Brad J. Hall,[1] *B.S.; Aashish R. Parikh*[1]*; and Jennifer S. Brodbelt,*[1] *Ph.D.*

Aqueous Phase Hexylchloroformate Derivatization and Solid Phase Microextraction: Determination of Benzoylecgonine in Urine by Gas Chromatography-Quadrupole Ion Trap Mass Spectrometry

REFERENCE: Hall BJ, Parikh AR, Brodbelt JS. Aqueous phase hexylchloroformate derivatization and solid phase microextraction: determination of benzoylecgonine in urine by gas chromatography-quadrupole ion trap mass spectrometry. J Forensic Sci 1999;44(3):527–534.

ABSTRACT: A derivatization/solid phase microextraction (SPME) method for the determination of benzoylecgonine in urine was developed. The derivatization is conducted directly in 1 mL of urine while sonicating for 3 min with 12 μL of hexyl chloroformate and 70 μL of a mixture containing acetonitrile:water:hexanol:2-dimethylaminopyridine (5:2:2:1 v/v), yielding benzoylecgonine hexyl ester (BHE) as the product. After the 3 min period, an aliquot of 250 μL is transferred to a vial for SPME. After the desired extraction time the 100 μm polydimethylsiloxane SPME fiber was transferred to the GC-MS for separation and analysis with a quadrupole ion trap mass spectrometer. The hexyl chloroformate derivatization and SPME procedures were optimized for compatibility and sensitivity. The method was found linear for 0.10 to 20.0 μg/mL ($r^2 = 0.999$) of benzoylecgonine in urine using benzoylecgonine-d_3 as an internal standard (1.5 μg/mL). Intra-day precisions were 8.8 and 6.8% RSD for 0.30 μg/mL and 17 μg/mL benzoylecgonine standards in urine ($n = 6$), respectively. Inter-day precision ($n = 3$) were ≤3.3% RSD, indicating good reproducibility. A detection limit of 0.03 μg/mL (S/N = 3) was achieved, thus making the SPME method a simplified alternative to SPE for GC-MS confirmation after EMIT tests for benzoylecgonine which have a cutoff of 0.30 μg/mL. Quantitative results by SPME and SPE of two clinical urine specimens known positive for cocaine by EMIT were in excellent agreement. Benzoylecgonine was detected by the derivatization/SPME method in 22 out of 22 other urine specimens known positive for cocaine.

KEYWORDS: forensic science, forensic toxicology, benzoylecgonine, cocaine metabolite, hexyl chloroformate, chloroformate derivatization, solid phase microextraction (SPME), gas chromatography-mass spectrometry

Solid phase microextraction (SPME) has become increasingly recognized an alternative to conventional extraction techniques such as solid phase extraction and liquid-liquid extraction due to the initial development aimed towards the solvent-free extraction and analysis of a variety of volatile and semi-volatile environmental contaminants in aqueous matrices. The advantages of SPME are gained from the ingenious design of a device which incorporates a polymeric coating on a fused silica fiber which acts solely as an extraction, concentrating, and injection device. Therefore, SPME methods greatly simplify the analysis in terms of labor and time while in turn eliminating the use of organic solvent in the extraction. Detailed discussions of the SPME process and theory are given in the following references (1–3).

Recently, SPME has been extended to the analysis of drugs of abuse in biological fluids in order to develop simplified procedures while retaining good selectivity and sensitivity. The studies include the analysis of amphetamines (4–8), benzodiazepines (9,10), phencyclidine (11), tricyclic antidepressants (12,13), ethanol (14), barbiturates (15,16), cannabinoids (17) cyanide (18), and corticosteroids (19). Applying SPME to a more complex matrix such as urine or saliva immediately complicates the method due to the presence of endogenous components and increased viscosity. In these cases, the importance of sample pretreatment and comprehensive SPME method development are essential to enhancing the partition of a target analyte into the SPME fiber. The application of derivatization procedures prior to or during SPME has been reported as one such method of sample pretreatment to enhance recoveries (6,20–23). Derivatizing polar drugs prior to analysis serves a two fold purpose: first increasing the partition of the drug by the SPME polymer, and second improving the chromatographic performance.

The goal of this study was to develop a derivatization/SPME method for the rapid and sensitive identification and quantitation of an important metabolite of cocaine found in urine, benzoylecgonine. Currently, the accepted method for benzoylecgonine analysis from urine involves extraction with a copolymeric solid phase extraction (SPE) column which has been shown to be an effective method in many previous studies (24–26). However, the SPE step is time-consuming and requires additional concentration and post-derivatization steps to achieve low detection levels. The method developed herein for benzoylecgonine is based on an alkyl chloroformate reaction in an aqueous medium used since the mid-seventies for the derivatization of amino acids and amines. Many groups have extensively studied the alkyl chloroformate derivatization procedure and developed successful methodologies to derivative alkyl carboxylic acids (27), 2-hydroxyalkyl carboxylic acids (28),

[1] Ph.D. candidate, graduate student in chemistry, undergraduate research assistant, and associate professor of chemistry, respectively. The University of Texas at Austin, Department of Chemistry and Biochemistry, Austin, TX.

Received 14 May 1998; and in revised form 14 Aug. 1998; accepted 27 Aug. 1998.

Copyright © 1999 by ASTM International

amino acids (29–31), amines (32), and phenolic acids (33). In addition, Minero and coworkers have reported on the effectiveness of using hexyl chloroformate and conducting the derivatization reaction under sonication to enhance the reaction yields (33). Methyl chlorformate derivatization followed by liquid-liquid extraction was recently reported by Jonsson and coworkers to analyze amphetamine and related drugs in urine (34). Ugland and coworkers have been the first group to describe the use of aqueous alkyl chloroformate derivatization in conjunction with SPME. In that study, propyl- and butyl chloroformate were used to derivatize amphetamine and methamphetamine directly in urine with subsequent extraction of the derivatives by direct-immersion SPME (6). Taking all of these reports into consideration, a method to extend the alkyl chloroformate procedure to a carboxylic acid metabolite, benzoylecgonine, directly in urine was developed. Benzoylecgonine is known to be a highly hydrophilic compound and exhibits poor chromatographic properties. Hexyl chloroformate was chosen as the derivatization reagent to enhance both the compatibility of benzoylecgonine with SPME and provided excellent separation on the capillary GC column. This study focuses on the optimization of the derivatization reaction conditions as well as the SPME parameters which influence extraction. The procedure is evaluated as a simplified alternative method to the confirmation and quantitation of benzoylecgonine in urine through the analysis of controlled standards and clinically obtained urine specimens known positive for cocaine.

Experimental

Materials

Benzoylecgonine as a pure solid was obtained from Sigma (St. Louis, MO) and used without further purification. Benzoylecgonine-d_3 for use as an internal standard was obtained as a 100 μg/mL solution in methanol from Radian International (Austin, TX). Since the derivatization product (referred to as benzoylecgonine hexyl ester (BHE) throughout the manuscript) was not commercially available, it was synthesized by a modified procedure previously reported for the synthesis of cocaethylene, as ethyl analog of cocaine (35). Details of the synthetic procedure may be obtained by contacting the authors of the manuscript herein. Ethyl chloroformate, hexyl chloroformate, hexanol, and 2-dimethylaminopyridine were purchased from Aldrich Chemical (Milwaukee, WI). Bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) was purchased from Supelco (Bellefonte, PA USA). A pH 4, 7, and 10 buffer solutions were obtained from Spectrum Chemical (Gardena, California). All other reagents were obtained from EM Science (Gibbstown, NJ). Deionized water was prepared by using a Barnstead NanoPure water purification system. Clinical urine samples which had tested positive for cocaine and/or metabolite presence by an enzyme multiplied immunoassay technique (EMIT) with a cutoff of 300 ng/mL were obtained from a local private drug testing lab.

Chloroformate Derivatization Procedure

For chloroformate derivatization the following procedure was optimized for reaction yield and compatibility with SPME. All steps of the reaction were conducted in a chemical hood in an uncapped 4 mL glass vial. To 1 mL of pure water or urine placed under intense sonic agitation in a Branson model 3200 sonicator operated at a water temperature of 42°C, was added 70 μL of a mixture of acetonitrile, water, hexanol, and 2-dimethylaminopyridine (5:2:2:1 v/v). Subsequently, 12 μL of hexyl chloroformate was added and the reaction was allowed to proceed for 3 min with sonication applied during the whole course. After this 3 min time period a 250 μL aliquot of the reaction mixture was then added to a vial for SPME, containing 2 mL of deionized water and 2 mL of pH 7 buffer.

Solid Phase and Liquid-Liquid Extraction Procedures

For method comparison studies and derivatization yield determinations a conventional solid phase extraction (SPE) method was employed using 300 mg Bond Elut Certify extraction columns from Varian (Harbor City, CA USA). The method was based on a previously published procedure with slight modification and briefly described below (26). To 0.8 − 3 mL of urine or chloroformate reaction mixture was added 3 mL of deionized water and 2 mL of pH 6 phosphate buffer. The SPE columns were conditioned with 2 mL of methanol followed by 2 mL of pH 6 phosphate buffer. The dilute urine or reaction mixture was added to the column followed by 3 mL of deionized water and 2 mL of 0.1 M HCL. The column was aspirated to dryness for 5 min. Three mL of methanol was added and the column was again aspirated to dryness. The analytes were then eluted by the addition of 3 × 1 mL methylene chloride:isopropanol:concentrated aqueous ammonium hydroxide (80:20:2 v/v). The elutant was evaporated to dryness under nitrogen. One milliliter of methylene chloride was added and the elutant was evaporated to dryness a second time. For chloroformate derivatization yield determination the dried elutant was reconstituted in acetonitrile and 1 μL was injected into the gas chromatograph for analysis. For benzoylecgonine measurement, the dried elutant was reconstituted in 20 μL of acetonitrile and 100 μL of BSTFA-TMCS. This mixture was heated for 30 min at 60°C to form the trimethylsilyl derivative of benzoylecgonine. One milliliter was injected into the gas chromatograph.

Liquid-liquid extractions of the derivatization reaction mixture were conducted at an alkaline pH with two portions of diethyl ether. The diethyl ether was evaporated under a gentle stream of nitrogen and the residue reconstituted in 1 mL of acetonitrile. One 1 μL was injected into the GC for analysis.

SPME Apparatus and Procedures

SPME experiments were performed with a manual fiber holder supplied from Supelco. A 100 μm polydimethylsiloxane (PDMS) fiber (Supelco) was utilized for all SPME procedures. SPME was typically performed for 10 min at an elevated temperature of 55°C to enhance short extraction time recovery. Air bubbles observed on the fiber when extracting at 55°C were removed by rapidly retracting and re-exposing the fiber after about 1 min into the extraction. The fiber was subsequently transferred to the injector port of the gas chromatograph for a 7 min desorption at 280°C.

The effect of salt on the SPME efficiency was investigated by adding differing amounts of a saturated salt solution to the SPME vials containing equal amounts of BHE prepared from derivatization of benzoylecgonine. Area counts obtained for a 20, 40, and 70% saturated salt solution were compared to a 0% salt solution to calculate a relative effect. Relative to the 0% salt solution, there was no effect observed up to the 70% saturated salt. Thus the addition of salt to the SPME vials as a means of enhancing SPME efficiency was not considered further. The effect of pH on SPME effi-

ciency was determined by comparing extractions in pH 4, 7, and 10 solutions. In both the salt and pH effect experiments SPME was performed for 10 min at room temperature and experiments were duplicated.

Instrumental Methods

Gas chromatographic-mass spectrometric analysis was carried out on a Varian Saturn 4D GC-MS system (Sugar Land, TX). Separations were conducted on a 30 m HP-5MS capillary column (0.25 mm ID, 0.25 μm d_f) (Hewlett Packard, Wilmington, DE). The GC column temperature program consisted of a 0.10 min hold at 60°C followed by a 25°C/min ramp to 270°C and holding at 270°C for 5.50 min. A Varian septum programmable injector (Varian 1093 SPI) was operated at an isothermal temperature of 280°C for SPME desorption and direct injections. A 0.75 mm ID inlet sleeve (Varian) which accepts any commercially available SPME fiber was used in the injection port. The transfer line temperature was maintained at 270°C.

The Saturn system is equipped with a quadrupole ion trap detector which was run in electron ionization (EI) mode and automatic gain control applied. A filament/multiplier delay of 10 min was applied. The ion trap manifold was set at 200°C for all experiments. The mass spectrometer was operated in the full scan mode between 78 and 385 amu. Benzoylecgonine and benzoylecgonine-d_3 were quantitated as the BHE derivative using the following ions: (82^+, 252^+, 373^+) and (85^+, 255^+, 376^+), respectively.

Results and Discussion

Optimization of the Chloroformate Derivatization Procedure

Aqueous alkyl chloroformate derivatization procedures may be made to occur under different conditions depending on the functionality (i.e., amine, carboxylic acid, or hydroxyl) which will undergo derivatization. Derivatization of amine and hydroxyl groups with alkyl chloroformates have been conducted simply in the presence of a bicarbonate buffer (6,27). In the case of alkyl carboxylic acids the derivatization was found to proceed only if a mixture of acetonitrile/hexanol/pyridine was added to the reaction mixture (27). Further studies indicated that alkyl carboxylic acids, hydroxyl groups alpha to a carboxylic acid, and phenolic hydroxyls were efficiently derivatized with hexyl chloroformate in the presence of pyridine under sonication (33).

For derivatization of benzoylecgonine in urine, it was found critical to add the acetonitrile/alcohol/pyridine mixture and conduct the reaction under sonication. For initial verification that the derivatization was proceeding, ethyl chloroformate was utilized as the derivatizing agent which would convert benzoylecgonine into cocaethylene. In this way the presence of derivatized benzoylecgonine could be identified based on the retention time and mass spectrum of a standard cocaethylene solution. A decision was reached to alter the derivatizing agent to hexyl chloroformate based on the following reasoning. First, the derivatization product could be distinguished from cocaethylene. Second, addition of a hexyl group to benzoylecgonine effectively converts the structure to a much nonpolar form, thus increasing the partition coefficient to the PDMS SPME fiber. Finally, hexyl chloroformate has been reported to be more reactive than shorter chain alkyl chloroformates for the derivatization of carboxylic acids (33). The overall derivatization reaction scheme using hexyl chloroformate is illustrated in Fig. 1. Sonication provides the intense mixing necessary for the relatively hydrophobic hexanol and hexyl chloroformate to interact with the benzoylecgonine in the aqueous phase. Efficient mixing is noted by the formation of an emulsion turning the water or urine solution cloudy. Figure 2 illustrates the mass spectra of (A.) benzoylecgonine derivatized in urine with hexyl chloroformate, and (B.) pure synthesized benzoylecgonine hexyl ester (BHE). This comparison yielded final structural confirmation of the hexyl chloroformate derivative of benzoylecgonine. The decision to use 2-dimethylaminopyridine instead of pyridine was based on a reduction of the background by the former around m/z 82, which is a mass used to monitor and quantitate the benzoylecgonine derivative with electron ionization.

The optimized reaction conditions are summarized in Table 1. These conditions were found to provide an adequate yield of derivatized product which could then be subjected to SPME analysis. Initially it was found that conducting the reaction at an elevated temperature of approximately 42°C allowed the reaction to proceed faster and yield a greater amount of derivatized benzoylecgonine in the same time frame of a room temperature reaction. Based on SPME tests and both liquid-liquid and solid phase extraction of the reaction mixture, the signal increased by a factor of 3.5 − 5.0 by conducting the reaction at 42°C. Derivatization yield was estimated by performing a liquid-liquid extraction and SPE on the reaction mixture and comparing the signal to a calibration curve generated from synthesized BHE. Under the optimized derivatization conditions, an approximately 25–35% yield was found when conducting the reaction in a urine samples spiked with benzoylecgonine. Due to differing compositions of urine, this yield may be ex-

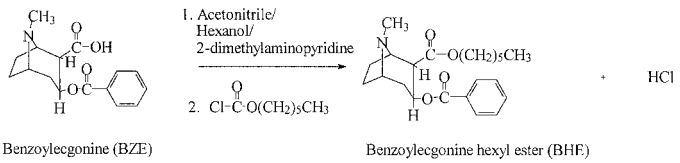

FIG. 1—*Aqueous phase hexyl chloroformate derivatization for benzoylecgonine (BZE). The reaction is conducted under sonication. The name benzoylecgonine hexyl ester (BHE) is given to identify the derivatization product.*

TABLE 1—*Optimized hexyl chloroformate derivatization and SPME procedures.*

Hexyl Chloroformate Derivatization	
Urine	1 mL
Acetonitrile/water/hexanol/2-DMAP (5:2:2:1 by vol)	70 μL
Hexyl chloroformate	12 μL
Mixing method	Sonication
Reaction time	3 min (while sonicating)
Reaction temperature	42°C sonicator water bath
SPME	
Solution for SPME	250 μL derivatized reaction mixture + 2.0 mL of deionized water + 2.0 mL of pH 7 buffer
SPME fiber	100 μm PDMS
Extraction temperature	55°C
Extraction time	10 min with magnetic stir bar agitation
Desorption time	7 min
Desorption temperature	280°C

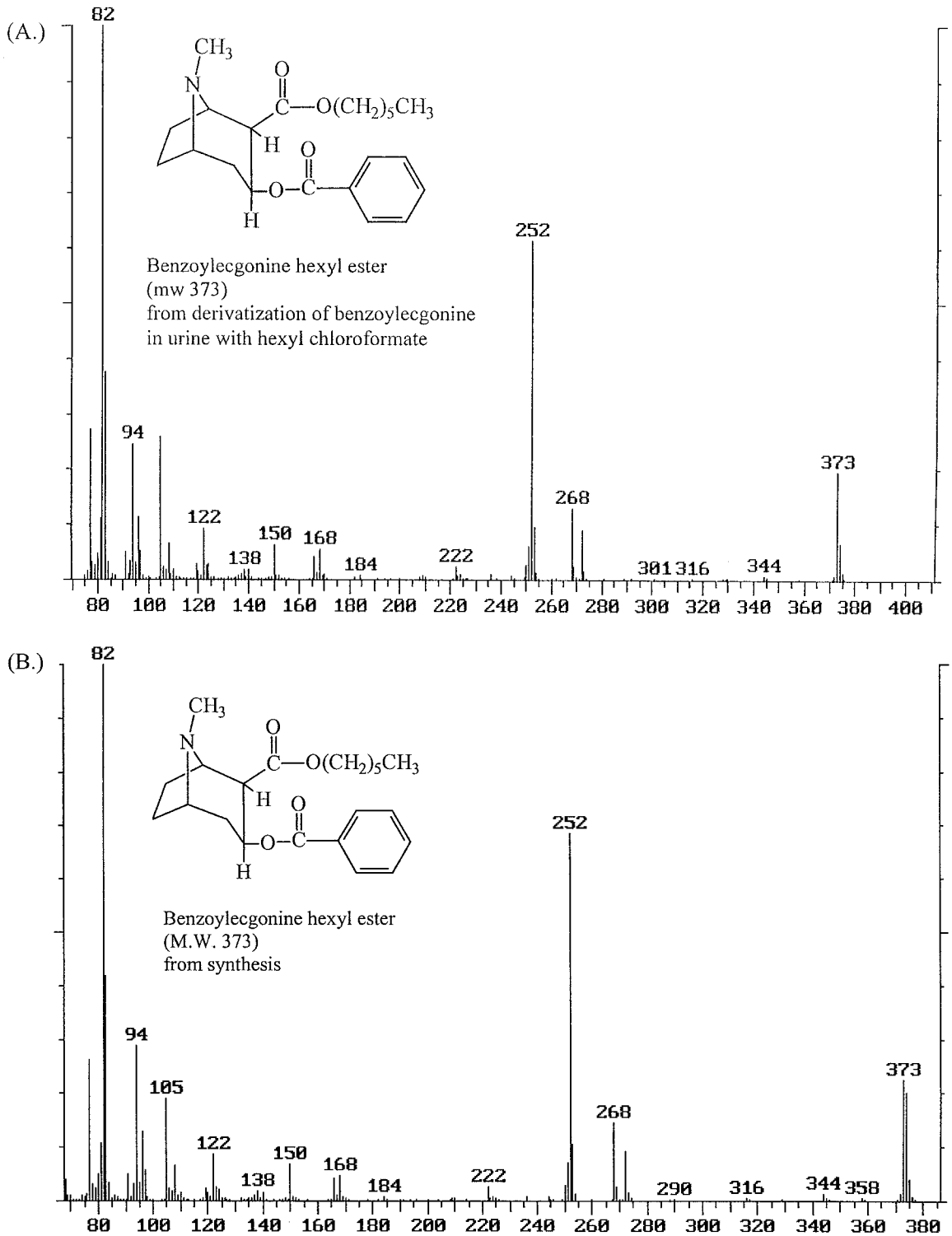

FIG. 2—*(A.) Electron ionization mass spectrum for benzoylecgonine hexyl ester (BHE) as the hexyl chloroformate derivative of benzoylecgonine (BZE) produced from derivatization in urine. (B.) Electron ionization mass spectrum for BHE as the pure substance synthesized from benzoylecgonine.*

pected to vary slightly. As discussed later in the quantitation section, this factor facilitates the need for standard addition or internal standard calibration. Using larger urine amounts than 1 mL did not offer substantial increase in product. This factor indicated that increasing the aqueous content of the reaction mixture slowed the derivatization reaction. Using a 1 mL volume of urine and the volumes of the reactants listed in Table 1, the reaction was allowed to proceed for 3 min. At that point an aliquot was taken for SPME analysis.

Optimization of SPME Parameters

The hexyl chloroformate derivatization procedure outlined above produces a reaction mixture relatively high in organic content from the presence of acetonitrile, hexanol, and 2-dimethylaminopyridine which may interfere with the extraction and limit the SPME fiber lifetime. Therefore it was found to be prudent to dilute the reaction mixture with pure water before SPME was performed to minimize the effects of the organics while maintaining good detection levels. Table 1 lists both the optimized solution conditions and SPME parameters. Evaluating the extraction efficiency at different pH values showed essentially the same recovery at pH 7 and pH 10. However, at pH 4 the signal dramatically dropped, a decrease attributed to ionization of the BHE molecule, thus giving it a higher affinity toward the aqueous phase. A neutral pH was chosen for the studies due to potential hydrolysis of BHE at elevated pH values.

SPME time profiles were conducted at two temperatures to determine the optimum extraction time and temperature, as well as equilibrium time for extraction of BHE with the PDMS fiber. The results are illustrated in Fig. 3. Conducting SPME at room temperature and 55°C yields equilibrium times of approximately 120 and 60 min, respectively. In accord with SPME theory, the amount extracted at equilibrium is less when conducting the extraction at an elevated temperature, since absorption to the fiber is an exothermic process. However, at lower extraction times the amount absorbed by the fiber is higher when extracting at 55°C resultant from a decreasing size of the boundary layer surrounding the fiber surface. Thus extraction proceeds more rapidly and equilibrium is reached faster. Extracting for 10 min at 55°C increases the amount extracted by about four times relative to room temperature. Therefore, a 10 min extraction time at 55°C was utilized for quantitative studies to enhance detectability. A 7 min desorption time at 280°C was found satisfactory to bring the carryover of BHE from the SPME fiber to an acceptable level of ≤1%.

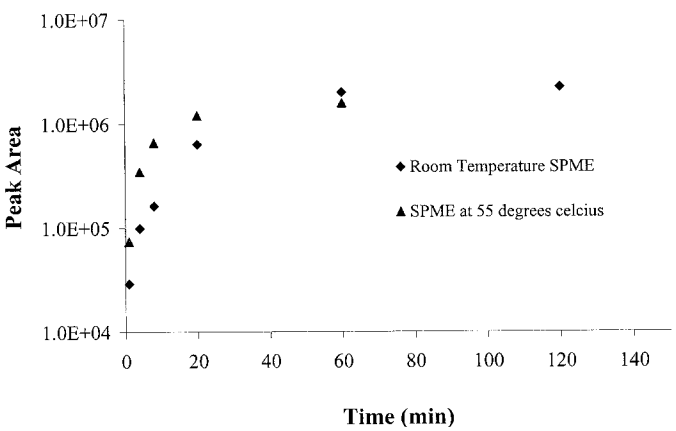

FIG. 3—*Peak area vs. extraction time for benzoylecgonine hexyl ester (BHE). Extractions were conducted at two different temperatures to evaluate the effect of heating on the SPME step. Solutions for SPME tests were prepared by derivatizing benzoylecgonine with hexyl chloroformate in deionized water. A 100 μm PDMS fiber was used for this study.*

Calibration and Quantitation Results

Using the optimized conditions outlined in Table 1, a five point calibration curve was established in the range from 0.10–20 μg/mL of BZE in urine utilizing benzoylecgonine-d_3 at a concentration of 1.5 μg/mL as the internal standard. The resultant linear equation for the derivative of BZE was $y = 0.4703x + 0.144$ ($r^2 = 0.999$). Intra-day and inter-day precisions were determined by analyzing replicates of control standards of benzoylecgonine prepared in urine at both 0.3 and 17 μg/mL, again utilizing benzoylecgonine-d_3 at a concentration of 1.5 μg/mL as the internal standard in both cases. Intra-day precision results were 8.8 and 6.8% RSD for the low and high concentration standards ($n = 6$), respectively. Inter-day precisions over a three day period, analyzing four replicates each day, were 2.2 and 3.3% RSD for the low and high concentration standards, respectively, indicating good reproducibility. A detection limit, defined as the concentration of benzoylecgonine spiked in urine yielding a signal-to-noise of 3, was found to be 0.03 μg/mL, substantially lower than the EMIT cutoff of 0.30 μg/mL. A single chloroformate derivatization mixture was divided into five SPME vials to determine the inherent precision of the SPME procedure. Measuring the area counts for the five replicate determinations resulted in a 17% RSD (in absence of internal standard) indicating that the SPME process has good repeatability. In fact, the PDMS fiber was found to be rugged and reusable for at least 50 extractions. No visual degradation or decrease in fiber performance was detected throughout at least 50 uses.

The SPME method was then applied to the detection and quantitation of benzoylecgonine in two urine specimens known positive for cocaine by conventional immunoassay tests conducted at a private drug testing laboratory. Figure 4 illustrates the chromatograms with inset mass spectra obtained for the two specimens. In order to show the different ways in which the data was evaluated, Fig. 4 (A) is shown as a total ion chromatogram while Fig. 4 (B) represents a reconstructed selected ion plot for BHE. Rapid identification of benzoylecgonine in both specimens, as the hexyl ester derivative, could be confirmed. The level of benzoylecgonine in the specimens was quantified by both the SPME technique with comparison to the conventional SPE method. Internal standard calibration curves were generated for both techniques by spiking drug free urine with benzoylecgonine and benzoylecgonine-d_3 and processing the standards in the same manner as the samples. Table 2 summarizes the results. Excellent agreement was found between the two methods indicating SPME to be an accurate means of quantitation. Twenty-two other cocaine positive urine specimens obtained from the same private drug testing laboratory were subjected to qualitative confirmation of benzoylecgonine by the hexyl chloroformate derivatization/SPME method. In all cases, benzoylecgonine was confirmed clearly above the baseline. Overall, the SPME method was found to be less laborious than SPE and confirmation of EMIT results were rapidly attained. With the appropriate autosampler which are commercially available from Varian and Leap Technologies (Carrboro, NC), the SPME extraction and injection procedure may be automated, thus only requiring the initial manual chloroformate derivatization procedure to be performed.

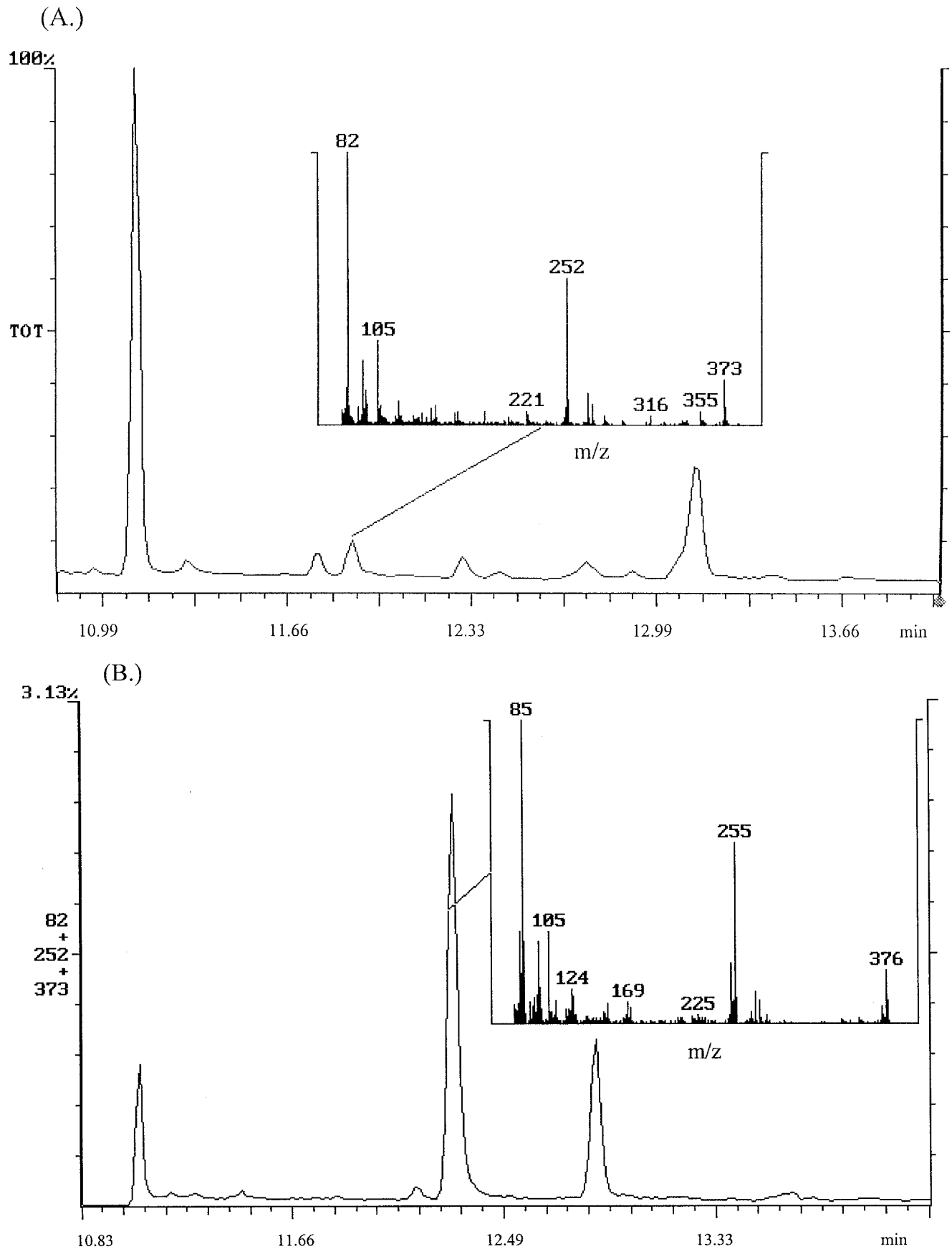

FIG. 4—(A.) *Total positive ion chromatogram for urine specimen 1 after performing the hexyl chloroformate derivatization and SPME procedures. The mass spectrum inset indicates the presence in benzoylecgonine (BZE) by identification of the characteristic ions from benzoylecgonine hexyl ester (BHE).* (B.) *Reconstructed selected positive ion chromatogram for the three characteristic ions of BHE after derivatization and SPME of urine specimen 2. For this run, BZE-*d_3 *was added to the urine prior to the procedures which results in ion signals at m/z* 376^+, 255^+, *and* 85^+ *which are present in the mass spectrum inset as well as ions representative of BHE.*

TABLE 2—*SPME and SPE quantitation results for benzoylecgonine in clinical urine specimens.*

	SPME*		SPE†	
	BZE (μg/mL)	% RSD (n = 3)	BZE (μg/mL)	% RSD (n = 3)
Specimen 1	3.3	7.8	3.6	6.3
Specimen 2	7.1	3.8	7.3	6.9

* Conditions for chloroformate derivatization and SPME conditions were used as outlined in Table 1. Specimen 1 was diluted 1:1 with deionized water prior to derivatization and performing SPME. % RSD represents triplicate aliquot determinations from the urine samples.

† SPE was performed with Varian Bond Elut Certify cartridges as outlined in the experimental section. Samples for SPE were run in triplicate, and duplicate injections of both standards and samples were performed on the gas chromatograph. For both the SPME and SPE method benzoylecgonine-d_3 was present as an internal standard throughout the experimental procedures.

Conclusions

A successful derivatization/SPME procedure for the determination of benzoylecgonine (BZE) in urine has been described. The derivatization of BZE is accomplished with hexyl chloroformate, with the appropriate catalyst, directly in urine after which an aliquot is taken for SPME and GC-MS analysis. The derivative formed is the n-hexyl ester of benzoylecgonine, referred to as benzoylecgonine hexyl ester (BHE). Optimization of the derivatization and SPME conditions were found to be crucial part to the development of a sensitive method. In addition, mass spectrometric detection was beneficial for both verification and quantitation of BHE by examination of the characteristic electron ionization mass spectrum. SPME was found to be an ideal method of extracting and introducing the benzoylecgonine derivative into the gas chromatograph because the extraction and concentration all occur on the SPME fiber. With conventional SPE methods large volumes of organic solvent are required for conditioning the column and eluting the desired analytes. In addition, time-consuming evaporative steps of the SPE elutant are crucial for both concentration and complete removal of water, which if present will adversely effect post-derivatization methods (i.e., BSTFA-TCMS derivatization). However, SPE does offer advantages in terms of identifying other potentially useful cocaine metabolites such as ecgonine methyl ester. Although SPME was a useful technique for this method, other extraction methods may be utilized after derivatization of benzoylecgonine directly in the urine with chloroformate reagents. Further investigation of other reagents such as halogenated chloroformates, with the appropriate halogenated alcohol, in conjunction with negative ion chemical ionization mass spectrometry may enhance the sensitivity of the technique to trace levels.

Acknowledgments

The authors wish to thank Esther Kempen for her advice in the synthesis of benzoylecgonine hexyl ester. Support from the National Science Foundation (CHE-9357422 and CHE-9421447) and the Welch Foundation (F-1155) are acknowledged.

References

1. Louch D, Motlagh S, Pawliszyn J. Dynamics of organic compound extraction from water using liquid-coated fused silica fibers. Anal Chem 1992;64:1187–99.
2. Zhang Z, Yang MJ, Pawliszyn J. Solid-phase microextraction. Anal Chem 1994;66:844A–53A.
3. Pawliszyn J. Solid phase microextraction theory and practice. Wiley-VCH, Inc.: New York, 1997.
4. Yashiki M, Kojima T, Miyazaki T, Nagasawa N, Iwasaki Y, Hara K. Detection of amphetamines in urine using headspace solid phase microextraction and chemical ionization selected ion monitoring. Forensic Sci Int 1995;76:168–77.
5. Nagasawa N, Yashiki M, Iwasaki Y, Hara K, Kohima T. Forensic Sci Int 1996;78:95–102.
6. Ugland HG, Krogh M, Rasmussen KE. Aqueous alkylchloroformate derivatisation and solid-phase microextraction: determination of amphetamines in urine by capillary gas chromatography. J Chromatogr B 1997;701:29–38.
7. Lord HL, Pawliszyn J. Method optimization for the analysis of amphetamines in urine by solid-phase microextraction. Anal Chem 1997; 69:3899–906.
8. Battu C, Marquet P, Fauconnet AL, Lacassie E, Lachâtre G. Screening procedure for 21 amphetamine-related compounds in urine using solid-phase microextraction and gas chromatography-mass spectrometry. J Chromatogr Sci 1998;36:1–7.
9. Krogh M, Grefslie H, Rasmussen KE. Solvent modified solid-phase microextraction for the determination of diazepam in human plasma samples by capillary gas chromatography. J Chromatogr B 1997;689: 357–64.
10. Luo Y, Pan L, Pawliszyn J. Determination of five benzodiazepines in aqueous solution and biological fluids using solid-phase microextraction and Carbowax/DVB fiber coating. J Microcolumn Sep. 1998;10(2): 193–201.
11. Ishii A, Seno H, Kumazawa T, Watanabe K, Hattori H, Suzuki O. Simple extraction of phencyclidine from human body fluids by headspace solid-phase microextraction. Chromatographia 1996;43:331–3.
12. Lee X, Kumazawa T, Sato K. Detection of tricyclic antidepressants in whole blood by headspace solid-phase microextraction and capillary gas chromatography. J Chromatogr Sci 1997;35:302–8.
13. Ulrich S, Martens J. Solid-phase microextraction with capillary gas-liquid chomatography and nitrogen-phosphorus selective detection for the assay of antidepressant drugs in human plasma. J Chromatogr B 1997; 696:217–34.
14. Penton Z. Blood alcohol determination with automated solid-phase microextraction (SPME): a comparison with static headspace sampling. Can Soc Forens Sci J 1997;30:7–12.
15. Li S, Weber S. Determination of barbiturates by solid-phase microextraction and capillary electrophoresis. Anal Chem 1997;68:1217–22.
16. Hall B, Brodbelt J. Determination of barbiturates by solid-phase microextraction (SPME) and ion trap gas chromatography-mass spectrometry. J Chromatogr A 1997;777:275–82.
17. Hall B, Satterfield-Doerr M, Parikh A, Brodbelt J. Determination of cannabinoids in water and human saliva by solid-phase microextraction and quadrupole ion trap gas chromatography-mass spectrometry. Anal Chem 1998;70:1788–96.
18. Takekawa K, Oya M, Kido A, Suzuki O. Analysis of cyanide in blood by headspace solid-phase microextraction (SPME) and capillary gas chromatography. Chromatographia 1998;47:209–14.
19. Volmer D, Hui J. Rapid determination of corticosteroids in urine by combined solid phase microextraction/liquid chromatography/mass spectrometry. Rapid Communications in Mass Spectrometry. 1997; 11:1926–34.
20. Pan L, Adams M, Pawliszyn J. Determination of fatty acids using solid-phase microextraction. Anal Chem 1995;67:4396–403.
21. Clark TJ, Bunch JE. Derivatization solid-phase microextraction gas chromatographic-mass spectrometric determination of organic acids in tobacco. J Chromatogr Sci 1997;35:209–12.
22. Pan L, Pawliszyn J. Derivatization/solid-phase microextraction: new approach to polar analytes. Anal Chem 1997;69:196–205.
23. Okeyo P, Rentz SM, Snow NH. Analysis of steroids from human serum by SPME with headspace derivatization and GC/MS. J High Resol Chromatogr 1997;20:171–3.
24. 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: 353–8.
25. Jennison TA, Jones CW, Wozniak E, Urry FM. The reliability of a solid-phase extraction system for the analysis of benzoylecgonine in urine. J Chromatogr Sci 1994;32:126–31.

26. 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;40:1299–305.
27. Hušek P. Derivatization and gas chromatographic determination of hydroxycarboxylic acids treated with chloroformates. J Chromatogr 1991;547:307–14.
28. Husěk P, Huang Z, Sweeley CC. Gas chromatographic determination of amines, aminoalcohols and acids after treatment with alkyl chloroformates. Anal Chim Acta 1992;259:185–92.
29. Hušek P. Rapid derivatization and gas chromatographic determination of amino acids. J Chromatogr 1991;552:289–99.
30. Vatankhah M, Moini M. Characterization of fluorinated ethylchloroformate derivatives of protein amino acids using positive and negative chemical ionization gas chromatography/mass spectrometry. Biol Mass Spectrom 1994;23:277–82.
31. Simpson JT, Torok DS, Markey SP. Pentofluorobenzyl chloroformate derivatization for enhancement of detection of amino acids or alcohols by electron capture negative ion chemical ionization mass spectrometry. J Am Soc Mass Spectrom 1995;6:525–8.
32. Lundh T, Åkesson B. Gas chromatographic determination of primary and secondary low-molecular-mass aliphatic amines in urine using derivatization with isobutyl chloroformate. J Chromatogr 1993;617: 191–6.
33. Minero C, Vincenti M, Lago S, Pelizzetti E. Determination of trace amounts of highly hydrophilic compounds in water by direct derivatization and gas chromatography-mass spectrometry. Fresenius J Anal Chem 1994;350:403–9.
34. Jonsson J, Kronstrand R, Hatanpää M. A convenient derivatization method for the determination of amphetamine and related drugs in urine. J Forensic Sci 1996;41:148–51.
35. Brzezinski MR, Christian CD, Lin M, Dean RA, Bosron WF, Harper ET. Convenient synthesis of benzoylecgonine ethyl ester, a homolog of cocaine. Synth Commun 1992;22:1027–32.

Additional information and reprints requests:
Jennifer S. Brodbelt, Ph.D.
The University of Texas at Austin
Department of Chemistry and Biochemistry
Austin, TX 78712-1167
Office: (512) 471-0028
Fax: (512) 471-8696
Email:jbrodbelt@mail.utexas.edu