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Gas Chromatography/Mass Spectrometry for the Determination of Cocaine and Benzoylecgonine over a Wide Concentration Range (<0.005–5 mg/dL) in Postmortem Blood

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ABSTRACT: The determination of cocaine and benzoylecgonine in blood by gas chromatography/mass spectrometry using selected ion monitoring was investigated. The method involved an extraction using rotation of an Amberlite® XAD-2-blood mixture and the use of deuterated internal standards with derivatization of benzoylecgonine using diazopropane. Standard curves with logarithmic-transformed data were constructed over the concentration range from 0.005 to 5 mg/dL. The use of a wide concentration range precludes using smaller sample sizes in repeated extractions to conform to a narrower concentration range of standards. The recovery of the extraction method was 85% for both cocaine and benzoylecgonine. The method was applied primarily to postmortem blood samples from 180 forensic cases and was statistically evaluated. The limit of detection for cocaine or benzoylecgonine extractions from 1 mL forensic blood samples is 0.00025 mg/dL. The method is routinely reliable and applicable to forensic toxicology investigations.

KEYWORDS: toxicology, cocaine, GC/MS, diazopropane, statistics, postmortem, blood, chromatographic analysis

Cocaine is one of the most widely publicized drugs of abuse throughout North America. Changes in the formulation, transportation, marketing and demand for cocaine are indicated in forensic cases, and can increase the demands placed on the method of assay by the forensic toxicologist. For example, we recently determined a cocaine concentration of 7 mg/dL[2] in the postmortem blood of a person who consumed imported rum, unaware that its contents had been saturated with cocaine. Case reports of similar or higher concentrations of cocaine in postmortem blood exist [*1–3*], however, the development of versatile methods for the determination of cocaine over a wide concentration range (several orders of magnitude) has not been reported. Traditional practices involve time-

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[2]One mg/dL is equivalent to 10^{+4} ng/mL, 10^{+1} μg/mL, 10^{+1} mg/L and 32.96 and 34.54 μmol/L for cocaine and benzoylecgonine respectively.

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consuming repeated extractions using smaller sample sizes to conform to a narrower and unnecessarily restrictive concentration range of standards.

In the Province of Ontario (10 million inhabitants), there has been a significant increase in the number of cases analyzed by this laboratory with (a) potentially fatal concentrations of cocaine in blood (>0.05 mg/dL) and (b) very high concentrations of benzoylecgonine, the principal metabolite of cocaine (>0.5 mg/dL). Fifty-seven percent of the determinations of cocaine (N = 343) and twenty-one percent with benzoylecgonine (N = 695) have been in these concentration ranges (Table 1).

For the accurate determination of drugs in biological fluids, concentrations of extracted drug standards should include and extend beyond those found in cases so that interpolation, rather than extrapolation, is used in the calculation of a case concentration. In addition, concentrations of drug standards at the maximum therapeutic and minimum fatal concentrations would facilitate interpretations. The maximum concentration of cocaine in surgical patients following nasal topical application has been reported as 0.047 mg/dL [*36*].

For the determination of cocaine and benzoylecgonine in forensic cases, the ideal analytical method should be specific, statistically defined (accuracy, precision and sensitivity), and routinely reliable. Cocaine and benzoylecgonine have been determined quantitatively in biological fluids using gas chromatography (GC) with flame ionization detection (GC/FID) [*4–6*], nitrogen-phosphorus detection (GC/NP) [*7–9*] and mass spectrometry (GC/MS) [*7,10–21*], high pressure liquid chromatography (HPLC) [*22–24*], and thin layer chromatography (TLC) [*6,25*]. For postmortem whole-blood samples, the methods for the analysis of cocaine and benzoylecgonine [*7,9,18,19*] have been reported with only (a) a few selected case findings [*1–3,7*] and (b) a comparison of blood, brain and liver concentrations in 37 cases [*19*].

GC/MS with single ion monitoring (GC/MS/SIM) is the preferred technique for a cocaine determination in postmortem blood because of its specificity at low detection limits (ng/mL) and capability for analyzing deuterated analytes that have been incorporated into the assay as internal standards.

Internal standards used in GC/MS analyses of cocaine include benzoylecgonine n-butyl-ester, m-toluylecgonine and m-toluylecgonine methyl-ester, ketamine, d_3- and d_5-

TABLE 1—*Cocaine and benzoylecgonine determinations from 1985 to 1992.*

	Number of Determinations[a]							
	Cocaine				Benzoylecgonine			
		Concentration (mg/dL)				Concentration (mg/dL)		
Year	Total	>0.05[b]	>0.5	>2	Total	>0.2[b]	>0.5	>2
1985	13	6	1	0	32	10	3	1
1986	28	10	0	0	51	15	8	0
1987	33	13	8	0	63	21	14	0
1988	51	26	12	2	93	43	26	2
1989	71	34	14	4	117	57	29	9
1990	26	12	4	0	85	25	12	3
1991	54	17	6	3	107	39	17	2
1992	67	17	6	2	147	39	17	4

[a]Number of Determinations refer to samples (blood etc., not urine) indicated for cocaine or benzoylecgonine by radioimmunoassay and quantitatively confirmed by GC/NP [*26*] or GC/MS/SIM [this work].

[b]The number of determinations of cocaine and benzoylecgonine less than or equal to 0.05 and 0.2 mg/dL respectively may be calculated by subtraction from the total.

cocaine and benzoylecgonine. The deuterated internal standards [*19,20*] are preferred because of their analogous chemical properties to the analytes.

For analysis by gas chromatography, it is necessary to modify benzoylecgonine into a more volatile compound that will better transfer onto the chromatographic column. The derivatization of the carboxylic acid group of benzoylecgonine into an alkyl-ester has been used because it provides a compound with good chromatographic properties. The methyl-ester derivative of benzoylecgonine is cocaine.

Derivatization of benzoylecgonine into cocaine [*26*] and longer chain alkyl esters [*6*] has been reported. The latter approach allows the gas chromatographic analysis of cocaine and the benzoylecgonine derivative from the same injection because these compounds elute at different times. Higher alkyl derivatives of benzoylecgonine have included ethyl, n-propyl, 2-propyl and 1-butyl-esters. The determination of benzoylecgonine as its ethyl-ester is not practical for forensic purposes because benzoylecgonine ethyl-ester (also known as cocaethylene or ethylcocaine) has been shown recently to be a significant metabolite of cocaine [*27*].

Derivatization of benzoylecgonine into benzoylecgonine n-propyl-ester has been performed using iodopropane, and N,N-dimethylformamide dipropylacetal. However, we found the highest conversion efficiency using the latter reagent was 70% and that many by-products from that derivatization were detectable. Derivatizations using diazomethane for converting a carboxylic acid into a methyl-ester are characteristically fast, clean and complete (100%) [*28,29*].

In this work, we present a GC/MS/SIM method for the determination of cocaine and benzoylecgonine that uses diazopropane, and compare this with a GC/NP method that uses diazomethane. With synthesized d_5 internal standards, we demonstrate the simultaneous determination of cocaine and benzoylecgonine using 1 mL of ante- and postmortem bloods over a wide concentration range (<0.005 to 5 mg/dL).

Materials and Methods

GC/MS/SIM Instrumentation

A gas chromatograph (Hewlett-Packard (HP), Model 5890) was equipped with a mass selective detector (HP, Model 5970). The molecular ions monitored had m/z values of 272, 277, 303, and 308 for cocaine, and 272, 277, 331, and 336 for benzoylecgonine. The GC/MS instrument contained a pre-column of deactivated fused-silica of 0.25 mm ID attached to a chromatographic column (DB-5; J & W Scientific, 0.25 mm ID, 0.1 μm film thickness) that was 30 m long. The column temperature was programmed as follows: 100°C isothermal for 2 min, then increased 18°C per min until 290°C, then isothermal for 2 min. The helium flow rate was approximately 0.75 mL/min. The injector and transfer line temperatures were 270°C and 290°C respectively. One microliter of toluene extract was injected. The retention times of cocaine and benzoylecgonine 1-propyl-ester were 12.28 and 13.05 min respectively.

GC/NP Instrumentation

A gas chromatograph (HP, Model 5710) was connected to an associated data interface (Spectra Physics (SP), Model 4020), data sampler (SP), data processor (SP, Model 4000) and printer (SP, Model 4060). The gas chromatograph was equipped with two columns: (a) DB-1; J & W Scientific, 0.53 mm ID, 1.5 μm film thickness and (b) FFAP; HP, 0.53 mm ID, 1 μm film thickness; both were 10 m long. The carrier gas flow rates of helium were 20 mL/min for the DB-1 column and 30 mL/min for the FFAP column. The flow rates of hydrogen and air through the NP-detectors were 15 and 40 mL/min respectively.

The temperature of the injector and detector were 250°C and 300°C respectively. The column temperature was programmed as follows: (a) for the DB-1 column; 180°C isothermal for 2 min, then increased 8°C per minute until 210°C, then isothermal for 2 min and (b) for the FFAP column; 170°C isothermal for 2 min, then increased 8°C per min until 210°C, then isothermal for 2 min. Two microliters of the analyte extract in toluene were injected. The retention times for cocaine were 3.18 and 6.92 min on the DB-1 and FFAP columns, respectively, and 4.50 and 5.71 min for benzoylecgonine 1-propyl-ester.

Material Preparations

Deuterated cocaine and benzoylecgonine were synthesized by a method similar to previous reports [*20,30*]. Briefly, cocaine hydrochloride was converted into ecgonine using aqueous HCl. This was then isolated and converted into ecgonine-methyl-ester hydrochloride using an ethereal solution of diazomethane. d_5-cocaine was prepared by reacting d_5-benzoyl chloride (prepared from reacting d_5-benzoic acid with $SOCl_2$) with ecgonine-methyl-ester in dried pyridine. The d_5-benzoylecgonine was prepared by refluxing an aqueous solution of d_5-cocaine. Preparative TLC and GC/MS were used for isolation and identification of products.

Diazomethane was prepared as an ethereal ethanolic solution by a distillation reaction of Diazald® (N-methyl-N-nitroso-p-toluenesulfonamide, Aldrich) using a commercially available kit. Safety considerations for preparing and handling diazomethane solutions are described elsewhere [*31–33*].

Diazopropane was prepared by a method similar to that for diazomethane except the precursor (3-nitro-1-nitroso-1-propylguanidine (Aldrich/Sigma)) required a 1:5 (v/v) methanol:diethyl ether solution to dissolve it. The diazopropane solution was orange and the diazomethane solution was yellow.

The diazomethane and diazopropane solutions were stored in glass bottles covered with aluminum foil and were tightly capped with a teflon stopper. These solutions were stored at −4°C and were useable for at least 6 months when stored as described.

Drug Standards

Stock solutions of cocaine and benzoylecgonine were prepared by dissolving cocaine hydrochloride (Health and Welfare Canada) or benzoylecgonine tetrahydrate (Altech Applied Science Labs, PA) in methanol to give effective concentrations of 40 mg/dL and 160 mg/dL respectively. Working solutions were prepared by mixing and diluting solutions to give nominal cocaine concentrations of 20, 2 and 0.2 mg/dL and benzoylecgonine concentrations of 80, 8 and 0.8 mg/dL.

Stock solutions of deuterated cocaine and benzoylecgonine were prepared by dissolving d_5-cocaine hydrochloride and anhydrous d_5-benzoylecgonine in methanol. Subsequent mixing and diluting provided an internal standard solution with concentrations of 2 mg/dL for d_5-cocaine and 8 mg/dL for d_5-benzoylecgonine.

Stock and working solutions were stored in capped and sealed glass vials at −4°C. Glass-distilled solvents were used throughout.

The cocaine standard solution was analyzed and found to contain 1.0% ± 0.2 (N = 3) benzoylecgonine and 0.12% ± 0.01 (N = 3) benzoylecgonine ethyl-ester. Benzoylecgonine had a specified purity of greater than 99% and no detectable amount of cocaine (<0.02%).

The deuterated cocaine and benzoylecgonine standard solutions were analyzed for nondeuterated (analyte) forms. They contained 0.47% ± 0.06 (N = 5) h_5-cocaine and 1.11% ± 0.04 (N = 5) of h_5-benzoylecgonine.

Sample Preparation

For GC/MS analysis, 1 mL of blood and 50 μL of internal standard solution were briefly vortexed inside a 15 mL disposable screw cap tube and allowed to stand for 30 minutes. The concentrations of analyte standard in blood were prepared by adding an additional 25 μL of the working standard solution to give concentrations of 0.005, 0.05 and 0.5 mg/dL for cocaine and 0.02, 0.2 and 2.0 mg/dL for benzoylecgonine. Two mL of purified [*34*] Amberlite® XAD-2 resin (Rohm & Haas, BDH Chemicals) slurry (buffered at pH 8.4) and 2 mL of pH 8.4-buffer solution (pHydron pH, Micro Essential Lab, NY) was then added and the contents rotated for 30 min on a Roto-Rack™ (Glas-Col, IA). The resin-blood mixture was filtered through an empty plastic column (Clini-Screen Cartridges; Brinkmann (Canada) Ltd.) containing a cotton plug, and the trapped resin was washed with pH 8.4-buffer solution (approximately 30 mL) until the effluent was colorless. The columns were placed in a 15 mL round-bottom tube and centrifuged at 700 × g for 20 min to eliminate excess water and facilitate transfer of the resin beads. The resin mixture was quantitatively transferred to a Teflon-lined screw-capped glass tube (16 × 125 mm), 3 mL of 1:3 (v/v) 2-propanol/chloroform solution was added, and the contents were rotated for 30 min. The resin mixture was centrifuged at 700 × g for 5 min and filtered through a Pasteur pipette that contained a small cotton plug into a similar clean tube. After the solvent was evaporated with a stream of helium at room temperature, 1 mL of an ethereal solution of diazopropane was added, the tube was capped, briefly vortexed and allowed to stand for 30 min. After evaporating this solution to dryness with a stream of helium at room temperature, 4 mL of 1:3 (v/v) 2-propanol/chloroform solution was added. After a brief vortex, 2 mL of 0.2 N H_2SO_4 was added and the contents vortexed for 45 s and centrifuged for 5 min. The acidic aqueous (top) layer was transferred to a smaller glass tube (13 × 100 mm) and made alkaline (pH 8.5) with the dropwise addition of saturated sodium bicarbonate solution. One mL of toluene was added, the contents vortexed for 45 s and centrifuged. The upper toluene layer was transferred into micro vials (1.1 CTV Chromacol, Diamed Lab, Toronto), concentrated to a volume of approximately 0.2 mL using a stream of helium, and subsequently capped. Samples were easily and routinely concentrated to approximately 20 μL for trace level confirmations. Quantitative results were still obtainable for at least 3 weeks from extracts stored under refrigeration (4°C).

For GC/NP analysis, a similar extraction method to that for GC/MS/SIM was used with the following exceptions. First, two 2 mL aliquots of blood were required, one for the determination of cocaine, and the other for benzoylecgonine. Second, no internal standards were coextracted. Third, an ethereal solution of diazomethane was used to convert benzoylecgonine into cocaine, which was additive to the cocaine previously in the sample. Fourth, the final volume of toluene extract remained at 1 mL. The concentration of benzoylecgonine was calculated by subtracting the determined cocaine concentration from the sum of concentrations of cocaine inherent in the sample and that produced from the derivatization of benzoylecgonine.

Results and Discussion

Recovery

The recovery of cocaine and benzoylecgonine from preserved whole-blood (Red Cross) was determined at four different concentrations from 0.005 to 5 mg/dL, repeated in four batches, using diazopropane and GC/NP analysis. No internal standards were coextracted. The recovery of the batches were consistent and averaged 84% ± 5 (range 79 to 88%) for cocaine and 86% ± 4 (range 83 to 88%) for benzoylecgonine.

Using GC/NP analysis, with confirmation by GC/MS full-scan analysis, reacting equal amounts of benzoylecgonine with diazomethane and diazopropane produced chromatographic peaks with equivalent areas (within experimental uncertainty of ±1%) for cocaine and benzoylecgonine n-propyl-ester. No significant change was found from methylating or propylating cocaine, or benzoylecgonine ethyl-ester, after accounting for the 1% of benzoylecgonine.

Accuracy

Drug-free blood was spiked with both cocaine and benzoylecgonine to achieve concentrations from 0.005 to 5 mg/dL. For analyte concentrations of 1 mg/dL and higher, the concentration of internal standard was analytically ten-fold higher than for all lower analyte concentrations and the toluene extract was not concentrated. The GC/MS/SIM determination was performed on eight different analyte concentrations, each repeated five times, for a total of 40 analyte determinations.

To mathematically describe the relationship between the amount of analyte found (AF) from analyte added (AA), it was preferable to initially perform a logarithmic transformation on the measured ion abundance ratios (IAR) and associated concentrations of analyte standard before calculating the linear least squares regression fit. This transformation removes a bias that larger numbers can impart to the regression. As well, the logarithmic transformation is useful in treating heteroscedastic data where standard deviations are proportional to mean values [*35*]. If the logarithmic transformation was not initially performed on the wide-spanning data, concentrations found using either IAR of cocaine or benzoylecgonine for the lowest analyte standards had negative values, which are not reasonable. Table 2 summarizes the parameter values of the two linear fits obtained from analyzing the *same* data (IAR versus analyte concentration) by linear-least squares regression using (i) logarithmic-transformed and (ii) nontransformed data. The standard errors in the slopes are comparable, but differ by two orders of magnitude in the y-intercepts, indicating the preference of the logarithmic-transformation approach.

Table 3 lists the concentrations of analyte found (AF) from analyte added (AA) to blood, with values plotted in Figure 1. Regression parameter values from linear regression fits of the AF versus AA data in Table 3 are given in Table 4. Again, the logarithmic transformation of data allows a better description of the amount of analyte found versus

TABLE 2—*Regression parameter values for ion abundance ratio versus cocaine and benzoylecgonine concentration in blood from 0.005 to 5 mg/dL (*N = *40).*

		Cocaine		Benzoylecgonine	
Mass ratio		272/277	303/308	272/277	331/336
(i)	m	1.006 ± 0.010	0.9949 ± 0.0085	0.9951 ± 0.0048	0.9994 ± 0.0057
	b	3.525 ± 0.028	3.505 ± 0.023	3.251 ± 0.013	3.257 ± 0.015
	R^2	0.9961	0.9972	0.9991	0.9988
(ii)	m′	33.91 ± 0.24	31.91 ± 0.27	25.348 ± 0.083	25.741 ± 0.118
	b′	0.63 ± 0.46	1.18 ± 0.53	0.383 ± 0.161	0.362 ± 0.229
	R^2	0.9982	0.9973	0.9996	0.9992

Parametric equations are: (i) Ln(IAR) = mLn(C) + b and (ii) IAR = m′C + b′ where IAR is the Ion Abundance Ratio, C is the concentration of analyte, and m, b, and R^2 are the slope, y-intercept and coefficient of determination for the regression. The uncertainties listed are the standard errors. Comparison of R^2 values for (i) and (ii) demonstrate the results may be considered linear using either equation and that R^2 values are not significantly changed in regression using logarithmic-transformed data.

TABLE 3—*Cocaine and benzoylecgonine concentrations found from that added to blood using regressions of logarithmic-transformed and nontransformed data (N = 40).*

Added (mg/dL)	Found (mg/dL) ± s			
	Cocaine		Benzoylecgonine	
Mass ratio	303/308	272/277	331/336	272/277
	With Log-Transformed Data			
5.0 E-3	6.05 ± 0.08 E-3	6.11 ± 0.59 E-3	5.60 ± 0.42 E-3	5.38 ± 0.34 E-3
2.0 E-2	1.72 ± 0.05 E-2	1.77 ± 0.11 E-2	1.88 ± 0.08 E-2	1.86 ± 0.12 E-2
5.0 E-2	4.37 ± 0.38 E-2	4.49 ± 0.90 E-2	4.52 ± 0.39 E-2	4.90 ± 0.50 E-2
2.0 E-1	2.06 ± 0.16 E-1	1.92 ± 0.32 E-1	1.98 ± 0.16 E-1	2.01 ± 0.18 E-1
5.0 E-1	5.22 ± 0.25 E-1	5.43 ± 0.52 E-1	5.21 ± 0.20 E-1	5.05 ± 0.24 E-1
1.0	1.04 ± 0.03	1.01 ± 0.03	1.01 ± 0.03	1.02 ± 0.02
2.0	2.08 ± 0.08	2.10 ± 0.10	2.07 ± 0.02	2.04 ± 0.04
5.0	4.77 ± 0.15	4.85 ± 0.09	4.92 ± 0.09	4.92 ± 0.02
	With non-Transformed Data			
5.0 E-3	−1.06 ± 0.06 E-2	−2.76 ± 0.08 E-2	−9.05 ± 0.34 E-3	−7.56 ± 0.42 E-3
2.0 E-2	−1.71 ± 0.05 E-2	−1.58 ± 0.05 E-2	4.64 ± 1.22 E-3	5.76 ± 0.76 E-3
5.0 E-2	2.73 ± 0.89 E-2	1.20 ± 0.40 E-2	3.59 ± 0.51 E-2	3.24 ± 0.39 E-2
2.0 E-1	1.73 ± 0.32 E-1	1.82 ± 0.16 E-1	1.91 ± 0.19 E-1	1.87 ± 0.16 E-1
5.0 E-1	5.26 ± 0.52 E-1	5.12 ± 0.25 E-1	5.01 ± 0.24 E-1	5.12 ± 0.20 E-1
1.0	9.97 ± 0.30 E-1	1.05 ± 0.03	1.02 ± 0.02	1.01 ± 0.03
2.0	2.12 ± 0.10	2.13 ± 0.08	2.06 ± 0.04	2.07 ± 0.01
5.0	4.95 ± 0.09	4.92 ± 0.15	4.97 ± 0.02	4.97 ± 0.10

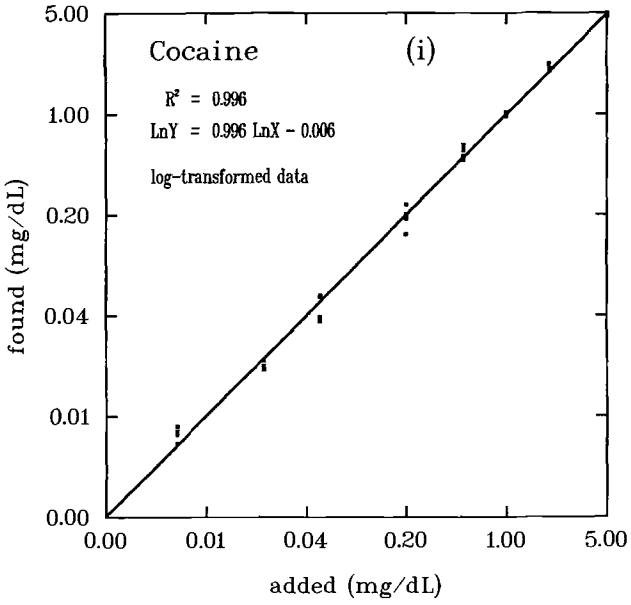
The uncertainties listed (s) are the standard deviations.

the amount added. As an example, the amount found (concentration) computed for the lowest cocaine standard (0.005 mg/dL) using the regression parameter values in Table 4 for m/z 272 and 277 is: (i) 0.0051 mg/dL using the logarithmic-transformation approach, (ii) 0.0070 mg/dL using the nontransformed data, and (iii) 0.030 mg/dL using nontransformed data originating from a logarithmic transformation of IAR versus concentration data. The regressions and example illustrate that regression to the logarithmic-transformed data provides both computational accuracy and precision for the lowest data measurement, whereas neither attribute is obtained using the nontransformed data approach.

GC/MS/SIM Versus GC/NP Methods

The GC/MS/SIM and GC/NP methods of determination for cocaine and benzoylecgonine were compared using simultaneous extractions on 27 different postmortem blood samples. These bloods were analyzed in three batches of nine samples. All blood samples had first been screened negative for cocaine metabolite using a radioimmunoassay (RIA) method (Roche Diagnostic Systems, NJ); limit of detection of 0.0005 mg/dL), but did contain other drugs and their metabolites. Each blood sample within a batch was independently spiked with one of three concentrations of both cocaine and benzoylecgonine, therefore each batch contained nine combinations of two analyte concentrations. The nominal concentrations in blood were chosen for operational purposes as 0.005, 0.05 and 0.5 mg/dL for cocaine and 0.02, 0.2 and 2.0 mg/dL for benzoylecgonine.

In the comparison of cocaine concentrations (N = 36) found from the two methods (Figs. 2a and 2b), 44% of measurements were within ±5%, 88% were within ±15%,



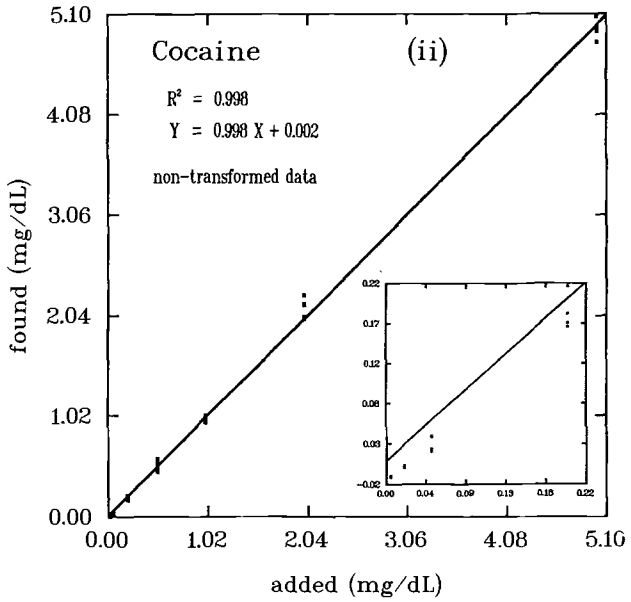


FIG. 1—*Amount of cocaine found* (Y) *from that added* (X) *to blood using (i) logarithmic-transformed data and (ii) nontransformed data. The lines shown are computed from regression parameter values (Table 4) for cocaine using the mass ratio of 272/277. The inset in (ii) displays the inadequacy of linear regression on nontransformed data when the range is extended to low concentrations.*

TABLE 4—*Regression parameter values for cocaine and benzoylecgonine found from that added in blood from 0.005 to 5 mg/dL (N = 40).*

		Cocaine		Benzoylecgonine	
Mass ratio		272/277	303/308	272/277	331/336
	m	0.9960 ± 0.0102	0.9972 ± 0.0085	0.9991 ± 0.0048	0.9988 ± 0.0057
(i)	b	−0.0060 ± 0.027	−0.0042 ± 0.023	−0.0014 ± 0.013	−0.0019 ± 0.015
	R^2	0.9960	0.9972	0.9991	0.9988
	m′	0.9982 ± 0.0069	0.9973 ± 0.0084	0.9996 ± 0.0033	0.9992 ± 0.0046
(ii)	b′	0.0020 ± 0.0135	0.0030 ± 0.0164	0.0004 ± 0.0064	0.0009 ± 0.0089
	R^2	0.9982	0.9973	0.9996	0.9992
	m	0.9765 ± 0.0072	0.9587 ± 0.0080	0.9864 ± 0.0030	0.9885 ± 0.0045
(iii)	b	0.025 ± 0.014	0.034 ± 0.016	0.0121 ± 0.0059	0.0143 ± 0.0088
	R^2	0.9979	0.9974	0.9996	0.9992

Parametric equations are: (i) Ln(AF) = mLn(AA) + b, (ii) AF = m′AA + b′ and (iii) AF = mAA + b where AF and AA are the amounts of analyte found and added, respectively.

and all were within ±25%. Similarly, for the benzoylecgonine concentrations, 40% were within ±5%, 80% were within ±15%, and all were within ±25%.

In the three blood samples spiked with the highest cocaine and lowest benzoylecgonine concentrations, an artificially higher concentration is expected for benzoylecgonine because of its presence in the cocaine standard employed. These samples were found to have 27% (range 23% to 33%) more benzoylecgonine than samples spiked with either the lowest or middle cocaine standards and the lowest benzoylecgonine standard. This result corresponds to a 1.1% proportion of benzoylecgonine in the cocaine standard, which is within the experimental uncertainty for the determination of benzoylecgonine in the cocaine standard (1.11% ± 0.04).

Applying the GC/MS/SIM method to preserved Red Cross blood (N = 12 over four batches), the deviations in concentrations computed using the single ion abundance ratios (that is, SIAR-concentration) from the average of the two SIAR-concentrations (MS-average concentration) for extracted analyte standards were all within ±10% for cocaine (68% were within ±5%) and all were within ±5% (85% within ±3%) for benzoylecgonine.

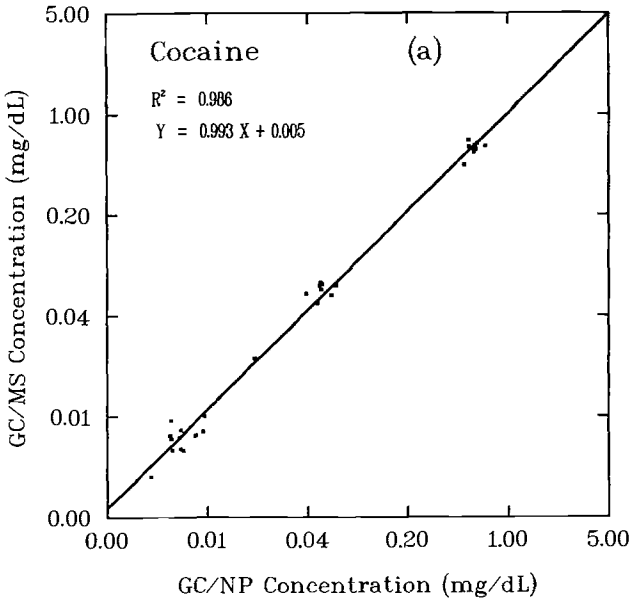
Thus the concentrations of cocaine and benzoylecgonine determined in spiked postmortem bloods by the GC/MS/SIM method using diazopropane were comparable to those by a GC/NP method using diazomethane.

Extractions of Forensic Samples

Fourteen extractions of forensic toxicology cases were performed over eleven months. Cocaine and benzoylecgonine were found in 112 and 180 samples, respectively. A total of 83% of these samples were obtained postmortem.

All cocaine and benzoylecgonine standards (N = 42 each) in the forensic assays had deviations in SIAR-concentrations from their average within ±5% with two exceptions, one at −7% for cocaine, and one at +9% for benzoylecgonine.

For the extracted analyte standards, the coefficient of variation (CV) was approximately 3% for all cocaine standards and the two lowest benzoylecgonine standards (Table 5). The consistency of the CV, in contrast to the standard deviation (σ), supports the use of a log-log model for linear regression, in contrast to the linear-linear model [*35*]. The



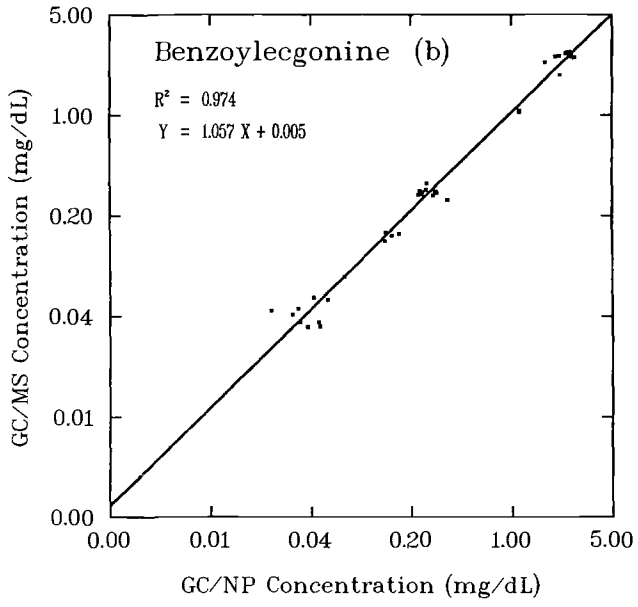


FIG. 2—*Concentration of* (a) *cocaine and* (b) *benzoylecgonine determined in postmortem blood using the GC/MS* (Y) *and GC/NP* (X) *methods of analysis in this work. The lines shown are computed from a linear regression.*

TABLE 5—*Variability in concentration of extracted cocaine and benzoylecgonine standards from blood in fourteen assays over eleven months.*

Cocaine			Benzoylecgonine		
Concentration mg/dL	%CV	Range (σ) min, max	Concentration mg/dL	%CV	Range (σ) min, max
5.0 E-3	2.5	−1.5, +1.2	2.0 E-2	2.8	−1.3, +1.6
5.0 E-2	3.2	−1.1, +1.7	2.0 E-1	2.8	−1.7, +1.4
5.0 E-1	3.1	−2.4, +1.4	2.0	7.9	−1.7, +1.5

σ is the standard deviation.

relative range of variation in all standard concentrations for both analytes (N = 84) were consistent, all being within $\pm 1.7\sigma$, with one exception at -2.4σ.

Table 6 indicates the percentage of cases with analyte concentrations in ranges of deviation of SIAR-concentrations from MS-averaged concentrations found in the fourteen assays. In the concentration range of the analyte standards used, 94% and 80% of cocaine and benzoylecgonine cases respectively had deviations within ±10% of the MS-averaged concentration.

For the cocaine cases, the largest relative deviation in SIAR-concentrations, within the standard range, occurred with findings of 0.062 and 0.079 mg/dL using m/z 272 and 303 respectively. At lower (extrapolated) concentrations of cocaine, five cases (all with poor signals) out of 27 cases had deviations greater than 30%, which corresponded to concentration differences from 3 to 22 ng/mL. The highest absolute deviations of those five cases were found with extract volumes of 200 µL, while smaller relative deviations were found with volumes of 20 µL. At higher (extrapolated) concentrations of cocaine, all cases less than 5 mg/dL (N = 6) had deviations within ±10%. In the extremely high range, only one case involving a stomach content extract had a deviation greater than 10%, the SIAR-concentrations being 5.0 and 6.9 mg/dL using m/z 272 and 303 respectively.

For benzoylecgonine cases, discernible interferences were originally encountered in some poor-quality postmortem bloods for m/z 272 in 6% of cases with concentrations

TABLE 6—*Variability in cocaine and benzoylecgonine SIAR-concentrations from MS-averaged concentration in forensic cases.*

		Percent Cases with Concentration				
Concentration (mg/dL)	Total Number	±5%	±10%	±25%	±35%	Interference
Cocaine						
0.005 to 0.6	49	61	94	100	100	nd
>0.001 and <0.005	26	35	42	88	96	nd
>0.6	11	36	73	91	100	nd
≤0.001	27	15	33	63	81	nd
Benzoylecgonine						
0.02 to 2.0	126	57	80	94	95	6
>0.004 and <0.02	32	50	69	81	94	6
>2.0	4	25	75	100	100	nd
≤0.004	18	22	28	44	72	28

within the standard range. In those cases, the chromatographic peak was noticeably increased in retention time, and caused a higher SIAR-concentration using m/z 272. The proportion of cases affected by this single interference was reduced by using a longer chromatographic column (30 m instead of 15 m) with a thinner film thickness (1 μm instead of 2.5 μm). At lower (extrapolated) concentrations of benzoylecgonine, any deviations greater than 30% could be assigned to the interference with m/z 272.

In trace level confirmations for cases where analyte concentrations are less than the lowest standard but significantly different (10σ) from the blank, both case and blank (internal standard spiked into blood) extracts were concentrated to a final toluene volume of 20 μL in nine of the fourteen assays. The lowest cocaine concentrations, estimated by extrapolating, averaged 2.5 ± 0.4 ng/mL (N = 25), with a range of deviation from -2.4σ to $+2.1\sigma$. The lowest benzoylecgonine concentrations averaged 19.6 ± 2.3 ng/mL (N = 16), with a range of deviation from -1.5σ to $+2.3\sigma$. These two concentrations correspond to 0.48% ± 0.08 and 1.0% ± 0.1 respectively of the concentrations of deuterated cocaine and benzoylecgonine internal standards that were used in the extractions. These percentages are in excellent agreement with the previously determined amounts of nondeuterated (analyte) forms in the deuterated internal standards. This quality of agreement implies that lower analyte concentrations can be reliably estimated, however we would report cocaine and benzoylecgonine concentrations of statistical significance less than the lowest extracted standard as "traces."

One group of cases (N = 13) were re-analyzed. Eight of the thirteen samples contained cocaine while all contained benzoylecgonine. With some notable exceptions, all cocaine (N = 7) and benzoylecgonine (N = 12) determinations had deviations in SIAR-concentrations from the average of the two extractions within ±10% (71% and 92% respectively of these cases were within ±5%). This assay included one case with stomach contents that had 1.3 mg/dL of cocaine. The cocaine case of exception was a sample without preservative in which a concentration of 0.005 mg/dL was not detected in the second extraction 10 days later. The benzoylecgonine case of exception involved an interference with m/z 272 that increased that SIAR-concentration by 0.03 mg/dL in 10 days. The other SIAR-concentration varied by less than 5%. Another benzoylecgonine case with an interference had an unchanged concentration over that time.

Thus using two ion abundance ratios, the concentrations of cocaine and benzoylecgonine determined in both (a) mainly postmortem bloods from forensic toxicology investigations and (b) spiked blood standards were in excellent agreement.

Conclusion

The method of determination of cocaine and benzoylecgonine by GC/MS/SIM developed using (a) rotation of an XAD-2-blood mixture and (b) derivatization by diazopropane was found routinely reliable in application to 180 forensic toxicology investigations. Standard curves using logarithmic-transformed data allowed determinations of cocaine and benzoylecgonine from postmortem blood over a wide concentration range (<0.005–5 mg/dL) and to a low limit of detection (0.00025 mg/dL).

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