TECHNICAL NOTE

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GC/MS Quantitation of Benzoylecgonine Following Liquid-Liquid Extraction of Urine

REFERENCE: Gerlits, J., **"GC/MS Quantitation of Benzoylecgonine Following Liquid-Liquid Extraction of Urine,"** *Journal of Forensic Sciences*, JFSCA, Vol. 38, No. 5, September 1993, pp. 1210–1214.

ABSTRACT: An analytical procedure for the determination of benzoylecgonine in urine by GC/MS using a two step liquid-liquid extraction scheme has been developed. Benzoylecgonine is one of the principle metabolites of cocaine. The extraction uses a wash step prior to extraction and derivatization and requires 0.5 mL of urine. Benzoylecgonine is extracted in good % recovery at a basic pH by using 4 mL of dichloromethane. The method is accurate and sensitive and allows the quantitation of benzoylecgonine in urine at or below the National Institute on Drug Abuse confirmation cutoff level of 150 ng/mL.

KEYWORDS: toxicology, cocaine, metabolite, derivatization

Benzoylecgonine (BE) is one of the principle metabolites of cocaine present in the urine of cocaine users [*1–3*]. BE is polar and hydrophilic and so liquid-liquid extraction procedures must address the problem of low % recovery. Variations have involved extractive alkylation [*4,5*], or extraction with polar solvent mixtures [*6–10*]. These procedures require back-extraction or cleanup steps after extraction and derivatization to remove interferences from the sample.

Extraction of BE from human urine can be accomplished by the use of a simple two-step scheme. This scheme involves the use of a wash step prior to extraction, utilizing the hydrophilic nature of BE to clean up the sample without lowering the % recovery. The wash step should use a solvent mixture of low polarity. The phase ratio of the extraction step can then be adjusted to optimize % recovery. Derivatization should not require further partitioning, separation, or purification steps. No further purification steps are necessary after converting BE to the t-butyldimethylsilyl derivative.

Experimental

An internal standard (I.S.) of deuterated benzoylecgonine (BE-D_3) was spiked into all samples at a concentration of 400 ng/mL. A calibration curve consisting of 75, 150,

Received for publication 5 June 1992; revised manuscript received 11 Feb. 1993; accepted for publication 12 Feb. 1993.

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300, and 1000 ng/mL of BE was prepared by spiking urine with a standard solution (1 mg/mL BE in methanol) and extracted for each batch. All glassware was silanized. All solvents were HPLC Grade. All other chemicals were analytical reagent grade. BE tetrahydrate was obtained from AllTech-Applied Science Labs. BE-D_3 was obtained from Sigma Chemical Company.

Add 0.5 mL of urine, 50 μL of I.S. solution (4000 ng/mL BE-D_3 in isopropanol), 0.1 mL of 4.0 M potassium phosphate buffer (pH = 12.1), and 2 mL of wash solvent [1:1 :1 (v/v/v) methylene chloride:hexane:diethyl ether] to a 13 by 100-mm screw-cap culture tube. Cap the tubes and mix on a mechanical shaker for 5 minutes. Centrifuge each tube for five minutes at 1000 rpm. Aspirate and discard the organic layer. Add 4 mL of methylene chloride. Cap the tubes and mix on a mechanical shaker for 5 minutes. Centrifuge each tube for five minutes at 1000 rpm. Aspirate and discard the aqueous layer. Transfer the remaining methylene chloride solution to a clean 13 by 100-mm screw-cap culture tube. Evaporate the solvent under air or nitrogen using a heating block at 55°C. To the dried extract in each tube, add 100 μL of derivatizing agent [1:1 Acetonitrile: N-Methyl-N-t-butyldimethylsilyl trifluoroacetamide (MTBSTFA) with 1% t-butyldimethylchlorosilane (TBDMCS)]. Cap each tube and heat for 15 min at 55°C using a heating block. Inject 2 μL of each sample into the GC/MS.

Instrumentation

The GC/MS system was a Hewlett Packard 5890 Gas Chromatograph/5790B Mass Selective Detector (MSD). The capillary column was a 12-m HP-1. The injection port was set at 250°C and the transfer line was set at 260°C. The oven was initially held at 170°C for 0.5 min, then programmed at 25°C/min to 260°C. Purge valve was turned on at 0.5 min. The MS was operated in the electron impact single ion monitoring (SIM) mode. Ions monitored were 282, 346, and 403 for derivatized BE, and 285, 349, and 406 for the internal standard.

Results

The mass spectrum of the t-butyldimethylsilyl derivative of BE (BE-TBDMS) is shown in Fig. 1. Operating the mass spectrometer in SIM mode improves the sensitivity of the system. Extracted ion profiles for each of the six ions monitored for BE-TBDMS and I.S. extracted from urine spiked at a concentration of 150 ng/mL are illustrated in Fig. 2.

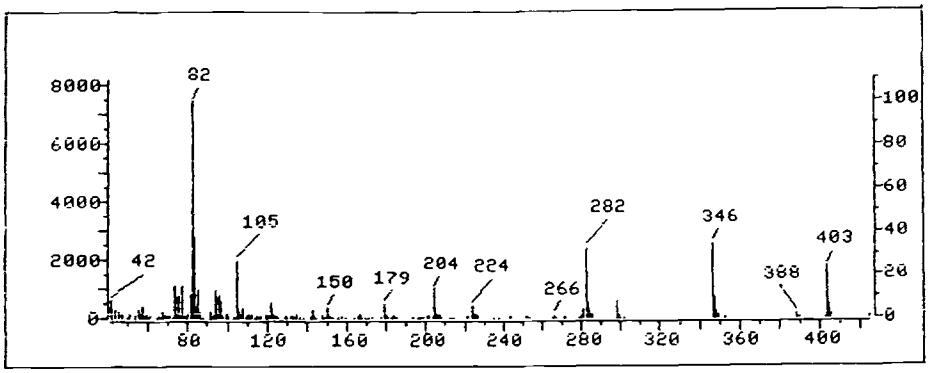

FIG. 1—*Mass spectrum of BE-TBDMS.*

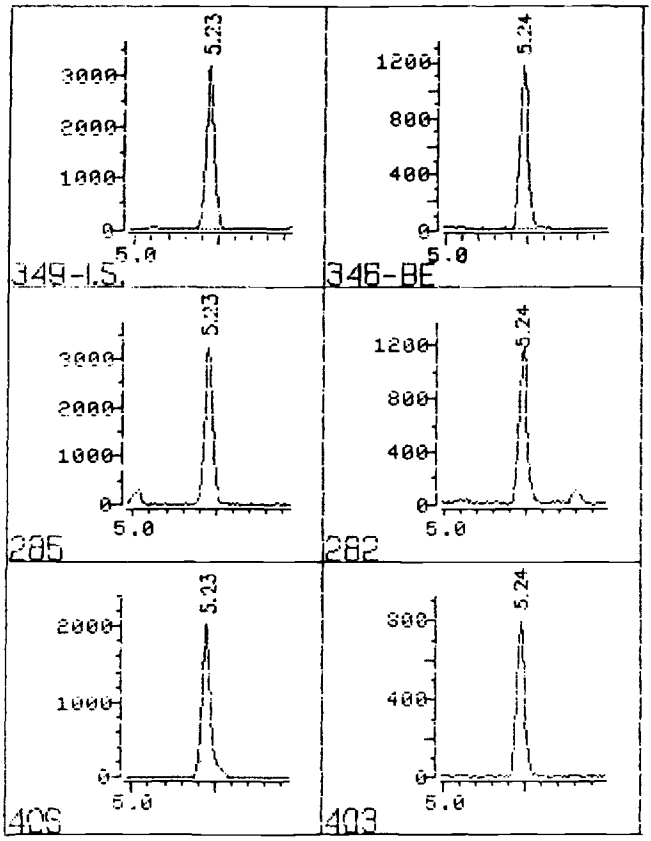

FIG. 2—*Extracted ion profiles for derivatized BE and BE-D3.*

A calibration plot for BE was constructed by plotting the [m/z = 346]/[m/z = 349] ratio of area versus BE concentration (ng/mL) using data for the range 75–1000 ng/mL. A linear (least squares) regression model was used to fit the data. The correlation coefficient was 0.9999 (Fig. 3). Ions 282 and 403 were used as secondary qualifier ions.

The precision of the method was evaluated by the analysis of control samples independently spiked at 150 ng/mL. Within-run and between-run precision were determined by analyzing the control material seven times. Within-run the mean concentration found was, BE at 142 ng/mL (CV = 3.0%). Between-run the mean concentration found was, BE at 145 ng/mL (CV = 2.7%).

The % recovery was evaluated in seven replicates. A urine solution of BE at 300 ng/mL was extracted according to the procedure, using isopropanol (50 μL) without I.S. Steps involving separation and transfer of solutions were done carefully to avoid losses. After extraction, the I.S. was added to the methylene chloride. The extracts were dried and derivatized and analyzed by GC/MS. The overall % recovery varied from 67 to 69%. This compares with 65% recovery reported using Wallace's method [*10*], and 65% [*6*] and 80% [*8*] recovery reported using modifications thereof.

Cocaine can be present in urine samples which contain the metabolite BE. In order to test the stability of cocaine at the basic pH of the extraction, on nine different days cocaine was spiked at a concentration of 2000 ng/mL into a urine solution containing 150 ng/mL of BE and analyzed according to the procedure. The average BE concentration

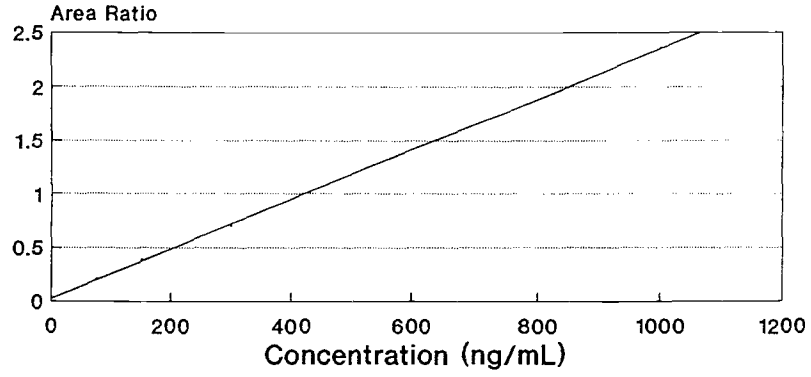

FIG. 3—*Benzoylecgonine.*

found was 161 ng/mL (CV = 5.1%). Cocaine is usually present in much lower concentration than BE, and hydrolysis of cocaine to BE appears to be limited to approximately 0.5% in this procedure.

Potentially interfering compounds are washed out of the sample by an organic solvent mixture in which BE has a relatively low solubility. A basic pH was used for the wash and extraction steps as this eliminated interferences and converted the amine functional group to the conjugate base form. This procedure has been used to confirm the presence of BE in the urine of thousands of cocaine users over a period of three years. No chromatographic interferences due to drugs or endogenous urine components were encountered. Interference studies indicates that the following drugs will not interfere with the procedure: lidocaine, amphetamine, methamphetamine, MDMA, MDA, MDE, ephedrine, pseudoephedrine, phentermine, chlordiazepoxide, nordiazepam, oxazepam, codeine, morphine, phencyclidine, scopolamine.

The present guidelines of the National Institute on Drug Abuse call for a cutoff concentration of BE of 150 ng/mL for GC/MS confirmation in urine. The procedure was found to be an accurate, reliable means for the identification and quantitation of BE at these levels.

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