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Joint Meeting

Society of Forensic Toxicologist, Inc.
And
Southwestern Association of Toxicologist, Inc.

THE CAPILLARY GAS CHROMATOGRAPHIC DETERMINATION OF TRAZODONE IN BIOLOGICAL SPECIMENS, Melecita Archuleta, W. H. Anderson, Office of the Chief Medical Examiner, 901 N. Stonewall, Oklahoma City, Oklahoma 73117.

A method for the analysis of trazodone in biological specimens using capillary gas chromatography will be presented. The method includes extraction of the drug and internal standard with 1% isopropanol in n-butyl chloride followed by chromatography on a short methyl silicon capillary column using hydrogen as a carrier gas. This combination of extraction solvent and chromatographic condition was determined to be both accurate and precise. Additional qualitative data will be briefly examined to augment the incorporation of trazodone into screening procedures.

DETECTION OF IBUPROFEN AND METABOLITES IN BIOLOGICAL SAMPLES

J. Vogel, M. Catinella, and C.N. Hodnett

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A relatively new prescription analgesic, Ibuprofen, has just become an OTC drug, and its use will further increase. Its involvement in our clinical and forensic cases is increasing. What will be discussed are analytical results generated with routine drug screening procedures (Thin Layer Chromatography and Gas Chromatography), a confirmatory procedure (Gas Chromatography/Mass Spectroscopy), and a quantitative procedure (High Performance Liquid Chromatography).

BLOOD AND TISSUE HALOPERIDOL AND REDUCED HALOPERIDOL CONCENTRATIONS FROM A HALOPERIDOL COMPLICATED DEATH - Stuart C. Bogema, Beverly Ronald and Jimmy L. Bailey, American Medical Laboratories, 11091 Main Street, Fairfax, VA 22030

A 32 year old male died of possible heat stroke complicated by possible haloperidol (HAL) overdose. HAL and its reduced metabolite (RHAL) have been quantitated in blood, liver, heart, kidney and brain. All tissues were homogenized on a Brinkmann Polytron with a final dilution of 1:90 with deionized water (DIW). Blood was diluted 1:3 with DIW. Standards containing HAL and RHAL at 20 and 100 ng/ml were used. Three (3) ml of standard or sample were mixed with 250 ng of chlorhaloperidol (ISTD) and 1 ml 0.5M bicarbonate buffer, pH 10.5. Each mixture was added to a 5 ml Jet-Tube® (Harlen Assoc., Pittsburgh, PA). Each tube was then eluted three times with 10 ml of hexane:isoamyl alcohol (99:1, HIA). This solvent was then extracted with 1 ml of 0.1N H_2SO_4 and then discarded. The acid extracts were made alkaline with 0.2 ml of 5N NaOH and extracted with 2 ml of HIA. The organic was evaporated. Each extract was redissolved in 50 mcl of HIA. Ten mcl portions were analyzed on a HP 5880 GC with NPD. A 2 foot 3% SP-2250 column with 40 ml/min He flow was used with initial temperature of 210°C for 0.1 min, then 20°C/min to 290°C. Quantitation was performed by peak height ratio to ISTD and comparison to the standards.

	BLOOD	BRAIN	KIDNEY	LIVER	HEART
HAL	67	832	871	2194	843
RHAL	249	2418	2659	6389	1595
HAL/RAL	3.72	2.91	3.05	2.91	1.89

All concentrations are ng/ml or ng/g.

SINGLE-SHOT MULTI-DRUG ANALYSIS, aka GETTING MAXIMUM OUTPUT FROM MINIMUM SAMPLE INPUT. H. K. Garber, New York State Police Crime Laboratory, Bldg 22, State Campus, Albany, NY 12226

Samples submitted in forensic drug analysis cases and in forensic toxicology cases often require quantitation of more than one drug. In such multi-drug cases (and especially for small samples), laboratories which rely upon several standard quantitation methods, each developed to handle one drug, risk consuming the entire sample before all drugs have been quantitated. A viable alternative: develop a SINGLE-SHOT MULTI-DRUG ANALYSIS scheme to simultaneously quantitate all the drugs present in samples from a given case (or at least as many drugs as possible). HPLC & GLC are excellent techniques to use because of their separation ability and rapidity. This talk discusses four forensic toxicology cases which were expedited using GLC versions of single-shot multi-drug analysis.

ULTRACELLULAR MEMBRANE ABNORMALITIES IN CNS AND HEPATIC CELLS INDUCED BY NEUROPSYCHOTROPIC DRUGS.
L. Roizin, J.C. Liu, M.A. Kaufman and N. Willson. Neuropathology & Neurotoxicology, N.Y.S. Psychiatric Institute; Pathology, College of Physicians & Surgeons, Columbia University, New York City.

Membranous structures are the most prominent morphological features of many CNS and liver cell organelles. Their component membranes are variable both in normal and pathologic states. The present report details pathologic changes involving membranous structures of organelles in CNS and liver cells of humans, monkeys, and rats exposed to phenothiazines, heroin and LSD-25. Electron microscope studies of the cerebral cortex, basal ganglia and hypothalamus and liver were carried out following glutaraldehyde and Millonig fixation by perfusion and immersion procedures. The ultracellular abnormalities included polymorphism of mitochondria, lysosomes and multivesicular bodies, as well as the occurrence of abnormal membranous arrays in cell bodies and synapses. In some places membranous elements surround individual or groups of organelles. At times they were aggregated to form lamellar structures. These qualitative and quantitative ultracellular changes were significantly more frequent following drug exposure than in controls. Biochemical analysis (gas chromatography and radioimmunoassay methods) of the CNS and liver tissues revealed varying concentrations of the aforementioned drugs. On the basis of these findings we hypothesize that these membranous changes (membranopathy) are manifestations of drug induced chemogenic lesion.

CHEMICAL TESTS EMPLOYED IN CERTIFICATION OF BREATH ANALYZER AMPOULES.
H. K. Garber, New York State Police Crime Laboratory, Bldg 22, State Campus, Albany, NY 12226

As the number of breath alcohol analyses performed continues to rise, so does the demand for certified breath analyzer ampoules. Because ampoule manufacturers are reluctant to testify in court regarding the composition of the ampoule reagent, the task of certification falls upon government agencies.

This talk begins with an overview of suitable chemical tests, then presents the particular specifications & tests employed by our lab. While developing our tests, we overcame several tricky inorganic chemistry problems posed by the nature of the ampoule reagent, not to mention confusion inherent in the question "what do you mean by 50% sulfuric acid?"

THE TOXICOLOGIST AS AN EXPERT WITNESS IN CASES OF SUBSTANCE ABUSE.
Roger P. Maickel, Dept. of Pharmacology & Toxicology, School of Pharmacy & Pharmacal Sciences, Purdue Univ. West Lafayette, IN 47907

The expert witness in substance abuse trials often plays a key role in the format and presentation of the case. Since the vast majority of toxicologists (and pharmacologists, as well) have had no formal training in such efforts, they often find themselves in somewhat tenuous situations in working with the other members of the criminal justice system, not to mention being uncomfortable on the witness stand. One can easily identify four categories of material that need to be dealt with in preparing for such a situation, in giving testimony, and in responding to cross-examination. One must be familiar with: (1) the chemistry; (2) the pharmacology, both pharmacokinetics and pharmacodynamics; (3) the toxicology, including drug interactions; and, (4) the therapeutic uses and dosages of the agent(s). In addition, one can identify three groups of people to whom this material must be communicated in a meaningful fashion: (1) the attorneys; (2) the jury; and, (3) the judge. The entire process may be considered as a transfer of information; the basic principles of this process can be clearly delineated.

THE USE OF STANDARD ADDITION IN THE ANALYSIS OF BRAIN TISSUE ALCOHOLS AND OTHER EXOTIC TESTS IN THE TOXICOLOGY LABORATORY,
R. F. Foery, G. E. Clement, J. Havassy, Toxicology Department, Lehigh Valley Hospital Center, 1200 S. Cedar Crest Blvd., Allentown, Pa. 18105

The toxicology laboratory is occasionally requested to perform analyses on less common samples and/or for less common substances. In these situations, laboratory personnel are often faced with the difficult and time-consuming task of selecting and preparing, if available, appropriate materials for standards and controls. Careful application of standard addition protocols can often provide simple and efficient solutions to these problems.

In the case of an analysis request on an unusual sample, we describe a method for the quantitative determination of ethanol and other volatile substances in brain tissue using a headspace GLC procedure on spiked samples of randomly selected brain tissue segments. N-propanol is the internal standard. Sample preparation is not time-consuming nor complicated and positive and/or negative controls can easily be prepared from blank (zero ethanol) brain tissue.

In the case of the unusual or rarely requested analyte, a similar approach may be applied in the absence of severe sample limitation. We describe a standard addition HPLC method for the analysis of plasma protriplyline (Vivactil) levels (a less commonly requested analysis in our laboratory).

EVALUATION OF THE ABBOTT RADIATIVE ENERGY ATTENTUATION METHOD FOR THE QUANTITATION OF ETHANOL IN BLOOD, SERUM AND URINE AND COMPARISON TO GAS-LIQUID CHROMATOGRAPHY AND THE DU PONT ACA

Cary, P.L., Whitter, P.D., and Johnson, C.A.; Toxicology Laboratory, University of Missouri-Columbia, Missouri.

A radiative energy attentuation (REA) method for quantitation of ethanol has been developed by Abbott Diagnostics for use on the TDx analyzer. We have evaluated this ethanol assay for precision and accuracy and compared it to gas-liquid chromatography and the Du Pont ACA. The three method comparison evaluated parallel determinations of 156 blood, 92 serum and 54 urine samples containing a wide range of ethanol concentrations. Within-assay CVs averaged 2.2%. Between-assay CVs ranged from 1.6 to 6.0 % for serum and blood controls at 50, 100 and 250 mg/dL. Linear regression analysis of the REA method correlated well with the reference methods: $r > 0.994$. Analytical data related to qauntitation of ethanol in "fresh" blood versus postmortem blood is reviewed. Forensic use of the REA method is discussed.

DETECTION AND CONFIRMATION OF 11-NOR-Δ^9-TETRAHYDROCANNABINOL-9-CARBOXYLIC ACID IN URINE

C. A. Sutheimer*, R. Yarborough, B. R. Hepler and I. Sunshine
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Both a thin layer chromatographic procedure (Toxi-Lab) and two homogeneous immunoassays with differing sensitivity limits (Emit-st, Emit-d.a.u.) were used to test 525 urine specimens for metabolites of tetrahydrocannabinol. Negative results were obtained for 283 specimens. The Emit-st was positive for 184 specimens, the Emit-d.a.u. for 47 additional specimens. The Toxi-Lab results were positive for all these 231 specimens. An additional 11 specimens were positive by Emit-d.a.u. and negative by Toxi-Lab. GC/MS analyses of 5 of these indicated that the 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid concentration for these specimens was within the range of 7.4 ± 2.2 ng/ml, well below the lower detection limit of the Toxi-Lab procedure. Using both Emit-d.a.u. and Toxi-Lab assures a reliable THC result at THC concentrations above 25 ng/ml.

QUANTITATION OF SUCCINYLCHOLINE CHLORIDE BY GC-NPD

Kathleen A. Baldwin, M.S. and Robert B. Forney, Jr., Ph.D., Medical College of Ohio, C.S. 10008, Toledo, OH 43699.

A gas-liquid chromatographic procedure utilizing nitrogen phosphorous detection and SPB-1 capillary column separation was established to detect and quantitate succinylcholine chloride (SCh) in the demethylated form. The injection port temperature was set at 310°C, column temperature programmed; 180-240°C at 20°/min. The retention times are 2 mins for SCh and 1.5 mins for Decamethonium bromide (Deca). SCh is extracted as the ion-pair of the quaternary ammonium compound and chemically demethylated with sodium benzenethiolate to the tertiary (volatile form) derivative as previously described (J. Anal. Tox. 6, 115-119, 1982). Deca was chosen as the internal standard since its structure is similar to SCh and it contains nitrogen. Deca is quantitatively extracted along with SCh giving a linear response in the range of 25 to 1000 ng. The internal standard is chemically stable and gives no peaks that interfere with SCh analyses. The advantages of the NPD method include rapid analysis and greater availability. The method has been applied to the detection and quantitation of SCh in tissue and plasma of dogs following 1 or 5 mg/kg intravenous injections.

QUANTITATIVE ANALYSIS AND IDENTIFICATION OF DELTA-9-TETRAHYDROCANNABINOL AND ITS PRIMARY ACID METABOLITE IN BLOOD AND URINE BY ELECTRON IMPACT GAS CHROMATOGGRAPHY-MASS SPECTROMETRY.

R. Hugh Granger, Robert V. Blanke, Medical College of VA, Dept. of Pathology, MCV Station-Box 597, Richmond, Virginia.

A simple and reliable method for the forensic identification and quantitation of Δ^9 Tetrahydrocannabinol and 11-Nor-9-carboxy-Δ^9-Tetrahydrocannabinol in post mortem blood, whole blood, serum, and urine is presented in detail. This analysis requires initial protein precipitation or alkaline hydrolysis followed by an additional solvent partitioning cleanup step. The isolated cannabinoid compounds are simultaneously methylated as ether and/or ester linkages and concentrated for analysis. The measurement of the cannabinoids is accomplished using selected ion monitoring of characteristic ions produced by electron impact mass spectrometry. Identification of the eluting chromatographic peaks is achieved by comparison of the corresponding mass ratios of the deuterated internal standards with those of the suspected cannabinoid fraction. Gas chromatographic separation is performed using a capillary fused silica column and a 1:20 split injection with direct interface into the mass spectrometer.

FORENSIC STANDARDS FOR THE DETERMINATION OF 11-NOR-DELTA-9-THC CARBOXYLIC ACID METABOLITE IN BIOLOGICAL SPECIMENS.

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Experience during the past year with numerous military laboratory inspections/ courts martials and civilian board hearings/ court trials has clearly demonstrated the need for specific forensic standards for the validation of procedures used for the confirmation of the major THC metabolite in human specimens. These investigations and testimonies have also shown that documentation of intra/inter laboratory QA/QC is essential to proper presentation of the scientific data for each specific case.

This presentation will discuss the analytical parameters involved in the GLC and GC/MS metabolite determinations. The main parameters include LOD(limit of detection), LOQ(limit of quantitation), "cutoffs", resolution, shoulders,coeluting peaks, interference probability, probability of confirmation and QA/QC charts such as Shewhart and Youden. The 1983 report of the U.S. Army Surgeon General's Blue Ribbon Panel for Urinalysis Drug Abuse Testing will be presented as a initiating model for this discussion.

DETERMINATION OF THIORIDAZINE 5-SULFOXIDE DIASTEREOISOMERS IN HUMAN SERUM AND URINE.

Paul W. Hale, Jr. and Alphonse Poklis, Departments of Pharmacology and Pathology, St. Louis University School of Medicine, 1402 S. Grand, St. Louis, MO 63104

The neuroleptic thioridazine exists as a racemic mixture of enantiomers with an asymmetric carbon at position 2 in the piperidyl ring. Oxidation of the ring sulfur atom creates another chiral center; thus thioridazine 5-sulfoxide (T5SO) exists as two diastereoisomeric pairs of enantiomers. T5SO has recently been associated with thioridazine cardiotoxicity. We have isolated and identified T5SO diastereoisomeric pairs of enantiomers in serum and urine from 7 patients receiving thioridazine. Nine TLC systems were evaluated for resolution of thioridazine, sulforidazine, T5SO and mesoridazine. Only two resolved the T5SO pairs from other metabolites. Serum extracts and eluted T5SO TLC spots were chromatographed by gradient flow HPLC using a 15 cm silica column with UV detection at 279 nm. Eluted spots produced single peaks at 8.1 and 11.7 min. corresponding to each T5SO pair. Recoveries of the fast and slow eluting T5SO pairs from serum averaged 97% and 103% respectively. Approximately equal concentrations of each T5SO pair were found in serum and urine, indicating no stereoselectivity in disposition or clearance of the T5SO isomers.