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The Determination of Cocaine and Its Major Metabolite, Benzoylecgonine, in Postmortem Fluids and Tissues by Computerized Gas Chromatography/Mass Spectrometry

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ABSTRACT: An analytical procedure for the simultaneous determination of both cocaine and benzoylecgonine in postmortem fluid and tissue samples has been developed by using computerized gas chromatography/mass spectrometry and gas chromatography using a nitrogen/phosphorus (N/P) detector. Both methods are accurate and sensitive and allow the determination of tissue concentrations of cocaine and benzoylecgonine as low as 0.015 μg/mL.

KEYWORDS: toxicology, cocaine, chemical analysis

Cocaine is a powerful central nervous system stimulant [*1*]. Overdose causes anxiety, depression, and confusion, and can eventually lead to convulsions, respiratory arrest, cardiac arrest, and death [*1,2*].

Although cocaine has some use as a topical anesthetic [*1*], it is widely used as a stimulant by drug abusers. Recent reports have shown that cocaine is used by 1.6% of the Los Angeles County probationers and 10 to 24.1% of narcotic addicts, and that 23 people died because of cocaine overdose in Los Angeles County in 1980.

Cocaine is metabolized very rapidly, largely to benzoylecgonine with trace amounts of other metabolites [*3*]. The degradation of cocaine to benzoylecgonine continues outside the body, especially if the pH of the biological sample is alkaline. Thus, most postmortem fluids and tissues of cocaine users will contain both cocaine and benzoylecgonine.

A method for analyzing for both cocaine and benzoylecgonine is presented here in which the high specificity and sensitivity of computerized gas chromatography/mass spectrometry and gas chromatography with a nitrogen/phosphorus (N/P) detector are employed. Concentrations of cocaine and benzoylecgonine as low as 0.015 μg/mL can be accurately detected without interferences.

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Materials and Methods

Chemicals

Cocaine hydrochloride (HCl) and benzoylecgonine can be obtained from Applied Science and proadifin (SKF 525-A) can be obtained from Smith Kline and French Laboratories.

Sample Preparation

The extraction method employed is a modification of Wallace's method [4] for extracting cocaine and benzoylecgonine from biological samples. The procedure is as follows:

To a 50-mL glass stoppered centrifuge tube add 25 mL of 20% (v/v) ethanol/chloroform. For fluids, add 5 mL of sample fluid (blood, bile, and urine); for tissue, add 10 mL of a tissue/distilled water homogenate (1:1). Stopper the tube and shake it vigorously for 5 min, then centrifuge the tube for 5 min at 2000 rpm. Aspirate the water (upper) layer, then filter the organic solvent into a 100-mL beaker through sodium sulfate and glass wool. Concentrate the solution by placing the beaker on a 55°C hot plate under a stream of air. When the solvent level is below 10 mL, transfer it into a 13-mL centrifuge tube and evaporate until dryness. Add 0.6 mL of ethanol/sulfuric acid (2:1) to the centrifuge tube, and vortex the tube to ensure complete dissolution of the solvent residue. Stopper the centrifuge tube and incubate it at 85°C for 10 min. Remove the tube from the water bath and allow it to cool. Wash the solution twice with 10-mL portions of ethyl ether (1-min vigorous shaking followed by brief centrifugation and aspiration of the ethyl ether layer). Evaporate the remaining ethyl ether under a stream of air at 55°C for 20 min. Then add 2.5 mL of 3.6*N* sodium carbonate solution to neutralize the sulfuric acid and raise the pH to 9. This final pH can be checked with pH indicator paper. Add 0.2 mL of chloroform including 1 μg/mL of proadifin (SKF 525-A) as an external standard. The mixture is vortexed for 1 min, followed by centrifugation for 1 min at 2000 rpm. The sample is ready for analysis by gas chromatography/mass spectrometry (GC/MS) or N/P gas-liquid chromatography (GLC).

Computerized Gas Chromatography/Mass Spectrometry

A Finnigan 1020 automated computerized gas chromatograph/mass spectrometer equipped with multiple ion (MI) detection and a Nova 4-based Finnigan data system were used for the sample analysis.

The computerized GC/MS (C-GC/MS) analysis of cocaine and benzoylecgonine was performed with a 1.8-m by 2-mm inside diameter silanized glass column packed with 3% OV-1 on Chrom Q 100-200 mesh. The carrier gas was 18 to 20 mL/mm helium. The injector temperature was 250°C, the column temperature was 230°C, and the GC/MS separator temperature was 250°C.

The following four ions were monitored by the MI procedure:

- Mass-to-charge ratio (m/z) 82 for cocaine and ethylbenzoylecgonine,
- Mass-to-charge ratio (m/z) 86 for SKF 525-A,
- Mass-to-charge ratio (m/z) 182 for cocaine, and
- Mass-to-charge ratio (m/z) 196 for ethylbenzoylecgonine.

For sample analysis 2 μL of chloroform containing the cocaine and benzoylecgonine derivative (cocaethylene), were injected. The cocaine and cocaethylene elute from the gas chromatograph with retention times of 3.15 and 3.48 min, respectively (Fig. 1).

N/P Gas Chromatographic Analysis

A Hewlett-Packard Model 5840A gas chromatograph equipped with an N/P detector was used for sample analysis. The N/P GC analysis of cocaine and benzoylecgonine was performed

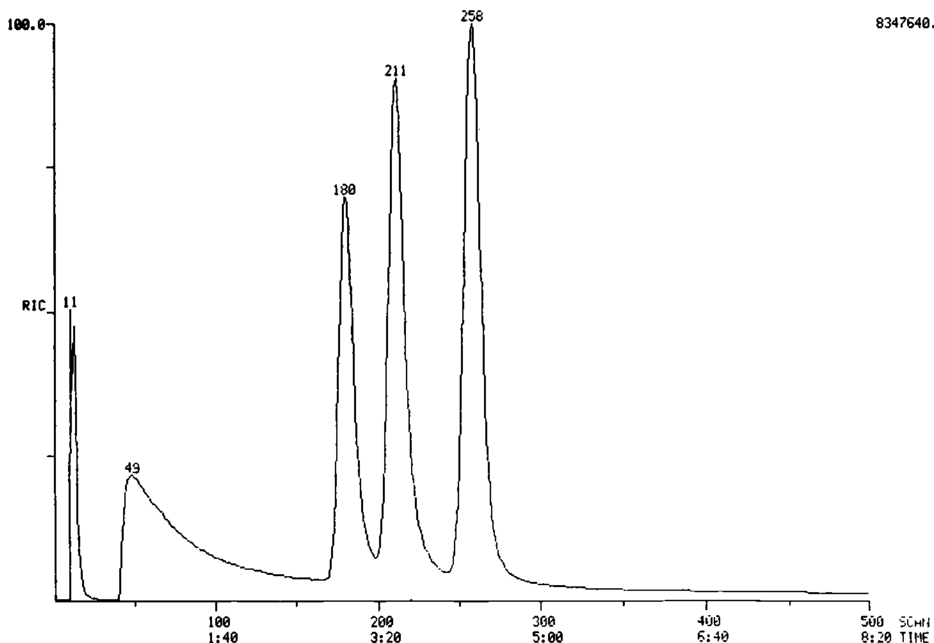

FIG. 1—*Reconstructed ion chromatogram of (*m/z *82, 86, 182, 196) cocaine (180), ethylbenzoylecgonine (211), and SKF 525-A (258).*

with a 1.8-m by 2-mm inside diameter silanized glass column packed with 3% OV-1 on Chrom Q 100-120 mesh. The carrier gas was 30-mL/min helium. The injector temperature was 250°C, the column temperature was 223°C, and the detector temperature was 300°C.

For sample analysis, 2 μL of chloroform containing the cocaine and benzoylecgonine derivative were injected. Peak heights were used for quantitation.

Results and Discussion

A method has been developed for the quantitative analysis of cocaine and benzoylecgonine in biological samples down to very low concentrations (0.015 μg/mL) with a very high degree of accuracy by using C-GC/MS and GC with an N/P detector.

Many methods have been developed for the analysis of cocaine in biological samples [*4-15*]. The analysis of biological samples for the presence of cocaine presents many difficulties [*5,6*]. Blood, usually employed for forensic science analysis, contains only small amounts ($<$5.5 μg/mL) of cocaine (Table 1). Cocaine is extensively metabolized into benzoylecgonine, which is generally present in much larger concentrations than cocaine itself [*4,7*]. The amount of cocaine present in a sample will continue to decrease over time because of continued degradation to benzoylecgonine in the sample container, especially if the pH of the tissue is alkaline and the container is stored in a warm place [*9,16*].

Benzoylecgonine is not easily analyzed for, since it is extremely soluble in water and is therefore difficult to extract into most organic solvents. Even when extracted it chromatographs very poorly by either GC or thin-layer chromatography (TLC). Special extraction procedures and derivitization techniques are required for its analysis.

Best results in the analysis of biological samples to determine the extent of cocaine intoxication are obtained by a procedure that analyzes for both cocaine and benzoylecgonine. Methods have been published in which simultaneous determinations of cocaine and ben-

TABLE 1—*Blood concentrations of cocaine in 13 coroner's cases where cocaine was the only drug detected.*

Cocaine Concentrations, μg/mL	Number of Cases
0.2-1.0	7
1.1-3.0	3
3.1-5.5	3
>5.5	0

zoylecgonine were made on urine samples by TLC analysis [*8-9*], radioimmunoassay [*10*], and by GLC [*11-13*]. Some of these methods are not quantitative, others do not differentiate between benzoylecgonine and cocaine, and others require duplicate extractions to determine the two compounds. A GC/MS method has been reported [*14*] in which benzoylecgonine is detected in urine after conversion to ethylbenzoylecgonine.

The extraction procedure presented here is a modification of a procedure developed by Wallace [*4*]. In the presented procedure, postmortem blood and other tissues were extracted rather than just urine. Ethylation was used rather than methylation, which allowed determination of both cocaine and benzoylecgonine simultaneously rather than in separate chromatographic analyses. The techniques of GC/MS and N/P GLC, which are quite specific, were used rather than just flame ionization detector GLC, thereby reducing the possibility of interferences.

We used SKF 525-A as an external standard on the basis of relative retention time to cocaine and ethylbenzoylecgonine. It served better than the butylanthroquinone used for this purpose by Wallace [*4*] because it did not induce changes in the sensitivity of the gas chromatograph to cocaine. Although it was not subjected to the ethylation procedure, it did go through the final basic extraction step.

Excellent analytical results were obtained. The cocaine recovery averaged over 91% and the benzoylecgonine recovery averaged nearly 65% for the concentrations studied. In these studies the N/P GC peak heights of nonextracted standards were compared with those of extracted, ethylated standards. As should be expected, benzoylecgonine was somewhat difficult to extract.

Cocaine was found to be stable during the extraction procedure since no ethylbenzoylecgonine was found in the chromatograms of the recovery study experiments. Cocaine, however, does break down into benzoylecgonine with the passage of time, both inside and outside the body, particularly under alkaline conditions [*9,16*]. Thus, a more realistic test of the procedure would be to determine both cocaine and benzoylecgonine in cocaine standards where degradation had been allowed to take place. To do this, cocaine standards were prepared and then allowed to stand for two weeks at room temperature before their analysis. The results (Table 2) show a combined cocaine/benzoylecgonine recovery averaging 99.5%, while the recovery of cocaine itself averaged just over 82%. Note that in the very low cocaine concentrations, benzoylecgonine was detected, but was below measurable concentration levels.

In our procedure, benzoylecgonine is converted to its ethyl derivative. Derivitization procedures have been developed previously for converting benzoylecgonine to alkyl derivatives [*9,11*]. Conversion of benzoylecgonine to cocaine, however, does not allow differentiation between cocaine and benzoylecgonine in the biological samples. Conversion of benzoylecgonine to ethylbenzoylecgonine allows such differentiation and is superior to other alkyl derivitization methods. Isopropylation and *n*-propylation were found to yield two peaks; *n*-butylation was found to have an inconveniently long retention time. Analyzed seven days later, the ethylbenzoylecgonine in chloroform was stable. The concentration had not changed.

The reconstructed ion chromatogram (RIC) of *m/z* (82, 86, 182, 196) of cocaine, ethyl-

TABLE 2—*Accuracy and precision data for the analysis of cocaine in blood.*[a]

Added Cocaine Concentration, μg/mL	Mean Measured Cocaine Concentration, μg/mL	Standard Deviation	Coefficient of Variation, %	Cocaine Recovery, %	Benzoylecgonine Concentration, μg/mL	Total Measured Cocaine and Benzoylecgonine, μg/mL	Percent Detection of Cocaine and Benzoylecgonine
1.0	0.9328 (7)[b]	0.0180	1.93	93.28	0.0657	0.9985	99.85
0.5	0.4386 (7)	0.0282	3.33	87.71	0.052	0.4906	98.12
0.25	0.2326 (7)	0.0269	2.89	93.07	0.279	0.2605	104.20
0.125	0.105 (7)	0.0180	2.26	84.45	0.269	0.1319	105.52
0.0625	0.04570(7)	0.0299	4.07	73.24	0.0104	0.0561	89.76
0.0312	0.02301(7)	0.0489	6.64	73.62	P[c]	...	...
0.0156	0.01082(6)	0.0360	5.20	69.01	P[c]	...	...

[a] Standards prepared two weeks before analysis by C-GC/MS.
[b] Number of determinations in parentheses.
[c] Present, but too small to quantitate.

TABLE 3—*Accuracy and precision data for the analysis of benzoylecgonine in blood.*[a]

Added Benzoylecgonine Concentration, μg/mL	Mean Measured Benzoylecgonine Concentration, μg/mL	Standard Deviation	Coefficient of Variation, %
1.0	0.9526 (7)[b]	0.0410	4.30
0.5	0.4690 (7)	0.0316	3.36
0.25	0.2311 (7)	0.0591	6.40
0.125	0.1221 (6)	0.0551	5.60
0.0625	0.0571 (5)	0.0581	6.36
0.0312	0.0299 (3)	0.0672	7.00
0.0156	0.01358(3)	0.0581	6.68

[a]Determined by C-GC/MS.
[b]Number of determinations in parentheses.

benzoylecgonine (cocaethylene), and SKF 525-A is shown in Fig. 1, demonstrating that the three substances are well separated from one another by this procedure.

The extraction residues can be analyzed by either C-GC/MS or by N/P GC. Cocaine and ethylbenzoylecgonine concentrations were found to be linear in GC/MS studies over the concentration range studied (0.0156 to 1.0 μg/mL). Additionally, excellent C-GC/MS accuracy and precision were obtained in various analytical studies of both cocaine and benzoylecgonine, as illustrated by Tables 2 and 3.

Both the C-GC/MS and N/P GLC methods have many advantages over other methods. They provide the analyst with a specific, sensitive, and reliable method for detecting and quantitating cocaine and benzoylecgonine simultaneously in blood, bile, urine, and liver tissue.

Several precautions should be kept in mind when performing this analytical procedure. First, the biological samples should be analyzed as quickly as possible following their removal from the body to prevent cocaine degradation to obtain an accurate measurement of the cocaine itself present in the sample [*16*]. Second, during the procedure, the final extraction pH should be close to 9; this extraction should be performed rapidly after alkalinization to minimize degradation. Third, the total concentration of cocaine products can be determined by adding the concentration of cocaine and ethylbenzoylecgonine. Finally, extraction residues with cocaine or ethylbenzoylecgonine concentrations greater than 0.1 μg/mL should be diluted with a chloroform/SKF 525-A solution so that the concentration will be within the linear range.

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