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FLUORESCENCE POLARIZATION IMMUNOASSAY OF VITREOUS HUMOR TO DETECT DRUG USE

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FLUORESCENCE POLARIZATION IMMUNOASSAY OF
VITREOUS HUMOR TO DETECT DRUG USE

BY

NANCY ROUND HALEY

A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

IN

PHARMACOLOGY AND TOXICOLOGY

UNIVERSITY OF RHODE ISLAND

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MASTER OF SCIENCE THESIS
OF
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APPROVED:

Thesis Committee

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1991

ABSTRACT

In recent years the number of postmortem cases in which cocaine involvement is suspected has increased considerably. This has created a need for a rapid screening procedure for the postmortem samples. The present study examined the usefulness of vitreous humor specimens, analyzed by a rapid immunological assay, as a possible alternative to the more conventional and relatively slow gas chromatography/mass spectrometry of blood samples to screen for antemortem cocaine use.

Medical examiner cases occurring over a six month period were reviewed and forty-eight cases which were determined to be drug related or drug induced deaths were analyzed. Post-mortem concentrations of cocaine and its hydrolysis product, benzoylecgonine, recovered from blood, were compared to the concentrations detected in vitreous humor. These specimens were screened via a rapid fluorescence polarization immunoassay (FPIA) and also analyzed by gas chromatography/ mass spectrometry (GC/MS) for a quantitation of cocaine and its primary hydrolysis product, benzoylecgonine.

An assessment of FPIA to screen postmortem vitreous humor samples was carried out. The sensitivity of this method was 0.03 mcg/ml. The vitreous humor cocaine metabolite levels by FPIA and GC/MS demonstrated a correlation coefficient of 0.7. Blood and vitreous humor benzoylecgonine levels from the same postmortem case analyzed by GC/MS showed a correlation coef-

ficient of 0.5.

Significant correlation of the FPIA results with the GC/MS results demonstrate that the FPIA analysis of vitreous humor samples appears to be a valid screening technique to detect antemortem cocaine use. The vitreous humor was found to be a clean and stable body fluid which was suitable for this technique. It is concluded that the screening of postmortem vitreous humor for cocaine use by this FPIA method is a useful and reliable screening technique in forensic toxicology.

ACKNOWLEDGEMENTS

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I. INTRODUCTION

Forensic toxicology is the study of poison isolation, identification, and quantitation from biological specimens for legal purposes. Most drug analyses in forensic toxicology are performed on urine, blood, or other tissue. During the multi-step drug extraction processes, the samples are susceptible to emulsification and pipetting errors. Blood samples are also degraded by postmortem changes. The combination of all these factors may result in loss of sensitivity and poor reproducibility in an assay.

Another postmortem fluid which is readily available but is often ignored is vitreous humor. In comparison with blood and urine, it is not as susceptible to major postmortem changes. The vitreous is compartmentalized and isolated, which makes it a suitable toxicological material, even after embalming or putrefaction, when blood may be unavailable (Coe, and Sherman, 1970).

This study investigated three main areas relating to fluorescence polarization immunoassay (FPIA) technology on vitreous humor specimens in determining drug use prior to death. First, the data generated was used to determine if elevated levels of cocaine and/or metabolites in blood are accompanied by elevated levels in vitreous humor. Secondly, this information coupled with the time between drug use and death (if available) might allow a calculation as to how

rapidly cocaine and/or metabolites diffuse into vitreous humor specimens (Jones and Pounder, 1987). And lastly, this research further demonstrated that FPIA screening of vitreous humor specimens is valid in forensic toxicology.

II. LITERATURE REVIEW

Vitreous humor is the fluid material which fills the posterior cavity of the eye. It is present at a volume of 2 to 2.5 milliliters in each eye (Logan and Stafford, 1989). The vitreous (hyaloid body) occupies about 80% of the volume of the ocular globe. It provides structural support and maintains intraocular pressure .

Chemically, the vitreous humor is a mucopolysaccharide gel composed of hyaluronic acid and collagen, and it has a water content of between 98 and 99.7% (Table 1). This table shows the relative simplicity and stability of vitreous humor. Vitreous humor is a clean biological fluid containing less protein than urine, and it can be collected with ease. In the forensic domain, the primary use of vitreous humor to date has been to aid in the postmortem diagnosis of disease, the analysis of electrolyte composition and enzymatic activity to determine "time-since-death" determinations (Devgun and Dunbar, 1986).

Vitreous is a partitioned fluid; but the amount of water movement is very high ($T_{1/2} = 10-15$ minutes). This tends to make one expect that drug levels might be expected to show good correlation with corresponding blood levels. This is true of ethanol determinations and vitreous humor samples which are often analyzed postmortem for alcohol concentration. The mean ratio of vitreous humor to blood ethanol concentration is 1.17 (Caplan and Levine, 1990).

Table 1. Comparison of the Composition of Three Postmortem Body Fluids

Putrified Blood (pH 4-9)	Urine (pH 4-9)	Vitreous Humor (pH 7-7.8)
Water (20-70%)	Water (96-99.9%)	Water (98- 99.7%)
Few cells+debris	Glucose	Glucose
Lysate	Protein	Hyaluronic Acid
Clots	Ammonia	Anions/cations
Denatured clots	Creatinine	Collagen
Bacterial debris	Urea	
Denatured protein	Uric acid	
Fat droplets	Steroids	
Steroids	Pigments	
Putrefactive base	Amino Acids	
Protein microagglutinates		
Enzymes		

The use of vitreous humor for toxicological analysis was first published involving ethanol determinations (Sturner and Coumbis, 1966). In this publication the vitreous humor ethanol levels were compared to blood ethanol levels, obtained simultaneously at autopsy. There have been various reports subsequent to this, verifying the usefulness and accuracy of this approach (Coe and Sherman, 1970, Felby and Olsen, 1969, Ziminski et.al., 1984).

In the literature, various drug distributions studies do mention vitreous drug levels, but little is said about the relation of these levels to blood levels or their ultimate interpretation. A study on alcohol levels illustrated that when patients have long survival times, they reveal blood/vitreous values approaching unity (Sturner and Garriot, 1975). There is speculation that the ratios reached at equilibrium depend on the drug's lipid solubility, plasma protein binding, and solubility in vitreous humor.

The substances that have been studied include such drugs as barbiturates, meprobamate, salicylates, ethchlorvynol, digoxin, quinine, and lithium (Felby and Olsen, 1969). Other studies have also appeared that relate levels of such drugs as barbiturates, methadone and morphine in vitreous humor to other tissues and fluids (Ziminski et. al., 1984). Quantitative postmortem determinations of vitreous drug levels have been reported rather sporadically in the literature.

The physical properties of vitreous humor make it a very good sample material for initial drug screening in postmortem

cases. Active transport of some compounds into the eye has been observed in some cases. This has been observed in some tests conducted on rabbit eyes with delta-9-tetrahydrocannabinol transport (Sorensen, 1971). Most compounds are believed to enter the eye through passive diffusion, a process limited by their lipophilicity and their charge at physiological pH.

There are many advantages to postmortem toxicological analysis of vitreous humor. In most cases, the specimen is easily obtainable at autopsy and obtainable without the necessity of an autopsy. Vitreous humor is often the best or only sample available; especially in cases of severe burning, when the body has been embalmed, in cases of advanced decomposition, and in cases of severe trauma. Vitreous is a clear, serous specimen and it is easy to work with analytically. It is also found in an isolated anatomical position. In addition, the lack of any metabolic activity in the eye suggests that vitreous levels might give a more accurate indication of drug concentrations present in the body at the time of death. Therefore, in the absence of a valid blood specimen, the vitreous humor specimen may permit toxicological interpretation. However, there must be a wealth of data available relating vitreous levels to blood levels before such interpretation would be possible.

The pharmacokinetics of any drug deals with the study of the time course of the drug and its metabolites in the body following administration. Every drug is subject to the

pharmacokinetic variables of absorption, distribution, metabolism, and excretion. The determination of the drug plasma levels is highly individual and depends upon these variables .

In the human body, the drug, cocaine, is rapidly metabolized ($T_{1/2} = 0.7$ to 1.5 hours) to ecgonine methyl ester, ecgonine and other fragments by serum cholinesterase and liver esterases (Baselt and Cravey, 1989). Cocaine is broken down to benzoylecgonine by chemical hydrolysis (Figure 1). These processes probably continue postmortem in the blood, liver and in drawn blood "in vitro". Enzymatic breakdown can be inhibited by any number of enzyme inhibitors, including fluoride, organophosphate pesticides, physostigmine, and heavy metals. Hydrolysis can also be slowed by refrigeration or freezing and by decreasing the pH of the sample below neutral. Refrigeration retards both processes, while freezing blood samples containing sodium fluoride can preserve the cocaine for extended periods of time.

One would expect cocaine in the blood to hydrolyze extensively between the time of death and the time when the autopsy blood specimens are taken. If survival time from dosage exceeds 48 hours, it would most likely result in a negative result, based on the elimination kinetics of the principal cocaine metabolite, benzoylecgonine. The mean urinary elimination half-life of benzoylecgonine has been estimated to be 4.5 hours (range, 2.8 to 10.5 hours) (Ambre et. al., 1988). Elimination rates are dependent, in part, on

Fig. 1. Cocaine Metabolism showing Formation of Metabolites

dosage, as well as other factors. Ambre in 1985 devised a nomogram based on published kinetic studies. Assuming a urinary output of 1 ml/min, this nomogram would predict that at least 0.35 mg/L or 0.35 mcg/ml of benzoylecgonine should be detectable in the urine for up to 48 hours following a 100mg or greater dose. These and other studies suggest that little metabolite would be detectable in urine by a homogeneous enzyme immunoassay with a detection limit of 0.30 mcg/ml beyond 48 hours from the last cocaine dose of 100mg.

In one review of the cocaine literature, it was evident that death from cocaine is rapid and unpredictable (Smart and Anglin, 1987). None of the papers examined were able to illustrate how to translate postmortem values into actual doses taken. Many of the authors state that following an overdose of cocaine, death is astonishingly rapid (Welti and Fishbain, 1985, Welti and Wright, 1979, Mittleman and Welti, 1984). Thus, it may not yet be possible to state the lethal dose of cocaine with any degree of certainty.

It has been suggested that brain tissue may be the better sample for cocaine determination rather than postmortem blood or liver tissue (Sphiehler and Reed, 1985). Cocaine is known to enter and leave the brain rapidly. Some researchers feel that there is an active transport of cocaine into the brain tissue, rather than a passive diffusion. At peak plasma concentrations, the brain cocaine concentration is just over four times the plasma or serum concentration. This brain/blood ratio is the most frequent median found for

cocaine in cocaine overdose fatalities. As blood cocaine concentrations fall slightly more rapidly than brain tissue concentrations, the brain/blood ratio increases. This increase reaches a peak of approximately 10 (range 8 to 12) between one and two hours after cocaine administration. These authors recommend brain tissue for cocaine determinations over postmortem blood or liver tissue. Brain is a better matrix than blood for cocaine analysis because the parent drug is more stable in the brain's lipid-rich environment. However, the technical difficulties in getting the tissue prepared for analysis may outweigh the accuracy of the brain tissue for cocaine concentration.

The examination of three human brains in the study by Browne, illustrated that cocaine and benzoylecgonine appear to be distributed throughout the different regions of the organ (Browne et al., 1991). This was in agreement with the findings of an earlier study by Spiehler and Reed (Spiehler and Reed, 1985). The concentrations of cocaine found in the brain were generally four to eight times greater than that found in blood. The addition of this study to the archives further suggests that brain tissue is a better sample than either blood or liver for cocaine determinations. However, working with brain tissue is difficult, as stated earlier. The presence in the brain of large concentrations of fats and other endogenous materials, make use of this specimen difficult. This material necessitates the use of lengthy and laborious extraction procedures.

One paper in the literature demonstrated the usefulness of testing decomposed human remains for cocaine in all cases where the presence is suspected (Manhoff et. al., 1991). Cocaine is generally quite labile and has a plasma half-life of less than one hour. It is known that in vivo it is rapidly hydrolyzed by enzymatic and nonenzymatic reactions. Despite this, it has been found that cocaine and its metabolites are frequently detectable in decomposed human remains. Cocaine has been detected even in advanced cases of decomposition and mummification.

Recent data now suggests that blood cocaine concentrations change significantly during the interval between death and autopsy . The change appears to be dependent upon the site from which the blood is sampled. Cocaine concentrations in the heart, aorta, and femoral vein blood increase during the interval between death and autopsy, while the subclavian vein blood decreases (Hearn et. al., 1991). This study emphasizes the difficulty in attempting to estimate the perimortem concentration of cocaine in the blood from any postmortem data. The data implies that cocaine accumulates in some tissues, such as the brain and the liver, and these demonstrate higher cocaine concentrations than in the blood.

In another study where the two eyes were sampled at different postmortem intervals, markedly different cocaine levels were demonstrated (Beno and Kriewall, 1989). In this study also, postmortem release is the only reasonable explanation for this unusual finding. In a case study of

cocaine involved in a fatal poisoning, cocaine tissue concentrations and body fluid concentrations were reported in the literature for this case (Poklis et. al., 1985). The blood level of cocaine was 1.8 mcg/ml and vitreous humor level was 2.4 mcg/ml. The blood/vitreous ratio in this case for the parent drug cocaine was 0.75. There was no benzoylecgonine levels reported in this case report as the procedure used did not analyze for benzoylecgonine. The survival time was known to be less than 2 hours and the drug was intravenously administered.

The incidence of cocaine abuse has arisen dramatically over recent years. This has directly resulted in a major increase in deaths attributed to cocaine overdose as well as to increased findings of cocaine during the screening of biological samples for drugs of abuse. There are numerous methods in the literature describing the analysis of cocaine and its hydrolysis product, benzoylecgonine in body fluids, using high performance liquid chromatography (HPLC) and gas chromatography/mass spectrometry (GC/MS) (Chinn et. al., 1980, Evans and Morarity, 1980, Griesmer et. al., 1983, Logan and Stafford, 1989, Tebbett and McCartney, 1988). All of these methods require that the drugs be first extracted from the biological matrix prior to chromatographic analysis.

Extraction of cocaine and benzoylecgonine is generally by liquid-liquid extraction procedures. Liquid-liquid extraction normally involves the use of volumes of solvents, numerous centrifugation, back extraction and evaporation steps before

the sample is suitable for analysis. Such methods can provide clean extracts with good recoveries, however the method is time consuming, often requiring several hours to extract a single sample. In addition, emulsion formation with some organic solvents may hinder phase separation, resulting in inconsistent recovery (Tebbett and Mc Cartney, 1988).

The prevalence of illegal drug use in this country, as stated earlier, has necessitated the need to detect and identify drugs of abuse. This has brought about the relatively new types of screening procedures. The accepted approach is to use preliminary screening tests, followed by more specific confirmatory assays. A wide variety of drug screening methods, each with its peculiar advantages and drawbacks may be utilized, including radioimmunoassay (RIA), enzyme immunoassay (EIA), thin-layer chromatography (TLC), gas chromatography (GC), high performance liquid chromatography (HPLC), and fluorescence polarization immunoassay (FPIA). FPIA has been used by the Abbott TD_x instrument since 1981 for therapeutic drug monitoring and since 1987 for the screening of abused drugs (Jolley, 1981 and Caplan et. al., 1987). A derivative of the TD_x, the AD_x, which is dedicated solely to screening specimens for the major classes of abused drugs, was introduced in 1988. (Caplan and Levine 1988). And its entry into the forensic field has, therefore, been relatively recent (Table 2).

Immunoassays are based on specific antigen-antibody reactions. The primary advantage of immunoassay is its

Table 2. FPIA Cocaine Metabolites Assay Sensitivity and Specificity

1. Good Screening Method
2. Benzoylecgonine Target Compound (inactive metabolite)
3. Some Cross-Reactivity
4. Semi-Quantitative

TEST CPD.	CONC. ADDED (mcg/ml)	CONC. FOUND (mcg/ml)	% CROSS- REACTIVITY
Cocaine	100.0	0.79	0.8
	10.0	0.04	0.4
	1.0	ND	-
Ecgonine	10.0	0.03	0.3
	1.0	ND	-
Ecgonine	100.0	0.03	<0.1
Methyl Ester	10.0	ND	-

ND = None Detected

(Concentration less than the sensitivity of assay 0.03 mcg/ml)

ability to detect small concentrations of drugs in body fluids and organs. This technique offers the best approach for detecting low drug levels associated with recreational drug use. The advantages of FPIA over other immunoassay methods such as radioimmunoassay (RIA) or enzyme multiplied immunoassay technique (EMIT) are nonradioactivity and stable calibration curves.

Drug detection by FPIA uses an inverse relationship between polarization and concentration. The exceptional sensitivity and precision at low concentrations minimize false-positive and false-negative results (Cone et. al., 1988). The Abbott instrument, the ADx, uses competitive binding and fluorescence polarization for detecting drugs of abuse in urine. A known amount of fluorescein-labeled drug competes with free drug in the sample for antibody binding sites. When bound to the antibody, the labelled drug rotates more slowly: thus, high levels of polarization correspond to low sample drug levels. Conversely, when the drug is free in the solution, it rotates more rapidly. Therefore, low levels of polarization correspond to high drug sample levels. The ADx measures polarization values and calculates the concentration from a previously stored calibration curve. The technology affords the screening of large numbers of samples at a time and yields a semi-quantitative result.

III. EXPERIMENTAL

Medical examiner cases from January 1989 to June of 1989 were reviewed. Pertinent data was recorded in spreadsheet format to easily refer to the cases and for summarizing results. There were 307 cases included in this investigative study, all analyses were recorded as well as medical examiner data of significance. The data that was recorded on the spread-sheet was as follows: the medical examiner case number, age, sex, race, cause of death, manner of death, survival time, vitreous humor specimen available, urine specimen available, urine immunoassay results, urine GC/MS results, blood specimen available, blood immunoassay results, blood GC/MS results (Appendix A). The cases that were selected to have vitreous samples analyzed met the following criteria: the vitreous sample had not previously been analyzed, and the case had cocaine and/or metabolites detected in blood or urine samples.

Forty-eight vitreous humor samples were selected for this study. The vitreous specimens had been stored at -20° C. since being received in the laboratory from the medical examiner. There were four negative controls which were included in the study ;there was no cocaine and/or metabolites detected in the bloods or urines of these cases.

The vitreous humor specimens were analyzed by fluorescence polarization immunoassay (FPIA) without any pretreatment, dilution, or centrifugation. The Abbott AD_x analyzer

was employed for the FPIA analysis. The calibrators, controls, and reagents were supplied by Abbott Laboratories. The instrument was operated in accordance with the Abbott operators manual for this instrument. Six calibrators were assayed and a valid calibration curve was derived by averaging the net polarization values (Figure 2). The assay requires a 50 microliter sample to be run on the assay. The assertion was that the simplistic composition of vitreous humor specimen would allow for this facilitated screening technique to be utilized. The sensitivity for the cocaine metabolite (benzoylecgonine) assay was lowered to a threshold which allowed for low level positives in vitreous to be identified. This presumptive screening process has already been mentioned as one which detects the primary metabolite of cocaine, benzoylecgonine, in human urine specimens. The reagent system demonstrates selective reactivity and high specificity. Benzoylecgonine has a reactivity of 100%, whereas cocaine, ecgonine methyl ester and ecgonine cross react with the assay at less than 1%. The technique has superior sensitivity which allows for an adjustable minimum allowable threshold. Below this threshold, the safety zone extends to the stated sensitivity of the assay (McCord and McCutcheon, 1988). The stated minimum allowable threshold for the cocaine/benzoylecgonine assay is 0.30 micrograms per milliliter (mcg/ml) and the sensitivity of the assay is 0.03 mcg/ml or 30 nanograms per milliliter which is based on the specifications of the instrument.

<u>Calibrator</u>	<u>Concentration ng/mL</u>	<u>Calibrator AVG P Values</u>
A	0.0	217.14
B	300	172.39
C	1000	133.23
D	2000	112.33
E	3000	100.35
F	5000	84.08

Fig. 2. FPIA Calibration Curve demonstrating Inverse Relationship between Polarization and Concentration

In forensic toxicology work, any preliminary or presumptive positive on an initial screening test must be confirmed by a confirmatory test. It is generally agreed in the forensic community that gas chromatography coupled with mass spectrometry (GC/MS) offers the best means for the unequivocal identification of drugs and their metabolites in biological materials.

A Hewlett-Packard 5890 gas chromatograph interfaced with a Hewlett-Packard 5970 Series mass selective detector (MSD) was used for GC/MS analysis. The data system in use was the HP RTE-A computer system. The MSD was operated in the electron impact (EI) mode at 70 eV with an ion source temperature of 280°C and an m/z range from 40 to 500. The instrument was autotuned with perfluorotributylamine (PFTBA) (Appendix C). The gas chromatograph column was an HP-1 coated fused-silica 12 m x 0.2 mm x 0.33-um film thickness. The GC was operated on a temperature program from 60° to 250°C, increasing at a rate of 30°/min and the injection was splitless. The MSD was operated using electron impact ionization and in the selected ion monitoring mode (SIM). The ions monitored for each compound were 213 m/z and 210 m/z for benzoylecgonine-D₃ (BZE-D₃) and benzoylecgonine (BZE), respectively and 185 m/z and 182 m/z for cocaine-D₃ (COC-D₃) and cocaine (COC) (Figures 3,4). In the selected ion monitoring mode the MSD can be set to monitor only those ions that are characteristic of the specific compounds. Since selective scanning permits the mass analyzer to dwell longer

Fig. 3. Mass Spectra of Benzoyllecgonine and Benzoyllecgonine-D3

Fig. 4. Mass Spectra of Cocaine and Cocaine-D3

on specific masses, this mode is far more sensitive than total ion scanning. It permits analysis of selected compounds at very low levels in very complex mixtures - mixtures that may be too complex for resolution by gas chromatography alone. The retention times obtained in the total ion chromatogram (TIC) were 9.11/9.12 and 9.66/9.67 min. for cocaine and benzoylecgonine, respectively (Figure 5). All standards were specialty analytical standards obtained from Radian Corporation. These were pure standards in methanol at a concentration of 10 mcg/ml. The standards included cocaine, trideuterated cocaine (cocaine-D₃), benzoylecgonine, and trideuterated benzoylecgonine (benzoylecgonine-D₃). These standards were diluted accordingly to allow for a standard curve calibration from 25 ng/ml to 1.0 mcg/ml. There were three levels of control urine specimens run along with the research samples to validate the derived calibration curve. These controls (CON-DOA) were purchased from Diagnostics Products Corporation (DPC) and were in the following concentrations: 0.18, 0.36, and 1.5 mcg/ml.

The confirmation procedure required the solid phase extraction (SPE) of the standards, controls, and samples. This was accomplished by the use of Extrelut^R extraction columns purchased from Analytichem International. These are disposable extraction columns designed for rapid sample preparation by a highly efficient liquid/liquid extraction. The SPE columns contain a specially modified form of diatomaceous earth. The analytes in the aqueous samples are

Data File: ^72542::D3
 Name: ME 1186-89 VH
 Misc: EXTRELOT EXTRACTION

Quant Output File: ^72542::D5
 Instrument ID: TOX-11

BTL#43

Id File: IDNANC::D5
 Title: COCAINE/BENZOYLECGONINE/METHYLECGONINE QUANTITATION
 Last Calibration: 910813 07:51 Last Qcal Time: <none>

Operator ID: TOX1
 Quant Time : 910816 15:02
 Injected at: 910726 16:23

Fig. 5. Total Ion Chromatogram (TIC) of Extracted Vitreous Humor Sample

adsorbed and distributed into a thin film over the hydrophilic packing. In this manner, background interferences are removed from the analysis.

All standards, controls and samples were analyzed by the same cocaine/benzoylecgonine procedure (Appendix D) adapted from Isenschmid (Isenschmid et. al., 1988). Into a clean 10 ml test tube, 1 ml of aqueous sample, 2 ml of phosphate buffer (pH 7.0), and 100 microliters of internal standards (COC-D₃ and BZE-D₃ at concentrations of 500 nanograms each) are pipetted. This chemical cocktail is vortexed and added to an SPE extraction column. The mixture is adsorbed for 4 to 5 minutes. The analytes are eluted into a disposable 15 ml centrifuge tube with two aliquots of 9:1 chloroform:isopropanol. The sample is then evaporated to dryness in a heated waterbath (50-60°C) under a stream of nitrogen to prevent oxidation. After dryness, the benzoylecgonine (if present) is derivatized to n-propyl cocaine by boiling over an open flame with 100 microliters of n,n-dimethylformamide-di-n-propyl acetal for 30 seconds (Figure 6). When cool, 1 ml of 1N sulfuric acid is added to the tube and it is mixed well. The sample is cleaned up to remove impurities when 3 ml of toluene: heptane: isoamyl alcohol (76:20:4) is added to the aqueous portion, mixed and the organic layer is discarded. The aqueous portion is next neutralized with 2 ml of sodium carbonate buffer (pH 9.0). The sample is extracted with 3 ml of the toluene:heptane: isoamyl alcohol mixture, mixed and the organic phase is transferred to a clean conical centrifuge

Fig. 6.
REACTION FOR THE DERIVATIZATION OF BENZOYLECGONINE TO
N-PROPYL COCAINE

Compound # 1 Calib File: CFNANC:05

Compound: COCAINE
 Istd: COCAINE-03

File: >719A2 >719A3 >719A4 >719A5 >719A6 >719A7 >719A8 >719A9 >719B0 >719B1
 Conc: .0250 .0500 .0750 .100 .250 .500 .750 1.00 2.50 5.00
 Rf: 1.5479 1.4967 1.4160 1.4180 1.3995 1.3667 1.2718 1.3549 1.1345 1.1564

Average of 10 Rfs: 1.3563 (9.89 % Rad) Rx: .0000000 Ry: .0000000
 1st Degree Equation: $y = -.101261 + .8729581(x)$
 1st Degree Corr Coef: .9993153
 2nd Degree Equation: $y = -.074080 + .8408837(x) + .0028910(x^2)$
 2nd Degree Corr Coef: .9993689

In the above equations:

$$y = \frac{\text{Conc Std}}{\text{Conc Istd}} \quad x = \frac{\text{Area Std}}{\text{Area Istd}}$$

Istd Conc for all calibration points is: .500

Fig. 7. Calibration Curve of Cocaine by GC/MS

Compound # 2 Calib File: CFNANC:05

Compound: BENZOYLECGONINE PROPYL DER
 Istd: BENZOYLECGONINE PROPYL DERIV.

File: >719A2 >719A3 >719A4 >719A5 >719A6 >719A7 >719A8 >719A9 >719B0 >719B1
 Conc: .0250 .0500 .0750 .100 .250 .500 .750 1.00 2.50 5.00
 Rf: .89390 .83442 .79362 .81493 .85639 .87263 .82032 .80217 .90439 .92177

Average of 10 Rfs: .85145 (5.31 % Rad) Rx: .000000 Ry: .000000
 1st Degree Equation: $y = .0685702 + 1.083609(x)$
 1st Degree Corr Coef: .9996432
 2nd Degree Equation: $y = .0251851 + 1.158802(x) + -.008517(x^2)$
 2nd Degree Corr Coef: .9998112

In the above equations:

$$y = \frac{\text{Conc Std}}{\text{Conc Istd}} \quad x = \frac{\text{Area Std}}{\text{Area Istd}}$$

Istd Conc for all calibration points is: .500

Fig. 8. Calibration Curve of Benzoylecgonine by GC/MS

tube and evaporated to dryness under a stream of nitrogen. The dried sample is then reconstituted with 100 ul of chloroform and 3 ul are injected into the GC/MS for mass spectral analysis.

Standard curves for cocaine and benzoylecgonine were established by running series of standards from 25 to 1000 ng/ml (Figures 7,8). The calibration curve for benzoylecgonine produced a slope of 1.083, zero intercepts and a correlation coefficient of 0.999. The procedure was determined to be reliably linear for concentrations between 0.030 and 1.00 mcg/ml. Quantitations for the analytes were achieved by comparing the abundance of the major ions and retention times of the selected ion chromatogram of the analytes with the corresponding internal standard (Table 3).

QUANT REPORT Page 1

Operator ID: TOX1 Quant Rev: 7 Quant Time: 910816 15:02
Output File: ^72542::05 Injected at: 910726 16:23
Data File: >72542::03 Dilution Factor: 1.00000
Name: ME 1186-89 UM Instrument ID: TOX-11
Misc: EXTRELUT EXTRACTION BTL#43

ID File: IDNANC::05
Title: COCAINE/BENZOYLECGONINE/METHYLECGONINE QUANTITATION
Last Calibration: 910813 07:51 Last Qcal Time: <none>

	Compound	R.T.	Q ion	Area	Conc	Units	q
1)	*COCAINE-D3	9.11	185.2	42983	.500	UG/ML	82
2)	COCAINE	9.12	182.2	2879	.0180	UG/ML	90
3)	*BENZOYLECGONINE PROPYL DERIV.	9.66	213.2	29260	.500	UG/ML	84
4)	BENZOYLECGONINE PROPYL DER	9.67	210.2	26084	.541	UG/ML	86

* Compound is ISTD

Table 3. GC/MS Quantitation Report showing Retention times, Ion Abundance and Calculated Concentrations

IV. RESULTS

From the 307 cases researched, 48 were selected to be included in this study. These cases were selected to analyze for the concentration of benzoylecgonine in the vitreous humors and to compare these to the blood concentrations of the same analyte. Of these selected 48 cases, 3 were determined to have vitreous samples that were of insufficient quantity to allow for FPIA and GC/MS determinations. This allowed for 45 samples to be selected to be analyzed by FPIA and GC/MS. These samples included four negative controls, which gave FPIA readings of zero.

For the purpose of this study, any FPIA reading greater than zero was initially considered a positive, which required further testing. Of the total cases reviewed ($n = 307$), 41 vitreous results by FPIA were obtained, which represented approximately 13% of the total cases. These vitreous positives by FPIA were separated into three categories (Table 4). The first category which represented 7.3% of the 41 samples had FPIA readings > 0 but < 0.03 mcg/ml. The second category which comprised 41.5% of the samples had FPIA readings $>$ or $=$ to 0.03 mcg/ml but less than 0.30 mcg/ml (the standard threshold level). The third category was the vitreous humors with FPIA readings $>$ or $= 0.30$ mcg/ml, and this accounted for 51.2% of the cases.

The majority of the vitreous humors tested were above the standard threshold level of 0.30 mcg/ml. However, a signi-

Table 4. Medical Examiner Cases with Vitreous Humor
Samples Analyzed for Cocaine Metabolites

87% Medical Examiner Cases - Vitreous Humor Samples
negative

13% Medical Examiner Cases - Vitreous Humor Samples
> 0 mcg/ml (n=41)

7.3% FPIA < 0.03 mcg/ml
41.5% FPIA readings > or = 0.03 mcg/ml
51.2% FPIA readings > or = 0.30 mcg/ml

ficant number of samples, 41%, fell between greater than 0.03 mcg/ml and less than 0.30 mcg/ml. If only vitreous humor was analyzed for these cases, these would not have been further investigated under conventional forensic toxicology guidelines.

The vitreous humor initial screening results were compared with benzoylecgonine levels determined by GC/MS. An attempt was made to see if a correlation existed between the FPIA technique (which screens primarily for benzoylecgonine) and the benzoylecgonine level determined by solid phase extraction and analysis by GC/MS (Table 5). The correlation coefficient for the two assays was 0.701 (Table 6). Although there was relationship between the two assays, there were several outliers (Figure 9). A correlation is a mathematical statistic that indicates the degree to which two variables are associated. If the correlation is squared (which varies from 0.0 to 1.0), then one gets a mathematical description of the proportion of one variable that explains the occurrence of the second variable. The scatter plot and the regression line are shown in Figure 9.

The GC/MS results of the vitreous humor samples were then compared with the GC/MS results of the corresponding blood samples (Table 7). Specifically, the benzoylecgonine quantitations were compared to calculate the correlation coefficient between the two analyses. The correlation between these two biological specimens for the same analyte was 0.510 (Table 8). The scatter plot and the regression line are shown

Table 5. Data showing Vitreous Humor Sample I.D. #, FPIA Reading, and GC/MS Benzoyllecgonine Quantitation

I.D. #	FPIA	GC/MS	I.D.#	FPIA	GCMS
1 ME0122	0.00	0.00	39 ME1838	1.05	1.02
2 ME0354	0.00	0.00	40 ME0814	1.08	1.29
3 ME0118	0.00	0.00	41 ME1530	1.29	2.71
4 ME0311	0.00	0.00	42 ME1935	1.38	0.83
5 ME1665	0.01	0.00	43 ME0006	1.80	2.38
6 ME0453	0.02	0.03	44 ME0260	2.01	2.87
7 ME1557	0.02	0.23	45 ME1901	2.59	0.90
8 ME0328	0.03	0.09			
9 ME1571	0.03	0.04	Units = mcg/ml		
10 ME0976	0.03	0.01			
11 ME0878	0.03	0.03			
12 ME0443	0.05	0.27			
13 ME0965	0.05	0.29			
14 ME2027	0.08	0.03			
15 ME1653	0.10	0.21			
16 ME1090	0.11	0.17			
17 ME0027	0.11	0.16			
18 ME0536	0.14	0.26			
19 ME0092	0.18	0.29			
20 ME0007	0.21	1.71			
21 ME1229	0.23	0.24			
22 ME0727	0.24	1.07			
23 ME1676	0.24	0.31			
24 ME0820	0.26	1.16			
25 ME1512	0.33	0.39			
26 ME1812	0.33	0.57			
27 ME1440	0.41	1.07			
28 ME2009	0.50	0.17			
29 ME0175	0.51	1.11			
30 ME1991	0.51	0.65			
31 ME1899	0.52	0.47			
32 ME0156	0.54	1.28			
33 ME0287	0.80	0.86			
34 ME1811	0.85	0.41			
35 ME0286	0.90	1.01			
36 ME1186	0.98	0.54			
37 ME2005	0.99	1.23			
38 ME1855	1.02	0.58			

Table 6. Linear Regression Analysis of Vitreous Humor
FPIA vs. GC/MS Results

Regression Output:

Constant	0.118228
Std Err of Y Est	0.429233
R Squared	0.497603
No. of Observations	45
Degrees of Freedom	43
X Coefficient(s)	0.595707
Std. Err of Coef.	0.091281
Correlation coefficient	0.701

Comparison of Vitreous Humor Levels FPIA vs. GC/MS

Fig.9. Scatter plot of Vitreous Humor Levels by FPIA vs. GC/MS

Table 7. Data of Vitreous Humor Benzoylecgonine Levels
by GC/MS and Blood Benzoylecgonine Levels by GC/MS

I.D. #	Vitreous Humor GC/MS	Blood GC/MS		I.D. #	Vitreous Humor GC/MS	Blood GC/MS
1 ME1665	0.00	0.00	*0.00 BZE IN URINE	41 ME0814	1.29	1.52
2 ME0122	0.00	0.00	*NEG CONTROL	42 ME0007	1.71	1.34
3 ME0354	0.00	0.00	*NEG CONTROL	43 ME0006	2.38	0.56
4 ME0311	0.00	0.00	*NEG CONTROL	44 ME1530	2.71	2.03
5 ME0118	0.00	0.00	*NEG CONTROL	45 ME0260	2.87	0.60
6 ME0976	0.01	0.06				
7 ME2027	0.03	0.33				
8 ME0878	0.03	0.10				
9 ME1571	0.04	0.07				
10 ME0328	0.09	0.10				
11 ME0027	0.12	0.46				
12 ME2009	0.17	0.00	*0.40 BZE IN URINE			
13 ME1090	0.17	1.12				
14 ME1653	0.21	0.14				
15 ME1557	0.23	0.04				
16 ME1229	0.24	0.13				
17 ME0536	0.26	0.59				
18 ME0443	0.27	0.07				
19 ME0965	0.29	0.72				
20 ME0092	0.29	0.12				
21 ME1676	0.31	0.76				
22 ME0453	0.03	0.00	*0.34 BZE IN URINE			
23 ME1512	0.39	0.45				
24 ME1811	0.41	0.42				
25 ME1899	0.47	0.42				
26 ME1186	0.54	0.56				
27 ME1812	0.57	0.25				
28 ME1855	0.58	0.53				
29 ME1991	0.65	0.71				
30 ME1935	0.83	0.82				
31 ME0287	0.86	1.92				
32 ME1901	0.90	0.77				
33 ME0286	1.01	0.34				
34 ME1838	1.02	2.19				
35 ME0727	1.07	0.60				
36 ME1440	1.07	1.42				
37 ME0175	1.11	1.60				
38 ME0820	1.16	0.29				
39 ME2005	1.23	4.52				
40 ME0156	1.28	1.06				

Units = mcg/ml

Table 8. Linear Regression Analysis of Vitreous Humor Benzoylecgonine Levels
by GC/MS and Blood Benzoylecgonine Levels by GC/MS

Regression Output:

Constant	0.355201
Std Err of Y Est	0.617418
R Squared	0.260023
No. of Observations	45
Degrees of Freedom	43
X Coefficient(s)	0.426367
Std. Err. of Coef.	0.111333
Correlation Coefficient	0.51

Comparison of Vitreous Humor and Blood Benzoyllecgonine Levels by GC/MS

Fig. 10. Scatter plot of Vitreous Humor and Blood Benzoyllecgonine levels by GC/MS

in Figure 10.

All four negative controls correlated well by FPIA and GC/MS, therefore exhibiting no false positives. In two cases, the FPIA results on vitreous humor were positive, yet the corresponding blood samples were negative. In these two cases, the urines were positive for benzoylecgonine, therefore it might indicate a lag time in the elimination of the drug. This has been demonstrated in the laboratory for other drugs, such as ethanol (Caplan and Levine, 1990). One case gave a positive FPIA result in vitreous (0.01 mcg/ml), and no benzoylecgonine was detected by GC/MS. Technically, due to the limits of the assay this is below the 0.03 mcg/ml sensitivity of the assay and therefore would not be considered a true false positive. Both the blood and the urine in this case were negative for the presence of benzoylecgonine (Table 9).

The confirmation analyses provided full concordance data between the two assays. The DPC controls run to validate the GC/MS calibration curve gave accurate quantitations. (Table 10).

Table 9. Summary of Analyses of Data from
Vitreous Humors by FPIA and GC/MS.

- * 307 Medical Examiner Cases reviewed
- * 48 Cases selected for vitreous humor analyses
- * 3 Cases - Vitreous humors had insufficient quantity
for GC/MS quantitations
- * No false negatives by FPIA
- * No false positives at 0.03 mcg/ml cut-off by FPIA:
 - (2 Cases - Vitreous humors - FPIA positive
GC/MS positive
Blood samples negative * Urines positive *)
 - (1 Case - Vitreous Humor - FPIA result 0.01 mcg/ml
GC/MS none detected)

Table 10. Accuracy check with Diagnostics Products Corp.
GC/MS Control Samples

	TARGET (NG/ML)	ACTUAL	VARIATION
CON-DOA LEVEL1	180	185	+/- 5 NG/ML
CON-DOA LEVEL2	360	362	+/- 2 NG/ML
CON-DOA LEVEL3	1500	1550	+/- 50 NG/ML
(GC/MS ANALYSIS)			

An additional study was conducted to determine the possible explanation for the variation in some cases between the vitreous humor FPIA result and the GC/MS result for benzoylecgonine. The first assumption was that the variation in the vitreous FPIA result was due to the viscosity factor. This factor could cause the variation in the instrumental pipetting which occurs in the FPIA technique.

A pooled reserve of human vitreous humor was obtained from samples to be discarded by the Acting Chief Medical Examiner. The pooled vitreous samples equaled a volume of 50 milliliters. The pooled vitreous humor was run by FPIA initially with no drug added, to ascertain the blank reading to insure that there were no drugs present that would give a positive reading. The sample was run in 20 repetitions and the results were consistently negative. The pooled sample was then spiked with benzoylecgonine standard to attain a concentration of 1.6 mcg/ml. This sample was separated into two equal volumes, one was untreated and one was treated with 10% trichloroacetic acid (TCA). The TCA could potentially remove the protein, thereby possibly eliminating the viscosity factor as a cause of the inconsistencies. However, the CVs for both aliquots were 2.9% and 3.3% respectively (Table 11). The aliquot treated with 10% TCA demonstrated a reduced positive result. The mean for these 20 repetitions was 1.07 mcg/ml, as opposed to the aliquot mean of 1.61 mcg/ml for the untreated.

It was thought that the addition of a proteolytic agent would eliminate the viscosity factor and allow for better

Table 11. Precision Study of pooled Vitreous Humor samples-
untreated and treated with 10% Trichloroacetic Acid

	UNTREATED VH	W/ 10% TCA
TARGET VALUE (MCG/ML)	1.60	*
MEAN	1.61	1.07
STD.DEV.	4.6	3.5
COEF. OF VAR. (%)	2.9	3.3
# OF TRIALS	20	20
MEAN NET POLARIZATION VALUE BY FPIA	112.46	*129.86
		* ALTERED NET POLARIZATION READING BY FPIA: QUANTITATION LOWERED

correlation between the vitreous humor FPIA result and the vitreous humor benzoylecgonine result by GC/MS. Trichloroacetic acid was selected as the agent, since it is commonly used in the laboratory to precipitate out proteins found in blood samples. The selection of 10% TCA was not a good choice since the solution could react with benzoylecgonine (a tertiary amino carboxylic acid) and produce a salt, thus interfering with the extraction of the analyte. The phenyl ester on benzoylecgonine could also be de-esterified and the resulting product, ecgonine, does not react significantly with the immunoassay. The agent (10% TCA) also exhibits some fluorescence, which would adversely affect the FPIA analysis, by causing a decrease in the quantitation. There were no inconsistencies exhibited by either aliquots, and they yielded low CV's. The most unusual finding was that the addition of 10% TCA increased the net polarization and therefore decreased the cocaine metabolite concentration by FPIA. The study could not duplicate the individual viscosity problem experienced when sampling the low volume vitreous samples.

V. DISCUSSION

Fluorescence polarization immunoassay of vitreous humor as a screening process provided a qualitative prediction of the final GC/MS results. There were no false positives or false negatives by using this screening process. The initial perception of a false positive ultimately proved to be below the limits of the assay's cut-off level.

The correlation coefficient between vitreous humor benzoylecgonine levels and blood benzoylecgonine levels was 0.5. Ideally, if the drug substance is distributed by simple diffusion, at equilibrium the blood and vitreous humor levels should be identical. Studies by Coe, indicated more variation between blood and vitreous humor levels (Coe and Apple, 1985). He also indicated potential difficulty in separating toxic from therapeutic levels of drugs based on vitreous humor levels alone.

From the data generated by this research and those of others, several generalizations may be made concerning the concentration of drugs in vitreous humor. The more water soluble drugs and those least affected by protein-binding factors in the blood, would be expected to readily diffuse from the blood into the vitreous humor. The drugs must have sufficient lipid solubility to penetrate the blood-vitreous barrier. In some cases in the study, the high water solubility of benzoylecgonine permitted high concentrations to be found in the vitreous, sometimes even higher than in the

blood. There are other factors causing the variation in the distribution of the metabolite in the blood and vitreous humor. Survival times of the decedents is a definite factor, along with the time since the administration of the drug, the presence of other drugs in the system, the varying states of decomposition, and also the perimortem interval. It was demonstrated by Beno that by sampling vitreous at different perimortem intervals, the drug content of one vitreous humor could vary greatly from the other vitreous (Beno and Kriewall, 1989). This variation could be due to the leaching of the drug from the high concentrations of the drug in the brain tissue.

The history in most of the cases did not afford the survival time information. In the majority of the cases, the survival time was unknown and therefore did not clarify the disparity between the two fluids. As mentioned in other studies, it had been predicted that when survival times were lengthy, the blood/vitreous ratio of an analyte might approach unity. However, this research did not clarify that assumption. Since vitreous humor is a partitioned fluid, with a $T_{1/2}$ of 10 to 15 minutes, the author anticipated a good correlation with corresponding blood levels. Also, since the molecular weights of benzoylecgonine and cocaine are relatively low, 289 and 303 respectively, these low molecular compounds could diffuse rather easily. However, the ratio varied a great deal between the two biological fluids. There are more factors involved, and the most important probably

being the difference between dosing and the time of death as indicated in the study by Poklis (Poklis et al., 1985).

The most troublesome finding was the variation between the vitreous FPIA result and the vitreous GC/MS benzoylecgonine quantitation. The FPIA technique is a semi-quantitative technique which reacts 100% with the compound benzoylecgonine. However, cross reactivity with other compounds does occur at a low percentage. The other compounds if present in varying high concentrations, may be causing the apparent discrepancy between the two assays. The additional study with 10% TCA did not clarify the problem, since there were no inconsistencies demonstrated that could be attributed to the viscosity factor. The variation appears to be one which must be explained on a case by case basis. The outstanding outliers in the study were reviewed and most demonstrated no known survival time or time since dosing based on the history.

The biggest liability in the screening of vitreous humor is the lack of sufficient sample volume to repeat analyses and generate statistical data on a case by case basis. The low sample volume did not allow for repetitive analyses, which then would allow for CVs to be calculated on each sample. This is not true of blood and urine, which when available, are usually of sufficient volume.

One point for discussion is the validity of testing infants and children by vitreous humor for cocaine exposure in the absence of a urine sample. Lowering the threshold

as was done in this study, to a level of 0.03 mcg/ml would be necessary to detect the low levels of cocaine and metabolites found in infants and children. In 25 cases reviewed of maternal substance abuse, all showed benzoylecgonine levels in the blood of the fetuses and neonates in the range of 0.12 to 3.2 mcg/ml (Sweeney, 1991). The routine drug screening of infants and children would now appear to be a modern necessity.

VI. SUMMARY AND CONCLUSIONS

The goal of the research was to provide a valid assessment of the fluorescence polarization immunoassay technique in detecting cocaine and/or its metabolites in vitreous humor. In all the cases reviewed, when cocaine and metabolites were present in the blood, cocaine metabolites were positive by FPIA in the vitreous humor. The research showed vitreous humor to be a reliable postmortem specimen for detecting antemortem cocaine use. It was demonstrated that elevated cocaine metabolite (benzoylecgonine) levels in blood are accompanied by elevated cocaine metabolite levels in vitreous humor. The research illustrated that the cocaine metabolites assay by FPIA has sufficient specificity and sensitivity to be a valid screening technique for vitreous samples at the low threshold of 0.03 mcg/ml.

The screening of vitreous humors by FPIA when the threshold is lowered, is a useful tool in forensic toxicology. The technique, however, is just a screening one and not meant to replace the confirmation of the result. When the absolute concentration of benzoylecgonine is critical for the interpretation of results, an alternate, more specific methodology should be used for an accurate concentration; GC/MS is such an alternative.

Vitreous humor specimens provide an additional medium for analysis in postmortem cases. These specimens can be screened rapidly and relatively inexpensively by fluorescence

polarization immunoassay for qualitative results. There are more studies necessary on the ability to detect various drugs in vitreous humor. In the future, vitreous drug concentrations when compared with blood concentrations, may allow for the assessment of the time between administration of the substance and death.

APPENDIX A.

COMPARATIVE TOXICOLOGY OF VITREOUS HUMOR AND BLOOD

#	CASES	CAUSE	MAN.		SURV.	VH	V.H. FPIA	V.H. GC/MS	BL. GC/MS
		OF	OF	A/R/S	TIME	AV	RESULTS	RESULTS	RESULTS
		DEATH	DEATH		(HR.)			(MCG/ML)	(MCG/ML)

JANUARY '89

1	ME0004-89	PEND	PEND	5M/W/M	NONE	Y	NEG		NDD
2	ME0005-89	ALC	NAT	58/W/M	UNK	Y	N/A		N/A
3	ME0006-89*	INJ	MVA	23/W/M	<1	Y	*1.80 COC/METS	2.38 BZE	0.05COC/0.56BZE
4	ME0007-89*	PEND	PEND	35/H/M	UNK	Y	*0.21 COC/METS	1.71 BZE	0.33COC/1.34BZE
5	ME0010-89	INJ	HOM	46/W/M	NONE	Y	N/A		N/A
6	ME0014-89	ASHD	NAT	64/B/F	NONE	Y	NEG		N/A
7	ME0017-89	COI	ACC	76/W/F	0.5	Y	BARBS	N/A	<1MCG/ML BUTAL
8	ME0027-89*	PEND	PEND	37/W/M	NONE	Y	*0.11 COC/METS	0.16 BZE	NEG/COC/0.46BZE
9	ME0028-89	INJ	ACC	75/W/F	NONE	Y	N/A		NEG FLURAZEPAM
10	ME0034-89	INJ	ACC	42/W/M	NONE	Y	NEG		N/A
11	ME0047-89	INJ	MVA	63/W/F	8.0	Y	PPX	N/A	0.24PPX/0.68NPA
12	ME0062-89	ASHD	NAT	57/W/M	NONE	Y	NEG		NDD
13	ME0066-89	SIDS	SIDS	1M/W/M	MINS	Y	N/A		QNS
14	ME0076-89	PEND	PEND	11M/W/M	NONE	Y	NEG		N/A
15	ME0080-89	OD	SUIC	43/W/M	NONE	N	N/A		1.6ATP/0.8NTP/1.5FLX
16	ME0083-89	INJ	MVA	83/W/F	NONE	Y	N/A		0.06NORDIAZ
17	ME0092-89*	ASPX	SUIC	27/W/M	NONE	Y	*0.18 COC/METS	0.30 BZE	0.03COC/0.12BZE/0.12FME
18	ME0118-89*	INJ	MVA	30/W/F	MINS	Y	*NEG/LOW	0.00 BZE	NDD
19	ME0122-89*	PEND	PEND	33/W/M	NONE	Y	*NEG/LOW	0.00 BZE	NDD
20	ME0139-89	INJ	SUIC	64/W/F	30DAYS	Y	NEG		NDD
21	ME0150-89	GSW	SUIC	84/W/M	NONE	Y	N/A		N/A
22	ME0156-89*	PEND	PEND	35/B/M	8.0	Y	*0.54 COC/METS	1.28 BZE	NEG/COC/1.06BZE
23	ME0159-89	ASHD	NAT	51/W/F	NONE	Y	NEG		N/A

24	ME0175-89	ASPX	SUIC	37/W/F	NONE	Y	*0.51 COCC.METS	1.11 BZE	0.33COCC/0.53EME/1.61BZE
25	ME0183-89	ASPX	ACC'	79/W/F	NONE	Y	NEG		N/A
26	ME0199-89	DIS	NAT	47/W/M	3.0	Y	N/A		ME:PROBAMATE
27	ME0206-89	INJ	MVA	82/W/F	6 WKS	Y	N/A		0.31TOT.MOR./0.15FREE MOR
28	ME0240-89	COI	ACC'	43/W/M	NONE	Y	N/A		N/A
29	ME0247-89	ASPX	SUIC'	19/W/M	NONE	Y	N/A		N/A
30	ME0249-89	PEND	SIDS	3M/W/F	0.5	N	N/A		N/A
31	ME0252-89	ASPX	MVA	15/W/F	NONE	Y	N/A		N/A
32	ME0260-89	GSW	SUIC	28/W/M	MINS	Y	*2.01 COCC.METS	2.87 BZE	0.05COCC/0.60EME/2.51BZE
33	ME0261-89	PEND	PEND	56/B/M	NONE	Y	NEG		N/D/D
34	ME0265-89	INJ	ACC'	46/W/F	0.5	Y	NEG		N/D/D
35	ME0278-89	PEND	PEND	7WK/W/M	NONE	N	N/A		N/D/D
36	ME0286-89	ASPX	PEND	23/W/M	NONE	Y	*0.90 COCC.METS	1.01 BZE	0.32COCC/0.34BZE
37	ME0287-89	PEND	PEND	39/B/M	NONE	Y	*0.80 COCC.METS	0.86 BZE	NEGCOCC/1.92BZE
38	ME0289-89	ASHD	NAT	40/W/M	NONE	Y	N/A		N/A
39	ME0294-89	COI'	ACC'	83/W/F	12DAYS	Y	N/A		0.05TOTMOR/12.00PH
40	ME0299-89	INJ	ACC'	80/W/F	1.0	Y	NEG		N/D/D
41	ME0301-89	PEND	PEND	11M/B/M	MINS	Y	NEG		N/A
42	ME0310-89	ALC'	PEND	43/W/F	NONE	Y	N/A		N/A
43	ME0311-89	INJ	MVA	23/W/M	NONE	Y	*0.00 COCC.METS	0.00 BZE	N/A
44	ME0312-89	ASHD	NAT	39/W/M	NONE	Y	N/A		N/A
45	ME0318-89	DIS	NAT	7/W/F	NONE	Y	N/A		14.6PHB
46	ME0328-89	GSW	SUIC	37/W/F	NONE	Y	*0.03 COCC.METS	0.09 BZE	0.08COCC/0.10BZE
47	ME0331-89	ASHD	NAT	61/W/M	NONE	Y	N/A		N/A
48	ME0341-89	PEND	PEND	50/W/F	NONE	N	N/A		QNS
49	ME0345-89	OD	SUIC	64/W/M	NONE	Y	N/A		3.92PTB
50	ME0346-89	DIS	NAT	52/W/M	MINS	N	N/A		QNS
51	ME0354-89	INJ	ACC'	30/W/M	NONE	Y	*LOW COCC.ME	0.00 BZE	2.0 PTM
52	ME0362-89	PEND	PEND	18/W/M	NONE	Y	N/A		N/D/D
53	ME0367-89	GSW	HOM	20/C/F	NONE	Y	N/A		N/A

54	ME0368-89	GSW	SUIC	23/O/M	NONE	Y	N/A		N/A
55	ME0373-89	DIS	PEND	46/W/M	NONE	Y	N/A		9.7 P11B
56	ME0380-89	SIDS	PEND	4M/W/M	NONE	N	N/A		1.1DC/C/PM
FEBRUARY '8									
57	ME0395-89	GSW	SUIC	48/W/M	NONE	Y	N/A		N/A
58	ME0403-89	ALC	NAT	82/W/M	UNKNOWN	Y	NEG		N/A
59	ME0434-89	CCT	DMVA	17/W/M	<1.0	Y	NEG		NDD
60	ME0436-89	INJ	ACC	17/W/M	NONE	Y	N/A		N/A
61	ME0438-89	ASPX	SUIC	19/W/M	NONE	Y	N/A		N/A
62	ME0443-89*	OD	PEND	31/W/M	NONE	Y	*0.05 COC.METS	0.27 BZE	NEG/COC/0.07BZE
63	ME0446-89	SURG	SURG	83/W/F	NONE	Y	N/A		N/A
64	ME0452-89	INJ	ACC	32/W/F	NONE	Y	N/A		N/A
65	ME0453-89*	INJ	ACC	30/B/M	NONE	Y	*0.02 COC.METS	0.03 BZE	COC/BZE
66	ME0464-89	PEND	PEND	42/W/M	NONE	Y	N/A		N/A
67	ME0486-89	INJ	ACC	39/W/F	NONE	Y	NEG		N/A
68	ME0512-89	ASHD	ACC	51/W/M	NONE	Y	NEG		N/A
69	ME0534-89	INJ	ACC	22/W/M	NONE	Y	NEG		THC/THCC
70	ME0535-89	CCT	ACC	56/W/F	NONE	Y	N/A		N/A
71	ME0536-89*	DROW	SUIC	27/H/M	NONE	Y	*0.14 COC.METS	0.26 BZE	0.16COC/0.59BZE
72	ME0538-89	ASPX	SUIC	21/W/M	NONE	Y	N/A		N/A
73	ME0540-89	ASHD	NAT	71/W/M	NONE	Y	N/A		N/A
74	ME0542-89	ASHD	NAT	43/W/M	<1.0	Y	N/A		N/A
75	ME0545-89	CCT	PEND	41/W/M	3DAYS	Y	NEG		N/A
76	ME0546-89	ALC	ALC	55/B/M	NONE	N	N/A		N/A
77	ME0547-89	SIDS	PEND	54/W/M	<1.0	Y	EMBAL MED		N/A
78	ME0548-89	INJ	ACC	34/W/M	8DAYS	Y	BARBS		9.0P11B
79	ME0549-89	ASHD	NAT	78/W/F	NONE	N	N/A		N/A
80	ME0550-89	CCT	ACC	31/H/M	<1.0	Y	NEG		N/A
81	ME0565-89	ASHD	NAT	65/W/M	NONE	Y	N/A		N/A
82	ME0585-89	PEND	PEND	90/W/F	NONE	Y	N/A		N/A

83	ME0592-89*	GSW	SUIC	32/W/M	NONE	Y	*QNS	QNS	NEG/COC/0.33BZE
84	ME0597-89	CCT	ACC	23/W/M	<1.0	Y	N/A		N/A
85	ME0616-89	CCT	ACC	43/W/M	MINS	Y	N/A		N/A
86	ME0638-89	ASHD	NAT	70/W/M	NONE	Y	N/A		N/A
87	ME0641-89	GSW	SUIC	69/W/M	NONE	Y	N/A		N/A
88	ME0642-89	ASHD	NAT	71/W/M	NONE	Y	NEG		N/D
89	ME0647-89	PEND	PEND	45/W/M	NONE	Y	N/A		N/A
90	ME0658-89	COI	ACC	28/W/M	NONE	Y	N/A		CHLORPHENIRAMINE
91	ME0660-89	DROWN	ACC	32/W/M	NONE	Y	NEG		LID
92	ME0661-89	EXPOS	ACC	77/W/M	NONE	Y	N/A		N/A
93	ME0683-89	DIS	NAT	45/B/M	10HRS	Y	N/A		N/A
94	ME0688-89	GSW	SUIC	46/W/M	NONE	Y	N/A		0.27NORDIAZ
95	ME0690-89	INJ	ACC	57/W/F	<2HRS	Y	N/A		N/A
96	ME0701-89	PEND	ACC	38/W/M	NONE	Y	N/A		N/A
97	ME0707-89	INJ	ACC	55/W/F	?	Y	N/A		N/A
98	ME0708-89	ALC	NAT	65/W/M	NONE	Y	N/A		DOXYLAMINE
99	ME0727-89*	GSW	SUIC	34/W/M	NONE	Y	*0.24 COC/METS	1.07 BZE	0.07COC/0.60BZE
100	ME0728-89	ALC	NAT	52/B/M	3 DAYS	Y	NEG		N/A
101	ME0747-89	DROWN	ACC	33/W/M	NONE	Y	BARBS		6.2PHENO/5.9PHENY
	MARCH '89								
102	ME0762-89	ASPX	ACC	11MO/O/	NONE	N	N/A		N/A
103	ME0763-89	SEIZ	NAT	24/W/F	NONE	Y	NEG		6.4CARB
104	ME0791-89	INJ	ACC	79/W/F	10 DAYS	Y	NEG		0.42TUTMOR
105	ME0798-89	PEND	PEND	38WK/W/	NONE	N	N/A		N/A
106	ME0796-89	GSW	HOM	39/W/M	MINS	Y	N/A		N/A
107	ME0801-89	GSW	HOM	23/B/M	10.5 HRS	Y	NEG		N/A
108	ME0804-89	ASHD	NAT	61/W/M	NONE	Y	NEG		N/A
109	ME0807-89	ASHD	NAT	43/W/M	NONE	Y	N/A		N/A
110	ME0814-89*	OD	PEND	41/W/M	NONE	Y	*1.08 COC/METS	1.29 BZE	1.97COC/1.52BZE
111	ME0820-89*	OD	PEND	26/W/M	36 HRS	Y	*0.26 COC/METS	1.16 BZE	0.08COC/0.29BZE

112	ME0822-89	ASPX	SUIC	20/O/M	2 HRS	Y	N/A		1.10/PHENY
113	ME0863-89	COI	ACC	9MO/W/M	NONE	Y	QNS		NDID
114	ME0886-89	INJ	SUIC	34/W/M	0.5 HRS	Y	N/A		10.0AMOBARBITAL
115	ME0878-89*	OD	UNCL ASS	26/W/M	16 DAYS	Y	*0.03 COCAINE	0.03 BZE	*BIL-0.19COCA/0.10BZE
116	ME0892-89	INJ	ACC	59/W/F	40 MINS.	Y	N/A		NDID
117	ME0893-89	DROWN	ACC	82/W/M	NONE	Y	N/A		NDID
118	ME0895-89	CCIT	ACC	19/W/M	NONE	Y	N/A		N/A
119	ME0905-89	CCIT	ACC	73/W/F	NONE	Y	OPIATES		0.05FRETICOD/0.31TICOD
120	ME0912-89	PEND	PEND	67/B/M	NONE	N	N/A		0.023HIALOPERIDOL
121	ME0913-89	PEND	PEND	75/W/F	NONE	Y	N/A		N/A
122	ME0922-89	ASPX	ACC	13MO/W/	NONE	Y	N/A		NDID
123	ME0939-89	DROWN	ACC	24/W/M	NONE	Y	N/A		N/A
124	ME0940-89	DROWN	SUIC	56/W/M	NONE	Y	NEG		0.07FLUOXETINE/0.13NORFLUOX
125	ME0945-89	PEND	PEND	31/B/M	NONE	Y	N/A		N/A
126	ME0960-89	ASHD	ACC	41/W/M	NONE	Y	N/A		NDID
127	ME0965-89*	ASTM	PEND	35/W/M	NONE	Y	*0.05 COCAINE	0.29 BZE	0.11COCA/0.72BZE
128	ME0976-89*	OD	PEND	19/W/M	NONE	Y	*0.03 COCAINE	0.01 BZE	0.09COCA/0.06BZE
129	ME0983-89	ASPX	SUIC	22/W/M	NONE	Y	N/A		N/A
130	ME0985-89	CCIT	ACC	34/W/F	>7 HRS	Y	NEG		N/A
131	ME0986-89	CCIT	ACC	64/W/F	NONE	Y	N/A		N/A
132	ME0996-89	DROWN	ACC	11/W/M	NONE	Y	N/A		N/A
133	ME0997-89	DROWN	ACC	11/M/W	NONE	Y	N/A		N/A
134	ME1001-89	ASPX	ACC	80/W/M	MINS	Y	NEG		NDID
135	ME1005-89	CCIT	ACC	85/W/M	DAYS	N	N/A		N/A
136	ME1015-89	ALC	NAT	45/B/M	NONE	Y	N/A		0.055ALPRAZOLAM
137	ME1020-89	ASHD	ACC	50/W/M	NONE	Y	N/A		NDID
138	ME1041-89*	PEND	PEND	31WK/W/	NONE	Y	*QNS	QNS	0.30COCA/1.12BZE
139	ME1042-89	GSW	HOM	17/W/M	MINS	Y	N/A		4.1NG/ML TIC/16THCC
140	ME1047-89	INJ	ACC	28/B/M	NONE	Y	N/A		N/A
141	ME1051-89	ASHD	NAT	63/B/M	NONE	Y	N/A		N/A

171	ME1267-89	ALC	PEND	68/W/F	NONE	Y	N/A	N/A
172	ME1274-89	DIS	NAT	23/B/M	1 DAY	Y	NEG	11.6 PHENYTOIN
173	ME1298-89	DIS	NAT	70/W/M	UNKNOWN	Y	N/A	N/A
174	ME1299-89	INJ	ACC	58/W/M	NONE	Y	N/A	N/A
175	ME1300-89	PEND	PEND	42/W/F	NONE	Y	QNS	0.95 METHADONE/1.1 DOCAINE
176	ME1312-89	CCIT	PEND	30/B/F	NONE	Y	N/A	N/A
177	ME1313-89	INJ	ACC	59/W/M	<1 HR	Y	N/A	N/A
178	ME1314-89	ASHD	NAT	64/W/M	NONE	Y	N/A	N/A
179	ME1323-89	ASHD	NAT	53/B/M	NONE	Y	NEG	N/A
180	ME1325-89	ASHD	ACC	76/W/M	NONE	Y	N/A	0.2 DIAZEP./0.2 NORID/0.2 TEMAZEPAM
181	ME1327-89	SIDS	PEND	1MO/W/M	NONE	N	N/A	NID
182	ME1328-89	DROWN	ACC	82/W/F	NONE	Y	N/A	2.9 PHENOBARBITAL
183	ME1336-89	CCIT	ACC	32WK/W/	NONE	Y	N/A	N/A
184	ME1338-89	INJ	ACC	23/W/M	NONE	Y	N/A	N/A
185	ME1343-89	INJ	HOM	29/W/M	NONE	Y	N/A	N/A
186	ME1365-89	GSW	SUIC	80/W/M	NONE	Y	NEG	NID
187	ME1370-89	DIS	NAT	78/W/F	21 DAYS	Y	N/A	N/A
188	ME1380-89	DIS	NAT	43/W/M	NONE	Y	N/A	15.5 PHENOBARBITAL/19.5 PHENYTOIN
189	ME1383-89	ASHD	NAT	62/W/M	NONE	Y	N/A	N/A
190	ME1408-89	INJ	ACC	59/W/M	1 HR	Y	NEG	NID
191	ME1409-89	OD	UNCLASS	29/W/M	NONE	Y	N/A	0.5 PRO/0.7 NPA/0.12 FRIEMOR/0.41 TOT
192	ME1411-89	OD	UNCLASS	31/W/M	NONE	Y	N/A	0.23 FRIEMOR/1.36 TOTMOR
193	ME1413-89	DROWN	ACC	26/W/M	NONE	Y	N/A	N/A
194	ME1415-89	OD	SUIC	39/W/F	3 HRS	Y	N/A	6.79 IMIPRAMINE/9.85 DESIPRAMINE
195	ME1426-89	DIS	NAT	34/W/F	4 HRS	Y	BARBS	8.0 PHENOBARBITAL
196	ME1440-89	OD	UNCLASS	29/W/M	< 1 HR	Y	*0.41 COCAINE	1.07 BZE 0.08 COC/1.42 BZE
197	ME1442-89	GSW	SUIC	18/W/M	< 1 HR	Y	N/A	N/A
198	ME1461-89	OD	UNCLASS	42/W/F	NONE	Y	N/A	0.76 TOTMORPHINE
199	ME1463-89	ASHD	NAT	33/W/F	1 HR	Y	N/A	NID
200	ME1464-89	ALC	NAT	71/B/M	10 HRS	Y	NEG	NID

201	ME1473-89	SIDS	PEND	2.5MO/W/	NONE	N	N/A		NDD
202	ME1491-89	CCT	ACC	20/W/M	>4.5 DAYS	N	N/A		N/A
203	ME1497-89*	PEND	PEND	6WK/W/M	NONE	Y	*0.02 COC.METS	QNS	0.08COC/NEGBZE
204	ME1505-89	ASPX	ACC	57/W/M	NONE	Y	N/A		0.3NORTRIPTYLINB
205	ME1506-89	SIDS	PEND	2MO/W/M	NONE	Y	QNS		QNS
206	ME1507-89	GSW	HOM	49/W/F	NONE	Y	N/A		N/A
207	ME1508-89	GSW	SUIC	47/W/M	NONE	Y	NEG		NDD
208	ME1511-89	ALC	UNCLASS	34/W/M	NONE	Y	N/A		N/A
209	ME1512-89*	GSW	HOM	27/W/M	NONE	Y	*0.33 COC.METS	0.39 BZE	0.15COC/0.45BZE
210	ME1513-89	PEND	ACC	16/W/M	3 DAYS	Y	N/A		QNS
211	ME1530-89*	GSW	HOM	23/B/M	0.5 HR	Y	*1.29 COC.METS	2.71 BZE	0.09COC/2.03BZE
212	ME1547-89	GSW	HOM	22/W/M	NONE	Y	NEG		NDD
213	ME1548-89	OD	UNCLASS	14/W/F	NONE	Y	N/A		1.7AMITRIPTYLINB/2.2NORTRIP.
214	ME1555-89	CVA	NAT	59/W/F	NONE	Y	NEG		NDD
215	ME1557-89*	ANBU	NAT	29/W/M	<1 HR	Y	0.02 COC.METS.	0.02 BZE	0.09TOTMOR./0.02FREBCOD/0.24TOT.
216	ME1567-89	INJ	ACC	3/W/M	NONE	Y	NEG		N/A
217	ME1571-89*	CCT	ACC	25/W/M	NONE	Y	*0.03 COC.METS	0.49 BZE	NEGCOX/0.07BZE
218	ME1572-89	INJ	ACC	18/W/F	5DAYS	Y	N/A		0.03DIAZEPAM/<0.01NORDIAZEPAM
219	ME1600-89	GSW	SUIC	53/W/M	NONE	Y	N/A		N/A
220	ME1605-89	GSW	SUIC	32/W/M	NONE	Y	N/A		N/A
221	ME1609-89	PEND	PEND	71/W/F	<1 HR	Y	BARBS		13.0 MCG/ML PHENO
222	ME1613-89	INJ	MVA	86/W/F	MINS	Y	NEG		DEXTROMETHORPHAN
223	ME1616-89	INJ	ACC	31/W/M	<1 HR	Y	N/A		N/A
224	ME1626-89	ASHD	NAT	52/W/M	NONE	Y	NEG		<2.5 MCG/ML PHENYTOIN
225	ME1633-89	INJ	MVA	72/W/M	NONE	Y	N/A		N/A
226	ME1648-89	INJ	ACC	22/W/M	MINS	Y	N/A		NDD
227	ME1650-89	ASPX	SUIC	17/W/M	MINS	Y	NEG		1.1DOCAINE
228	ME1653-89*	OD	SUIC	40/B/F	NONE	Y	*0.10 COC.METS	0.21 BZE	NEG COX/0.14 BZE/0.71PROP
229	ME1665-89*	PEND	PEND	42/W/M	NONE	Y	*0.01 COC.METS	0.00 BZE	N/A
230	ME1666-89	INJ	MVA	24/W/F	2.5 HRS	Y	N/A		N/A

231	ME1668-89	GSW	SUIC	41/W/M	NONE	Y	N/A		N/A
232	ME1671-89	ASUD	NAT	57/W/M	NONE	Y	N/A		N/A
233	ME1676-89*	INJ	HOM	26/W/F	NONE	Y	*0.24 COCAINE	0.31 BZE	0.62COCA/0.76BZE
234	ME1687-89	DIS	NAT	67/W/M	NONE	Y	NEG		<2.5 MCCG/ML PHENYTOIN
235	ME1692-89	INJ	HOM	53/W/M	NONE	Y	N/A		9.3MCCG/ML PHENO
236	ME1696-89	ANIEU	NAT	37/W/M	NONE	Y	N/A		N/A
237	ME1697-89	INJ	ACC	58/W/M	NONE	Y	NEG		0.21DIA/0.3NOR/38FLX/0.03NORFLX
238	ME1710-89	INJ	ACC	2/W/F	NONE	Y	N/A		N/A
239	ME1713-89	OD	ACC	26/W/F	NONE	Y	N/A		1.5PRO/1.1NPA/.37CYCLOBENZ.
240	ME1714-89	INJ	HOM	35/B/F	NONE	Y	N/A		14.8 PHENO
241	ME1719-89	INJ	ACC	41/W/M	NONE	Y	N/A		N/A
242	ME1741-89	INJ	MVA	34/W/M	1 HR	Y	NEG		ETHOCAINE
243	ME1743-89	CCCT	MVA	21/W/M	30 HRS	Y	N/A		NDD
244	ME1745-89	ASPX	SUIC	16/W/M	NONE	Y	NEG		NDD
245	ME1753-89	INJ	MVA	21/W/M	NONE	Y	N/A		N/A
246	ME1754-89	ASUD	NAT	45/W/M	NONE	Y	N/A		N/A
247	ME1780-89	ALC	NAT	56/W/M	NONE	Y	N/A		N/A
248	ME1801-89	GSW	HOM	58/W/M	1 HR	Y	N/A		N/A
249	ME1811-89*	INJ	MVA	39/W/M	NONE	Y	*0.85 COCAINE	0.41 BZE	0.42BZE/NEG COCA
250	ME1812-89*	INJ	MVA	29/W/F	NONE	Y	*0.33 COCAINE	0.57 BZE	0.25 BZE
251	ME1822-89	PEND	PEND	1M/B/M	NONE	Y	N/A		QNS
252	ME1825-89	DIS	NAT	49/W/M	8 HRS	Y	N/A		N/A
253	ME1826-89	CCCT	MVA	16/W/F	NONE	Y	N/A		N/A
254	ME1829-89	CO	ACC	36/W/M	NONE	Y	N/A		N/A
255	ME1834-89	DIS	NAT	47/W/M	1 HR	Y	NEG		0.16FLX/0.14NFX/0.21DIA
256	ME1838-89*	OD	UNCL	33/W/M	NONE	Y	*1.05 COCAINE	1.02 BZE	3.99COCA/2.19BZE
257	ME1839-89	INJ	MVA	31/W/M	1 HR	Y	N/A		NDD
258	ME1841-89	GSW	HOM	62/W/F	NONE	N	N/A		0.6NORDIAZE/PAM
259	ME1855-89*	ASPX	SUIC	42/B/M	NONE	Y	*1.01 COCAINE	0.58BZE	0.09COCA/0.53BZE
260	ME1856-89	GSW	HOM	35/B/M	1.5 HRS	Y	NEG		ETHOCAINE

	JUNE 1989									
261	ME1864-89	BLBC	ACC	30/W/M	NONE	Y	N/A			N/A
262	ME1899-89*	GSW	HOM	32/H/M	1.5 HRS	Y	*0.52 COC.METS	0.47 BZE		0.22COC/0.47BZE
263	ME1901-89*	IVDA	UNCLA	27/W/M	NONE	Y	*2.59 COC.METS	0.90 BZE		0.11COC/0.77BZE
264	ME1905-89	ASHD	NAT	36/W/M	MINS	Y	N/A			N/A
265	ME1908-89	CCT	ACC	48/W/M	NONE	Y	N/A			N/A
266	ME1935-89*	INJ	SUIC	25/W/M	MINS	Y	*1.38 COC.METS	0.83 BZE		0.16COC/0.82BZE
267	ME1948-89	CCT	HOM	45/W/M	NONE	Y	N/A			N/A
268	ME1952-89	CCT	ACC	66/W/M	1 WK.	Y	N/A			N/A
269	ME1953-89	ASHD	NAT	41/W/M	NONE	Y	N/A			N/A
270	ME1970-89	INJ	ACC	34/W/M	NONE	Y	N/A			N/A
271	ME1981-89	DIS	NAT	2/W/M	NONE	Y	N/A			N/A
272	ME1985-89	ASHD	NAT	64/W/M	NONE	Y	N/A			N/A
273	ME1991-89*	CCT	MVA	30/B/F	NONE	Y	*0.51 COC.METS	0.65 BZE		0.17COC/0.71BZE
274	ME1995-89	DIS	NAT	21/W/M	NONE	Y	BARBS			NDD
275	ME2002-89	PEND	PEND	90/W/M	6 HRS	Y	N/A			23.0 THEOPHYLLINE
276	ME2162-89	INJ	MVA	24/W/M	NONE	Y	N/A			N/A
277	ME2164-89	ASHD	NAT	31/W/M	MINS	Y	N/A			0.07FRIEMOR/0.09TOT.MOR
278	ME2166-89	DIS	NAT	63/W/M	1 HR.	Y	NEG			NDD
279	ME2171-89	ASPX	SUIC	25/B/M	NONE	Y	N/A			NDD
280	ME2193-89	CCT	ACC	81/W/F	9 DAYS	Y	NEG			NDD
281	ME2215-89	INJ	ACC	75/W/M	25 DAYS	Y	NEG			N/A
283	ME2003-89	SUDS	PEND	50/W/M	NONE	Y	N/A			N/A
284	ME2005-89*	OD	UNCLASS	31/W/F	NONE	Y	*0.99 COC.METS	1.23 BZE		14.75COC/4.52BZE
285	ME2009-89*	GSW	SUIC	21/W/M	NONE	Y	*0.50 COC.METS	0.17 BZE		NEGCOX/NEGIBZE
286	ME2006-89	INJ	ACC	21/W/M	NONE	Y	N/A			N/A
287	ME2014-89	OD	ASPX	37/W/M	NONE	N	N/A			N/A
288	ME2023-89	ASHD	NAT	60/W/M	NONE	Y	N/A			N/A
289	ME2027-89*	ASPX	SUIC	28/W/M	NONE	Y	*0.08 COC.METS	0.03 BZE		0.21COC/0.33BZE
290	ME2039-89	ASHD	NAT	72/W/F	NONE	Y	N/A			N/A

291	MF2052-89	GSW	HOM	19/W/F	NONE	N	N/A	NEG COCAINE/URINE
292	MF2048-89	SHDS	PEND	6WK/C/F	NONE	N	N/A	N/D
293	MF2056-89	DROW	ACC	62/B/M	NONE	Y	NEG	N/D
294	MF2057-89	CCCT	ACC	90/W/F	4 HRS	Y	N/A	N/D
295	MF2062-89	ASHD	NAT	32/W/F	HOURS	Y	N/A	N/D
296	MF2072-89	ASHD	NAT	74/W/M	NONE	Y	N/A	N/A
297	MF2074-89	DROW	ACC	43/W/M	NONE	Y	N/A	N/A
298	MF2087-89	CCCT	ACC	17/W/M	NONE	Y	N/A	N/A
299	MF2111-89	OD	ACC	35/W/M	NONE	Y	N/A	2.3C ARBAMAZEPINE
300	MF2109-89	DIS	NAT	43/B/F	NONE	Y	N/A	N/A
301	MF2123-89	OD	ACC	38/W/F	NONE	Y	N/A	1.50FEPICODINE/1.77TOTCOD
302	MF2117-89	PEND	PEND	54/W/M	NONE	Y	N/A	0.60MEPERIDINE/LID
303	MF2126-89	DROW	ACC	38/W/M	NONE	Y	N/A	N/A
304	MF2132-89	DROW	ACC	62/B/M	NONE	N	N/A	N/A
305	MF2136-89	CCCT	MVA	32/W/M	NONE	Y	NEG	N/D
306	MF2153-89	OD	ACC	53/W/F	NONE	Y	N/A	8.71DOXEFIN
307	MF2156-89	GSW	SUIC	90/W/M	6 DAYS	N	N/A	N/A

APPENDIX B.

CAUSE OF DEATH MASTER LIST

ACC	ACCIDENTAL
ALC	ALCOHOLISM (ACUTE/CHRONIC)
ANEU	ANEURYSM
ASHD	ARTERIOSCLEROTIC HEART DISEASE
ASPX	ASPHYXIA
ASTM	ASTHMA
CCT	CRANIO-CEREBRAL TRAUMA
COI	CARBON MONOXIDE INTOXICATION
COPD	CHRONIC OCCLUDED PULMONARY DISEASE
DIS	DISEASE
DMVA	DRIVER/MOTOR VEHICLE ACCIDENT
DROW	DROWNING
ELECT	ELECTROCUTION
EXSG	EXSANGUINATION
GSW	GUN SHOT WOUND
HGSW	HOMICIDE GUN SHOT WOUND
HOM	HOMICIDE
IDDM	INSULIN DEPENDENT DIABETES MELLITUS
IVDA	INTRAVENOUS DRUG ABUSE
LIV	LIVER NECROSIS
MVA	MOTOR VEHICLE ACCIDENT
NAT	NATURAL DEATH
OD	OVERDOSE
PEND	PENDING
RHD	RHEUMATIC HEART DISEASE
SGSW	SUICIDE/GUN SHOT WOUND
SIDS	SUDDEN INFANT DEATH SYNDROME
SUDS	SUDDEN UNEXPLAINED DEATH SYNDROME
SUIC	SUICIDE
SURG	SURGICAL MISADVENTURE

>>>>AUTOTUNE REPORT<<<<

DATE: 5/21/91 11:20 Tuned from previous values
 SYSTEM#2: 5970(TOX-II) SERIAL NO.: 2716A10133
 TUNE TYPE: EI MODE: POSITIVE

	Repller Volts	IonPcs Volts	EMvlt Volts	Gain DACs	Offset DACs	EntLens mV/AMU	X-ray Volts	Mass Gain	Mass Offset
Initial:	10.2	0	2693	166	82	65	108	122	15
Final:	10.2	0	2835	166	82	60	120	122	15

Mass	Abs.Abund	Rel.Abund	Trgt.Abund	Iso.Mass	Iso.Abund	Iso.Ratio	Trgt.Ratio
69.00	131141	100.00%	100.00%	70.02	1421	1.08%	1.08%
218.95	104416	79.62%	35.00%	219.95	4608	4.41%	4.32%
502.00	9546	7.28%	2.00%	503.00	991	10.39%	10.11%

Autotune finished

Mass	Abs. Abund	Rel. Ratio	Trgt. Ratio	Iso. Mass	Iso. Abund	Iso. Ratio	Trgt. Ratio
69.00	125168	100.00%	100.00%	70.00	1364	1.09%	1.08%
218.95	96584	77.17%	35.00%	219.95	4651	4.82%	4.32%
502.00	7832	6.26%	2.00%	503.10	746	9.53%	10.11%

APPENDIX C.

APPENDIX D.

COCAINE/BENZOYLECGONINE PROCEDURE¹

- 1) ADD TO A CLEAN TEST TUBE:
INTERNAL STANDARD (500 NG D3-COCAINE)
(500 NG D3-BENZOYLECGONINE)

1 ML OF SPECIMEN (BLOOD, URINE, GASTRIC, ETC)
2 ML PHOSPHATE BUFFER PH 7.0
- 2) MIX AND ADD TO EXTRELUT EXTRACTION COLUMN. LET ADSORB 4-5 MINS
- 3) ELUTE INTO A DISPOSABLE 15 ML CENTRIFUGE TUBE WITH TWO 8 ML ALIQUOTS OF 9:1 CHCL₃/ISOP
- 4) SPLIT SAMPLE AND EVAPORATE TO DRYNESS ON N-EVAP
- 5) DERIVATIZE BENZOYLECGONINE IN ONE TUBE TO N-PROPYL COCAINE BY BOILING OVER AN OPEN FLAME WITH N,N DIMETHYLFORMAMIDE DI-N-PROPYL ACETAL FOR 30 SECONDS
- 6) ADD 1ML H₂SO₄ TO TUBE.
- 7) CLEANUP STEP. ADD 3 ML OF TOLUENE:HEPTANE:ISOAMYL ALCOHOL (76:20:4) TO AQUEOUS AND VORTEX DISCARD ORGANIC.
- 8) NEUTRALIZE AQUEOUS WITH 2 ML SODIUM CARBONATE BUFFER PH 9.0
- 9) EXTRACT WITH 3 ML OF TOLUENE:HEPTANE:ISOAMYL ALCOHOL. TRANSFER ORGANIC PHASE TO A CLEAN CONICAL CENTRIFUGE TUBE AND EVAPORATE ON N-EVAP.
- 10) RECONSTITUTE WITH 100 UL CHCL₃ AND TRANSFER TO AN AUTOSAMPLER VIAL FOR MASS SPECTRAL ANALYSIS.

MASS SPECTROMETER PARAMETERS

METHOD- COCBEN::D5
INJECTION VOLUME- 3ul
IDFILE- IDBEC
FMGR TR FILE- SIMFX::D5

¹ ADAPTED FROM "A METHOD FOR THE SIMULTANEOUS DETERMINATION OF COCAINE, BENZOYLECGONINE AND ECGONINE METHYL ESTER IN BLOOD AND URINE USING GC/EIMS WITH DERIVATIZATION TO PRODUCE HIGH MASS MOLECULAR IONS" ISENSCHMID ET AL., JOUR. OF ANAL. TOX. 12:9-10;1988

APPENDIX E.

Correlation Coefficient:

$$r = \frac{S_{xy}}{\sqrt{S_{xx}} \sqrt{S_{yy}}}$$

$$\begin{aligned} \text{where } S_{xy} &= \sum (x - \bar{x})(y - \bar{y}) \\ S_{xx} &= \sum (x - \bar{x})^2, \quad S_{yy} = \sum (y - \bar{y})^2 \end{aligned}$$

The quantities S_{xx} and S_{yy} are the sums of squared deviations of the x observations, and the y observations, respectively, S_{xy} is the sum of cross products of the x deviations and the y deviations.

N.B.: An observed correlation between two variables may be spurious. That is, it may be caused by the influence of a third variable.

Standard Deviation:

$$s = \sqrt{\frac{\sum x^2 - 1/n (\sum x)^2}{n - 1}}$$

Coefficient of Variation:

$$CV \% = \frac{s}{\bar{x}}$$

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