

DOT/FAA/AM-03/23

Office of Aerospace Medicine
Washington, DC 20591

Analysis of Cocaine, Its Metabolites, Pyrolysis Products, and Ethanol Adducts in Postmortem Fluids and Tissues Using Zymark® Automated Solid-Phase Extraction and Gas Chromatography-Mass Spectrometry

Russell J. Lewis
Robert D. Johnson
Michael K. Angier
Roxane M. Ritter
Civil Aerospace Medical Institute
Federal Aviation Administration
Oklahoma City, OK 73125

Holly S. Drilling
Shyla D. Williams
University of Central Oklahoma
Edmond, OK 73034

December 2003

Final Report

This document is available to the public
through the National Technical Information
Service, Springfield, VA 22161.



U.S. Department
of Transportation

**Federal Aviation
Administration**

NOTICE

This document is disseminated under the sponsorship of the U.S. Department of Transportation in the interest of information exchange. The United States Government assumes no liability for the contents thereof.

Technical Report Documentation Page

1. Report No. DOT/FAA/AM-03/23		2. Government Accession No.		3. Recipient's Catalog No.	
4. Title and Subtitle Analysis of Cocaine, Its Metabolites, Pyrolysis Products, and Ethanol Adducts in Postmortem Fluids and Tissues Using Zymark [®] Automated Solid-Phase Extraction and Gas Chromatography-Mass Spectrometry				5. Report Date December 2003	
				6. Performing Organization Code	
7. Author(s) Lewis RJ ¹ , Johnson RD ¹ , Angier MK ¹ , Ritter RM ² , Drilling HS ² , Williams SD ²				8. Performing Organization Report No.	
9. Performing Organization Name and Address ¹ FAA Civil Aerospace Medical Institute P.O. Box 25082 Oklahoma City, OK 73125 ² University of Central Oklahoma Edmond, OK 73034				10. Work Unit No. (TRAIS)	
				11. Contract or Grant No.	
12. Sponsoring Agency name and Address Office of Aerospace Medicine Federal Aviation Administration 800 Independence Ave., S.W. Washington, DC 20591				13. Type of Report and Period Covered	
				14. Sponsoring Agency Code	
15. Supplemental Notes This work was accomplished under the approved task AM-B-03-TOX-204.					
16. Abstract Cocaine (COC) is one of the most widely abused illicit drugs in America. COC abuse transcends all social, racial, and economic boundaries. Following the introduction in the mid-1980s of a new form of cocaine called "crack," cocaine use has been on the rise. Because of its intense "high," crack smoking has become very popular. Despite its popularity, crack smoking is a particularly dangerous form of COC use. Additionally, COC and ethanol are frequently used together, resulting in the formation of a biologically active molecule that is nearly as psychoactive as COC but produces a longer lasting and toxic effect. Demonstrating the presence or absence of COC and COC-related molecules in postmortem fluids and/or tissues can have serious legal consequences and may help determine the cause of impairment and/or death. We have developed a simple method for the simultaneous determination of COC and the COC metabolites benzoylecgonine, norbenzoylecgonine, ecgonine methyl ester, ecgonine, and norcocaine, as well as anhydroecgonine methyl ester (a unique byproduct of COC smoking), cocaethylene (a molecule formed by the concurrent use of COC and ethanol) and their related metabolites, anhydroecgonine, norcocaethylene, and ecgonine ethyl ester. This method incorporates a Zymark [®] RapidTrace [™] automated solid-phase extraction system, gas chromatography/mass spectrometry, and PFP/PFPA derivatives. The lower limits of detection ranged from 0.78 - 12.5 ng/mL, and the linear dynamic range for most analytes was 0.78 - 3200 ng/mL. The extraction efficiencies were from 26 - 84%, with the exception of anhydroecgonine and ecgonine, which were from 1 - 4%. We applied this method to 5 aviation fatalities. This method has proven to be simple, robust, and accurate for the simultaneous determination of COC and 11 COC metabolites in postmortem fluids and tissues.					
17. Key Words Forensic Toxicology, Cocaine, Pyrolysis Products, Zymark, Cocaine Ethanol Adducts, Aircraft Accident Investigation			18. Distribution Statement Document is available to the public through the National Technical Information Service Springfield, VA 22161		
19. Security Classif. (of this report) Unclassified		20. Security Classif. (of this page) Unclassified		21. No. of Pages 16	22. Price

ANALYSIS OF COCAINE, ITS METABOLITES, PYROLYSIS PRODUCTS, AND ETHANOL ADDUCTS IN POSTMORTEM FLUIDS AND TISSUES USING ZYMARK[®] AUTOMATED SOLID-PHASE EXTRACTION AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY

INTRODUCTION

The Federal Aviation Administration's (FAA's) Civil Aerospace Medical Institute (CAMI) is responsible, under Department of Transportation Orders 8020.11A and 1100.2C, to "conduct toxicologic analysis on specimens from ... aircraft accident fatalities" and "investigate ... general aviation and air carrier accidents and search for biomedical and clinical causes of the accidents, including evidence of ... chemical (use)." Therefore, following an aviation accident, samples are collected at autopsy and sent to CAMI's Forensic Toxicology Research Laboratory where toxicological analysis is conducted on various postmortem fluids and tissues. Occasionally during a toxicological evaluation, cocaine is detected in postmortem specimens from civil aviation accident victims.

Cocaine (COC) is one of the most widely abused illicit drugs in America. COC abuse transcends all social, racial, and economic boundaries. It is available in two primary forms: COC hydrochloride, a white crystalline powder that can be snorted, swallowed, or injected, and "crack," COC hydrochloride that has been processed into its freebase form through a reaction with either ammonia or bicarbonate (1). The intensity of the euphoric high derived from COC use is strongly dependent upon the route of administration. COC administered intranasally results in euphoria more slowly than when injecting COC or smoking crack (2). Crack use produces a "high" much more rapidly than other methods of COC administration (2, 3). The rapid rate by which crack affects the user has resulted in a dramatic increase in its abuse (2). Despite its popularity, smoking crack is an extremely dangerous method for the introduction of COC into the body. Reports of sudden death from smoking crack are not uncommon (2). This is primarily due to the unpredictable, sometimes fatal, quantities of COC delivered to the bloodstream via inhalation of COC-saturated smoke.

The presence of COC, its metabolites, pyrolysis products and/or ethanol adducts in biological fluids and tissues provide markers of COC use and its possible route of administration (4-6). Numerous COC-related compounds and their unique transformation pathways are shown in Figure 1. The catabolism of COC results primarily in the ester hydrolysis products benzoylecgonine (BE) and, to a lesser extent, ecgonine methyl ester (EME) (7). *In vivo*,

BE is predominantly produced from chemical hydrolysis of COC while EME results from enzymatic hydrolysis (8, 9). Other COC metabolites, which appear at lower levels than BE or EME, include norcocaine (NCOC), norbenzoylecgonine (NBE), *m*-hydroxybenzoylecgonine (HBE), and ecgonine (E). When COC is smoked, a unique pyrolysis product, anhydroecgonine methyl ester (AEME), is formed. AEME and its metabolite, anhydroecgonine (AE), have been used as indicators of crack use (4, 5, 10-13). The concurrent use of COC and ethanol results in a biologically active molecule, cocaethylene (CE), and two non-active metabolites of CE, norcocaethylene (NCE), and ecgonine ethyl ester (EEE). COC and ethanol are frequently taken together, due to the effects of CE, which is nearly as psychoactive as COC but produces a much longer lasting high (14-16). CE is even more toxic than COC, and its potency results in an increased risk of death due to overdose (17).

Identification and quantitation of COC, CE, AEME, and their related metabolites in postmortem fluids and tissues are important aspects of forensic toxicology and may provide crucial information in determining the cause of impairment and/or death. Described herein is a rapid, automated procedure for the single-step extraction and simultaneous determination of COC and its metabolites BE, NCOC, NBE, HBE, EME, and E, as well as the pyrolysis products AEME and AE and the ethanol adducts CE, NCE, and EEE in postmortem fluids and tissues using a Zymark[®] RapidTrace[™] solid-phase extraction (SPE) system and gas chromatography with mass spectrometry (GC/MS). While many methods exist for the identification and quantitation of COC and COC-related compounds (18-29), none offer the sensitivity of this method combined with the simultaneous extraction and analysis of all 12 related COC compounds discussed above.

MATERIALS AND METHODS

Chemicals and Reagents

All aqueous solutions were prepared using double deionized water (DDW), which was obtained from a Milli-QT_{plus} Ultra-Pure Reagent Water System (Millipore[®], Continental Water Systems, El Paso, TX). All chemicals were purchased in the highest possible purity

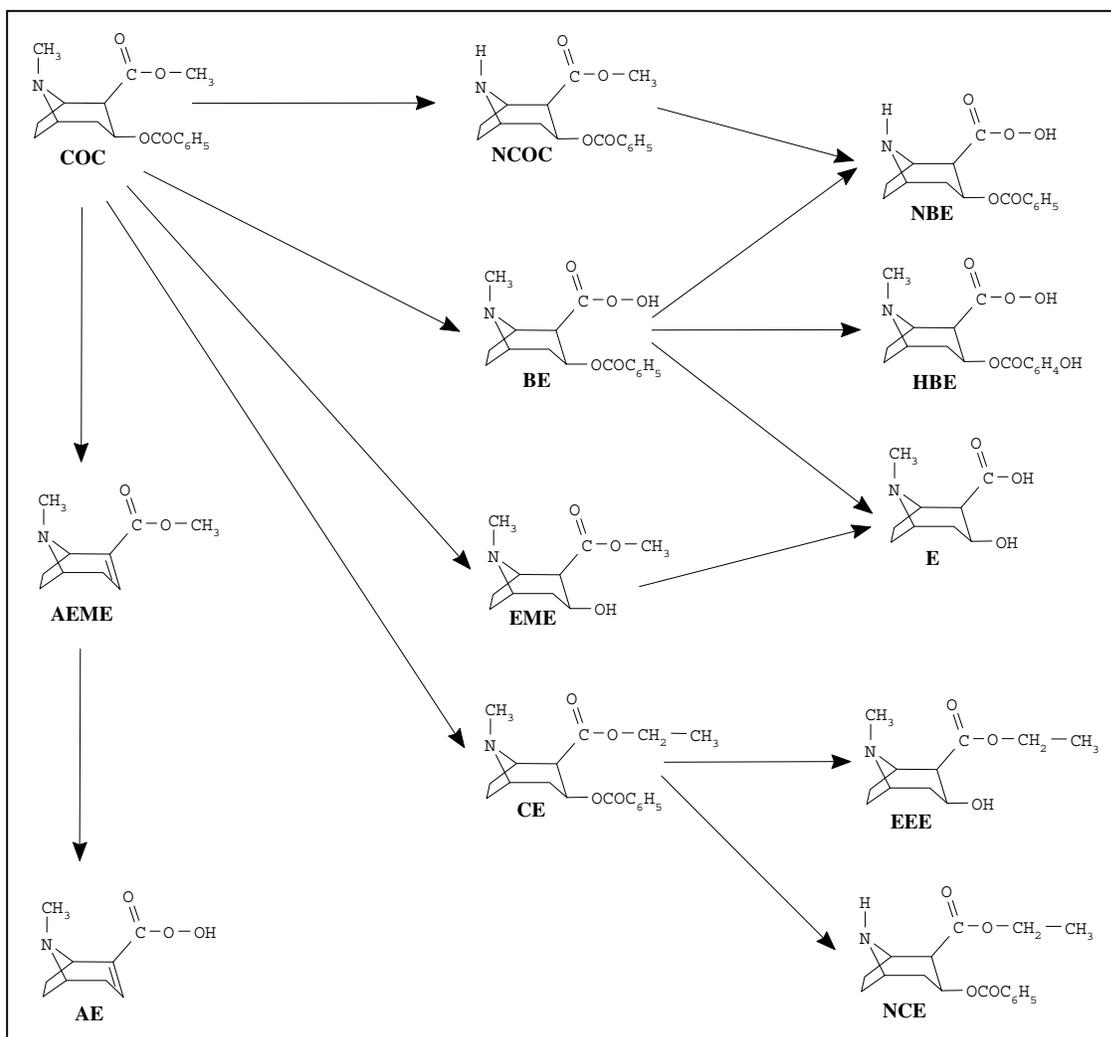


Figure 1. COC metabolites, ethanol adducts and pyrolysis products.

and used without any further purification. COC, BE, NBE, HBE, EME, E, NCOC, CE, NCE, EEE, AEME, and AE were purchased from Cerilliant (Cerilliant Corp., Round Rock, TX) as 1.00 mg/mL sealed glass ampules. COC- d_3 , BE- d_3 , EME- d_3 , and CE- d_3 were purchased from Cerilliant as 0.100 mg/mL sealed glass ampules. The derivatization reagents, pentafluoropropionic anhydride (PFPA), 2,2,3,3,3-pentafluoro-1-propanol (PFP), and BSTFA with 1% TMCS (TMS) were obtained from Pierce (Pierce Inc., Rockford, IL). Sodium acetate was purchased from Sigma (Sigma Chemical Co., St. Louis, MO). Methanol, acetonitrile, ammonium hydroxide, hydrochloric acid, methylene chloride, and isopropanol were purchased from Fisher (Fisher Scientific, Pittsburgh, PA). Ethyl acetate was purchased from Varian (Varian Inc., Palo Alto, CA).

Gas Chromatographic/Mass Spectrometric Conditions

All analyses were performed using a benchtop gas chromatograph/mass spectrometer (GC/MS), which consisted of a Hewlett Packard (HP) 6890 series GC, interfaced with a HP 5973 quadrupole MS (Agilent, Palo Alto, CA). The GC/MS was operated with a transfer line temperature of 280°C and a source temperature of 250°C. The MS was autotuned on a daily basis using perfluorotributylamine. The electron multiplier voltage was set at 106 eV above the autotune voltage. Chromatographic separation was achieved using a HP-ULTRA-1 crosslinked 100% methyl siloxane capillary column (12 m x 0.2 mm i.d., 0.33 μ m film thickness). Helium was employed as the carrier gas and used at a flow rate of 1.0 mL/min. A HP 6890 autosampler was used to inject 1

μL of extract into the GC/MS. The GC was equipped with a split/splitless injection port operated at 250°C in the splitless mode with a purge time of 0.5 min. The oven temperature profile was established as follows: $70 - 130^{\circ}\text{C}$ at $30^{\circ}\text{C}/\text{min}$, $130 - 140^{\circ}\text{C}$ at $5^{\circ}\text{C}/\text{min}$, $140 - 210^{\circ}\text{C}$ at $35^{\circ}\text{C}/\text{min}$, $210 - 222^{\circ}\text{C}$ at $4^{\circ}\text{C}/\text{min}$, and $222 - 290^{\circ}\text{C}$ at $45^{\circ}\text{C}/\text{min}$, with a final hold time of 0.49 min resulting in a total run time of 11 min. Initially, neat standards of each compound ($1 \mu\text{L}$ of a $100 \text{ ng}/\mu\text{L}$ solution) were injected individually and analyzed using the full scan mode of the GC/MS, which scanned from 50 to 600 AMU. Quantitation and qualifier ions used for each analyte were selected based on their abundance and mass-to-charge ratio (m/z). Because of their reproducibility and lack of interference, high mass ions were selected when possible. The ions chosen for each respective analyte can be seen in Table 1. Upon selection of unique ions, the MS was run in selected ion monitoring (SIM) mode with a dwell time of 20 msec.

Analyte concentrations were determined using an internal standard calibration procedure. Response factors were determined for each analyte present. The response factor was calculated by dividing the area of the analyte

peak by the area of the internal standard peak. Calibration curves were then prepared by plotting a linear regression of the analyte/internal standard response factor versus the analyte concentration for all calibrators analyzed. These calibration curves were then employed to determine concentrations of the various analytes present in both controls and specimens.

Sample Selection and Storage

A search of our toxicology laboratory's database identified 5 fatalities from separate aviation accidents from the previous 3 years that were reported positive for COC or BE and also had a majority of the desired biological tissues and fluids (blood, urine, liver, kidney, and muscle) available for analysis. In all cases, blood was stored at -20°C in tubes containing 1.0% (w/v) sodium fluoride/potassium oxalate until analysis. All other specimens were stored without preservation at -20°C until analysis. Blood COC or COC-related metabolite values determined in this study were compared with those previously determined. The values found in this study agreed with those previously reported, verifying no deterioration had occurred.

Table 1. Ions used for the analysis of COC and related compounds.

Compound	Derivative Formed	Ions (m/z)*	Retention Time
AE	PFP	270 , 299, 271	3.00
AEME	none	152 , 181, 166	3.34
E	PFP/PFPA	300 , 463, 314	3.71
d_3 -EME	none	185 , 348, 317	4.02
EME	PFPA	182 , 345, 314	4.03
EEE	PFPA	196 , 359, 314	4.53
d_3 -BE	none	303 , 424, 319	7.22
BE	PFP	300 , 421, 316	7.23
NBE	PFP/PFPA	312 , 431, 214	7.52
HBE	PFP/PFPA	300 , 583, 434	7.80
d_3 -COC	none	185 , 306, 275	7.85
COC	none	182 , 303, 272	7.86
d_3 -CE	none	199 , 320, 275	8.27
CE	none	196 , 317, 272	8.28
NCOC	PFPA	313 , 435, 214	8.33
NCE	PFPA	327 , 214, 105	8.72

* ions in bold used for quantitation.

Preparation of Standards

For each analysis, controls, and calibrators were prepared from the purchased 1.00 mg/mL drug standards using certified-negative whole blood as the diluent. Calibration curves were prepared by serial dilution at concentrations ranging from 0.78–3200 ng/mL for each analyte. Controls used for the determination of accuracy, precision, and analyte stability were prepared at 45 and 450 ng/mL. Controls and calibrators were prepared from separate methanolic drug standards to ensure the accuracy of the prepared calibration curves. Controls separately containing COC or BE were prepared. These COC and BE controls were prepared at concentrations of both 250 ng/mL and 1000 ng/mL. All controls were prepared in pools large enough to provide replicates for the entire study. The internal standard solution containing d_3 -COC, d_3 -BE, d_3 -CE and d_3 -EME was prepared at a concentration of 400 ng/mL in DDW.

Sample Preparation and Extraction Procedure

Postmortem fluid and tissue specimens, calibrators, and controls were prepared and extracted in the following manner. Tissue specimens were homogenized using a PRO250 post-mounted homogenizer (Pro Scientific, Oxford, CT). The generator used with this homogenizer was 10 mm in diameter and set to rotate at 22,000 rpm. Tissues were homogenized following a 1:2 dilution with 0.10 M acetate buffer pH 4.00. Three mL aliquots of specimen fluids, calibrators and controls, and 3.0 g aliquots of tissue homogenate were transferred to individual 16 x 150 mm screw top tubes. To each specimen, calibrator, and control, 1.0 mL of the internal standard mixture (400 ng) was added. Samples were vortexed briefly and allowed to stand at room temperature for 10 min. Eight mL of 0.10 M acetate buffer, pH 4.00, were added to each sample. The samples were then mixed on a rotary extractor that was set to rotate at 15 rpm for 20 min. Following rotation the samples were centrifuged at 1230xg for 45 min. The supernatant was then transferred to clean 16 x 100 mm culture tubes for extraction.

The samples were extracted using a Zymark® RapidTrace™ automated SPE system (Zymark Corp., Hokinton, MA). The SPE cartridges used were 3 mL Varian Bond Elute-Certify I with a 130 mg sorbent bed (Varian Inc., Palo Alto, CA). The RapidTrace™ was programmed with the following parameters: SPE cartridges were conditioned with 2.0 mL methanol, followed by 3.0 mL 0.10 M acetate buffer pH 4.00, both at a flow rate of 3 mL/min. Following conditioning, 8.0 mL of

sample was loaded on to each column at a flow rate of 1.5 mL/min. The SPE columns were then rinsed with 3.0 mL 0.10 M HCl at a flow rate of 3 mL/min, dried for 2 min with nitrogen at a pressure of 30 p.s.i., rinsed with 6.0 mL methanol at a flow rate of 3 mL/min and dried a final time for 2 min at a pressure of 30 p.s.i. The analytes were then eluted with 4.0 mL dichloromethane-isopropanol-ammonium hydroxide (80:20:2 v/v/v), which was prepared fresh daily, into 15 mL round-bottom, screw top tubes. To avoid carry over, the RapidTrace™ cannula was washed with 6.0 mL of methanol, and the RapidTrace™ column plunger was washed by sequentially passing 3.0 mL elution solvent and 6.0 mL water to waste after completion of each sample extraction.

Each sample eluent was evaporated to dryness in a water bath at 40°C under a stream of dry nitrogen. Once dryness was achieved, PFPA (50 µL) and PFP (50 µL) were added to each. The tubes were then capped tightly, vortexed briefly, and incubated in a heating block set to 70°C for 20 min. Samples were removed from the heating block and allowed to cool to room temperature. Two hundred µL of ethyl acetate was added to each sample to aid in the removal of the derivatizing reagent. The derivatizing reagent/ethyl acetate mixture was evaporated to dryness in a water bath at 40°C under a stream of dry nitrogen. Once samples were dry, they were promptly removed from the water bath to avoid unnecessary loss of any volatile metabolites. The samples were reconstituted in 50 µL ethyl acetate and transferred to GC autosampler vials for GC/MS analysis. All specimens were analyzed at one time to avoid inter-assay variations. Specimens with analyte concentrations above the associated calibration curves were diluted by an appropriate factor and re-extracted, so that the result fell within the calibration curve.

Extraction Efficiency

The method used for the determination of analyte recovery has previously been reported by Lewis et al. (30). Briefly described, two groups of controls, X and Y, prepared using certified-negative blood were extracted in the same manner as discussed above. Group X was spiked with a precisely known concentration of each analyte prior to extraction, while group Y was spiked with the same precisely known concentration of each analyte following extraction. Upon analysis, the average response factor obtained from group X was divided by the average response factor obtained from group Y to yield the percent recovery value ($100 * (X/Y) = \% \text{ recovery}$) for each of the compounds.

RESULTS AND DISCUSSION

Method Validation

The procedure described herein, which utilizes a Zy-mark® RapidTrace™ automated SPE system, PFP/PFPA derivatives, and GC/MS, provides a rapid, reproducible, and sensitive method for the determination of COC, BE, NBE, HBE, NCOC, EME, and E, as well as the pyrolysis products AEME and AE and the ethanol adducts CE, NCE, and EEE. All analyte peaks were completely resolved, with the exception of CE and NCOC. However, CE and NCOC each provide ions with unique m/z , so no interference was observed. Deuterated COC, BE, CE, and EME were used as internal standards for the quantitations performed in this study. For analytes with no deuterated analog available, the closest structurally related internal standard was employed. Surprisingly, even with such a simple extraction procedure, no analyte suffered interference from endogenous matrix components. A representative chromatogram demonstrating the separation of each of the 12 analytes is shown in Figure 2.

Initially, we investigated the use of BSTFA with 1% TMCS (TMS) as a derivatizing agent but found that it resulted in incomplete derivatization of secondary amines in the “nor” analytes. TMS was also found to be an unstable derivative for the analysis of the COC-related analytes. The use of PFP/PFPA as a derivatizing agent provided a more stable derivative (20, 31) with a significantly higher molecular weight and less background noise than TMS, which is generally used for COC-related

analyses. PFP derivatizes hydroxyl and secondary amine functional groups contained within many of the COC-related compounds. PFP derivatizes carboxylic acids contained within various COC-related compounds. The derivatives formed by the COC-related compounds can be seen in Table 1. Stability of these derivatives was determined by comparing the peak area of COC, which does not contain a functional group that will derivatize, to the peak areas of the derivatized analytes immediately following derivatization and periodically up to one week after derivatization. We found an insignificant amount of degradation for the derivatized analytes when stored at 4°C, with the exception of HBE. Derivatized HBE was not as stable as the other analytes when stored at 4°C, giving a response that was 25% of its original response within 48 h.

AEME is a pyrolysis product of COC and is, therefore, an excellent marker for the smoking of crack COC. It is, however, theoretically possible to convert COC to AEME under high temperatures encountered in the GC injection port (4, 32-34). The injector port of the GC/MS used in this study was maintained at 250°C. The high temperature in the injector port necessitated the evaluation of the production of AEME from the heated injector port thermal decomposition of COC. This evaluation was accomplished by injecting 1 µL of a 100 ng/µL neat COC standard and monitoring the formation of AEME. The production of AEME, monitored by collection of its base peak at m/z 152, was found to be $0.5 \pm 0.1\%$ ($n=4$) of the peak area of the COC base peak at m/z 182. This

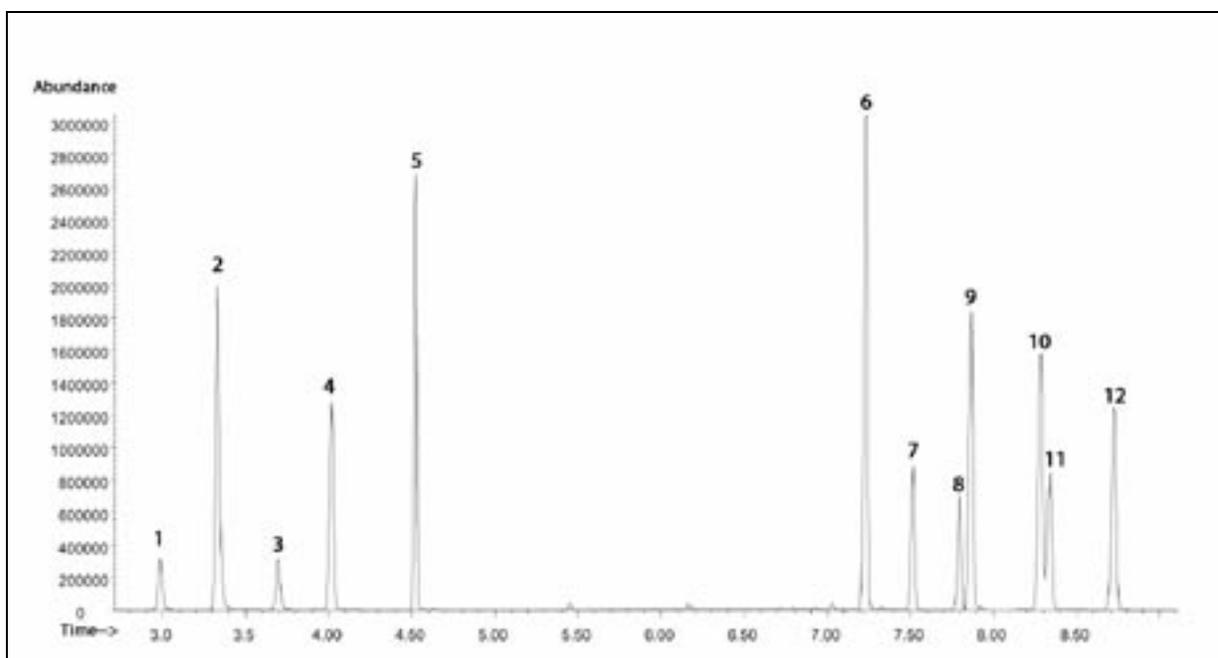


Figure 2. Selected ion chromatogram of the 800 ng/mL calibrator. The peak identification are as follows: 1. AE; 2. AEME; 3. E; 4. EME; 5. EEE; 6. BE; 7. NBE; 8. HBE; 9. COC; 10. CE; 11. NCOC; 12. NCE.

result agrees with other published findings (10, 19, 35). As a precautionary measure, we took steps to continually monitor the possible artifactual formation of AEME from COC throughout this study. All analyses contained numerous 1000 ng/mL and 250 ng/mL COC controls, which were monitored for the formation of AEME. The average conversion of COC to AEME for the 1000 ng/mL control was $0.7 \pm 0.1\%$ ($n=8$) and for the 250 ng/mL was $0.8 \pm 0.2\%$ ($n=4$). As can be seen in these data, there was no observed change in the percentage of conversion of COC to AEME over a broad COC concentration range. This lack of concentration dependence supports other published findings (19). Another route of artifactual AEME formation has also been suggested. The dehydration of EME in the heated injector port may form AEME (19). However, no AEME, as monitored by collection of its base peak at m/z 152, was detected following the injection of 1 μL of a 100 ng/ μL EME neat standard.

AE, a metabolite of AEME and marker for crack use, could potentially be formed from the thermal decomposition of BE (36, 37). Following the injection of 1 μL of a 100 ng/ μL neat standard of BE, the production of AE, monitored by collection of its base peak at m/z 270, was found to be $0.5 \pm 0.3\%$ ($n=4$) of the area of the BE base peak at m/z 300. This result also agrees well with other published findings (37). As a precautionary measure, we took steps to continually monitor the possible artifactual formation of AE from BE throughout this study. All analyses contained numerous 1000 ng/mL and 250 ng/mL BE controls, which were monitored for the formation of AE. The average conversion of BE to AE for the 1000 ng/mL control was $0.5 \pm 0.2\%$ ($n=8$) and for the 250 ng/mL was $0.5 \pm 0.1\%$ ($n=4$). As our data indicate, there was no observed change in the percentage of conversion of BE to AE at various BE concentrations.

While numerous studies investigating AEME and/or AE use an injector temperature of 250°C or greater (10, 19, 35, 37), a few reports recommend the use of cooler injector temperatures (12, 34, 37). To determine if there was a significant difference between 250°C and lower recommended temperatures, we simultaneously conducted the above conversion investigations at an injector port temperature of 210°C. We found no statistical difference between conversions found at 250°C and 210°C. Additionally, lower GC inlet temperatures have been reported to sacrifice GC/MS sensitivity (38). Therefore, we kept our injector temperature at 250°C for the entire study.

E is a di-ester cleavage metabolite of COC and has been proposed as a potential marker in postmortem specimens for the determination of COC use (39). Extreme caution must be used, however, when interpreting quantitative E

results. Following derivatization and subsequent analysis of a 100 ng/ μL EME neat standard, approximately 2% of the EME originally present was converted to E. While the hydroxyl group present on both EME and E derivatize with PFPA, only E should react with PFP since EME lacks the necessary carboxylic acid moiety. However, as is indicated by our results, EME appears to undergo a transesterification reaction with PFP during the derivatization process to form E. To discern other possible sources of E, we also investigated the formation of E from EEE and BE. EEE was found to consistently produce E at less than 0.6% of the original EEE concentration. Following derivatization, BE was also found to produce E but at levels of approximately 0.4% of the original BE concentration. While it appears that many COC metabolites can produce small amounts of E during the derivatization process, the presence of E, in the absence of EME and BE, or at concentrations substantially greater than that possible from artifactual formation, further supports the past use of COC.

The quantitation and qualifier ions for each analyte and internal standard examined in this study are listed in Table 1. Acceptability criteria employed for analyte identification and quantitation were as follows: 1) ion ratios for a given analyte, measured as the peak area of a qualifier ion divided by the peak area of the quantitation ion, were required to be within $\pm 20\%$ of the average of the ion ratios for each respective calibrator used to construct the calibration curve for that analyte; 2) each ion monitored was required to have a minimum signal-to-noise ratio (S/N) of 5; and 3) the analyte was required to have a retention time within ± 0.20 min of the average retention time for each respective calibrator used to construct the calibration curve for that analyte. Any analyte not meeting these criteria was reported as either being negative or inconclusive.

The linear dynamic range (LDR) for each analyte is presented in Table 2. In general, the LDRs were approximately 0.78 - 3200 ng/mL. At concentrations greater than 1600 ng/mL, the responses for NCOC, NCE, NBE, and E were not consistently linear. The correlation coefficients for the calibration curves used to ascertain LDR were all greater than 0.99 as demonstrated in Table 2. Additionally, Table 2 shows the lower limit of detection (LOD) and lower limit of quantitation (LOQ) determined for each analyte. The LOD was defined as the lowest analyte concentration detectable that meets the above-discussed identification criteria. The LOQ was defined as the lowest analyte concentration detectable that not only met all identification criteria discussed above but also had an experimentally determined concentration within $\pm 20\%$ of its prepared value. The LOD for these COC-related

Table 2. LOD, LOQ, LDR and recovery for COC-related compounds.*

Compound	LOD** (ng/mL)	LOQ (ng/mL)	LDR (ng/mL)	r ²	Recovery (%) [‡]	
					45 ng/mL	450 ng/mL
COC	0.78	0.78	0.78-3200	0.999	62 ± 4	71 ± 5
NCOC	0.78	1.56	1.56-1600	0.999	69 ± 7	83 ± 8
BE	0.78	0.78	0.78-3200	0.999	58 ± 2	64 ± 4
NBE	3.12	3.12	3.12-800	0.999	26 ± 3	29 ± 3
HBE	1.56	1.56	1.56-3200	0.993	43 ± 10	42 ± 9
EME	1.56	1.56	1.56-3200	0.998	43 ± 1	48 ± 2
E	12.5	25	25-1600	0.999	0.7 ± 0.1	1.6 ± 0.2
CE	0.78	0.78	0.78-3200	0.999	68 ± 5	71 ± 4
NCE	0.78	1.56	1.56-1600	0.998	68 ± 7	84 ± 8
EEE	0.78	0.78	0.78-3200	0.997	49 ± 4	57 ± 2
AEME	0.78	0.78	0.78-3200	0.998	75 ± 3	83 ± 8
AE	12.5	12.5	12.5-3200	0.999	2.5 ± 0.3	4.1 ± 0.4

* 3 mL blood volumes were used in the determination of these parameters.

** concentrations below 0.78 ng/mL were not examined.

‡ n=5 for each recovery group.

compounds ranged from 0.78 - 12.5 ng/mL. The LOQ for these COC-related compounds ranged from 0.78 - 25 ng/mL.

The average recoveries of COC and its metabolites at 45 ng/mL and 450 ng/mL ranged from 26 - 84%, with the exception of AE and E. These values are listed in Table 2. At both control concentrations, the recoveries for COC, BE, CE, NCOC, NCE, and AEME were above approximately 60%. The experimentally determined extraction efficiency values for these compounds agreed well with previously reported recoveries in whole blood (39-41). At both concentrations, AE and E exhibited poor recovery from whole blood. The experimentally determined extraction efficiencies for these compounds ranged from approximately 1 - 4%. Given the high polarity of AE and E, the poor recoveries are likely due to compound loss during the sample loading and HCl wash steps of the extraction.

Carryover from one sample to the next did not occur with either the Zymark[®] or the GC/MS. Carryover on the Zymark[®] was investigated by extracting a negative control following the 3200 ng/mL calibrator. Carryover on the GC/MS was initially investigated and subsequently monitored by the use of ethyl acetate solvent injections. An ethyl acetate blank injected following the 3200 ng/mL calibrator injection showed no carryover contamination.

Subsequently, ethyl acetate blanks were analyzed between each sample throughout the sample sequence to verify that no sample-to-sample contamination had occurred.

Intra-day (within day) and inter-day (between days) accuracy and precision were examined for this extraction. The accuracy was measured as the relative error between the experimentally determined and prepared concentrations of a sample. The precision was measured as the relative standard deviation (RSD) for the experimentally determined concentrations. Pools of controls, prepared using whole blood as the diluent, were created at 45 ng/mL and 450 ng/mL in volumes large enough to be used for the entire precision and accuracy investigation. These controls were stored at 4°C until analyzed. For intra-day analyses, a calibration curve was extracted, along with 5 replicates of each control concentration on Day 1 of the experiment. For the intra-day assay, all analytes at both concentrations yielded relative errors within 9% of the target concentration. Furthermore, all analytes had RSDs within 10%, with the exception of AEME and AE. The RSD for these COC pyrolysis products was as high as 12%. This is likely a result of compound loss during the various dry-down steps in the extraction process, since these two compounds are highly volatile. The intra-day assay results are shown in Table 3. These results demonstrate the exceptional precision and accuracy of this method.

Table 3. Intra-day and inter-day accuracy and precision.*

	Target (ng/mL)	Day 1			Day 3			Day 5		
		Mean (ng/mL)	RSD	%E	Mean (ng/mL)	RSD	%E	Mean (ng/mL)	RSD	%E
COC	45	45.3 ± 0.4	0.9	+0.7	43.8 ± 0.4	0.9	-3	43 ± 1	2	-4
	450	445 ± 2	0.4	-1	432 ± 3	0.7	-6	442 ± 3	0.7	-0.7
NCOC	45	47 ± 1	2	+4	47 ± 2	4	+4	49 ± 1	2	+9
	450	431 ± 12	3	-4	433 ± 16	4	-4	436 ± 6	1	-3
BE	45	48.4 ± 0.1	0.2	+8	48.6 ± 0.3	0.6	+8	48.8 ± 0.4	0.8	+8
	450	420 ± 2	0.4	-7	429 ± 5	1	-5	418 ± 1	0.2	-7
NBE	45	46 ± 3	7	+2	46 ± 4	9	+2	47 ± 4	9	+4
	450	419 ± 22	5	-7	434 ± 28	6	-4	448 ± 28	6	-0.7
HBE	45	47 ± 2	4	+4	45 ± 4	9	0	51 ± 2	4	+13
	450	417 ± 19	4	-7	422 ± 42	10	-6	419 ± 40	10	-7
EME	45	41.9 ± 0.1	0.2	-7	40.2 ± 0.2	0.5	-11	38.8 ± 0.3	0.8	-14
	450	465 ± 4	0.9	+3	455 ± 5	1	+1	456 ± 6	1	+1
E	45	47 ± 4	9	+4	47 ± 4	9	+4	48 ± 5	10	+7
	450	417 ± 40	9	-7	412 ± 43	10	-8	401 ± 35	9	-11
CE	45	44.8 ± 0.1	0.2	-0.4	43.9 ± 0.6	1	-2	42.8 ± 0.5	1	-5
	450	461 ± 5	1	+2	468 ± 10	2	+4	471 ± 12	3	+5
NCE	45	43 ± 1	2	-4	43 ± 3	7	-4	46 ± 1	2	+2
	450	427 ± 14	3	-5	439 ± 21	5	-2	427 ± 7	2	-2
EEE	45	41 ± 1	2	-9	39.5 ± 0.5	1	-12	38 ± 2	6	-16
	450	441 ± 4	0.9	-2	443 ± 6	1	-1	419 ± 20	5	-7
AEME	45	44 ± 1	2	-2	36 ± 1	3	-20	29 ± 2	7	-36
	450	464 ± 46	10	+3	434 ± 32	7	-4	345 ± 14	4	-23
AE	45	48 ± 5	11	+6	39 ± 6	15	-13	33 ± 6	18	-27
	450	464 ± 56	12	+3	439 ± 73	17	-17	397 ± 60	15	-12

* n=5 for all measurements

Accuracy measured as relative error (%E) from target concentration.

Precision measured as relative standard deviation (RSD) in replicate measurements.

Inter-day accuracy and precision were evaluated by extracting 5 replicates of each of two control concentrations on Days 3 and 5. The quantitative values determined on these days were based on the calibration curves originally prepared on Day 1. The results obtained after storage of each control lot at 4°C for 3 and 5 days can be seen in Table 3. For a majority of the COC-related analytes, the concentrations determined on Days 3 and 5 showed no significant difference from those obtained on Day 1. This agrees well with other published findings (42, 43). The RSDs for compounds with deuterated internal standards were all within 3% on Days 3 and 5. For all other compounds, the RSDs were within 10% on Days 3

and 5, with the exception of AEME and AE. Their RSDs ranged from 12 - 36%, but, again, this may be due to their extreme volatility as discussed above. Additionally, there was a substantial decrease in concentration observed over the course of 5 days for both AE and AEME. AE demonstrated an absolute decrease in concentration from Day 1 to Day 5 of 31% and 14% in the 45 ng/mL and 450 ng/mL whole blood controls, respectively. AEME showed a similar decrease of 34% and 26%, respectively. The apparent decrease in AE and AEME prepared in whole blood and stored at 4°C emphasizes the need for prompt analysis once forensic samples have been thawed (44).

Method Application: Postmortem Specimen Analysis

In fatal aviation accidents, specimens from accident victims are routinely sent to the Federal Aviation Administration's Forensic Toxicology Research Laboratory for toxicological analysis. Postmortem fluid and tissue samples obtained from 5 fatalities involved in separate aviation accidents over the past 3 years that had previously been screened

positive for COC and/or BE by GC/MS were re-examined using this new method to determine the presence of various COC analytes. The fluid and tissue samples selected for analysis were blood, urine, liver, kidney, and muscle. The 5 aviation fatalities chosen for this investigation had a majority, if not all, of the desired specimens available for analysis. The results of this analysis are shown in Table 4.

Table 4. COC and COC-related compound concentrations (ng/mL, ng/g) in postmortem fluids and tissues of 5 aviation fatalities.

Case 1	COC	NCOC	BE	NBE	HBE	EME	CE	NCE	EEE	AEME	AE
Blood	52	-	151	5	-	63	-	-	-	17	.*
Urine	659	14	6025	220	-	3427	39	34	35	126	4884
Liver	46	D	308	-	-	158	-	-	-	159	713
Kidney	83	-	517	-	-	343	-	-	-	207	.*
Muscle	35	-	145	-	-	46	-	-	-	22	.*
Case 2											
Blood	13	-	406	-	-	-	3	-	-	P**	P**
Urine	1031	-	41655	517	-	11177	D	-	641	434	16632
Liver	56	D	736	10	-	450	11	18	-	5595	37052
Kidney	94	-	1093	19	-	464	-	-	34	719	8581
Muscle	29	-	354	-	-	63	-	-	D	82	1237
Case 3											
Blood	D	-	621	4	-	99	-	-	39	6	181
Urine	348	D	8386	119	-	2837	255	19	432	29	3433
Liver	20	-	840	-	-	312	111	16	134	331	1937
Kidney	47	-	1454	-	-	493	56	-	135	185	1970
Muscle	15	-	612	-	-	98	14	-	30	21	435
Case 4											
Blood	10	-	847	8	-	112	20	D	83	32	927
Liver	13	-	971	-	-	279	-	-	168	193	2114
Kidney	31	-	1942	15	15	614	81	-	377	1214	5821
Muscle	41	-	1060	-	-	116	29	-	62	128	5903
Case 5											
Liver	136	-	1099	-	-	P**	-	-	-	P**	P**
Kidney	86	-	846	-	-	348	-	-	-	2268	43839
Muscle	49	-	462	-	-	84	-	-	-	111	3134

* AE area count was less than 5 times the conversion percentage determined for BE on that day.

** Compound was present (P) at substantial concentration but internal standard unsuitable for quantitation.

D=detected but below calibration curve, (-)=not detected.

E was not reported for any specimens since its area counts were consistently less than 5 times the conversion percentage.

COC, BE, and EME were found in all cases examined. Blood concentrations for COC, BE, and EME ranged from <0.78 - 52 ng/mL, 151 - 847 ng/mL and 0 - 122 ng/mL, respectively. Urine concentrations for COC, BE, and EME ranged from 348 - 1031 ng/mL, 6025 - 41655 ng/mL, and 2837 - 11177 ng/mL, respectively. Liver concentrations for COC, BE, and EME ranged from 13 - 136 ng/g, 308 - 1099 ng/g, and 158 - 450 ng/g, respectively. Kidney concentrations for COC, BE, and EME ranged from 31 - 94 ng/g, 517 - 1942 ng/g, and 343 - 614 ng/g, respectively. Muscle concentrations for COC, BE, and EME ranged from 15 - 49 ng/g, 145 - 1060 ng/g, and 46 - 116 ng/g, respectively. As can be seen from these data, there is no apparent correlation between COC, BE, and/or EME concentrations within any of the specimen types analyzed. The general trend observed for the highest to lowest concentration of COC, BE, and EME between specimen types analyzed was urine>kidney>liver>blood-muscle.

The identification of either AEME or AE is a good marker of crack use. However, since we previously showed that COC produces small amounts of artifactual AEME, and BE produces both AE and E, the interpretation of AEME, AE or E found in an actual case specimen must be approached cautiously. As previously described, whole blood controls containing COC and BE separately were run with each analysis. Following each analysis, the percentage of COC converted to AEME and the percentage of BE converted to both AE and E was determined. The percent conversion from one analyte to another was then multiplied by 5 to obtain a cutoff value, which was used to aid in determining whether AEME, AE or E found in a case specimen was actually present in the specimen or formed during analysis due to thermal decomposition of COC or BE in the injection port. A multiplication factor of 5 was chosen to provide a conservative cutoff that would ensure that any AE, AEME and/or E reported was real and not a false positive. During case analysis, the percent conversion, as determined by dividing the area count of the quantitation ion for AEME, AE or E by the area count of the quantitation ion for COC or BE, was found to be: COC to AEME = $0.7 \pm 0.1\%$ (n = 4); BE to AE = $0.5 \pm 0.2\%$ (n = 4); and BE to E = $0.2 \pm 0.2\%$ (n = 4). Therefore, a cutoff for AEME was established for this study at 3.5% of COC. For example, an AEME result having a detector response less than 3.5% of the COC detector response was reported as negative. The cutoffs for AE and E were established as 2.5% and 1.1%, respectively.

AEME and AE were detected in all 5 cases examined. More specifically, it should be noted that their responses were substantially above our established cutoffs. E was

detected in most of the cases examined but at levels below our established cutoff. Thus, the E observed may have been artifactually produced from BE and was reported as negative. Blood concentrations for AEME and AE ranged from 6 - 32 ng/mL and 0 - 927 ng/mL, respectively. Urine concentrations for AEME and AE ranged from 29 - 434 ng/mL and 3433 - 16632 ng/mL, respectively. Tissue concentrations for AEME and AE ranged from 22 - 5595 ng/g and 0 - 37052 ng/mL, respectively. We were unable to identify any correlation between AEME or AE and COC, BE or EME concentrations within any of the specimen types analyzed. Additionally, there was also no apparent trend observed for which specimen type contained the highest concentrations of AEME and AE. Since the recovery for AE is poor and BE, which can produce AE, is generally quite concentrated in typical COC-positive specimens, values reported for AE may be erroneously high. This is especially true in the presence of very high concentrations of BE. However, the true importance of the identification of AE is its presence as an indicator of crack use, not its specific specimen concentration.

CE, NCE, and EEE are good markers for the co-administration of COC and ethanol. CE, NCE, and EEE were found in 4 of the 5 cases investigated. Blood concentrations for CE, NCE, and EEE ranged from 0 - 20 ng/mL, 0 - <1.56 ng/mL, and 0 - 83 ng/mL, respectively. Urine concentrations for CE, NCE, and EEE ranged from <1.56 - 255 ng/mL, 0 - 34 ng/mL, and 35 - 641 ng/mL, respectively. Tissue concentrations for CE, NCE, and EEE ranged from 0 - 111 ng/g, 0 - 8 ng/g, and 0 - 377 ng/g, respectively. No apparent correlation was observed between CE, NCE or EEE, and COC, BE, or EME concentrations within or between specimen types tested.

Various other COC-related metabolites were also identified. NCOC was found at very low concentrations in 2 urine specimens and 2 liver specimens. NBE was found in various specimens in 4 out of the 5 cases analyzed. HBE was found in the kidney specimen of only 1 case. Since these metabolites are random in their presence and concentration, they are of little help in interpreting COC results, other than reaffirming the use of COC.

CONCLUSION

An automated method that is rapid, reliable, robust, and sensitive has been developed for the identification and subsequent quantitation of COC and 11 COC-related compounds in postmortem fluids and tissues. This method offers the ability to differentiate between smoking crack and intranasal/intravenous COC use and

is able to elucidate whether ethanol and COC were used simultaneously. Application of this procedure shows the effectiveness of GC/MS for the separation and subsequent detection of both underivatized and PFP/PFPA derivatized COC metabolites. One of the most important aspects of this method is the simultaneous analysis of 12 compounds using a single extraction procedure. The relative simplicity of this method should make the quantitation of previously obscure COC-related compounds more readily attainable for the field of forensic toxicology.

REFERENCES

1. Nakahara Y, Ishigami A. Inhalation efficiency of free-base cocaine by pyrolysis of "crack" and cocaine hydrochloride. *J Anal Toxicol* 1991;15(3):105-9.
2. Cone EJ. Pharmacokinetics and pharmacodynamics of cocaine. *J Anal Toxicol* 1995;19(6):459-78.
3. Perez-Reyes M, Di Guiseppi S, Ondrusek G, Jefcoat AR, Cook CE. Free-base cocaine smoking. *Clin Pharmacol Ther* 1982;32(4):459-65.
4. Jenkins AJ, Goldberger BA. Identification of unique cocaine metabolites and smoking by-products in postmortem blood and urine specimens. *J Forensic Sci* 1997;42(5):824-7.
5. Kintz P, Sengler C, Cirimele V, Mangin P. Evidence of crack use by anhydroecgonine methylester identification. *Hum Exp Toxicol* 1997;16(2):123-7.
6. Cone EJ, Sampson-Cone AH, Darwin WD, Huestis MA, Oyler JM. Urine testing for cocaine abuse: Metabolic and excretion patterns following different routes of administration and methods for detection of false-negative results. *J Anal Toxicol* 2003;27:386-401.
7. Isenschmid DS, Fischman MW, Foltin RW, Caplan YH. Concentration of cocaine and metabolites in plasma of humans following intravenous administration and smoking of cocaine. *J Anal Toxicol* 1992;16(5):311-4.
8. Stewart DJ, Inaba T, Tang BK, Kalow W. Hydrolysis of cocaine in human plasma by cholinesterase. *Life Sci* 1977;20(9):1557-63.
9. Toennes SW, Thiel M, Walther M, Kauert GF. Studies on metabolic pathways of cocaine and its metabolites using microsome preparations from rat organs. *Chem Res Toxicol* 2003;16(3):375-81.
10. Cone EJ, Hillsgrove M, Darwin WD. Simultaneous measurement of cocaine, cocaethylene, their metabolites, and "crack" pyrolysis products by gas chromatography-mass spectrometry. *Clin Chem* 1994;40(7 Pt 1):1299-305.
11. Jacob P, 3rd, Lewis ER, Elias-Baker BA, Jones RT. A pyrolysis product, anhydroecgonine methyl ester (methylecgonidine), is in the urine of cocaine smokers. *J Anal Toxicol* 1990;14(6):353-7.
12. Shimomura ET, Hodge GD, Paul BD. Examination of postmortem fluids and tissues for the presence of methylecgonidine, ecgonidine, cocaine, and benzoylecgonine using solid-phase extraction and gas chromatography-mass spectrometry. *Clin Chem* 2001;47(6):1040-7.
13. Jacob P, 3rd, Jones RT, Benowitz NL, Shulgin AT, Lewis ER, Elias-Baker BA. Cocaine smokers excrete a pyrolysis product, anhydroecgonine methyl ester. *J Toxicol Clin Toxicol* 1990;28(1):121-5.
14. Erzouki HK, Allen AC, Newman AH, Goldberg SR, Schindler CW. Effects of cocaine, cocaine metabolites and cocaine pyrolysis products on the hindbrain cardiac and respiratory centers of the rabbit. *Life Sci* 1995;57(20):1861-8.
15. Wu AH, Onigbinde TA, Johnson KG, Wimbish GH. Alcohol-specific cocaine metabolites in serum and urine of hospitalized patients. *J Anal Toxicol* 1992;16(2):132-6.
16. de la Torre R, Ortuno J, Gonzalez ML, Farre M, Cami J, Segura J. Determination of cocaine and its metabolites in human urine by gas chromatography/mass spectrometry after simultaneous use of cocaine and ethanol. *J Pharm Biomed Anal* 1995;13(3):305-12.
17. Dean RA, Harper ET, Dumaul N, Stoeckel DA, Bosron WF. Effects of ethanol on cocaine metabolism: Formation of cocaethylene and norcocaethylene. *Toxicol Appl Pharmacol* 1992;117(1):1-8.
18. Phillips DL, Tebbett IR, Bertholf RL. Comparison of HPLC and GC-MS for measurement of cocaine and metabolites in human urine. *J Anal Toxicol* 1996;20(5):305-8.
19. Toennes SW, Fandino AS, Kauert G. Gas chromatographic-mass spectrometric detection of anhydroecgonine methyl ester (methylecgonidine) in human serum as evidence of recent smoking of crack. *J Chromatogr B Biomed Sci Appl* 1999;735(1):127-32.

20. Abusada GM, Abukhalaf IK, Alford DD, Vinzon-Bautista I, Pramanik AK, Ansari NA, et al. Solid-phase extraction and GC/MS quantitation of cocaine, ecgonine methyl ester, benzoylecgonine, and cocaethylene from meconium, whole blood, and plasma. *J Anal Toxicol* 1993;17(6):353-8.
21. Browne SP, Tebbett IR, Moore CM, Dusick A, Covert R, Yee GT. Analysis of meconium for cocaine in neonates. *J Chromatogr* 1992;575(1):158-61.
22. Campora P, Bermejo AM, Taberero MJ, Fernandez P. Quantitation of cocaine and its major metabolites in human saliva using gas chromatography-positive chemical ionization-mass spectrometry (GC-PCI-MS). *J Anal Toxicol* 2003;27(5):270-4.
23. Fandino AS, Karas M, Toennes SW, Kauert G. Identification of anhydroecgonine methyl ester N-oxide, a new metabolite of anhydroecgonine methyl ester, using electrospray mass spectrometry. *J Mass Spectrom* 2002;37(5):525-32.
24. Hernandez A, Andollo W, Hearn WL. Analysis of cocaine and metabolites in brain using solid phase extraction and full-scanning gas chromatography/ion trap mass spectrometry. *Forensic Sci Int* 1994;65(3):149-56.
25. Karacic V, Skender L. Analysis of drugs of abuse in urine by gas chromatography/mass spectrometry: Experience and application. *Arh Hig Rada Toksikol* 2000;51(4):389-400.
26. Klette KL, Poch GK, Czarny R, Lau CO. Simultaneous GC-MS analysis of meta- and para-hydroxybenzoylecgonine and norbenzoylecgonine: a secondary method to corroborate cocaine ingestion using nonhydrolytic metabolites. *J Anal Toxicol* 2000;24(7):482-8.
27. Lewis DE, Moore CM, Leikin JB, Koller A. Meconium analysis for cocaine: a validation study and comparison with paired urine analysis. *J Anal Toxicol* 1995;19(3):148-50.
28. Mackey-Bojack S, Kloss J, Apple F. Cocaine, cocaine metabolite, and ethanol concentrations in postmortem blood and vitreous humor. *J Anal Toxicol* 2000;24(1):59-65.
29. Oyler J, Darwin WD, Preston KL, Suess P, Cone EJ. Cocaine disposition in meconium from newborns of cocaine-abusing mothers and urine of adult drug users. *J Anal Toxicol* 1996;20(6):453-62.
30. Lewis RJ, Johnson RD, Blank CL. A novel method for the determination of sildenafil (Viagra) and its metabolite (UK-103,320) in postmortem specimens using LC/MS/MS and LC/MS/MS/MS/MS. Department of Transportation, Federal Aviation Administration, DOT/FAA/AM-00/20 2000.¹
31. Stout PR, Gehlhausen JM, Horn CK, Klette KL. Evaluation of a solid-phase extraction method for benzoylecgonine urine analysis in a high-throughput forensic urine drug-testing laboratory. *J Anal Toxicol* 2002;26(7):401-5.
32. Fandino AS, Toennes SW, Kauert GF. Studies on in vitro degradation of anhydroecgonine methyl ester (methylecgonidine) in human plasma. *J Anal Toxicol* 2002;26(8):567-70.
33. Toennes SW, Fandino AS, Hesse FJ, Kauert GF. Artifact production in the assay of anhydroecgonine methyl ester in serum using gas chromatography-mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 2003;792(2):345-51.
34. Zhang JY, Foltz RL. Cocaine metabolism in man: Identification of four previously unreported cocaine metabolites in human urine. *J Anal Toxicol* 1990;14(4):201-5.
35. Kintz P, Cirimele V, Sengler C, Mangin P. Testing human hair and urine for anhydroecgonine methyl ester, a pyrolysis product of cocaine. *J Anal Toxicol* 1995;19(6):479-82.
36. Paul BD, McWhorter LK, Smith ML. Electron ionization mass fragmentometric detection of urinary ecgonidine, a hydrolytic product of methylecgonidine, as an indicator of smoking cocaine. *J Mass Spectrom* 1999;34(6):651-60.
37. Gonzalez ML, Carnicero M, de la Torre R, Ortuno J, Segura J. Influence of the injection technique on the thermal degradation of cocaine and its metabolites in gas chromatography. *J Chromatogr B Biomed Appl* 1995;664(2):317-27.
38. Grob K, Biedermann M. Video-taped sample evaporation in hot chambers simulating gas chromatography split and splitless injectors. II. Injection with band formation. *J Chromatogr A* 2000;897(1-2):247-58.

¹This publication and all Office of Aerospace Medicine technical reports are available in full-text from the Civil Aerospace Medical Institute's publications Web site: <http://www.cami.jccbi.gov/aam-400A/index.html>

39. Smirnow D, Logan BK. Analysis of ecgonine and other cocaine biotransformation products in postmortem whole blood by protein precipitation-extractive alkylation and GC-MS. *J Anal Toxicol* 1996;20(6):463-7.
40. Winecker RE, Goldberger BA, Tebbett IR, Behnke M, Eyler FD, Karlix JL, et al. Detection of cocaine and its metabolites in breast milk. *J Forensic Sci* 2001;46(5):1221-3.
41. Crouch DJ, Alburges ME, Spanbauer AC, Rollins DE, Moody DE. Analysis of cocaine and its metabolites from biological specimens using solid-phase extraction and positive ion chemical ionization mass spectrometry. *J Anal Toxicol* 1995;19(6):352-8.
42. Levine B, Ramcharitar V, Smialek JE. Stability of ecgonine methyl ester in postmortem urine specimens. *J Forensic Sci* 1996;41(1):126-8.
43. Moody DE, Monti KM, Spanbauer AC. Long-term stability of abused drugs and antiabuse chemotherapeutic agents stored at -20 degrees C. *J Anal Toxicol* 1999;23(6):535-40.
44. Fandino AS, Toennes SW, Kauert GF. Studies on hydrolytic and oxidative metabolic pathways of anhydroecgonine methyl ester (methylecgonidine) using microsomal preparations from rat organs. *Chem Res Toxicol* 2002;15(12):1543-8.

