philip M. Kemp[5], Mary E. McWilliams[3,4], Frances Nixon[1], Mary Jo Fitzgerald[1], and Roy R. Reeves[1], Departments of [1]Psychiatry, [2]Medicine, [3]Neurology and the [4]Center of Excellence in Clinical and Forensic Toxicology, LSU Medical Center, Shreveport, LA 71130 and the [5]Chief Medical Examiner's Office, Oklahoma City, OK*

Casual marijuana user-volunteers (N=4 male, 4 female; 18-35 y.o.) assigned to a double-blind, 3 x 3 random dosing protocol smoked three doses of marijuana (placebo, 1.77% THC, 3.54% THC). Drug was administered at weekly intervals using a computer controlled, paced-smoking procedure. Performance was evaluated before and after smoking at hourly intervals for eight hours post smoking. Blood was collected at 1 min intervals following initiation of smoking. Blood was also collected at 0, 0.5, 1 hour and hourly thereafter for 8 hours post smoking. Urine was collected as often as available on a similar time protocol. Performance as a simple tracking task was measured using a Pursuit Meter III device. Preliminary evaluation of data suggested that performance was impaired after smoking the 3.54% THC cigarette when compared with the placebo dose. Whether the performance impairment correlated with the excretion profiles of one or more of the cannabinoids monitored in this study is currently under statistical investigation. Protocol approved by LSUMCS Institutional Review Board for Human Research. Supported by NIDA Grant No. 05850.

Excretion Profile of Three Cannabinoid Metabolites After Smoking Marijuana

Joseph E. Manno[1,2,4], Imad K. Abukhalaf[1,4], Barbara R. Manno[2,4], Philip M. Kemp[5], Dempsey D. Alford[1,4], Mary E. McWilliams[3,4], Frances Nixon[2], Joel R. Mills[4], Christopher J. Achee[4], and Emily E. Robinson[4], Departments of [1]Medicine, [2]Psychiatry, [3]Neurology and the [4]Center of Excellence for Clinical and Forensic Toxicology, Louisiana State University Medical Center, Shreveport, LA 71130 and [5]Chief Medical Examiner's Office, Oklahoma City, OK*

Urine specimens were collected over a 30 hour period from eight healthy, occasional marijuana user-volunteers (18-35 y.o.) who smoked marijuana cigarettes containing 0% (placebo), 1.77%, and 3.54% Δ^9-tetrahydrocannabinol (THC). One mL of urine was incubated overnight at 37°C with β-glucuronidase enzyme from *E. Coli* (bacteria) to free the glucuronide-linked metabolites. Specimens were then extracted, derivatized and analyzed by gas chromatography/mass spectrometry for Δ^9-tetrahydrocannabinol (Δ^9-THC), 11-hydroxy-Δ^9-THC (11-OH-THC), and 11-nor-Δ^9-THC-9-carboxylic acid (Nor-COOH-THC) by the method of Kemp et al. (J Anal. Tox., 19:285-291; 19:292-298, 1995). Excretion profiles for the three metabolites suggested that Δ^9-THC concentration peaked earlier than 11-OH-THC whereas Nor-COOH-THC took longer to reach its peak concentration in urine. Urine Δ^9-THC concentrations returned to baseline (0 ng/mL) within 4 to 5 hours after smoking one marijuana cigarette. The concentrations of 11-OH-THC and Nor-COOH-THC remained above baseline after 30 hours post smoking. The protocol was approved by the LSUMCS Institutional Review Board for Human Research. Supported by NIDA Grant No. DA-05850.

Base and Enzymatic Hydrolysis of Cannabinoids in Human Urine

Imad K. Abukhalaf[1,3], Philip M. Kemp[4], Barbara R. Manno[2,3], Joseph E. Manno[1,2,3], and Dempsey D. Alford[1,3], Louisiana State University Medical Center, Departments of [1]Medicine, [2]Psychiatry and the [3]Center of Excellence for Clinical and Forensic Toxicology, Shreveport, LA 71130 and [4]Chief Medical Examiner's Office, Oklahoma City, OK*

Base and enzymatic hydrolysis were compared to examine their efficacy in hydrolyzing cannabinoid glucuronides in human urine. In addition to base hydrolysis, enzymatic hydrolysis of specimens was performed with three prototypes of β-glucuronidase from three different sources: *Escherichia coli* (bacteria), *Helix pomatia* (snail) or *Patella vulgata* (limpet) as described by Kemp *et al.* (J. Anal. Tox., 19:285-291; 19:292-298). Urine was obtained one hour after human subjects (N=6) each

smoked one marijuana cigarette containing 3.58% THC. Pooled urine (1mL) was buffered to the optimal pH for each form of the enzyme tested. The ß-glucuronidase was added to the specimens, incubated, extracted, and derivatized for gas chromatographic/mass spectrometric (GC/MS) analysis. Data revealed differences between base and enzymatic hydrolysis and the prototype of ß-glucuronidase used in terms of the concentration of the cannabinoids detected. The recovery of free 11-nor-Δ^9-THC-9-carboxylic acid (nor-COOH-THC) was comparable regardless of hydrolytic treatment. Data suggested that quantitative differences in the yield of Δ^9-tetrahydrocannabinol (Δ^9-THC) and 11-hydroxy-Δ^9-THC (11-OH-THC) was dependent upon ß-glucuronidase source. The protocol was approved by the LSUMCS Institutional Review Board for Human Research. Supported by NIDA Grant No. DA-05850.

Relationship of Alpha Brain Wave Activity and Three Cannabinoids Levels in Marijuana Smokers

Mary E. McWilliams[1,4], Philip M. Kemp[5], Imad K. Abukhalaf[2,4], Barbara R. Manno[3,4], Frederick A. Struve[3,4], Gloria Patrick[3], Frances E. Nixon[3], Mary Jo Fitzgerald[3], Roy R. Reeves,[3] and Joseph E. Manno[2,4], Departments of [1]Neurology, [2]Medicine, [3]Psychiatry and the [4]Center of Excellence in Clinical and Forensic Toxicology, Louisiana State University Medical Center, Shreveport, LA 71130 and the [5]Chief Medical Examiner's Office, Oklahoma City, OK*

Alterations in brain wave activity have been reported in humans as a result of exposure to the primary psychoactive component of marijuana, Δ^9-tetrahydrocannabinol (Δ^9-THC). Healthy volunteers (N=8; 18-35 y.o.), assigned to a double-blind 3 x 3 randomized dosing design, smoked marijuana cigarettes (0%, 1.77% and 3.58% Δ^9-THC) according to a computer controlled, paced-smoking protocol. Eyes closed, 21-channel topographic quantitative electroencephalograms (QEEG), using the International 10-20 Placement System, were recorded prior to smoking, during the first half of smoking (0-4 min), the second half of smoking (5-8 min) and at 4 post smoking periods. Blood and urine specimens were collected prior to smoking, immediately after smoking and hourly thereafter for 8 hours post smoking and analyzed for Δ^9-THC, and 11-nor-9-carboxy-Δ^9-THC by GC/MS. QEEG data indicated that temporal body fluid profiles of the Δ^9-THC and 11-hydroxy-Δ^9-THC and alpha wave activity (8-13 Hz) occurred during and after smoking. A dose dependent, significant increase in alpha activity was recorded by the pre-frontal and frontal electrodes during and after smoking the 3.54% Δ^9-THC cigarette. Alpha activity with this dose peaked during the second half of the smoking period and had not returned to baseline 4 hours post-smoking. The protocol was approved by the LSUMCS Institutional Review Board for Human Research. Supported by NIDA Grants No. DA 05850 and DA 06643.

The Stability of Morphine Glucuronide in Blood Samples

Hans Sachs, Ludwig von Meyer, and Gustav Drasch, Institute of Legal Medicine, Frauenlobstr 7a, 80046 Munich, Germany.*

Heroin and morphine are metabolized to morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G). Recently, free morphine concentrations in blood have been used to evaluate the effect of opiates, and the ratio of free morphine to morphine glucuronides has been used to estimate the time of application or the survival time, respectively. This is useful only if one assumes that morphine is the only active substance and that the glucuronides are stable during storage. However, M6G is as active pharmacologically as morphine. Recent experiments have shown that, during storage, glucuronides can be totally degraded and morphine levels increase. After chronic administration, M3G concentrations increase more than 10-fold relative to free morphine concentrations. As M3G is considered to be inactive, degradation of M3G to morphine during storage leads to artificially high concentrations of morphine and false estimations of survival time.

DCI/MS Confirmation of Urinary Demoxepam Identified by an HPLC Method

Herbert Essien, S. Jason Lai, Steve R. Binder and Dave L. King, Clinical Systems Division, Bio-Rad Laboratories, 4000 Alfred Nobel Drive, Hercules, CA 94547*

Demoxepam is a major metabolite of chlordiazepoxide. We have observed that demoxepam degrades in situ during GC/MS analysis, and the breakdown product is nordiazepam. In this study, we report the use of an off-line HPLC method followed by DCI/MS, desorption chemical ionization/mass spectrometry, confirmation of demoxepam in urine.

Ten benzodiazepine positive urine samples analyzed by the EMIT immunoassay were positive for demoxepam using a modified REMEDi-HS system. The urine samples were enzymatically hydrolyzed prior to HPLC analysis. The demoxepam peak fraction from the REMEDi-HS system was collected for DCI/MS analysis. A simple liquid/liquid extraction was performed to remove inorganic salts. The final extracts were applied to a DCI probe (Vacumetric, Inc.) for mass spectrometric analysis. A Hewlett-Packard 5989A mass spectrometer was used.

Standards of demoxepam and nordiazepam were also analyzed by DCI/MS, and their DCI mass spectra were different from each other. The molecular ions produced by demoxepam and nordiazepam were present in their mass spectra. The extraction efficiency of demoxepam from a spiked standard was 95%. The limit of detection of demoxepam by DCI/MS in the full scan mode was 400 ng/mL (40 ng on platinum wire) and 50 ng/mL (5 ng on platinum wire) in the selected ion monitoring mode. We believe that this is the first direct confirmation method reported for demoxepam.

Determination of Naltrexone and 6ß-Naltrexol in Plasma by Solid Phase Extraction and Gas Chromatography/ Negative Ion Chemical Ionization Mass Spectrometry

Wei Huang, David E. Moody and Rodger L. Foltz, Center for Human Toxicology, Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, UT 84112*

A previously reported method (K.M. Monti, R.L. Foltz and D.M. Chinn, J. Anal. Toxicol., 1991,15,136-40) for the determination of naltrexone and 6ß-naltrexol in plasma has been simplified by using solid phase extraction and a one-step derivatization, prior to analysis by gas

chromatography / negative ion chemical ionization mass spectrometry. The deuterated analogs of naltrexone (d_3) and 6ß-naltrexol (d_7) were used as internal standards. After solid phase extraction, the extracts were derivatized with pentafluoropropionic anhydride (PFPA) at room temperature to form predominately the bis-pentafluoropropionyl derivative of naltrexone and the tris-pentafluoropropionyl derivative of 6ß-naltrexol. The derivatized extracts were analyzed by selected ion monitoring ion currents at m/z 633 (naltrexone-d_0), m/z 636 (naltrexone-d_3), m/z 633 (6ß-naltrexol-d_0), and m/z 640 (6ß-naltrexol-d_7). To validate the assay, control plasma samples containing 0.3, 3, or 30 ng/mL of each analyte were analyzed for precision and accuracy with the following results: within run, the % of target concentrations were 102-117% for naltrexone and 113-117% for 6ß-naltrexol, and the C.V.'s were 3.6-7.0% for naltrexone and 3.4-5.6% for 6ß-naltrexol; between runs, the % of target concentrations were 111-117% for naltrexone and 110-120% for 6ß-naltrexol, and the C.V.'s were 2.7-10.6% for naltrexone and 1.9-8.7% for 6ß-naltrexol. The limit of quantitation for both analytes was 0.1 ng/mL with accuracy within 20% and % C.V.'s ≤ 15%. (Supported by NIDA contract N01-DA-1-9205.)

Quantitation of Alprazolam and α-Hydroxyalprazolam in Human Plasma and Rat Hair by Negative Ion Chemical Ionization GC/MS

Karin M. Höld[*1], *Dennis J. Crouch*[1], *Diana G. Wilkins*[1], *Douglas E. Rollins*[1] *and Dennis V. Canfield*[2], [1]*Center for Human Toxicology, University of Utah, Salt Lake City, UT 84112 and* [2]*Toxicology and Accident Research Laboratory, FAA Aeronautical Center, Oklahoma City, OK*

Alprazolam (Xanax®) is a relatively new and popular benzodiazepine. A sensitive and specific method has been developed for the quantitative determination of alprazolam (AL), and its major metabolite α-hydroxyalprazolam (OH-AL) in plasma and hair. After the addition of deuterium labeled internal standards, plasma samples were buffered to ~pH 9 with 1 mL of saturated sodium borate buffer, extracted into toluene:methylene chloride (7:3) and evaporated to dryness. Dried extract residues were treated with BSTFA containing 1% TMCS and analyzed on a Finnigan-MAT™ mass spectrometer operated in the negative-ion chemical ionization mode with methane as the reagent gas. Chromatographic separation was achieved on a Restek 200™ (15M x 0.25µ) capillary column with hydrogen as the carrier gas. The assay was linear from 0.25 to 50 ng/mL for both compounds. Intra-assay precision for AL at 0.5 ng/mL was 16.1% and 4.6% at 50 ng/mL. Intra-assay precision for OH-AL at 0.5 ng/mL was 15.8% and 4.2% at 50 ng/mL. The method was used to quantitate AL and OH-AL in human plasma samples collected after a single 2 mg oral dose of AL. Peak concentrations of 47.1 ng/mL of AL and 0.9 ng/mL of OH-AL were detected at 5 hours following the dose.

The method has also been used to determine if AL is deposited into hair. AL was administered to rats at 5 mg/kg twice a day, ip, for 5 days. Hair was collected prior to dosing and at 14 and 28 days, digested with 1N NaOH, extracted and analyzed as described above. The concentration of AL in the hair collected on day 14 ranged from 213-658 pg/mg.

Totally Automated Sequential Analysis of Tricyclic Antidepressants by Solid Phase Extraction Module System and Reverse Phase HPLC Analysis with a Base Deactivated C-18 Column

Mark McGuire[1*], *Steven H. Wong*[1,2], *Victor Skrinska*[1], *Kimber Fogelman*[3], *and Wayne Miles*[3], [1]*Department of Health Sciences-University of Wisconsin,* [2]*Department of Pathology-Medical College of Wisconsin, Milwaukee, WI 53226 and* [3]*Hewlett Packard, Little Falls, DE*

Amitriptyline (Ami) and nortripyline (Nor) are tricyclic antidepressants. Nor, also the demethylated metabolite of Ami, interferes with the transport, release and storage of catecholamines, and inhibits the activity of histamine, 5-hydroxytryptamine, and acetylcholine. Ami inhibits the re-uptakes of norepinephrine and serotonin. The current study evaluated their analysis by Hewlett-Packard Bench Supervisor software, automated PrepStation and HPLC system. The PrepStation software is a Microsoft Windows based program for sample preparation methods. The system is designed to handle sample preparation functions up to the point the sample is injected into the analyzer. Sample preparation for assay by solid phase extraction used disposable 100 mg DAU cartridges. The sample was mixed with the internal standard, clomipramine (Clo), and then applied to the DAU cartridge. Elution was performed with methylene chloride, isopropanol and conc. ammonium hydroxide (80:20:2) and an aliquot was injected into a Hypersil BDS-C18 column. Chromatographic parameters were: mobile phase, phosphate buffer (pH 4.7)/acetonitrile (6:4), 214nm, and 2 mL/min. Retention times of Nor, Ami, and Clo were 3.9, 4.5, and 6.5 min. Total extraction and analysis time was about 50 min/sample. Recoveries ranged from 30 to 50% and sensitivity was 25ng. Precision studies showed within-run CV's: n=10, Ami 2.1%, Nor 5.3%; and between-run CV's: n=22, 9.7% and 7.1%, respectively. Preliminary studies showed patients samples n=3 with Nor concentration ranged from 50 to 245 ng/mL, and Ami 92 to 251 ng/mL. This result shows that the procedure can be used for monitoring patients treated with Ami and Nor.

Simultaneous Solid Phase Extraction of Whole Blood and Urine for Cocaine, Benzoylecgonine and Morphine Using PFPA/HFIP Derivatization with GC/MS Confirmation

Laureen Marinetti-Sheff, Michigan State Police Crime Laboratory, Toxicology Subunit, 714 S. Harrison St., East Lansing, MI 48823

The objective of this study was to develop a simple, relatively clean extraction for biological specimens for cocaine, morphine and benzoylecgonine. The Worldwide Monitoring Corporation procedure "Cocaine and Benzoylecgonine in Serum, Plasma or Whole Blood for GC or GC/MS Confirmation Using 200 mg Clean Screen Extraction Column" was utilized with the following modifications: samples were loaded onto the column and drugs were eluted without aspiration; 4 mL of extraction solvent instead of 3 mL and the final eluent was split 1/3 and 2/3 with the former fraction derivatized with PFPA and HFIP and the latter fraction evaporated and injected neat on to the GC/MS in EI mode utilizing full scan.

It was determined that this procedure was excellent in the detection and identification of these three compounds. The limit of detection was 1 ng on column for cocaine,

HFIP derivative of benzoylecgonine and the di-PFPA derivative of morphine. Lower limits of detection were possible depending upon the condition of the sample. For example, if excessive amounts of biological co-extractives were not present, better detection limits could be achieved.

This procedure was performed with both antemortem and postmortem blood and urine specimens. Other drugs detected included the PFPA derivative of codeine, methylecgonine, ethylecgonine and the isopropyl derivative of benzoylecgonine*. Additional non-derivatized drugs were also detected in the neat fraction including codeine, diazepam, N-desmethyldiazepam, methadone, ethyl cocaine, cotinine, nicotine and carbamazepine.

The results indicated that confirmation of cocaine, benzoylecgonine and morphine can be accomplished in a simple, one step extraction procedure with detection limits as low as 1 ng on column. Recovery rates for morphine, cocaine and benzoylecgonine were as follows: 75%, 88% and 92%, respectively.

* The isopropyl derivative of benzoylecgonine arises during the elution process where the solvent mixture is methylene chloride, isopropyl alcohol and ammonium hydroxide. It is present in the neat fraction at concentrations greater than 20 ng on column.

A Practical Approach to Determination of Laboratory GC/MS Limits of Detection

Paula J. Underwood, Gerald E. Kannanen, and Edwin K. Armitage, Forensic Toxicology Drug Testing Laboratory, 2490 Wilson Street, Fort George G. Meade, MD 20755-5235*

Determination of Limit of Detection (LOD) values in a forensic laboratory serves a fundamental forensic requirement for assay performance. In addition to demonstrating assay capability, LOD values can also be used to fulfill certification requirements of a high volume forensic drug determination laboratory. Information outlined in "Limit of Detection (LOD)/Limit of Quantitation (LOQ): Comparison of the Empirical and the Statistical Methods Exemplified with GC-MS Assays of Abused Drugs" by D. A. Armbruster *et al.* was used as a starting point for our practical approach. We define the LOD as the lowest concentration of drug that the laboratory will detect in a sample with forensic certainty at a minimum of 85% of the time. Overall batch acceptance criteria included acceptable quantitation of control materials (within 20% of target), acceptable chromatography (symmetry, peak integration, peak shape, peak and baseline resolution), retention time within ±1% of the extracted standard and mass ion ratios within ± 20% of the extracted standard mass ion ratios. Individual specimen acceptance criteria are the same as the batch acceptance criteria excluding the quantitation requirement. Data was collected from all instruments, on different runs. A minimum of 10 data points were required per certified instrument with a minimum of 85% of data points being acceptable. Quantitation within ±20% of the LOD concentration was not required, but acceptable mass ratios are required. Data points with poor chromatography (internal standard failed mass ratios, interference of the baseline, i.e., shoulders, asymmetry and baseline resolution) were omitted from the acceptable rate calculation. Data points with good chromatography with failed mass ion ratios were included. With these criteria, we established the following LOD's: 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid - 2 ng/mL; benzoylecgonine - 5 ng/mL; phencyclidine - 2.5 ng/mL; amphetamine - 150 ng/mL; methamphetamine - 100 ng/mL; codeine - 500 ng/mL; morphine - 1000 ng/mL.

Chemical Profiling of "Ecstasy" Specimens

Katrin Bracher, Daniel Bourquin, Hans-Jörg S. Helmlin and Rudolf M. Brenneisen, Institute of Pharmacy, University of Berne, Baltzerstrasse 5, CH-3012 Berne, Switzerland.*

In Europe the hallucinogen 3,4-methylenedioxymethamphetamine (MDMA, "Ecstasy", "E", "Adam", "XTC") in addiction to Cannabis is the most abused illicit drug at all-night "rave" parties. The variable composition and content, which are usually unknown to the consumer, may produce unpredictable psychotropic and somatic effects and a high risk of intoxication. As part of an ongoing long-term analytical monitoring of "Ecstasy" street samples, we report the chemical profiles of 57 specimens confiscated in 1995 in Switzerland at rave events. After the photographic documentation, HPTLC was used for the initial qualitative screening, followed by GC/MS for identification and HPLC-DAD for quantitation. Additionally, identification of unknown compounds was performed by NMR after isolation.

Thirty specimens contained MDMA (0.05-154 mg) and/or the related compounds, 3,4-methylenedioxyethylamphetamine (MDEA, "Eve", "Love") and N-methyl-1-(3,4-methylene-dioxyphenyl)-2-butanamine (MDMB), as well as amphetamine and caffeine. Five specimens contained amphetamine and methamphetamine or amphetamine, ephedrine and caffeine. Other drugs (mainly analgesics and antidepressants) were identified in 14 specimens; e.g., paracetamol, ibuprofen, amitriptyline, tramadol, ephedrine, and norephedrine. In 8 specimens no psychoactive substance was detected. The study also showed that an identical logo on an "Ecstasy" tablet does not always mean identical composition and content.

Pharmacokinetic Profiles of Oral and Intravenous Psilocybin

Felix Hasler, Daniel Bourquin, Rudolf M. Brenneisen, Institute of Pharmacy, University of Berne, Baltzerstrasse 5, CH-3012 Berne, Switzerland and F.X. Vollenweider, Research Department, Psychiatric University Hospital, Zurich, Switzerland.*

To study the relationship between psilocybin (PY)-induced psychotic symptoms and cerebral energy metabolism, a PY model of psychosis using positron emission tomography (PET) and the radioligand ^{18}F-fluorodeoxyglucose (FDG) was established. One important experimental aspect was to correlate PY-induced psychosis and the plasma levels of psilocin (PI), the main active metabolite of the prodrug PY. Therefore, a procedure using reversed-phase high-performance liquid chromatography with electrochemical detection (HPLC-ECD) for accurate and sensitive determination of PI and 4-hydroxy-indole-3-acetic acid (4HIAA) in plasma was developed. A real analytical challenge was the extreme unstability of the phenolic PI and its low concentration in plasma. After stabilization with ascorbic acid the

lyophilized plasma were extracted by microdialysis. The analysis of PI was performed on 3µm-Spherisorb C_8 with column-switching. The mobile phase was 47% (v/v) water containing 0.3 M ammonium acetate buffered to pH 8.3 with concentrated ammonia and 53 % (v/v) methanol. For the determination of 4HIAA a 5µm-Lichrospher 100 RP-18 column was used. The mobile phase was 70 mM phosphoric acid containing 5.5 % (v/v) acetonitrile and 300µL/L hexylamine. After an oral dose of 15 mg PY the peak plasma concentrations of PI and 4HIAA were 12.3 (after 88 min) and 335 ng/mL (after 290 min). After an I.V. dose of 3 mg PY the peak plasma concentration of PI was 8.5 ng/mL after 19 min, 4HIAA was not detectable.

Plasma Profile After Intravenous Administration of 200 mg Diacetylmorphine HCl (Heroin Maintenance Program)

Daniel Bourquin, Barbara von Wattenwyl, Thomas Lehmann, and Rudolf M. Brenneisen, Institute of Pharmacy, University of Berne, Baltzerstrasse 5, CH-3012 Berne, Switzerland; Robert B. Hämmig, KODA-1, Department of Social Psychiatry, University of Berne, CH-3012 Berne, Switzerland; and Michael Bührer, Department of Anesthesia, University Hospital of Berne, CH-3012 Berne, Switzerland.*

The Swiss Federal Administration of Health started in 1994, as part of a 3-year pilot project, to dispense diacetylmorphine (heroin, DAM) to heavy heroin addicts (Heroin Maintenance Programs). Supporting pharmaceutical research is mainly focused on pharmacokinetic and pharmacodynamic aspects of different application forms (I.V., oral, pulmonalry, etc.) of high-doses of DAM.

Here we report about the methodology used to establish the plasma profile of a patient who received 200 mg DAM hydrochloride I.V. One-mL plasma samples were extracted by SPE and analyzed by HPLC-DAD. Within a 3h blood collection phase, the peak plasma concentrations of DAM, 6-monoacetylmorphine (MAM), morphine (M), morphine-6-O-glucuronide (M6G) and morphine-3-O-glucuronide (M3G) were 1494, 3410, 166, 430 and 3852 ng/mL, respectively. DAM could be detected up to 8 min, the DAM metabolites up to 180 min.

In addition, samples from a clinical intoxication case with M were analyzed. The HPLC data showed a perfect correlation of the time of M3G peak plasma level and the coma of the patient. After a total I.V. dose of 110 mg of M the peak plasma concentrations were 93, 687 and 6047 ng/mL for M, M6G and M3G, respectively.

Optimization of Urinary 11-Nor-9-Carboxy-Δ^9-Tetrahydrocannabinol Recovery Using C_{18} Solid Phase Extraction Columns

Leon R. Glass, Donald J. Rynbrandt and Yun S. Kwak, Pathology and Laboratory Medicine Service, 113-B, Veterans Administration Medical Center, 10000 Brecksville Road, Brecksville OH 44141*

The steps in the sample preparation process of urinary 11-Nor-9-carboxy-Δ^9-tetrahydrocannabinol, THC-COOH, using C_{18} solid phase extraction, SPE, columns were investigated. Five milliliter aliquots from a pool of 17 ng/mL THC-COOH in urine were used as the analyte for all studies. Deuterated standard; d_3-11-Nor-9-carboxy-Δ^9-tetrahydrocannabinol, d_3-THC-COOH; was introduced at separate points in the process to isolate the individual preparation steps in order to evaluate the loss of analyte at each phase. The TMS derivatized samples were analyzed by gas chromatography followed by electron impact mass spectrometry (GC/MS) using selective ion monitoring (SIM).

The parameters investigated included: sample pH, composition of wash solutions, column bed drying prior to elution, and eluant strength. The final conditions: 5 mL sample hydrolyzed with 0.2 mL 10N NaOH at 60°C for 30 min, adjusted to pH 3.0-3.3 with 10:1 glacial acetic acid/conc. HCl, was applied to SPE columns pretreated with 3 mL methanol, 3 mL deionized water, and 1 mL 0.1N HCl. The columns were washed with 3 mL deionized water followed by 2 mL of acetonitrile/0.1N HCl (30:70, v/v). The columns were dried by application of 5" Hg vacuum for 3 min. The dried columns were washed with 0.25 mL hexane and eluted with 3 mL hexane/ethyl acetate (1:1, v/v). The eluant was dried under a stream of dry, oil-free air in a 40°C heating block. The residue was reconstituted in 0.4 mL ethyl acetate/methanol (70:30, v/v) and transferred to an autosampler vial. The sample was redried as above, reconstituted with 0.05 mL of derivatization grade acetonitrile and capped. The samples were derivatized by the addition of 0.05 mL of BSTFA + 1% TMCS and heated for 45 min in a 70°C heating block. The derivatized samples were transferred to autosampler microvials and recapped for GC/MS analysis. These conditions provided 95% recovery, and 0.47 ng/mL as the limit of detection with an undamaged column and clean source.

A Reliable Method for the Detection, Confirmation and Quantitation of Cannabinoids in Blood

Christine R. Goodall and Barbara J. Basteyns, Wisconsin State Laboratory of Hygiene, Department of Toxicology, 465 Henry Mall, Madison, WI 53706*

A sensitive and reliable method was developed for the identification and quantitation of cannabinoids in blood. Samples were screened by fluorescence polarization immunoassay. The procedure used for confirmation and quantitation was a modification of a procedure published by Foltz, et al. Analysis was performed on a bench top mass selective detector using selected ion monitoring. The limits of detection were 0.2 ng/mL for Δ^9-tetrahydrocannabinol (THC) and 11-hydroxy-THC and 2 ng/mL for 11-nor-9-carboxy-THC. Extensive method validation is presented including within run variation, between run variation and results from external proficiency testing. Day to day variation resulted in CV's of 8-11% for THC and 11-12% for carboxy-THC. Standard deviation of duplicate results was very good. Sample stability was studied over a six month period. Data from a blind study of 217 samples showed a predictive value of 90% for a positive screening test and 99% for a negative screening test. The procedure is used routinely in the laboratory on samples from drivers issued a citation for impaired driving and also on postmortem blood from death investigations.

Adsorption Characteristics of Plastic Containers for Urinary Δ^9-THCA. Part II.

Robert F. Foery, Monica R. Artis, Ramakrishnan K. Seshadri, and Roger L. Rutter, National Laboratory Center, Inc., MedExpress, 4022 Willow Lake Blvd., Memphis, TN 38118*

The adsorption of 9-carboxy-11-nor-Δ^9-tetrahydrocannabinol (Δ^9-THCA) from biological matrices to plastic surfaces has been documented in the literature and related to container composition as well as to the ratio of specimen volume to surface area. In a previous collaborative study, we reported on the evaluation of a variety of polyolefin urine specimen containers commonly used in drug testing to determine surface adsorption characteristics as a function of the time lapse between date of manufacture and date of use.

Drug free urine (15 or 30 mL) spiked with various concentrations of Δ^9-THCA or commercial urine drug controls were introduced into 35-mL polypropylene containers which varied by date of manufacture. Spiked solutions were equilibrated at ambient temperature for 12 to 36 hours prior to GC/MS or FPIA analysis.

The data indicate that polypropylene containers, manufactured from Petrothene® PP8004-ZR (Quantum Chemical Corp.) over the period 1/29/93-3/2/94, gave recoveries greater than 99% at 60 ng THCA/mL (36 hrs) and greater than 96.4% at 120ng/mL (36 hrs) when compared to silylated glass (36hrs). Recovery appears to be independent of the age of the manufactured container.

Other experiments suggest that virgin polypropylene containers (0% polyethylene) give excellent recovery data for amphetamines, benzoylecgonine, opiates, and PCP.

Investigation of Cannabinoid Loss Using the TD_XFL_X®, A_XSYM®, and X-ray Photoelectron Spectroscopy

Kenneth D. W. Roth[1], Neal A. Siegel[2], Robert W. Johnson[2], Laszlo X. Litauszki[2], Lawrence Salvati[2], Charles A. Harrington[2], and Larry K. Wray[2], [1]Chemistry Department, Michigan State University, East Lansing, MI 48824-1322 and [2]Abbott Laboratories, 100 Abbott Park Road, Abbott Park, IL 60064-3500*

The loss of cannabinoids from aqueous solutions, presumably due to binding to solid surfaces, is a well-documented phenomenon. The amounts of cannabinoid which bind to a variety of surfaces were determined with the goal of minimizing handling losses. X-ray photoelectron spectroscopy was used to detect the presence of cannabinoids at solid surfaces. TD_XFL_X® and A_XSYM® instruments were used to measure the following losses of 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid from 100 ng/mL solutions when stored in various materials overnight:

Material	Loss from Water (ng/cm^2)	Loss from Urine (ng/cm^2)
Untreated glass	0.0	0.9
Polymethylmethacrylate	0.8	0.9
Silylated glass	1.1	1.1
Teflon	4.1	3.0
Polystyrene	3.9	3.4
Polypropylene (Type 1)	5.7	4.2
Polypropylene (Type 2)	7.2	5.0
High density polyethylene	9.7	3.8

The effect of this loss ranged from insignificant (0% concentration drop) to severe (46% drop).

Does Smoking Marijuana Produce Personnel Identification Failure When Tested by an Infra-Red Retinal Scanning Security Device?

Imad K. Abukhalaf[1,3], Barbara R. Manno[2,3], Philip M. Kemp[4], and Joseph E. Manno[1,3], Louisiana State University Medical Center, Departments of [1]Medicine, [2]Psychiatry and the [3]Center of Excellence for Clinical and Forensic Toxicology, Shreveport, LA 71130 and [4]Chief Medical Examiner's Office, Oklahoma City, OK*

Infra-red retinal scanning systems are currently available for monitoring employee identification as an integral part of workplace security. These systems utilize the unique patterns and spatial stability of the vasculature of the human retina to provide a means for individual identification analogous to fingerprinting. Anecdotal reporting indicate that some persons who have "failed" personal identification with such devices have subsequently tested positive for urine cannabinoids. We sought to determine if smoking marijuana results in retinal vascular changes preventing a positive individual identification. Subjects assigned to a 3 x 3 randomized dosing design smoked marijuana cigarettes (placebo, 1.77% THC and 3.54% THC) according to a computer controlled, paced-smoking protocol. Retinal scans (Ibex 90 Retinal Reader, Eyedentify, Inc., Baton Rouge, LA), blood and urine specimens were collected at baseline (before smoking) and at intervals up to 8 hours post-smoking. Plasma and urine specimens were analyzed for cannabinoid concentrations (Δ^9-THC, 11-Hydroxy-Δ^9-THC and Nor-COOH-Δ^9-THC) by GC/MS analysis (Kemp et al., J. Anal. Tox. 19:285-291; 19:292-298,1995). No relationship was found between smoking marijuana, a positive urine cannabinoid screen and failure to pass a personal identification test. Protocol was approved by the LSUMCS Investigational Review Board for Human Research. Supported by NIDA Grant No. DA 05850.

Discovery of Oleander Poisoning in a Child by Digoxin Immunoassays and Reversed-Phase HPLC

Saeed A. Jortani, R. Allen Helm, and Roland Valdes Jr., Department of Pathology, University of Louisville, Louisville, KY 40292 and Abhya Gupta, Prashant Joshi, and Zulfikarali Verjee, Hospital For Sick Children, Toronto, Canada.*

Ingestion of Nerium Oleander plant causes severe cardiac intoxication similar to digoxin poisoning. Oleandrin is the major cardiac glycoside present in the oleander plant. During the 1991 to 1993 period, 2383 incidents of exposure to this plant were reported to the American Association of Poison Control Centers. Use of Digibind to treat oleander intoxication has been suggested. We present the case of a 13-month old male presented to the Hospital For Sick Children in Toronto, Canada for lethargy, vomiting and an irregular heart beat with heart block. His clinical findings were consistent with cardiac glycoside intoxication. Analysis of his serum collected at the time of his presentation to the emergency room by TD_X digoxin assay yielded an apparent digoxin value of 16 nmol/L. Serum sample collected after 48 hours was analyzed by the TD_X, Stratus, On-Line, and ACS:180 digoxin assays which resulted in digoxin values of 1.7, 0.8, 0.6, and 0.0 nmol/L, respectively. Analysis of this sample by reversed-phase HPLC showed no digoxin;

however, there were 3 major peaks, two of which corresponded to oleandrin and oleandrigenin. Digoxin, oleandrin, and oleandrigenin separate with baseline resolution by this HPLC method. Added oleandrin and oleandrigenin standards to digoxin-free serum (up to 200 µM) measured different apparent digoxin readings among digoxin immunoassays. The ACS:180 was not affected by the presence of oleandrin or oleandrigenin. We conclude oleander poisoning should be considered while resolving discrepancies in digoxin results. Since oleandrin and its aglycone congener cross-reacted differently in digoxin immunoassays, we recommend that a combination of assays be used to rule out oleander glycosides in suspected cases.

Case Report: Fatal Ephedrine Intoxication

Ronald Backer, Donna Tautman, and Sybil Lowery, Harrison Laboratories, Inc., Midland, TX 79711 ; Charles M. Harvey, Jefferson County Morgue, Beaumont TX ; and Alphonse Poklis, Medical College of Virginia, Richmond, VA 23298-0165*

A 28 year old, white female with a history of two prior suicide attempts was found dead in her home by her common law husband. Autopsy findings were unremarkable with the exception of nine undissolved white tablets found in the gastric and duodenal contents. The tablets were found to contain ephedrine. Significant toxicological finding included ephedrine; blood, 11 mg/L; liver, 24 mg/kg; kidney, 14 mg/kg; brain, 8.9 mg/kg; and amitriptyline; blood, 0.33 mg/kg; liver 7.8 mg/kg. No other drugs or poisons were detected. Quantitation of ephedrine was by gas chromatography/mass spectrometry (GC/MS) following liquid/liquid extraction from alkaline samples and pentafluoropropionic acid derivatization. The ephedrine values far exceed those associated with therapeutic administration and are consistent with the few reported cases of severe ephedrine intoxication. The cause of death was determined to be fatal ephedrine intoxication and manner of death suicide.

Fluoxetine Fatal Poisoning - A Case Report

Gerald E. Clement, Joann Sell, Isidore Mihalakis, and Zachary Lysek, Health Network Laboratories, Lehigh Valley Hospital, 17th and Chew Street, Allentown, PA 18105*

The deceased was a 9 year old 45 lb male foster child with a history of fetal alcohol disorder, Tourette's syndrome and attention deficit disorder. His medications included Prozac 20 mg qid, Tylenol Jr. bid, Clonidine 0.8 mg daily, and Phenergan suppository 25 mg daily. He had a history of a seizure disorder. Events leading to his death were as follows: on Friday he was known to be looking forward to the weekend; in the evening he vomited and was unable to sit up; throughout the night he received Phenergan and Clonidine. Saturday was uneventful until 3:00 pm when he had a tonic clonic seizure. His temperature was 101°F and on Sunday at 12:00 am he had a second seizure; at 3:30 am he was very stiff with vocal noises; and at 3:40 am he had a violent seizure which resulted in transportation to the ER where he was pronounced dead at 4:43 pm. The laboratory analyses performed by National Medical Services (NMS) showed the following: blood promethazine 44 ng/mL, fluoxetine 21000 ng/mL, norfluoxetine 21000 ng/mL, clonidine 53 ng/mL, methylphenidate 39 ng/mL and metabolite 179 mg/mL. Fluoxetine and norfluoxetine concentrations in the following tissues were: gastric 53,38 µg/mL, liver 2800,2200 µg/g, brain 24,22 µg/g, and kidney 38,34 µg/g, respectively.

Could this be a case of Munchausen by Proxy? What contribution was made by the postmortem redistribution of fluoxetine from the tissues? How did the deceased ingest several hundred 20 mg tablets?

Distribution of Venlafaxine in Two Postmortem Cases

Barry Levine, Amanda J. Jenkins, Rebecca Jufer, and John E. Smialek, Office of the Chief Medical Examiner, State of Maryland, 111 Penn St., Baltimore, MD 21201*

Venlafaxine (V) is a second generation antidepressant approved for use in the United States in 1993. It is a derivative of phenethylamine and is structurally unrelated to first and other second generation antidepressants. Nevertheless, its mechanism of action is similar to other antidepressants; it inhibits the reuptake of presynaptic norepinephrine and serotonin. Its major routes of elimination involve O- and N-demethylation. The O-desmethylvenlafaxine (ODV) is biologically active. Therapeutic conditions of V and ODV are in the range of 0.2 and 0.4 mg/L, respectively.

Two cases of muitiple drug intoxication involving V are presented. V and ODV were identified by gas chromatography/nitrogen-phosphorus detection after alkaline extraction of the biological specimen. On a DB-5 column, V and ODV elute after bupropion and fluoxetine, but prior to first generation antidepressants, sertraline, amoxapine and trazodone. V and ODV were confirmed by full scan electron impact gas chromatography/mass spectrometry. The tissue distribution of V and ODV in the two cases was as follows:

	Case 1		Case 2	
Specimen	V	ODV	V	ODV
Heart blood (mg/L)	6.6	31	84	15
Subclavian blood (mg/L)	----	----	46	7.1
Bile (mg/L)	100	32	290	52
Urine (mg/L)	640	310	150	59
Liver (mg/kg)	34	54	430	140
Kidney (mg/kg)	----	----	210	43

In case 1, acetaminophen and diphenhydramine were found in the blood at 140 and 2.6 mg/L, respectively. In case 2, amitriptyline, nortriptyline and chlordiazepoxide were found in the blood at 2.8, 0.5, and 3.3 mg/L, respectively. In each case, the manner of death was suicide.

Unusual Death Due to Methamphetamine and Acetone Exposure: What is the Route of Exposure?

N. G. S. Rao and Nancy Wohlenberg, Toxicology Bureau, Scientific Laboratory Division, New Mexico Department of Health, 700 Camino de Salud NE, Albuquerque, NM 87196 ; Kurt B. Nolte, Office of the Medical Investigator, 700 Camino de Salud NE, Albuquerque, NM 87196 ; and Carolyn H. Revercomb, Bexas County Forensic Science Center, 7337 Louis Pasteur Dr., San Antonio, TX 78229*

An intoxication death involved high levels of methamphetamine and acetone. A suspected illicit drug manufacturer was found dead, supine on his bathroom floor in a puddle of dark brown liquid. This home was used for drug manufacturing. A few pieces of broken glass on the floor and moist brown material with a strong chemical smell on the jeans were observed at the scene. Several chemicals, including two 5 gallon acetone containers, were found at the scene.

Autopsy revealed light colored crystalline powder around the mouth and on the right hand. Analysis of the postmortem samples disclosed the following chemical agents.

Tissue	Acetone (gm %)	Isopropyl Alcohol (gm %)	Meth mg/L or /kg	Amp mg/L or /kg
Blood (F)	0.262	0.012	8.24	0.1
Blood (H)	0.193	0.033	43.74	0.16
Bile	0.201	0.08	22.44	0.34
Liver	0.105	0.134	67.84	0.94
Brain	0.217	0.01	28.16	0.00
Stomach Contents	0.216	0.043	3.88	0.00
Urine	0.091	0.00	37.62	0.62
Lung	----	---	28.88	0.00
Kidney	----	---	47.42	0.00
Vitreous	----	---	37.05	0.00

The cause of death was determined to be combined methamphetamine and isopropyl alcohol poisoning. Besides acetone and methamphetamine, the tissue also contained their metabolites isopropyl alcohol and amphetamine, and phenylpropanolamine, respectively. The blood and tissue concentrations of acetone and methamphetamine found in this case are among the highest found in the literature. These concentrations likely reflect dermal absorption and/or inhalation.

Evaluation of Oxazepam and Lorazepam ß-Glucuronide Primary Reference Materials

Carol L. O'Neal, and Alphonse Poklis, Medical College of Virginia, P. O. Box 980165, Richmond, VA 23298-0165*

The major metabolite of hydroxylated benzodiazepines is the ß-glucuronidated metabolite, thus a hydrolysis step is included in benzodiazepine analyses in urine. We evaluated the new Alltech (R,S) oxazepam and lorazepam ß-glucuronide primary reference materials for hydrolysis controls and proficiency testing. GC/MS analysis of the benzophenones following acid hydrolysis and of the parent drug following ß-glucuronidase hydrolysis revealed that the oxazepam ß-glucuronide material was only 54.0% pure, whereas the lorazepam ß-glucuronide material was >95% pure. Recovery after hydrolysis with ß-glucuronidase at 60°C for 2 hours and 22°C for 24 hours was compared. There was no difference in the recovery of lorazepam incubated under both conditions, but 15.3% less oxazepam was recovered after incubation at 22°C. HPLC analysis separated the R and S isomers of the glucuronides. The S isomer hydrolyzed faster than the R isomer, but at optimal conditions hydrolysis with ß-glucuronidase was complete. Alltech's glucuronide materials can be valuable as hydrolysis controls in method development and routine analyses, but each laboratory must validate the purity of the material and determine the acceptable reference ranges for in-house controls made from these materials.

Positive results on the Triage TCA Plus Panel for Drugs of Abuse were achieved when lorazepam glucuronide material was added at 154% of the cutoff concentration, but addition of the oxazepam glucuronide at >200% of the cutoff concentration did not produce a positive result. Discrepancies may be due to preferential binding of the antibodies to either the R or S isomers, because the Alltech materials do not contain the isomers in the same ratio as that excreted in human urine.

Testing for Cocaine and Opiate Use with the PharmChek™ Sweat Patch

Robert Fogerson and Jacqueline Sutliff, PharmChem Laboratories, 1505A O'Bien Drive, Menlo Park, CA 94025 ; and Cheryl Walker, Michigan Department of Corrections, 206 East Michigan Street, Lansing, MI 48809*

Drug abuse is a major concern of criminal justice agencies charged with the supervision of individuals in both prisons and residential settings. The PharmChek™ sweat patch is designed to provide continuous monitoring for drug use. This study was designed to assess the effectiveness of testing for evidence of drug use with the PharmChek™ sweat patch, as compared to more traditional methods of urine testing.,

Individuals under the supervision of the Michigan Department of Corrections, and subject to routine urine drug testing, were recruited to wear the PharmChek™ sweat patch for varying periods of time. Urine specimens were collected at times of patch application and removal, and at intervals between those times.

Sweat patches were analyzed for the presence of drugs by STC microplate immunoassay and GC/MS; urine specimens were analyzed by EMIT immunoassay and GC.

The analysis of the PharmChek™ sweat patches detected a greater number of cocaine users than was found through urine testing (97 patch positives vs. 22 urine positives). The sweat patch was able to specifically identify heroin use, rather than the broader indication of opiate use.

The PharmChek™ sweat patch offers both quantitative and qualitative advantages over urine based testing in monitoring drug use in a criminal justice population.

Sweat Eluate Analysis for Phencyclidine by STC Diagnostics PCP Micro-Plate EIA and GC/MS

John F. Fay and R. Sam Niedbala, STC Diagnostics, 1745 Eaton Avenue, Bethlehem, PA 18018 ; and Peter Lo and Robert Fogerson, PharmChem Laboratories, 1505A O'Brien Drive, Menlo Park, CA 94025*

The objective of this study was to evaluate sweat as a matrix in the STC Diagnostics PCP Micro-Plate EIA. Volunteers for this study were in probation and rehabilitation programs and had a history of PCP abuse. PharmChek™ sweat collection patches were applied to various body regions (i.e., upper arms, lower back, and chest) and worn for several days. PharmChek™ patches were collected over the duration of the study. The patches were extracted by the addition of 2.5 mL of 0.2 M acetate buffer containing methanol (pH 5.0), and rotated on an orbital shaker at 150 rpm for 30 minutes. The eluate was screened by EIA and confirmed by GC/MS. PCP

sample concentrations ranged from 0.6 to 75.7 ng/mL. There was 96% agreement (one false negative) between the EIA screen (10.0 ng/mL cutoff) and GC/MS (3.0 ng/mL) confirmation. The performance characteristics of the EIA make it well suited for low level PCP analysis. The assay uses a monoclonal antibody which exhibits 100% cross-reactivity towards PCP and approximately 30% cross-reactivity towards 4-phenyl-4-piperidino-cyclohexanol. The limit of detection (LOD) extrapolated from the standard curve was 0.80 ng/mL. Within-run and total precision over 20 days of testing calculated using the NCCLS EP5-T2 protocol yielded CV's ranging from 0.95-1.06% and 1.60-2.34%, respectively.

Determination of Colchicine in Human Biofluids by HPLC/ISP-MS

Antoine Tracqui, Pascal Kintz and Patrice Mangin, Institut de Médecine Légale, 11 rue Humann, 67085 Strasbourg, France.*

Specific determination of colchicine (COL) in biological samples is difficult since the drug is active at low levels and is not amenable to GC/MS analysis. To solve this problem we have developed an original method by HPLC with Ionspray/mass spectrometry (HPLC/ ISP-MS) for COL analysis in human blood, plasma or urine.

After single-step liquid-liquid extraction by dichloromethane at pH 8.0 with tofisopam (TOF) as an internal standard, solutes were separated on a 5-µm C18 Microbore (Alltech) column (250 x 1.0 mm, i.d.), using acetonitrile:2 mM NH_4COOH, pH 3 buffer (75:25, v/v) as the eluent (flow 50 µl/min). Detection was accomplished by a Perkin-Elmer Sciex API-100 mass analyzer equipped with a ISP interface (nebulizing and curtain gas: N_2 quality U; main settings: ISP, + 4.0 kV; orifice, + 50 V; electron multiplier, + 2.2 kV); MS data were collected as either TIC (*m/z* 100-500 or 380-405), or SIM at *m/z* 400 and 383 for COL and TOF, respectively. COL mass spectrum showed a prominent molecular ion $[M + H]^+$ at *m/z* 400. Increasing orifice potential failed to provide a significant fragmentation. Retention times were 2.70 and 4.53 min for COL and TOF, respectively. The method was linear (r = 0.998) over a concentration range 5 to 200 ng/mL. The LOD in SIM mode was 0.6 ng/mL COL, making the method convenient for both clinical and forensic purposes.

A Gas Chromatographic/Positive Ion Chemical Ionization-Mass Spectrometric Method for Determination of l-α-Acetylmethadol (LAAM), norLAAM and dinorLAAM

David E. Moody, Dennis J. Crouch, and Rodger L. Foltz, Center for Human Toxicology, Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, UT 84112*

l-α-Acetylmethadol (LAAM) is approved as a substitute for methadone for the treatment of opiate addiction. Analytical methods are needed to quantitate LAAM and its two psychoactive metabolites, noracetylmethadol (norLAAM) and dinoracetylmethadol (dinorLAAM), to support pharmacokinetic and other studies. We developed a gas chromatographic positive ion chemical ionization-mass spectrometric method for these analyses. The method used 0.5 mL of urine or 1.0 mL of plasma or tissue homogenate, deuterated (d_3) isotopomers as internal standards, methanolic denaturation of protein (for plasma and tissue), and extraction of the buffered sample with n-butyl chloride. For tissue homogenates, an acidic back-extraction was included. norLAAM and dinorLAAM were derivatized with trifluoroacetic anhydride. Chromatographic separation of LAAM, and derivatized norLAAM and dinorLAAM was achieved with a 5% phenyl methylsilicone capillary column. Positive ion chemical ionization detection using a methane:ammonia mixture as the reagent gas produced abundant protonated ions (MH^+) for LAAM (m/z 354) and LAAM-d_3 (m/z 357); and ammonia adduct ions (MNH_4^+) for the derivatized norLAAM (m/z 453), norLAAM-d_3 (m/z 456), dinorLAAM (m/z 439) and dinorLAAM-d_3 (m/z 442). The linear range of the calibration curves was matrix dependent; 5-300 ng/mL for plasma; 10-1000 ng/mL for urine; and 10-600 ng/g for tissue homogenates. The low calibrator was the validated limit of quantitation for each matrix. The method was precise and accurate with %CV's and % of targets within 15%. The method has been applied to the analysis of human urine and plasma samples; rat plasma, liver, and brain samples; and human liver microsomes following incubation with LAAM. (Supported by NIDA Contract N01-DA-1-9205.)

Excretion Profile of Immunoassay Cross-Reacting Substances Following Controlled Administration of Lysergic Acid Diethylamide

John T. Cody and Sandra Valtier, Clinical Investigations, Wilford Hall Medical Center, Lackland AFB, TX 78236-5319*

Testing lysergic acid diethylamide (LSD) as a part of a drug abuse detection/prevention program has been hampered by the difficulty of analysis due to the low concentrations excreted from the body, a reflection of the low amount of drug administered. Radioimmunoassay (RIA) has been used for the screening for LSD due to this technique's ability to detect low concentrations of analyte. However, numerous samples which screen positive for LSD either do not contain the drug, or the level is below the detection limits of commonly available confirmation assays. This begs the question whether the positive results are the result of LSD metabolites, or some other substance which cross-reacts with the antibodies used in the immunoassays. LSD (2 µg/kg) was administered to 5 non-human primates and urine samples were collected at 1, 2, 4, 8, 12, 18, 24, 36, 48, 72 and 96 hours post-dose to assess the excretion profile of RIA cross-reacting substances. Urine samples were analyzed using radioimmunoassay reagents from Roche Diagnostics and Diagnostic Products Corporation (DPC) following the manufacturers' protocols. GC/MS/MS analysis was accomplished as described by Nelson and Foltz (Anal. Chem., 64, 1578, 1992).

The Roche results showed all subjects positive ($\geq$ 500 pg/mL) at some point, with a positive reading as late as 24 hours for one subject. DPC showed no positive results after 4 hours and one subject had no positive results. Overall, use of the DPC assay resulted in fewer positive samples than seen with the Roche assay. LSD, measured by GC/MS/MS, showed concentrations dramatically less than both immunoassays, with the greatest differences from the Roche assay. These results demonstrate that screening urine samples for the presence of LSD is dramatically influenced by the LSD RIA assay.

Characterization of Anhydroecgonine Methyl Ester in Human Urine and Hair

Pascal Kintz and Patrice Mangin, Institut de Médecine Légale, 11 rue Humann, 67000 Strasbourg, France.

A method using gas chromatography coupled to mass spectrometry for the determination of the cocaine pyrolysis product, anhydroecgonine methyl ester, in urine and hair is described. The same procedure allows the simultaneous determination of cocaine, benzoylecgonine, ecgonine methyl ester and cocaethylene. The assay involved acid hydrolysis of hair, addition of deuterated internal standards, a 3-step liquid-liquid extraction and derivatization with BSTFA + 1% TMCS. Detector responses for analytes were linear over the concentration ranges of 0.2-50 ng/mg and 10-2000 ng/mL for hair and urine, respectively. Artifactual formation of anhydroecgonine methyl ester during GC injection was <1%. Anhydroecgonine methyl ester was tested for in 65 and 81 cases for hair and urine, respectively, where cocaine and/or benzoylecgonine was present. Concentrations of anhydroecgonine methyl ester ranged from 0.2 to 2.4 ng/mg (n = 7) and from 4 to 226 ng/mL (n = 12) in hair and urine, respectively. In conclusion, the presence of anhydroecgonine methyl ester was only observed in a few cases, clearly indicating that cocaine smoking is not frequent in France.

Comparison of *In Vitro* Binding of Morphine and Codeine to *In Vivo* Binding in Rat Hair

Robert Joseph, Tsung-Ping Su, and Edward J. Cone, Addiction Research Center, Baltimore, MD 21224 and Steven P. Gygi, Diana G. Wilkins, and Douglas E. Rollins, Center for Human Toxicology, University of Utah, Salt Lake City, UT 84112*

Assays have been developed in our laboratory to evaluate *in vitro* binding of drugs to hair. Preliminary studies have shown significant differences in cocaine binding between Caucasoid and Africoid hair, between male and female Africoid hair, and between dark colored and light colored hair. The ability to extrapolate *in vitro* data to whole animals has been hindered because of the lack of validation of *in vitro* binding techniques. In the present study, we compared *in vitro* and *in vivo* binding of morphine and codeine to rat hair, and investigated whether hair color affected binding. Studies performed *in vivo* involved i.p. administration of 40 mg/kg/day codeine for 5 days to hooded Long Evans (LE), Sprague Dawley (SD), and Dark Agouti (DA) rats. LE black, LE white, DA and SD hair were collected 9 days following the last dose and analyzed by GC/MS to determine morphine and codeine concentrations in hair. *In vitro* studies were performed with drug-free LE black, LE white, SD and DA hair collected from a separate group of rats. Total binding was determined by preparing separate hair suspensions that contained 7.86 µM of ^{3}H-morphine and 7.86 µM ^{3}H-codeine. Nonspecific binding suspensions were prepared with 205 µM codeine and 205 µM morphine. Specific binding responses represented the difference between total and nonspecific binding responses. The mean results (N=10) are listed in the following table:

Species (hair color)	Morphine	Codeine	Morphine	Codeine
	In vitro specific binding (Mean DPMs/mg hair)		*In vivo* concentration (Mean ng/mg hair)	
LE (black)	7379	10204	14.5	111.9
LE (white)	449	371	0.5	2.4
DA (brown)	3705	2739	0.5	6.0
SD (white)	180	89	0.3	1.0

These results demonstrated similar trends for *in vivo* and *in vitro* binding of codeine and morphine to rat hair (LE black > DA brown ≥ LE and SD white hair). These findings also provided preliminary evidence that supports the use of *in vitro* studies to evaluate the binding of drugs to hair.

Failure of Intravenous Calcium to Reverse the Effects of Verapamil Overdose: Fifteen Case Reports

Kevin S. Merigian, Stephen L. Winbery and Kari E. Blaho, Department of Emergency Medicine and Clinical Toxicology, UTMG, P.O. Box 63637, Memphis, TN 38163*

The wide spread use of calcium channel modulators in the treatment of hypertension and cardiovascular diseases has increased the incidence of their overdose. Calcium channel blockers produce their therapeutic and toxic effects by interacting with the a-1 subunit of the L-calcium channel. Their pharmacological effects include negative inotropy, dromotropy and chronotropy. Many have postulated that increasing calcium levels will reverse the toxic effects of these drugs. We report the results of treatment of 15 cases of verapamil overdose to examine the effectiveness of intravenous calcium and various pressor agents in reversing bradycardia, hypotension and cardiac conduction delays in verapamil overdose. Analysis of all cases reveals that calcium infusions alone were not effective in reversing the toxic effects of overdose. In three of the fifteen cases, there was an immediate increase in blood pressure and heart rate in response to calcium infusion but all were temporally related to beta-agonist administration. The apparent ineffectiveness of calcium infusions when used alone indicates that it is not the drug of choice in cases of calcium channel blocker overdose. Beta-agonists may increase the availability of calcium at the tissue level by promoting the formation of intracellular cAMP, thus reversing the effects of calcium channel blockers by an alternative mechanism. Based on these case reports, we conclude that calcium infusions are ineffective when used alone and should not be used as first line therapy in calcium channel blocker overdose.

A Comparison of the Pharmacokinetics and Abuse Liability of Cocaine, Heroin and Nicotine after Smoked and Intravenous Administration

Amanda J. Jenkins, Robert M. Keenan, Jack E. Henningfield, and Edward J. Cone, Addiction Research Center, NIDA, NIH, Baltimore MD 21224*

Cocaine, heroin and nicotine are known to be addictive drugs and yet there have been few studies comparing how differences in route of administration affect abuse liability. We conducted a series of clinical studies in which cocaine (0, 10, 20, and 40 mg), heroin (0, 3, 6, and 12 mg) and nicotine (0, 0.75, 1.5 and 3.0 mg) were administered to

healthy male volunteers in a controlled clinical setting. Each drug (salt) was administered intravenously in isotonic saline infused over 30 s. Smoked drug was administered with a computer assisted smoking device which delivered drug (base) in a single puff. Subjective indices of abuse liability such as the "Feel drug", and "Drug liking" scales on the subject Single Dose Questionnaire, and "Feel any drug effect", and "Feel any good drug effect" on the Visual Analogue Scales, were measured prior and periodically after drug administration. Blood or plasma samples were collected simultaneously with subjective measures. Samples were analyzed according to protocol for anhydroecgonine methyl ester, cocaine and metabolites, heroin, 6-acetylmorphine and morphine, and nicotine and cotinine by solid phase extraction-gas chromatography/mass spectrometry.

Subjective effects were reported immediately after drug administration by both routes for all three drugs. The times to reach maximal subjective effects were similar to the times of peak blood parent drug concentrations. The magnitude of subjective effects for each drug and peak blood drug concentrations were dose related. In addition, the magnitude of subjective effects were similar between the drugs across both routes of administration. These data indicated that the smoked route of administration was an effective means of drug delivery and produced subjective effects similar to intravenous administration. The combination of efficacy and ease of use makes the smoking route highly attractive to drug users as a drug delivery system.

The Analytical and Pharmacological Characterization of α-Benzyl-N-methylphenethylamine, An Impurity of Illicit Methamphetamine Synthesis

Karla A. Moore[1], Alphonse Poklis[1], William H. Soine[2], Joseph F. Borzelleca[2], Joseph J. Saady[1], and James C. Valentour[3], [1]Department of Pathology and [2]Department of Pharmacology/Toxicology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298-0165 and [3]Office of the Chief Medical Examiner, Commonwealth of Virginia, Richmond, VA 23219-3694*

Illicit synthesis of methamphetamine (METH) results in various contaminants. Few impurities have been studied in vivo and their pharmacology/toxicology is unknown. One such impurity is α-benzyl-N-methylphenethylamine (BNMPA). We investigated the hypothesis that BNMPA may be contributing to the apparent increased toxicity of METH and the utility of using BNMPA/metabolites as markers of illicit METH consumption.

We predicted the four major metabolites of BNMPA to be N-demethyl-α-benzylphenethylamine (N-demethyl-BNMPA), diphenyl-2-propanone, para-hydroxy-N-demethyl-BNMPA, para-hydroxy-BNMPA (p-OH-BNMPA), and diphenyl-2-propanol. We synthesized these compounds and developed a gas chromatography/mass spectrometry (GC/MS) detection method. We confirmed these as true metabolites in a study with a volunteer who ingested BNMPA. The utility of these compounds as markers of illicit METH consumption was confirmed when BNMPA metabolites were found in two of eighty urine samples from METH abusers. Additionally, a trace amount of p-OH-BNMPA was detected in the urine from a patient who died following METH consumption.

In mice, BNMPA alone caused convulsions at doses much lower than lethality. When combined with METH, it did not significantly alter METH-induced convulsions or spontaneous locomotor activity. These in vivo observations were supported by the in vitro demonstration that BNMPA and N-demethyl-BNMPA failed to displace compounds from the dopamine and serotonin transporter in striatal and cortical neuron membrane fragments, respectively. In voltage clamp studies of N-methyl-D-aspartate (NMDA) receptors expressed in *Xenopus* oocytes, BNMPA/N-demethyl-BNMPA (3, 10, 30 and 100 μM) inhibited NMDA-induced currents by 15, 29, 54 and 78%, respectively. Neither compound (100 μM) had an effect on gamma-amino-butyric acid (GABA)-induced current in neuronal cultures.

ONTRAK TESTCUP: A Novel, On-site, Multi-analyte Screen for the Detection of Abused Drugs

James Towt, S-C. Jane Tsai, Marta R. Hernandez, Alex D. Klimov, Cynthia V. Kravec, Susan L. Rouse, Haleema S. Subuhi, Barbara Twarowska and Salvatore J. Salamone, Roche Diagnostic Systems, Inc., 1080 US Highway 202, Somerville, NJ 08876*

We have developed a rapid, sensitive, simple to use, multi-analyte diagnostic device for the detection of drugs of abuse in urine, called ONTRAK TESTCUP. This device requires no sample or reagent handling and it also serves as the sample collection cup. TESTCUP contains immunochromatographic reagents that qualitatively and simultaneously detect the presence of benzoylecgonine, morphine and cannabinoids (THC) in urine. It is based on the principle of competition between the drug in the sample and membrane-immobilized drug-conjugate for anti-drug antibodies coated on blue-dyed microparticles. Each drug assay has its own strip which contains an antibody specific to benzoylecgonine, morphine or THC. A sample is collected in the TESTCUP, a lid is placed on it and a chamber at the top of the cup is filled with urine by inverting the cup for 5 seconds. Urine proceeds down immunochromatographic strips and the assays are developed. In approximately three to five minutes, the Test Valid bars appear, a decal is removed from the detection window and the results are interpreted. The appearance of a colored bar at the detection window for each drug indicates a negative result. The absence of color in any specific drug detection window indicates a positive result for that drug. If a positive result is obtained, the same device-cup can be sent out for GC/MS confirmation. When the precision of the TESTCUP was evaluated, the following results were obtained: ≥96%, ≥98% and ≥96% negative results for urine controls containing drug at 50% of its cutoff concentration for benzoylecgonine, morphine and THC, respectively; ≥97%, 100% and ≥98% positive results for urine controls containing drug at 120% of its cutoff concentration for benzoylecgonine, morphine and THC, respectively. Correlation of clinical sample results from TESTCUP versus GC/MS, ONTRAK and ONLINE were assessed. There was 100% agreement between samples prescreened positive by GC/MS and positive by TESTCUP for all three assays. There was 100% agreement between TESTCUP and ONTRAK results and between TESTCUP and ONLINE results when testing clinical samples positive and negative for cocaine (benzoylecgonine) or THC. Greater than 99% agreement was observed between TESTCUP and ONTRAK results and between TESTCUP

and ONLINE results when testing clinical samples positive and negative for morphine. The cross reactivity of the TESTCUP assays to related drugs and drug metabolites was also determined and the results were similar to those of ONTRAK and ONLINE.

Simultaneous Assay of Buprenorphine and Norbuprenorphine by Negative Chemical Ionization Tandem Mass Spectrometry

James J. Kuhlman, Jr., Joseph Magluilo, Jr., and Barry Levine, Division of Forensic Toxicology, Armed Forces Institute of Pathology, Washington, DC 20306 , and Edward J. Cone, Addiction Research Center, NIDA, NIH, Baltimore, MD 21224*

A method for the simultaneous measurement of buprenorphine (B) and its N-dealkylated metabolite, norbuprenorphine (NB), in human plasma was developed with negative chemical ionization tandem mass spectrometry. B and NB were extracted from biological fluids by solid phase extraction. The samples were derivatized with heptafluorobutyric anhydride and measured with negative chemical ionization tandem mass spectrometry. B formed a heptafluorobutyryl derivative and NB formed a bis-heptafluorobutyryl derivative. The LOQ for B was 0.20 ng/mL and the LOQ for NB was 0.03 ng/mL. B was linear from 0.15 ng/mL to 10 ng/mL and NB was linear between 0.016 ng/mL to 5 ng/mL. Between run and within run precision for B at 0.5 ng/mL was 13.8% and 9.8%, respectively. Between run and within run precision for NB at 0.5 ng/mL was 23.1% and 17.9%, respectively. The molecular anion for B was used as a precursor ion while the $[M-197]^-$ was used as a precursor for NB in tandem mass spectrometry. Product ion spectra from collision induced dissociation resulted principally from dissociations of the heptafluorobutyryl group. The method was applied to samples collected from a patient who received oral (40 mg) and subcutaneous (1 mg, 2 mg) B administrations. B plasma concentrations were measured from < 0.20 ng/mL to 8.7 ng/mL and NB plasma concentrations were measured from <0.03 to 3.26 ng/mL. This assay will be useful for the determination of the pharmacokinetics of buprenorphine in human subjects under controlled dosing conditions.

Meconium Drug Testing: Screening vs. Confirmatory Data

Christine M. Moore[1], Douglas E. Lewis[1], and Jerrold B. Leikin[1,2], [1]U.S. Drug Testing Laboratories, 2201 W. Campbell Park Drive, Chicago, IL 60612 and [2]Rush Poison Control Center, Rush Presbyterian-St. Luke's Hospital, Chicago, IL 60612

The analysis of meconium as an alternative to neonatal urine for the determination of fetal drug exposure is becoming increasingly popular since meconium provides a longer history of drug exposure than urine and is easier to collect. The complex nature of meconium coupled with the small amounts which are available for testing make it an extremely difficult specimen with which to work. It is necessary to operate the initial screening procedures at increased sensitivity levels in order to eliminate false negative results.

For two months, our laboratory correlated the number of screen positive meconium samples for the NIDA-5 panel of drugs against the number of positive results confirmed by gas chromatography/mass spectrometry (GC/MS). Our screening and confirmatory cut-off values were as follows: cocaine, opiates and phencyclidine, 25 and 5 ng/g; amphetamines, 100 and 5 ng/g; THC metabolite, 25 and 2 ng/g, respectively.

The confirmatory results were as follows: THC metabolite 56.1% (97 out of 173); cocaine metabolite(s) 59.2% (135 out of 228); opiates 56.7% (34 out of 60); amphetamines 25.7% (19 out of 74). Overall, 53.3% of screen positive samples subsequently confirmed using GC/MS.

Currently, many hospitals diagnose drug exposure *in utero* based on screen only data. Our results show this to be a misleading and incorrect practice.

GC/MS Determination of Amphetamine and Methamphetamine in Human Urine for 12 Hours Following Oral Administration of d-Methamphetamine: Lack of Evidence Supporting the Established Forensic Guidelines for Methamphetamine Confirmation

Jimmie L. Valentine[1], Gregory L. Kearns[1], and Carrie R. Valentine[2], [1]Department of Pediatrics, Section on Pediatric Clinical Pharmacology, University of Arkansas for Medical Sciences, Little Rock, AR 72202-3591 and [2]Division of Genetic Toxicology, National Center for Toxicological Research, Jefferson, AR 72079-9502*

Ten human volunteers, naive to amphetamines and divided into 2 groups of 5 each, were given an oral dose of 30 mg/70 kg of d-methamphetamine in one of two different paradigms; the initial dose at 0930 hours or the initial dose at 2130 hours. One week later each subject was crossed-over with regard to time, but given the same dose. A total of 214 urine specimens were collected either prior to dosing or at each micturition for a 12-hour period post-dose. Specimens were analyzed on a blind basis for methamphetamine and one of its metabolites, amphetamine, by SIM GC/MS using coinjection of extracted sample and PFPA. Approximately 20% of the d-methamphetamine was recovered unchanged from the urine specimens and 2% as amphetamine. Mean urine methamphetamine concentration in both groups reached a maximum within 4-6 hours and declined thereafter. A residual amount of methamphetamine was found in some pre-dose specimens at the cross-over evaluation reflecting that methamphetamine may be detected in urine for up to 7 days. Amphetamine concentration reached a plateau by 4-6 hours. Methamphetamine levels were consistently above the 500 ng/mL cutoff in most post-dosing specimens while amphetamine levels generally did not achieve the 200 ng/mL cutoff specified by SAMHSA guidelines for GC/MS confirmation of methamphetamine. Some specimens containing methamphetamine had no amphetamine metabolite. The current guidelines would have resulted in 90.2% of the specimens containing methamphetamine being ruled negative by confirmation following either night or day administration. These findings suggest that the current SAMHSA guidelines select for individual metabolic variations and that GC/MS confirmation of methamphetamine will result in most occasional users being ruled negative following an oral dose of methamphetamine while some will be ruled positive.

A Novel Approach to Treating Acute Cocaine Overdose: A Case Report

Stephen L. Winbery, Kevin S. Merigian and Kari E. Blaho, Department of Emergency Medicine and Clinical Toxicology, UTMG, P.O. Box 63637, Memphis, TN 38163*

The treatment of acute cocaine overdose is currently controversial because of insufficient data. It has been reported that β-blockers are relatively contraindicated as a treatment. We present a case describing a novel, but effective method of treating cocaine overdose. An 18 year old male was brought to the emergency department by police after ingesting a massive quantity of crack cocaine (8 bricks) to avoid arrest. On arrival his blood pressure was 230/180, pulse was 133, he was disoriented, agitated diaphoretic and paranoid. He had no other complaints. Control of blood pressure was achieved with the intravenous administration of a calcium channel blocker, nicardipine (50 mg/hr). The tachycardia persisted however, and intravenous esmolol was added (30 mg bolus, 3 mg/min). Lorazepam (2 mg, IV) was also given for control of the CNS symptoms of cocaine overdose. The patient was admitted to the ICU with blood pressure and heart rate improving. He was discharged after 24 hours of observation with no apparent after effects of the cocaine overdose.

The ideal antidote for the cardiovascular complications of cocaine overdose should be rapid acting, have minimal negative inotropic effects and should decrease afterload. The novel approach reported here fits these criteria. The use of calcium channel modulators affords peripheral dilation with minimal negative inotropy. Esmolol is a short acting, selective β-antagonist that blocks the chronotropic actions of cocaine and reduces oxygen demand. There are no reports of the concurrent use of β-blockers and calcium channel modulators in cocaine overdose. Since cocaine increases calcium flow into the myocyte, calcium overload may be one of the mechanisms of cocaine toxicity. Based on the molecular actions of cocaine, the use of a β-blocker and a calcium channel blocker are rational and effective therapeutic choices.

Interpretation of Cocaine Concentrations in Postmortem Blood

Ashraf Mozayani and James Valentour, Division of Forensic Science, 1 North 14th Street, Richmond, VA 23219*

According to 1994 FBI statistics, Richmond was ranked as the second highest city for homicides per capita in the nation. The most frequently found drug in these cases was cocaine, which was present in 30% (49 cases) of all homicide victims (162 cases). The rise in cocaine positive homicide cases in Richmond also paralleled its appearance in other types of death investigations throughout the state of Virginia with a greater than 20% increase of cocaine positive cases per year since 1988. The following table presents the appearance of cocaine in medical examiners' cases in 1994 in Virginia. Cases where benzoylecgonine was found to the exclusion of cocaine were not considered here.

Appearance of Cocaine in Postmortem Blood Samples in Virginia in 1994.

Manner of Death	# of Cocaine Positive Cases	Blood Cocaine Concentrations (mg/L) Range	Mean±SD	Mode
Accident	30	0.01-13.3	0.93±2.75	0.02
Homicide	57	0.02-0.65	0.15±0.12	0.20
Suicide	7	0.01-0.69	0.18±0.24	0.23
Natural	5	0.05-0.17	0.11±0.04	N/A
Undetermined	1	0.01	----	----
Pending	1	0.23	----	----

We found that cocaine concentration in blood often did not appear to be related to the manner of death. Median and, to a large extent, mean concentrations of cocaine were similar between groups of victims of homicide, accident, suicide or even natural causes.

Urinary Excretion Profile of Cocaine, Metabolites and Pyrolysis Product After Smoked Cocaine

Rebecca A. Jufer, Jonathan M. Oyler, and Edward J. Cone, Addiction Research Center, NIDA, NIH, Baltimore, MD 21224*

Smoking has become an increasingly popular route for cocaine self-administration. Despite widespread use by this route, the urinary excretion profile of cocaine metabolites has not been fully described. We analyzed urine samples collected for 72 hours following administration of 42 mg of cocaine base by the smoked route to male subjects. Urine samples were analyzed for anhydroecgonine methyl ester (AEME), ecgonine methyl ester (EME), cocaine (COC), benzoylecgonine (BE), benzoylnorecgonine (BNE), and norcocaine. Cocaine and metabolites were extracted by solid phase extraction followed by GC/MS analysis in the SIM mode. Limits of detection for each analyte were approximately 1 ng/mL for AEME, COC, and BE and 5 ng/mL for BNE. The Sigma minus method was utilized to determine excretion half-lives in urine. Cocaine and metabolites were detected at different times. Mean time ranges of detection (hr) of each analyte were as follows: AEME (6.00-6.75); EME (1.50-61.50); COC (1.50-21.83); BE (1.50-61.50); and BNE (4.35-49.00). Mean pharmacokinetic parameters for each analyte are tabulated below for two subjects:

	AEME	EME	COC	BE	BNE
Half-life (hr-1)	ND	4.9	2.9	4.8	5.7
% Dose excreted	0.04	6.39	0.60	9.74	0.45
Peak conc. (ng/mL)	36.5	1646	392	3306	104.9
Peak time (hr)	3.0	5.8	3.6	8.0	9.5

ND = not determined

These data demonstrated that the early appearance of AEME in urine is indicative of the smoked route of cocaine administration. However, since AEME only appeared in the urine of one of three subjects and at only very low concentrations, the use of AEME in urine as a marker for the smoked route of administration is limited. It was also noted that the urinary excretion half-lives for EME, BE and BNE were significantly greater than that observed for cocaine. This indicated that the rate limiting step of clearance was the excretion of these metabolites rather than their formation from cocaine.

Urinary Excretion and Kinetic Profile of m-Hydroxybenzoylecgonine in Urine After Cocaine Administration to Adult Males

Jonathan M. Oyler, Rebecca A. Jufer, William D. Darwin, and Edward J. Cone, Addiction Research Center, NIDA, NIH, P.O. Box 5180, Baltimore, MD 21224*

A metabolite of cocaine, m-hydroxybenzoylecgonine (m-HOBE), has been identified in meconium from cocaine-exposed babies. Recently this metabolite was reported to be a more reliable indicator of fetal cocaine exposure than benzoylecgonine. However, the origin of m-HOBE (mother or fetus) remains unclear. We examined the urinary excretion pattern and kinetic profile of m-HOBE in adult male subjects following the administration of cocaine by the smoked (42 mg) and intranasal (32 mg) routes. Urine samples were collected for 72 hours after drug administration and analyzed by solid phase extraction followed by gas chromatography/ mass spectrometry. Chromatographic retention times and mass spectra were compared to those of an analytical standard. Positive identification was accomplished in the scan mode, and quantitation was performed in the SIM mode. Deuterated benzoylecgonine was used as the internal standard. The lower limit of quantitation for m-HOBE was 5 ng/mL. Standard curves were linear across a concentration range of 5-500 ng/mL with correlation coefficients ≥0.985. Half-lives were determined by the Sigma minus method. Mean pharmacokinetic parameters of m-HOBE for both routes of cocaine administration for 3 subjects are tabulated below:

	Detection time (hr)	% Dose	Half-life (hr^{-1})	Peak conc. (ng/mL)	T_{max} (hr)
Smoked	3.0-53.6	0.67	7.89	302.0	7.91
Intranasal	7.2-55.2	0.30	7.42	40.9	7.17

These data indicated that m-HOBE is a minor metabolite of cocaine that is excreted by adult males over a relatively long period of time. Also, the excretion profile and pharmacokinetics imply that m-HOBE formation and excretion is independent of the route of cocaine administration. The longer half-life of m-HOBE than its possible precursor benzoylecgonine indicated that the rate limiting step in the clearance of this novel metabolite was excretion rather than formation.

The Incidence of Psychotropic Drugs, Opiates and Alcohol in Fatally Injured Drivers: A Prospective Study in Northern France

Marc Deveaux[1], Thierry Prangere[2], Jean-Claude Marson[2], Patrick Goldstein[2], and Didier Gosset[1], [1]Institut de Médecine Légale, place Variet, 59000 Lille, France and [2]Médecine Nucléaire et SAMU, C.H.R.U., 59037 Lille Cédex, France.*

Although the main cause of fatal road accidents is undoubtedly alcohol consumption, the use of psychotropic drugs and narcotics has an adverse effect on road safety. Specific studies on fatally injured drivers are rare in France. This work describes the second one in the Region Nord-Pas de Calais (northern France).

Method: Within one year, blood samples obtained at time of death from 103 drivers and pedestrians killed in traffic accidents were analyzed for alcohol (BAC) by gas/chromatography, for tricyclic antidepressants (TA), barbiturates (BA) and benzodiazepines (BE) by FPIA, and for morphine (MO) by RIA, GC/MS was used to identify opiates (OP).

Results: Only 29% of the fatalities were studied. They were 85% men and 15% women. We distinguished 3 classes: pedestrians (12%), 2-wheeled vehicles (25%) and 4-wheeled vehicles (63%). 51% of BAC were greater than 0.1 g/L and 45% greater than the legal limit (0.7 g/L). Psychotropic drugs including OP were present in 39% of the cases; half of them were TA. Drugs and alcohol were present simultaneously in 19% of the individuals. Five samples were positive simultaneously in 19% of the individuals. Five samples were positive for MO, but 6-monoacetylmorphine was not found. Where data were available, results were compared with those of our previous study.

Validation of an Enzyme Immunoassay for Qualitative Detection of Cocaine in Sweat

Vina Spiehler, Spiehler and Associates, Newport Beach, CA 92663; Robert Fogerson, PharmChem Laboratories, Menlo Park, CA; Don Schoendorfer, Sudormed Inc., Santa Ana, CA; and John Fay and R. Sam Niedbala, STC Diagnostics, Bethlehem, PA*

A solid phase, enzyme immunoassay using microtiter plates was modified for analysis of cocaine in sweat. Sweat was collected with the PharmChek™ sweat patch and drugs were eluted from the collection pad of the patch. Sweat contains primarily parent cocaine. The assay was determined to have cross-reactivity for cocaine of 102% relative to 100% for the benzoylecgonine (BE) calibrators. Cross-reactivity with cocaethylene was 148%.

The optimum cutoff concentration for this modified assay was determined by Receiver Operating Characteristic (ROC) analysis to be 10 ng/mL of cocaine or BE equivalents. At this cutoff concentration the assay had 94.5% sensitivity and 99.1% specificity vs. GC/MS as the gold standard. The positive predictive value at a prevalence of 50% was 99%. Threshold analysis for positives suggested that the 95% confidence level for a positive result by the EIA was between 12.5 and 15 ng/mL and that quality control samples at 5 ng/mL and 15 ng/mL could be run with each batch to certify the precision around the cutoff concentration.

All positive samples must be confirmed by GC/MS before reporting. The sensitivity and specificity of the overall analysis system (sweat collection, immunoassay screen and GC/MS confirmation) was 86% and 97% using known cocaine dosing of volunteers as the gold standard. The positive predictive value of the overall system was 96.8% at a 50% prevalence.

Experiences with Glutaraldehyde Analysis

Ross H. Lowe and Yale H. Caplan, Corning National Center for Forensic Science, 1901 Sulphur Spring Rd., Baltimore, MD 21227*

Glutaraldehyde has been identified as the major component of UrinAid, a commercial product that when added to urine specimens has been demonstrated to be effective in preventing the detection of drugs of abuse in urine tested by EMIT™ methods.

Urine samples containing UrinAid can be presumptively identified by unusually negative absorbance readings in the EMIT assays. Colorimetric methods for the general detection of aliphatic aldehydes can be effective as an

initial screening test and GC/MS methods can definitively confirm the presence of the glutaraldehyde adulterant.

Our experience with a GC/MS method (Sansom et al., 1993) for the quantitative determination of glutaraldehyde in urine specimens was correlated with immunoassay and screening test results (N=16). The GC/MS method utilized a single step methylene chloride extraction. The extract was directly injected onto an HP-5 capillary column and glutaraldehyde eluted at approximately 2.1 minutes. Qualitative identification was obtained using full scan analysis and quantitation was accomplished in SIM. Linearity from 0.05 to 1.0% (v/v) concentrations was demonstrated and calibration curves showed an average correlation coefficient of 0.993 (N=6). The assay was simple and efficient and was accomplished with a commonly available column and instrumentation. Experiences with a screening test discriminated positives from negatives at a sensitivity of 0.05% (v/v).

The Disposition of Ethanol After Hemorrhage and Fluid Replacement in Rats

Frederick W. Fochtman, School of Pharmacy, Duquesne University, Pittsburgh, PA 15282 and Todd M. Probeck, MetPath Corning Clinical Labs, 875 Greentree Road, Pittsburgh, PA 15220*

Traumatic fatal and non-fatal accidents frequently involve the effects of ethanol. These accidents can result in varying degrees of blood loss with subsequent administration of intravenous fluids. Samples of blood for forensic interpretation could be drawn any time up to several hours after the accident and treatment. It was the objective of this study to determine if significant differences would occur in the blood alcohol concentration (BAC) after hemorrhage alone and hemorrhage with fluid replacement.

Male Wistar rats cannulated via the jugular vein were dosed orally with a dose of ethanol to achieve a BAC above 0.10% but not greater than 0.15%. After complete absorption of ethanol the rats were hemorrhaged via the cannula 10% and 25% of their blood volume. Normal saline was used for fluid replacement. At 10 min., 40 min. and 70 min. after hemorrhage, 0.2 mL of blood was drawn to measure ethanol. Ethanol concentrations were measured by gas chromatography on a carbowax column by direct injection.

Results of the study, showed there was no statistically significant difference in BAC between controls and hemorrhage alone and hemorrhage with fluid replacement. Furthermore, there was no difference in the rates of disposition between the groups.

In conclusion, it appears that hemorrhage alone or hemorrhage with fluid replacement up to 25% of blood volume has little or no effect on the blood ethanol during the post absorption period.

The Use of a Broad Spectrum Drug Identification System in Emergency Clinical Toxicology Screening

Ni-Chi Young[1], Keh-Sung Tsai[1], and S. Jason Lai[2], [1]National Taiwan University Hospital, Medical College, Department of Laboratory Medicine, Chung-Shan Road #6, Taipei, Taiwan, R.O.C. and [2]Bio-Rad Laboratories, Clinical Systems Division, 4000 Alfred Nobel Drive, Hercules, CA 94547

National Taiwan University Hospital has a capacity of 2000 beds, located in an international metropolis of 3 million people. From April 94 to March 95, we used REMEDi HS to analyze 501 emergency patient samples (39.7% serum, 59.9% urine, and 0.4% gastric fluid). Seventy five different drugs were detected and 65.9% of patient samples were found to contain one or more drugs. The percentages of drug-positive samples in serum and urine were 43.2% and 80.7%, respectively. Forty one different drugs were identified from serum samples and 67 different drugs were identified form urine samples. Therefore, without prior history of the patient, there was a higher likelihood to detect drugs in urine samples. The top twenty most frequently encountered drugs were in the following order: lidocaine, sulpiride, caffeine, chlorpheniramine, ephedrine, diphenhydramine, amphetamine, cimetidine, chlorpromazine, desipramine, ranitidine, flurazepam, carbamazine, trimethoprim, metoclopramide, erythromycin, morphine, propranolol, and demoxepam. Lidocaine is commonly used as a local anesthetic for catheters in patients.

From our experience, when dealing with a prior history of psychotic medical treatment or suspecting an overdose of benzodiazepines, both serum and urine samples should be analyzed in order to have a better understanding of the medication involved. A separate study was conducted on 16 patients who met this description. Both the serum and urine samples were analyzed. Seven different benzodiazepines; diazepam, bromazepam, estazolam, midazolam, flurazepam, nitrazepam, and temazepam; and ten different other drugs were detected in serum. One benzodiazepine, flurazepam, and sixteen other drugs were identified in urine samples. Meperidine appeared only in serum. There were ten other drugs that were only identified in the urine, including: benzhexol, procyclidine, codeine, metoclopramide, morphine, trazodone, propoxyphene, azacyclonol (metabolite of terfenadine), cimetidine and procainamide. Six other drugs were identified in both serum and urine samples, including: propranolol, sulpiride, lidocaine, chlorpheniramine, diphenhydramine and doxepin.

Another emerging concern is the high frequency of drug interactions due to polypharmacy among the geriatric population. Broad spectrum drug screening could assist in the determination of drug overdose, drug interactions, chronic effects, or noncompliance. In this study, a majority of the 75 different kinds of the detected drugs did not belong to the category of abused drugs, and no other assays were readily available for the detection of these medicines. In our experience, a broad spectrum drug identification system can produce valuable information for use in the emergency department.

Digoxin Monitoring by A_XSYM

Steven H. Y. Wong, Edward A. Sasse, Linda L. Herrick, and Debbie K. Cardoni, Clinical Toxicology and Therapeutic Drug Monitoring Section, Department of Pathology, Medical College of Wisconsin, Milwaukee, WI 53226 and Gloria Schock, Abbott Laboratories, Abbott Park, IL 60064*

The A_XSYM immunoassay analyzer was recently introduced as a batch/random analyzer, presently applicable for Therapeutic Drug Monitoring and Toxicology. Different from TD_X and IM_X analyzers, A_XSYM is capable of performing primary tube sampling,

multitasking and computer interfacing for sample accessioning and data processing. The present study evaluated the performance of digoxin monitoring by A_XSYM. Performance characteristics were evaluated for precision, curve stability, detection limit, correlation and interference. Prior to A_XSYM measurements, pretreatment of the samples was performed by mixing 400 mL of samples with 200 mL of precipitation reagent, followed by vortexing and centrifugation for 2 min to afford a clear supernatant. Then, the supernatant was immediately transferred to sample cups for analysis by FPIA on the A_XSYM. The reaction sequence and subsequent measurements were similar to those of FPIA using TD_X. Precision studies showed:

	Low	Medium	High
			Within-Run
Mean, mg/L	0.76	1.43	3.65
CV%	8.9	4.1	2.4
			Between-Run
Mean, mg/L	0.77	1.42	3.53
CV%	7.3	3.7	2.8

Calibration was stable up to 42 days. Detection limit was about 0.19 mg/L. Correlation study of 226 patient samples showed that A_XSYM = 0.9490TD_X - 0.0216 (r^2 = 0.9838). Samples analysis of renal, hepatic, pregnant patients and cord blood showed comparable results with TD_X, with the majority of the apparent concentrations <0.6 mg/L. In conclusion, the present study showed that digoxin may be readily monitored by A_XSYM with similar clinical efficacy to that of TD_X.

A Study of the Stability of Cocaine, Benzoylecgonine, Ecgonine Methyl Ester, Creatinine and Other Chemistries in Urine

Susan H. Dugan, Anthony G. Costantino, American Medical Laboratories, Inc., 14225 Newbrook Drive, Chantilly, VA 22021 ; Stuart C. Bogema, PDLA, South Plainfield, NJ. ; and Nicholas T. Lappas, The George Washington University, Washington, D.C.*

The stability of cocaine (COC), benzoylecgonine (BE), and ecgonine methyl ester (EME) in unpreserved urine samples was studied. Each urine sample was spiked with COC, BE and EME at 1000 ng/mL. The rate of degradation was found to be independent of the drug concentration and creatinine but dependent upon pH and temperature. At 25°C, EME and COC were <100 ng/mL by day 7 at pH 5, day 5 at pH 7 and by day 1.5 at pH 9. This degradation was delayed at 4°C to day 90 at pH's 5 and 7, day 7 at pH 9 and delayed further in frozen samples. BE concentrations increased 50% by day 7 at 25°C at all pH's, then decreased 50-77% by day 30. pH effects on degradation were delayed at 4°C and -15°C.

When COC only was added to urine at pH 9, no EME was detected by 60 days. COC decreased to <100 ng/mL by day 1 at 25°C, by day 7 at 4°C and by day 60 at -15°C. BE was not detected at 25°C at time 0 but was detected at 12 hours, and not until 24 hours at 4°C and 7 days at -15°C.

Urine chemistries were also measured to investigate any relationship between them and the stability of COC, BE and EME. Rates of degradation were similar at creatinine concentrations of 25 and 165 mg/dL. Creatinine concentration remained stable for the duration of this study.

"CyberTox": Toxicology Resources on the Internet

Diana Garside and Bruce A. Goldberger, University of Florida College of Medicine, P.O. Box 100275, Gainesville, FL 32610-0275*
[internet address - goldberg.pathology@mail.health.ufl.edu]

The Internet is a global network of connected computer systems located in government, education, military, and commercial institutions. Historically, the Internet was designed in readiness for doomsday by the U.S. Department of Defense in the event that a nuclear war would disrupt military command and control systems. Today, it is used as a giant bulletin board, so rich in resources that it provides its users with an almost infinite wealth of information from every walk of life; its diverse subjects range from recipe swapping to the latest advances in rocket science.

Amongst the realm of information available on the Internet, toxicology resources include the following World Wide Web (WWW) sites:

Resource Title	WWW Site
WWW Drug Information Server	http://www.paranoia.com/drugs/
Drug-Related Network Resources	http://hyperreal.com/drugs/faqs/resources.html
Internet Accessible Drug-Related Resources	http://www.ksu.edu/~vivaldi/druginfo

Using the Internet, the forensic toxicologist can update themselves on the latest licit and illicit drugs and "in vogue" drug habits, drug slang, drug sources, procedures on how to synthesize drugs, public opinions and attitudes, myths and misconceptions, and what is known about drugs and drug testing. A knowledge of this information is essential for combating drug abuse.

A selection of articles collected from "CyberTox" are presented, chosen to demonstrate information resources available to the toxicologist.

Computer File Format Translations: A Means of Producing Publication Quality Graphics and Text Translations in Forensic Research

William D. Darwin and Edward J. Cone, Addiction Research Center, NIDA, NIH, P.O. Box 5180, Baltimore, MD 21224*

Most analytical instruments used in the forensic laboratory have interactive computers interfaced for use as controllers and as data stations. Computer functions include: 1. data file (graphics) storage; 2. text file storage; 3. methods storage and; 4. other automated instrumental functions. Publication quality graphics from laboratory generated data are frequently needed for scientific reports (hard copy graphics) and for formal presentations (35-mm slides and transparencies). Graphic requirements in our laboratory include gas chromatographic tracings, GC/MS spectra and data illustrations. We have used a simple and convenient method of translating Hewlett-Packard Graphics Language (HPGL) files from HP ChemStations to Macintosh PICT format, the standard graphics format used for Macintosh applications. HiJaak Graphics Suite,

Ver. 3 (Inset Systems) was used with the HP DOS ChemStation for screen captures and graphics conversion. Once a screen capture has been executed, HiJaak allows the HPGL files to be translated into Computer Graphics Metafile (CGM) format. With Macintosh translation software, MacLink Plus, Ver. 8 (DataViz, Inc.), screen captures in CGM format can be translated into the PICT format. In PICT format, any Macintosh graphics application, such as MacDraw Pro, SoftShell ChemIntosh, CSC ChemDraw Plus or Adobe Persuasion can be used to annotate chromatograms and spectra by adding desired graphics and deleting unnecessary background to produce a finished product. This procedure is not exclusive for HPGL files. Many different types of translations are possible. Generally for graphics productions, it is easier to translate data graphics from a DOS environment to a Macintosh environment than in the opposite direction. This is a limitation of the software packages that convert graphics file formats. However, in general, text translations can be accomplished in any of a number of ways (e.g., DOS-to-DOS, DOS-to-Macintosh, Macintosh-to-DOS and Macintosh-to-Macintosh). These methods are used in our laboratory for production of publication quality graphics and for text translations on a routine basis.

A Model for Postdoctoral Education in Forensic Toxicology

George F. Jackson, Andrew P. Mason, Robert A. Middleberg, Carl Selavka, Dean F. Fritch and Robert F. Turk, National Medical Services, Inc., 2300 Stratford Avenue, Willow Grove, PA 19090*

Traditional methods for acquiring training in the field of forensic toxicology involved work experience / mentor system. We have expanded this basic concept and developed a novel postgraduate / fellowship for additional training in forensic toxicology. The duration of the training period is two years, during which the fellow receives comprehensive instructions in all aspects of laboratory operations. This includes rotation through the TDM, Environmental, Forensic, Criminalistics, and Research sections of the company. The duties performed by the fellow will be coordinated to coincide with the training received, and will be cumulative. The goals of the fellowship are to train the individual to become a fully qualified, independently acting Forensic Toxicologist.

A Custom Computer Program That Automatically Tracks GC/MS Quality Control Data on Hewlett-Packard UNIX ChemStation GC/MS Systems

William N. Bennett and Anthony Wu, Bayshore Clinical Laboratories, Brown Deer, WI 53223 and A. Wu & Associates, Inc., Brookfield, WI 53005*

We have written a custom computer program on the 5970 Hewlett-Packard UNIX ChemStation GC/MS system (Rev. B) which allows automatic tracking, (i.e., collection, storage and data reduction) of GC/MS quality control data for drugs of abuse testing. This integrated computer program was developed in our DHHS-certified laboratory to automate day-to-day GC/MS confirmation analyses and allow easy data reduction of associated quality controls. The program is composed of 3 major subprograms (all written in HP-UX series and can be copied to other LAN workstations) which control the following: 1) calculation, storage and summarizing/printing of data, 2) updating of QC means and lot numbers and 3) data reduction of previously stored or manually entered QC data. The program is written for a single-point calibration method and is capable of tracking (lot #, concentration, date placed in service, technologist) a Negative QC, Low QC, High QC, Positive QC and Blind QC on a per batch basis. The program requires very little input from the operator. QC and donor samples are monitored on a per sample basis whereby out-of-range parameters are automatically flagged as delineated by Westgard's l_{2S} rule. Parameters which are monitored include Retention Time Window (RTW), Ion Ratios (IR), and, for controls, Quantitative Values (QV). RTW is set at ± 2% (as defined by an unextracted QC) and IR and QV are set at ± 20% (or ± 2SD) of the calibrator and historical mean, respectively. A one-page data printout is produced for each sample and contains all relevant information regarding the integrity of the analysis. A one-page summary report is produced at the conclusion of a batch analysis and includes all relevant QC and donor sample information to allow easy review of the adequacy of the analysis. At any time interval (e.g., monthly), QC data can be reduced to give means, acceptable ranges, CV's, and other demographic information as well as a Levy-Jennings plot. The program is a better alternative to the expensive and cumbersome programs currently being marketed in the field of drugs of abuse analysis.

Differences in Two Radioimmunoassays for the Analysis of Lysergic Acid Diethylamide (LSD)

Sandra Valtier and John T. Cody, Clinical Investigations, Wilford Hall Medical Center, Lackland AFB, TX 78236-5319*

Screening urine samples for LSD with two commercially available radioimmunoassay (RIA) kits for LSD, Diagnostic Products Corporation (DPC) Coat-a-Count and Roche Abuscreen assays, often give different results, presumably due to differences in antibody cross-reactivity. Responses to LSD should result in essentially the same value. In reality, however, significant differences were seen in the response of the assays to LSD.

To assess the reason for these differences, calibrators and samples made from dilutions of calibrators from each kit, along with drug-free urine and urine containing various concentrations of LSD were analyzed following manufacturers' protocols. Results differed significantly between the two assays. Samples diluted from a DPC calibrator (to 750 pg/mL) gave results about 30% lower than the target concentration when assayed with the Roche kit and corresponding samples prepared from a Roche calibrator read above the target level by a similar percentage when assayed by the DPC kit. The percent deviation also varied with target concentration. GC/MS quantitation of the 500 pg/mL calibrators showed the Roche calibrator to be greater than 20% different than the DPC calibrator. Lacking a reference standard for LSD, it was not possible to demonstrate which calibrator was "correct"; however, it clearly demonstrated differences. Due to the dramatic differences in calibrator concentrations, samples with the same LSD concentration analyzed with these methods will yield different results.

Syva Emit® LSD Assay on the Hitachi 717

Peter Nguyen, Mae Hu, Ed Berger, Qing Lin, Yihshing Shih, Margaret Henson, Tom Kempe, Ken Gottwald, and Joan Centofanti, Behring Diagnostics Inc., 3403 Yerba Buena Road, San Jose, CA 95135*

A homogeneous enzyme immunoassay for the detection of LSD in human urine has been developed for use on the Hitachi 717. The assay cutoff is 0.5 ng/mL. The assay is packaged as a stable, two reagent liquid formulation which requires no reconstitution.

The Emit LSD assay Reagent 1 contains a monoclonal antibody to LSD and Reagent 2 contains a novel LSD enzyme conjugate. Calibrators consist of known amounts of LSD in a urine matrix. The sample, Reagent 1 and Reagent 2 volumes used on the Hitachi 717 are 20 µL, 153 µL and 77 µL, respectively. The assay demonstrates excellent within-run and total precision with CV's less than 1.5%. The separations between drug levels were maintained for at least 14 days after initial calibration.

Forty-eight samples, positive for LSD by the Abuscreen®* RIA method were also positive by the Emit LSD assay. One hundred and one Abuscreen® RIA negative samples were tested. One hundred samples were found to be negative by the Emit assay. One sample was positive by the Emit assay. Out of ten spiked samples, all samples containing more than 0.5 ng/mL LSD assayed positive, and all samples containing less than 0.5 ng/mL LSD assayed negative. The assay showed no clinically significant crossreactivity to compounds such as α-ergocryptine, ergotamine, lysergic acid, methysergide and serotonin. The assay did not crossreact with compounds associated with other drugs of abuse.

The Emit LSD assay provides an accurate and reliable method for high-volume screening for the presence of LSD.

Profile of Drug Use in California: A Composite of Vehicle and Health and Safety Code Drug Findings

William H. Phillips, Jr., California Department of Justice, Bureau of Forensic Services, 4949 Broadway Street, Sacramento, California 95820

This poster will illustrate the results of the analysis of blood and urine samples collected from impaired drivers in the State of California to include calendar years 1992 through 1994. The blood alcohol result will be compared with the drug finding in the same sample. The California Office of the Attorney General, Department of Justice, Division of Law Enforcement, provides comprehensive analysis of biological samples collected from impaired drivers that have been submitted from law enforcement agencies in 46 counties. If the blood alcohol concentration is below 0.08% the subject's sample is automatically submitted to the Toxicology Laboratory for drug analysis. The samples are screened by immunoassay for the presence of amphetamines, benzodiazepines, cocaine, marijuana, opiates, and phencyclidine. Marijuana is the most commonly encountered drug, found in 30% of the samples submitted in 1994. This is closely followed by methamphetamine found in 24% of blood and urine samples. A trend of increasing positive findings of marijuana and methamphetamine is apparent from 1992 through 1994. The poster will also compare results of testing samples submitted from subjects violating section 11550 of the California Health and Safety Code, being under the influence of controlled substances. This program is available on a fee for service basis, with expert testimony included as part of the service.

A Tabular Summary of Functional/Clinical Neuroanatomy, Neurotransmitter-Receptor Location, and Sites of Drug Action in the Human Brain

Chester Flaxmayer, Forensic Alcohol Science and Technology, P.O. Box 5658, Glendale, AZ 85312*

A summary of functional/clinical neuroanatomy, neurotransmitter-receptor location, and sites of drug action in the human brain, correlates observed behavior or physiological response to the influence of drugs or disease. We compiled a summary of functional/clinical neuroanatomy, neurotransmitter-receptor location and sites of drug action in the human brain. The tabular neuroanatomy is sufficiently detailed to allow one to localize particular behavior or physiological responses with the area of the brain believed to be responsible for them. In addition, neurotransmitter-receptor information is paired to anatomy and function, allowing one to predict behavioral or physiological changes that may occur from drugs that affect the specific neurotransmitter or from drugs that interact at the specific receptor site. For example, the superior colliculus and the substantia nigra, responsible for control of saccadic eye movements, contain $GABA_A$ receptors. Since benzodiazepines bind at $GABA_A$ receptors, this table allows one to predict that benzodiazepines will affect saccadic eye movements. Additional information covers general classes of drugs and the specific receptor activity that they exhibit. This allows one to estimate the behavioral or physiological changes that may occur from ingestion of the drug.

This material was collected from diverse sources in the fields of anatomy, neurobiology, neurochemistry, and toxicology, and provides a valuable resource for forensic toxicologists, medical personnel, and those working in related areas, allowing correlation of CNS-induced symptoms with drugs and, in some cases, disease.

Variations of Blood Digoxin Levels During Storage

Eric Revuelta[1], Marc Deveaux[1], Patrice Fialdes[2], Valéry Hedouin[1], Philippe Dewailly[2], and Didier Gosset[1], [1]Institut de Médecine Légale, place Variet, 59000 Lille, France and [2]Médecine Nucléaire et CSPA, C.H.R.U., 59037 Lille Cédex, France.*

Acute and chronic intoxications by digoxin are not infrequent. If therapeutic monitoring of digoxin can be easily achieved by radioimmunoassay, this method can be also used successfully in post-mortem determinations.

Aim of Study: To determine if different temperature storage conditions of blood samples has an influence on digoxin levels.

Material and Method: Blood samples (10 mL) were taken by venipuncture from 22 elderly patients treated with digoxin (0.06 to 0.25 mg per day) and from 5 control subjects who were not receiving digoxin. For each sample, determination of blood digoxin was performed after day 0, 7, 14 and 28 and at different storage temperatures (-20°C, +4°C and room temperature).

Results: Statistical analysis of the results did not show a significant difference between digoxin levels under the

different storage temperatures: therefore, this data indicate that digoxin is stable in blood samples stored for periods up to 28 days at temperatures between -20°C and room temperature.

Synthetic Strategies and Analytical Verification of Neat Forensic Reference Materials

H. Kenan Yaser, Michael A. Re, and Mitzi M. McDowell, Radian Corporation, P.O. Box 201088, Austin, TX 78720-1088*

A large number of neat forensic reference materials were synthesized. A variety of methods were developed for production, identification, and purity analysis of these compounds. A range of synthetic scenarios were encountered. Parent drugs such as Butalbital were synthesized to prove identity. Production of deuterated materials such as Phenobarbital-D_5 had to balance synthetic difficulty with user applicability. Compounds such as d-methamphetamine were synthesized within a framework of synthetic limitations unique to optically active compounds.

Identity and purity verification of finished standards was achieved using a variety of analytical tools. Instruments such as GC, HPLC, DSC, EA and Karl Fischer were used to confirm product purity. GC/MS, NMR, FTIR were used primarily for product identity. Each analytical instrument had specific strengths (e.g., DSC for analysis of inorganic impurities, NMR for solvent contamination, and Karl Fischer for water), yet each had limitations on the scope of their use. Thus, only a combination of data gave comprehensive results. In order to prepare high quality forensic reference standards the many questions relating to synthesis and analysis of neat forensic reference materials had to be satisfactorily addressed.

Description of a Screening Assay System to Detect Adulteration of Urine Samples

John W. Soper and Dennis V. Canfield, Civil Aeromedical Institute, Forensic Toxicology Research Section, P. O. Box 25082, Oklahoma City, OK 73125*

There is no current consensus about how urine screening adulterants are best detected. We have taken the approach that an adulteration assay should be designed to look for multiple adulteration methods, and should be able to flag an adulteration effort, even if a significant amount of drug is present in the urine.

Our basic methodology has involved adding PCP to Reagent B in the Syva PCP assay. We have found that the addition of methanol to the solution markedly increases the assay sensitivity, and we have also reversed the order of reagent addition. When the assay is performed, a *normal* urine should therefore produce a *high* absorbance change, while an *adulterated* sample should produce a *lowered* value.

The use of PCP, as described above, produced an inhibition of 83% by "Urinaide", while "Mary Jane Super Clean" (MJ), produced an inhibition of 48%. One vial of MJ in a typical urine sample is sufficient to cause a THC spike of 150 ng/mL to be rendered as negative, with absolutely no instrument flags being produced. This amount of MJ produced an inhibition of 30% with our adulteration assay.

Our original 'Urinaide' patient had a normal 'negative' Chem 1 value for THC, with a GC/MS value of 74 ng/mL THC. The AD_X value for this patient was 93 ng/mL. The adulteration assay was inhibited by 47% for this sample.

A Homogeneous Enzymatic Method (pH-Detect™) for Urine pH Determination

Cheng-I Lin, Bhanu P. Sharma and Yuh-geng Tsay, Diagnostic Reagents, Inc., 601 California Avenue, Sunnyvale, CA 94086*

Urine pH measurement is one of the easiest and most common method used in the clinical laboratory for detection of urine adulteration. Urine pH has been measured with pH paper, pH meter or an end point calorimetric method. We describe here a kinetic homogeneous enzymatic pH detection method (pH-Detect™)† suitable for automated chemistry analyzer application. The method was based on the relationship between glucose-6-phosphate dehydrogenase (G6PDH) enzymatic activity and pH. A two-point (pH 5 and pH 8) calibration curve was established for extrapolation of the urine pH with its respective enzymatic assay activity measured spectrophotometrically at 340 nm. Within-run precision and between-run precision using urine samples with pH ranging from 5 to 8 was less than 0.8 %. A 100.1% average recovery was observed with the assay using urine samples with pH ranging from pH 4 to pH 11. Clinical correlation against the pH meter method gave a linear regression of y (pH-Detect) = 1.4 x - 2.3 and a correlation coefficient (r) of 0.91.

The stable liquid ready-to-use enzymatic pH detection method is applicable for high volume urine screening for detection of sample adulteration.

(† Patent pending)

CEDIA® LSD Assay for Urine Drug Testing

Jeffrey E. Shindelman, Jill L. Brown, Deborah D. Motton, Paul W. Weingarten, Cindy A. Vistica, Mary C. Crenshaw, Davis G. Harris, Neal F. Bellet, William A. Coty, and Pyare L. Khanna, Microgenics Corp., 2400 Bisso Lane, Concord, CA 94524 and Gerald F. Sigler, Boehringer Mannheim Corp., Indianapolis, IN 46250*

A novel homogeneous enzyme immunoassay for the detection of LSD in urine has been developed and applied to the Boehringer Mannheim/Hitachi 911 Automated Analyzer. In the CEDIA method, the enzyme β-galactosidase is split into two inactive fragments: a large fragment (EA) and a smaller polypeptide (ED), which can spontaneously recombine to form active enzyme. LSD is covalently attached to each ED molecule so that enzyme formation is not affected; however, binding of the ED-LSD conjugate to anti-LSD antibodies inhibits reassociation of enzyme fragments. Analyte present in a sample competes with conjugate for binding to the antibody. Thus the amount of enzyme formed (as measured by the rate of substrate hydrolysis) is proportional to the analyte concentration. The assay is performed on the BM/Hitachi 911 using a fully automated format.

The CEDIA LSD Assay at 0.5 ng/mL cutoff has a range of 0 to 4.0 ng/mL, with intra-assay precision of 3.6% CV at cutoff, 4.0% CV at -25% of cutoff and 3.4% CV at +25% of cutoff. In a random study of 2012 LSD negative samples, 2010 samples tested negative and 2 tested positive, specificity 99.9%. The assay showed insignificant crossreactivity to related compounds such as ergotamine,

alpha ergocryptine, lysergic acid, and serotonin. Confirmed LSD positive samples by GC/MS/MS were tested by CEDIA and a commercial RIA method. Both assays found 35 samples to be greater than the 0.5 ng/mL cutoff and 4 to be below cutoff, sensitivity 88%. The CEDIA LSD assay is equal in performance to RIA, and has the advantage of being fully automated and eliminates the need for radioisotopes.

Improved CEDIA® DAU Benzodiazepine Assay for Urine Drug Testing

Neal F. Bellet, Heidi A. Scholz, Michael L. Opel, Elizabeth Padilla, F. Roark Galloway, and Pyare Khanna, Microgenics Corp., 2400 Bisso Lane, Concord, CA 94524*

We developed a homogeneous enzyme immunoassay for the detection of benzodiazepines in urine using CEDIA technology. In the assay, the enzyme ß-galactosidase was genetically engineered into two inactive parts: Enzyme Acceptor (EA) and Enzyme Donor (ED), which can spontaneously reassociate to form active enzyme. The drug derivative is covalently attached to ED so that enzyme formation is not affected; however, binding of the antibody to the ED-drug conjugate inhibits the reassociation with EA. The assay is performed on the Hitachi 717 as follows: 3 µL Sample plus 130 µL Reagent 1 containing EA and antibody are incubated for 5 min, then 130 µL Reagent 2 containing ED-drug conjugate and substrate (CPRG) is added. The subsequent changes in absorbance are used to determine the concentration of drug in the sample.

Using the CEDIA Benzodiazepine Assay, the following results were obtained: assay range from 0 to 5,000 ng/mL, separation of 110 mAU/min from 0 to 200 ng/mL and 150 mAU/min from 0 to 300 ng/mL, and dose CV's of less than 7%. There are two major improvements in this product versus the original CEDIA Benzodiazepine Assay. Specificity of the assay has been substantially improved, including the elimination of unconfirmable positive results due to sertraline and its metabolites. In addition, the sensitivity to samples containing clonazepam (Clonapin) and flunitrazepam (Rohypnol) has been significantly improved. Several samples containing clonazepam or flunitrazepam, which tested negative with the current CEDIA and EMIT II® products, tested positive with the new CEDIA Benzodiazepine Assay. The CEDIA Benzodiazepine Assay provided a rapid, convenient and effective method of screening for the presence of benzodiazepines in urine.

A Comparison Study of an Abuscreen OnLine® Immunoassay for the Detection of Propoxyphene in Urine with the Syva Emit II Propoxyphene Assay

John Irving, Scott Brewington and Jim King, Lab Corp of America, 1120 Stateline Road, South Haven, MS 38671*

The OnLine technology is a highly sensitive immunoassay which has been developed for the detection of propoxyphene and nor-propoxyphene in urine. The assay uses an immunogen and labeled derivative coupled with the nitrogen position of nor-propoxyphene. This immunoassay is based on the Kinetic Interaction of Microparticles in a Solution (KIMS) principle where the drug content in a urine sample is directly proportional to the inhibition of microparticle aggregation. Typical quantitative within-run precision on large analyzers such as the Hitachi 717, 747, and the Olympus AU 800 at X=cut-off of 300 ng/mL, at 0.5X, 0.8X, 1.0X and 1.2X was ≤5%. Cross reactivity to the primary metabolite, nor-propoxyphene, was ≥70% and cross reactivity to para-hydroxypropoxyphene was ≥20%. Urine specimens (N=5150) were randomly screened on the Olympus 800 with both the OnLine and Syva Emit II propoxyphene immunoassays. Fourteen of these samples screened positive in both assays and were confirmed by GC/MS for propoxyphene and/or nor-propoxyphene. An additional six samples screened positive by OnLine and were negative by Emit II, and six of these samples were confirmed by GC/MS to contain nor-propoxyphene.

To demonstrate the reliability of this assay it was important to use a GC/MS procedure that detected both propoxyphene and nor-propoxyphene. It can therefore be concluded that when using the OnLine screening assay a laboratory's ability to detect clinically positive samples should increase.

Comparison of Production Efficiencies Between Syva EMIT® and Microgenics CEDIA® DAU Reagents in a High Volume Toxicology Laboratory

Robert F. Foery, Cindy G. Stewart, Michael S. Tornatore, and Roger L. Rutter, National Laboratory Center, Inc., MedExpress, 4022 Willow Lake Blvd., Memphis, TN 38118*

The decision process for a major reagent change in a forensic laboratory may involve the study of technical literature supplied by the manufacturer, a site visit to a laboratory utilizing the new reagent system, a review of data from FDA clinical trials and/or alpha/beta site evaluations as well as the laboratory's own evaluation (however limited) of proposed versus existing reagents. In the final analysis, however, an actual production audit of new reagent versus old reagent may offer the best information about the validity of the decision.

In March, 1995, MedExpress/NLC, Inc. implemented a significant change in screening reagents from EMIT® to CEDIA®. A three month production audit of DOT and non-DOT EMIT® screening data (April-June, 1994) versus CEDIA® screening data (April-June, 1995) indicated major cost savings resulting from the use of the CEDIA® reagent system.

Preliminary data analyses indicated that the CEDIA® reagent stability resulted in a significant reduction (greater than 95%) in the number of repeat analyses due to control failures, when compared to EMIT® on the Hitachi-747/200. Additional benefits included: reduction (greater than 25%) in the number of AMP/METH re-screens, increased absorbance rate separation between the 75% and 125% controls, increased assay dynamic range resulting in fewer GC/MS repeats due to dilutional inaccuracy as well as shorter and more simplified reagent reconstitution protocols.

High-Pressure Liquid Chromatographic Analysis of Pilocarpine in Urine

Robert D. Williams and Ickbok K. Juhng, Ohio State University Medical Center, 1214 Kinnear Road, Columbus, OH 43212*

Pilocarpine (Isopto® Carpine, Alcon Inc.) is a cholinergic agent which is primarily used in 0.5-4% ophthalmic solutions to control intraocular pressures in the treatment of open-angle glaucoma. Pilocarpine hydrochloride (Salagen®, MGI Pharmaceuticals) was approved in 1994 by the FDA for oral use as a salivary gland stimulant in radiation-induced xerostomia. Oral pilocarpine can produce several adverse effects including sweating, nausea, dizziness, rhinitis, and vasodilation. Analysis of pilocarpine in plasma and aqueous humor by HPLC using multi-step liquid/liquid extraction procedures have been published, however, no assays have been reported for its determination in urine.

A high-pressure liquid chromatography method for the on-line purification and analysis of pilocarpine in urine was established using the Bio-Rad Remedi™ HS. A 1 mL aliquot of sample was added to 200 µl of chlorpheniramine internal standard (10 µg/mL) and assayed. A 50-fold linear dynamic range was demonstrated from the limit of detection (200 ng/mL) to the upper limit of linearity (10 µg/mL) with a correlation coefficient (r) of 0.999. The within-run coefficient of variation was 1.6% for 1 µg/mL samples (n=10) and 7.3% for between-run (n=20). The routine chromatographic conditions provided for the differentiation of pilocarpine from its pharmacologically inactive *trans* stereoisomer, isopilocarpine. Phenobarbital, oxycodone, meperidine, hydrocodone, caffeine and endogenous compounds in the specimens analyzed did not interfere with the assay. Thus, this selective assay is sensitive at detecting pilocarpine and represents the first procedure established for its quantitation in urine.

Improved Chromatographic Separation of Opiates as TMS Derivatives by Formation of the Oxime-TMS Derivatives of Hydrocodone and Hydromorphone

Randal Clouette and Gary H. Wimbish, Laboratory Specialists, Inc., 113 Jarrell Drive, Belle Chasse, LA 70037

Oxime derivatives of hydrocodone and hydromorphone can be formed during enzymatic hydrolysis by the addition of hydroxylamine hydrochloride. The hydrolyzed/derivatized samples can then be extracted with a conventional liquid-liquid extraction procedure. Treatment with MSTFA forms the TMS derivatives of codeine and morphine while hydrocodone and hydromorphone are silylated to form their corresponding oxime-TMS derivatives. Urine samples submitted for confirmations are subjected to an enzymatic hydrolysis to liberate glucuronide conjugated opiates. Simultaneously, any hydrocodone or hydromorphone which may be present is converted to its corresponding oxime by the addition of a small amount of hydroxylamine hydrochloride along with the glucuronidase. The hydroxylamine reacts with the ketone at the C-6 position of these drugs to form an oxime. The oxime leads to different retention time and spectra when analyzed by GC/MS.

Using this modification, we were able to achieve complete chromatographic resolution of these common opiates while decreasing instrumental analysis time. Full spectral scans of the oxime-TMS derivatives of hydrocodone and hydromorphone provided satisfactory ions for SIM monitoring. The changes in elution order caused by the pretreatment also led to complete chromatographic resolution of commonly encountered opiates.

Methadone and Propoxyphene Assays with Hitachi 717 Analyzer

Parisa Khosropour and Tom Chia, Diagnostic Reagents, Inc., 601 California Avenue, Sunnyvale, CA 94086*

Instrument application on a Hitachi 717 analyzer has been developed for homogeneous methadone and propoxyphene enzyme immunoassay. Typical instrument parameters used for these assays include 10-15 µL sample, 125 µL antibody/substrate reagent, 125 µL enzyme conjugate reagent, 37°C assay temperature, 30-35 reading cycle and 340 nm primary wavelength. Within-run and between-run precision for both assays are generally ≤1%. The reagents are stable for twelve months when stored refrigerated before or after bottle opening. Methadone antibody exhibits equal specificity toward methadone and long acting l-α-acetylmethadol (LAAM). The propoxyphene assay not only recognizes the parent drug but also shows high specificity to its major metabolite, norpropoxyphene. No cross reactivity to other commonly encountered medications is observed with both assays. Sensitivity for both methadone and propoxyphene assays are 10 ng/mL and 15 ng/mL, respectively. Both assays correlate well with commercial EIA assay methods using clinical urine specimens.

The liquid ready-to-use methadone and propoxyphene assays are suitable for routine clinical use with the Hitachi 717 clinical chemistry analyzer for the detection of methadone and propoxyphene in human urine.

Solid Phase Extraction of Marijuana Metabolite Using Micro-Bed Sorbents

Lisa O'Dell, Kathy Rymut, Tony Darpino, and Barbara Fryer, United Chemical Technologies, Inc., 2731 Bartram Road, Bristol, PA 19020*

We describe a packed micro-bed SPE method for the extraction of THC-COOH utilizing 80% less total solvent volume and significantly faster throughput than traditionally packed SPE columns. THC-COOH was added to certified negative urine at a concentration of 15 ng/mL. The 1 mL sample was treated with 0.1 mL 10N NaOH and 0.6 mL glacial acetic acid. Methanol, deionized water, and 0.1M HCl (0.5 mL each) was used to condition the micro-bed cartridge. The prepared sample was loaded on the column and 0.5 mL of deionized water and 1 mL 0.1M HCl/acetonitrile (70/30) were added to wash away interferences. The column was dried for 3 mins before 0.1 mL of hexane was added. THC-COOH was eluted with 2 x 0.5 mL of hexane/ethyl acetate (75/25). Internal standard, D_3-THC-COOH, was added to the eluate, dried and derivatized using BSTFA with 1% TMCS. Analysis was achieved by GC/MS in the SIM mode using a RT_X-1 capillary column with a temperature program of 150°-325°C at 25°C/min. The average absolute recovery of THC-COOH was greater than 90%.

Pre-Employment Urine Testing for Drugs of Abuse and the Medical Review Officer: Six Years Experience

Elkin Simson, Long Island Jewish Medical Center, The Long Island Campus for the Albert Einstein College of Medicine, 270-05 76 Avenue, New Hyde Park, NY 11040

Six years of pre-employment urine testing for drugs of abuse at a large tertiary care medical center is reviewed to assess the role of the Medical Review Officer (MRO) in this process.

All applicants for employment at Long Island Jewish Medical Center to whom a job offer has been made are required to pass a urine drug test. For the 6 years from January 1989 to December 1994, urine samples from 8699 applicants were tested for amphetamines, barbiturates, benzodiazepines, cocaine metabolite, opiates, and phencyclidine. Screening was by enzyme immunoassay, with confirmation of presumptive positives by gas chromatography/mass spectrometry. All positive results were reviewed by the MRO (the author) and a medical explanation for each positive result was sought by examination of supplied prescriptions, telephone conversations and personal interviews.

Of the 8699 urines tested, 216 had a total of 262 confirmed positives for an applicant positive rate of 2.5%. Of these 216 applicants, 111 (51%) were cleared for employment after MRO review. The MRO verified 59 positives (27%) and these applicants were not employed. Forty applicants (19%) who had a plausible explanation for the use of prescription drugs but with inadequate prescription evidence to support the positive finding, or who described ingestion of poppy seeds to explain a positive morphine result, agreed to random urine testing during the probation period of employment; none failed random urine testing. Six applicants with confirmed positive results withdrew their applications before MRO review was complete.

This experience confirms the important role of the MRO in pre-employment urine testing.

The Detection and Quantitation of Fluoxetine and Norfluoxetine in Postmortem Specimens

Iain M. McIntyre, Allison Peace, Christopher V. King, Matthew J. Lynch and Olaf H. Drummer, Victorian Institute of Forensic Pathology and Department of Forensic Medicine, Monash University, Southbank, Victoria 3006, Australia.*

Fluoxetine (Prozac®) is an antidepressant with specific neuronal inhibitory effects on serotonin receptors. Fluoxetine is rapidly metabolised to its desmethyl metabolite, norfluoxetine, which has pharmacological activity equivalent to the parent drug. An HPLC method is described for postmortem blood, liver, bile and vitreous humor. We also provide information on the concentrations detected in these postmortem tissues.

Fluoxetine was initially detected in postmortem blood in butyl chloride extracts using capillary gas chromatography with confirmation by mass spectrometry. Quantitation was then achieved by HPLC with dual UV detection at 210 nm and 254 nm. Extracts were prepared by alkalization of tissues with Na_2CO_3 and extraction with butyl chloride. Drugs were back extracted with dilute phosphoric acid. An aliquot was injected into the HPLC.

Fluoxetine and norfluoxetine separated chromatographically from other antidepressants and commonly encountered drugs. The blood detection limits were 0.02 mg/L, and the blood calibration curves were linear to at least 2.5 mg/L.

Thirty postmortem cases were identified with fluoxetine/norfluoxetine in the calendar year of 1994. These cases were then studied in detail. The causes of death were: suicide (non-drug related) in 13 cases; drug related (mixed drug toxicity) in 12 cases; and other causes in 5 cases. Femoral blood concentrations were within the established therapeutic ranges in 19 cases: 0.17 ± 0.11 mg/L (fluoxetine) and 0.10 ± 0.09 mg/L (norfluoxetine). However, in 6 cases, femoral blood concentrations were greater than the therapeutic range: up to 1.1 mg/L (fluoxetine) and up to 0.57 mg/L (norfluoxetine). Blood was collected from other sites such as chest/subclavian in 5 cases.

Fluoxetine was not the sole drug of abuse, and was not considered a major contributory factor, in any of the deaths.

Time Dependent Changes and Site Dependence Differences in Postmortem Morphine Concentration in Man

Barry K. Logan and David Smirnow, Washington State Toxicology Laboratory, Department of Laboratory Medicine, University of Washington, 2203 Airport Way S., Seattle, WA 98134*

In 32 deaths involving morphine, left ventricular blood, femoral blood, and cisternal cerebrospinal fluid were collected as soon after death as possible, and were collected again, together with iliac blood, at the time of autopsy. Samples were analyzed in duplicate for morphine by coated tube radioimmunoassay (Coat-a-Count, DPC).

No evidence was found for changes in morphine concentration with respect to time at either central or peripheral sites, or in the cerebrospinal fluid. This suggests that the blood morphine level at the time of autopsy is representative of the perimortem blood morphine level even after several days.

Site dependent differences were apparent when the ventricular morphine level exceeded 0.30 mg/L, in which case the peripheral blood morphine was generally lower. This could be due either to concentration dependent release of morphine from cardiac muscle, or to incomplete distribution of the drug following an acute overdose. At peripheral sites, femoral and iliac blood morphine concentrations were well correlated with each other and stable with respect to time, and are recommended sampling sites for postmortem forensic toxicology.

Sequential Derivatization, Extraction, and Analysis of Polar Cocaine Metabolites in Postmortem Whole Blood by GC/MS

David Smirnow and Barry K. Logan, Washington State Toxicology Laboratory, Department of Laboratory Medicine, University of Washington, 2203 Airport Way S., Seattle, WA 98134*

The cocaine metabolites benzoylecgonine (BZE), benzoylnorecgonine (BZNE), norcocaine (NC), ecgonine methyl ester (EME), nor-EME, ecgonine ethyl ester (EEE), ecgonine (EC) and norecgonine (nor-EC) contain polar functional groups, and are poorly extracted from human tissues and fluids using common liquid/liquid extraction procedures Routine analysis does not detect many of these compounds, and as a result little is known about their toxicological significance.

A novel extractive derivatization procedure was developed to allow measurement of the above metabolites

and hydrolysis products in whole blood. Following an initial protein precipitation, sequential derivatization reactions blocked the polar functional groups and permitted extraction of the products into a common organic solvent.

Blood and plasma proteins were precipitated with a methanol/acetonitrile mixture. Following evaporation of the supernatant, sequential derivatizations were performed. The first was an alkylation using propyl iodide to alkylate carboxylic acids and secondary amines. This was followed in the same reaction vessel by the addition of para-nitrobenzoylchloride to esterify alcohols. The reaction mixture was then extracted with a routine n-butyl chloride procedure for basic drugs. The final extract in chloroform was analyzed by gas chromatography/mass spectrometry. The procedure is robust, specific and linear over the range 10 to 10,000 ng/mL, and allowed quantitation of those compounds for which standards were available.

Evaluation of the ANSYS SPEC PLUS® for the Analysis of Morphine, Codeine and Hydromorphone in Urine Using Electron Impact
Gas Chromatography/Mass Spectrometry.

Neil A. Fortner, Tim E. Johnson, Perry A. Fukui, and David S. Lindman, PharmChem Laboratories, Inc., 1505A O'Brien Drive, Menlo Park, CA 94025*

Opiates continue to be one of the most widely abused classes of drugs. Our experience with large criminal justice drug testing programs indicate that opiates represent up to 35% of the illicit drug positives. We have evaluated the ANSYS SPEC PLUS® solid phase extraction column for the analysis of morphine, codeine and hydromorphone in urine using electron impact gas chromatography/mass spectrometry. Following acid hydrolysis, samples were extracted using the SPEC PLUS columns, concentrated and derivatized using MBTFA to form the TFA derivative. Within run precision was 2% for morphine, 1% for codeine and 3% for hydromorphone. Day to day precision was 9% for morphine, 6% for codeine and 10% for hydromorphone. The LOD was 30 ng/mL for morphine, 40 ng/mL for codeine and 50 ng/mL for hydromorphone. LOQ was 50 ng/mL for all analytes. Linearity was 20,000 ng/mL for morphine and hydromorphone and 10,000 ng/mL for codeine. No carryover was observed at 20,000 ng/mL for morphine, codeine or hydromorphone. We conclude that the ANSYS SPEC PLUS® offers a reliable and cost effective alternative for the extraction of morphine, codeine and hydromorphone from urine specimens.

Recovery of Trace Level Pharmaceutical Residues in Biological Matrices by Supercritical Fluid Extraction

Robert J. Maxwell, Owen W. Parks, Alan R. Lightfield and Alida A. M. Stolker, U. S. Department of Agriculture, ARS Eastern Regional Research Center, 600 East Mermaid Lane, Philadelphia, PA 19118*

In this laboratory efforts are underway to develop methods for the isolation of veterinary pharmaceutical residues from biological matrices such as blood, tissue and urine by supercritical fluid extraction (SFE). Our objectives were to provide alternatives to the lengthy, solvent-intensive methods currently employed in regulatory laboratories. Initial efforts were focused on the design of an SFE for trace level analyte recovery, an instrument which is now available commercially. This instrument was used to develop methods for the recovery of analytes such as sulfonamides, nitrobenzamides and anabolic steroids from tissue and urine at ppb concentrations. Target analytes extracted by supercritical carbon dioxide were trapped free of co-extracted interferences by means of an innovative *in-line* technique. Using this approach, a multi-residue mixture of nortestosterone, testosterone and methyl testosterone was recovered from bovine urine at 12.5 ppb concentration with a mean of 92.5% and a relative standard deviation of 4.8%. The SFE technique described was simple to perform, used negligible amounts of organic solvents and appeared to be applicable to many classes of pharmaceutical compounds.

Problems Commonly Encountered with Preparation, Analysis and Stability of Analytical Reference Standards

Mitzi M. McDowell, Michael L. Brake, and H. Kenan Yaser, Radian Corporation, P.O. Box 201088, Austin, TX 78720-1088*

Ensuring accurate and reliable results in forensic analysis is dependent on the quality of analytical reference standards. After obtaining highly pure, neat reference materials, standard solutions were prepared following rigorous protocols for preparation and analysis. Certain factors were then considered to maintain their integrity and quality. During an ongoing stability testing program it was discovered that solvent selection and storage conditions affected long-term stability. Issues such as solubility, chemical degradation and isomerization were greatly influenced by the choice of solvent. It was determined by GC/MS analysis that cocaethylene and analogs were unstable in protic solvents such as methanol but remained stable in distilled acetonitrile. Methods of analysis of analytical reference standards were affected by isotope effects, fragmentation patterns and derivatization dilemmas. By utilizing GC/MS in the SIM and CI modes, it was concluded that the above variables do exist in the analysis of oxazepam-D_5 TMS and secobarbital-D_5. Consideration of the internal standard, derivative selected, and ions monitored reduced these problems. Derivatization techniques were also evaluated, and it was determined that many of the common problems encountered when using analytical reference standards were due to excessive, incomplete, or no derivatization.

S.O.F.T. CONVENIENT MULTI-PURPOSE FAX SHEET

FAX TO: 602-839-9106
SOCIETY OF FORENSIC TOXICOLOGISTS, INC.

FROM: _____

FAX NO.: _____

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Got a SOFT-related question? Need to update or correct your address or phone and FAX numbers? Finally get ABFT-certified and want to add those credentials after your name? How about requesting an application form for a colleague?

Make a copy of this sheet. Then note your request/s or comment/s below and FAX to the SOFT Administrative Services number above.

S.O.F.T. DENVER '96

October 14 - 18

Important information to help you plan:

WORKSHOPS topics in the planning stages:

Capillary Gas Chromatography
Drugs & Driving
Fundamentals of Medical Examiner Toxicology
Inhalants
Internet
New Concepts in Forensic Urine Drug Testing
Principals of Drug Metabolism

HOTEL Denver Marriott Tech Center
for reservations, call:
1-800-228-9290 or 303-779-1100
Room Rate = current Federal Govn't = $77 including tax

SHUTTLE SERVICE:
from Denver International Airport = $15 one way

Watch for pre-registration materials in the March issue of TOXTALK

Meeting Hosts:
Laurel J. Farrell or J. Robert Zettl
CDPHE - Division of Laboratories
P.O. Box 17123
Denver, CO 80217
303-691-4727 or 303-691-4738
FAX: 303-393-7881

SOCIETY OF FORENSIC TOXICOLOGISTS

DENVER '96 - October 14-18, 1996

Instructions for Abstract Preparation

General Instructions:

The program committee solicits abstracts on all forensic toxicology topics, but is especially interested in papers on the analysis of drugs and metabolites in biofluids and tissues, drug interactions, drug-metabolism, pharmacology, pharmacokinetics and case reports involving drug toxicity/overdose. An original and three copies of the abstract must be submitted on the official abstract form. All abstracts must also be submitted on computer disk. Any IBM or Macintosh word processing format, or ASCII format can be accommodated. Please label the disk with the presenting author's name, IBM or Macintosh, and the word processing program utilized. The deadline for submission of abstracts is June 1, 1996. Late abstracts will not be accepted. The presenting author will be required to register for the meeting.

Scientific papers selected for presentation will be divided into two groups: Platform Presentations (15 min including questions, limit of one per presenter) and Poster Presentations (4 ft high by 8 ft wide). Tack boards and thumb tacks will be provided. Only abstracts written in English will be considered.

Content of Abstract:

1. Author(s) name(s) and address(es)
2. Short specific title
3. Statement of paper's objectives
4. Statement of experimental design
5. Statement of methods, if pertinent
6. Summary of results.
7. Statement of conclusion

Format of Abstract:

Your abstract must be typed and submitted in a neat and legible format following the instructions and style provided in the sample below. Type the entire abstract within the boxed area, single spaced with 12 point font. Type the title in upper and lower case, followed by the author(s) name(s) and address(es). Use an asterisk to identify the presenting author. Separate the author(s) name(s) from the body of the abstract by a single blank line. Indent each paragraph three spaces. Identify three key words at the bottom of the abstract.

Notification of Acceptance:

The presenting author will be notified upon receipt of the abstract. Notification of acceptance of the abstract and selection of the type of presentation will be mailed or sent by facsimile to the presenting author no later than August 15, 1996.

Specific Instructions:

Complete the attached form and follow the sample provided below. Proofread all information provided. Return original, three additional copies, and an IBM or Macintosh disk with the abstract to:

Mail Address: Dr. Amanda Jenkins
Chemistry and Drug Metabolism Section
Addiction Research Center, NIDA, NIH
P.O. Box 5180
Baltimore, MD 21224

Street Address: Dr. Amanda Jenkins
Chemistry and Drug Metabolism Section
Addiction Research Center, NIDA, NIH
4940 Eastern Avenue, Building C
Baltimore, MD 21224

Submissions by Federal Express, Airborne, DHL, other commercial carriers or Priority Mail should utilize the street address.

Sample Abstract:

Title: Type upper and lower case. Use significant words descriptive of subject content.

Author(s) name(s) and address(es): Type upper and lower case; spell out first and last name and use middle initial, *e.g.* Amanda J. Jenkins. Specify presenting author with an asterisk. Specifying degrees is unnecessary.

Indent each paragraph three spaces. Type the entire abstract within the boxed area, single spaced in 12 point font. Do not use all capital letters. Capitalize and punctuate exactly as you wish the abstract to appear.

Key Words: Type three key words or short phrases in upper and lower case.

SOCIETY OF FORENSIC TOXICOLOGISTS

DENVER '96 - October 14-18, 1996

Abstract of Paper

Presenting Author Name and Mailing Address:

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Presentation Preference:

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Co-author(s) and Business Affiliation(s):

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Has this paper been presented before?

☐ yes ☐ no; if yes, where and when?

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Available Audio-Visual Equipment: 35 mm slide projector & pointer; overhead projector will not be provided

Mail Address: Dr. Amanda Jenkins
Chemistry and Drug Metabolism Section
Addiction Research Center, NIDA, NIH
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Baltimore, MD 21224

Street Address: Dr. Amanda Jenkins
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Addiction Research Center, NIDA, NIH
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The abstract review committee reserves the right of final placement of the paper. The presenting author will be required to register for the meeting.

_____

Signature of presenting author

The JCETT Newsletter Fall 1995

Joint Committee on Education and Training in Toxicology

What is the JCETT?

The JCETT was established in 1993 to coordinate, develop, publicize, and encourage the training and continuing educational activities of the members of its sponsoring organizations. Presently, JCETT is sponsored by the American Association for Clinical Chemistry (TDM-Tox Division), the American Academy of Forensic Sciences (Toxicology Section), the California Association of Toxicologists, and the Society of Forensic Toxicologists. The JCETT is composed of three members, each serving a three year term, appointed by each sponsoring organization.

Who are the JCETT Members?

Chair: Irving Sunshine, Ph.D.
Vice-Chair: Thomas Rosano, Ph.D.
Secretary: Laurel Farrell, B.S.

AACC Representatives
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Bruce Goldberger, Ph.D.
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CAT Representatives
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SOFT Representatives
W. Lee Hearn, Ph.D.
Chip Walls, B.S.
Vickie Watts, M.S.

Present & Future JCETT Activities

Toxicology Bibliography: Bibliographies, similar to those published in the CAT and SOFT newsletters, will be prepared on a quarterly basis and distributed to all sponsoring organizations. The JCETT will also investigate the dissemination of a cumulative bibliography on the Internet. This activity will be coordinated by Chip Walls and Rodger Foltz.

Visiting Scientist Program: A visiting scientist program has been established which includes a list of toxicology laboratories willing to accept visiting scientists. Laboratories or individuals interested in participating, either as a training facility or visiting scientist, respectively, should contact Wm. Lee Hearn (305-545-2450). A Visiting Scientist Application has been printed on the reverse side of the JCETT newsletter.

Newsletter: A bi-yearly JCETT newsletter will be published and distributed to all sponsoring organizations. This activity will be coordinated by Bruce Goldberger.

Educational Programs: The JCETT will disseminate information regarding Educational Programs including workshops, professional meetings, and specialized training programs of interest to clinical and forensic toxicologists. This activity will be coordinated by Halle Weingarten.

Lending Library: The JCETT will develop a system for inter-society loan from a central literature repository presently held by CAT. Materials will be available through a designated representative appointed by each sponsoring organization. This activity will be coordinated by Tom Rosano, Laurel Farrell, Judy Stewart (CAT), and Vickie Watts.

Visiting Scientist Application

Personal & Professional Information: (Please enclose your CV)

Name: _____ Lab Name: _____
Address: _____ Lab Address: _____
_____ _____
_____ _____
Home Phone: _____ Bus. Phone _____
Bus FAX _____

Toxicology Specialties of Your Laboratory

Postmortem ___ FUDT ___ Clinical ___ Human Performance ___ Other ___
How many years of laboratory experience do you have? ___
Your Analytical Skills - List instruments and techniques:

Immunoassay ___	EIA ___	RIA ___	FPIA ___	Other ___
TLC ___	Commercial Kit:	Yes ___	No ___	
GC ___	FID ___	NPD ___	ECD ___	Other ___
HPLC ___	UV ___	Diode Array ___	Fluorescence ___	
GC/MS ___	EI ___ CI ___	Quadrupole ___	Ion Trap ___	MS/MS ___ Other ___
UV/VIS ___	Fluorometer ___	Atomic Absorption ___		
FTIR ___	Computerized Record/Data Handling ___			

Experience Desired:

1. General approach to toxicology in other laboratories. Specialty (all that apply).
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   _____
   _____
   _____

2. Specific type of instrument or technique (list in order of importance.
   _____ _____
   _____ _____
   _____ _____
   _____ _____

3. Specific analysis (list in order of importance).
   _____ _____
   _____ _____
   _____ _____
   _____ _____

4. Other:
   _____
   _____
   _____
   _____

How many days do you wish to spend at the laboratory? _____

Return the completed form with all attachments to:
Wm. Lee Hearn, Ph.D., Dade County Medical Examiners Office,
Number One on Bob Hope Road, Miami, Florida 33136-1133