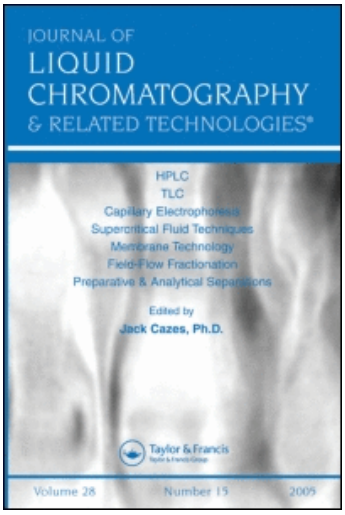

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Comparison of High Performance Liquid Chromatographic and GAS Chromatographic Analyses of Cocaine in Coca Leaves

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COMPARISON OF HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC AND GAS CHROMATOGRAPHIC ANALYSES OF COCAINE IN COCA LEAVES

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ABSTRACT

A high-performance liquid chromatographic (HPLC) method and a capillary gas chromatographic (GC) method are compared for the quantitative analysis of cocaine in Erythroxylum coca leaves. Cocaine was extracted by refluxing air-dried coca leaves (0.05-4.0 g) with 95% ethanol (30-100 mL) for 15 min. Aliquots of the crude ethanolic leaf extracts were removed for analysis while the remaining portions of the extracts were taken through a cleanup procedure. The mean cocaine contents determined by HPLC and GC methods in six replicated crude leaf (1.0 g) extracts were 0.56% and 0.54%, respectively. However, the cocaine contents in the cleanup extracts were 0.43% (HPLC) and 0.42% (GC). GC analysis gave better resolution of cocaine and related alkaloids, but HPLC analysis of cocaine was simpler and faster.

INTRODUCTION

Advanced chromatographic techniques are being used increasingly by analysts in the fields of forensic

chemistry, medicine, pharmacology and natural products to analyze cocaine and other alkaloids in plants and illicit coca products. The volume of studies in the literature on the analyses of cocaine and other drugs of abuse using GC/MS (gas chromatography/mass spectrometry) spectral analysis has expanded rapidly; however, fewer studies have appeared on the analyses of cocaine and related alkaloids in coca plants. Turner et al. (1) reported the first GC method for the routine analysis of cocaine and related alkaloids in coca leaves. Later, Turner et al. (2) and Rivier (3) independently reported on the GC/MS analysis of cocaine and cis- and trans-cinnamoylcocaine. Rivier analyzed the crude ethanolic leaf extract, whereas Turner carried the crude extract through an exhaustive cleanup procedure before analysis. More recently, LeBelle et al. (4) using HPLC and GC-MS identified and determined the amounts of norcocaine and cocaine in coca leaves and illicit cocaine samples. Lydon et al. (5) reported a capillary GC method for analyzing cocaine in crude coca leaf extracts. The present paper compares the accuracy, precision and sensitivity of HPLC and capillary GC methods that are used for the determination of cocaine in coca leaves.

EXPERIMENTAL SECTION

Chemicals: Cocaine hydrochloride, 99.9% purity, was obtained from Merck Chemical Division (Rahway,NJ). Absolute ethanol (The Warner Graham Co., Cockeysville, MD), chloroform (EM Science, Cherry Hill, NJ), acetonitrile (EM Science), triethylamine (Aldrich Chemical Co., Milwaukee, WI), tetrahydrofuran (EM Science) and methanol (EM Science) were standard reagent grade. The internal standard, 4-androstene-3,17-dione, was obtained from Aldrich Chemical Co. (Milwaukee, WI).

Plant Material: Erythroxylum coca leaves were collected from plants, which were grown from seeds under greenhouse conditions as described by Johnson and Elsohly (6).

Extraction: Air-dried leaves (0.05-4.0 g) were refluxed with 95% ethanol (30-100 ml) for 15 min as described earlier by Turner et al. (1). The extract was passed through Whatman No. 4 filter paper to remove all particulate matter. An aliquot (1 ml) of the filtrate was combined with 1-ml aliquot of 2000 ug/ml 4-androstene-3,17-dione and then was analyzed by GC. Another 1-ml aliquot of the filtrate without the internal standard was subsequently analyzed by HPLC. These samples were categorized as crude E. coca extracts in this investigation. The remaining filtrate was rotary evaporated at 55°C under vacuum in preparation for the cleanup procedure. The green residue was dissolved in 20 ml of chloroform and then transferred to a separatory funnel. The following steps were minor modifications of the original procedure(1). A 20-ml volume of 1.5% aqueous citric acid (w/v) was combined with the residue and shaken thoroughly in the vessel. The aqueous layer was collected and adjusted to about pH 7.0 with 10 ml 1.2 M sodium bicarbonate. A final pH of 8.8 was obtained more quickly with additions of 1.0 M sodium hydroxide which resulted in a smaller final volume near 50 ml. Cocaine was partitioned into 20 ml of chloroform, which was subsequently collected and dried over granular sodium sulfate. The solvent was removed by roto-evaporation and the cocaine residue was dissolved in 5 ml of methanol. An aliquot (1 ml) of the final methanolic solution was combined with 1-ml aliquot of the internal standard for GC analysis as described earlier. Similarly, an aliquot without the internal standard was analyzed by HPLC.

Gas Chromatography: GC analysis of cocaine was performed with a modified method of Lydon et al. (5) by using a

Hewlett-Packard Model 5890 instrument (Avondale,PA) equipped with a flame ionization detector, and a H-P 2934A integrator. A dimethylsilicone capillary column [DB-5, 15 m x 0.25 mm (i.d.), 0.25 μm film thickness] was obtained from J & W Scientific (Rancho Cordova, CA). Helium (99.95% purity) at approximately 60 cm/s linear velocity was used as the carrier gas. The injection and detector temperatures were 250 and 285°C, respectively. The temperature program consisted of an initial oven temperature of 70°C followed by a ramp rate of 25°C/min for 8 min and held at 280°C for 1 min. The HP 7673A auto-sampler was used to make 1-μl injections in a spitless mode. The HP 5890A GC Chemstation data system processed the chromatographic data such as peak areas, retention times of the various peaks and the amount of cocaine in each chromatogram. The quantitation of cocaine was performed with the internal standard, 4-androstene-3,17-dione. The calibration curve for cocaine was determined by varying the concentrations (20-1000 μg/ml) of cocaine dissolved in methanol containing 1000 μg/ml 4-androstene-3,17-dione.

High-Performance Liquid Chromatography: HPLC analysis of cocaine was performed with a modified method of LeBelle et al. (4) using a Spectra-Physics Model 8800 ternary pump (San Jose, CA) equipped with a Rheodyne Model 7125 valve fitted with a 5-μl loop. Cocaine was detected with a variable wavelength detector (Spectra-Physics, Model SP8440) operated at 240 nm. Chromatograms were recorded and processed on a Beckman Integrator (Model 427). Two sets of conditions were used for the HPLC determinations of cocaine: a C-8 reversed-phase column [12.5 cm x 4.6 mm (i.d.), 5-μm particles; R. E. Gourley Co., Laurel, MD] and a mobile phase consisting of acetonitrile: 1.0% triethylamine pH=4 (40:60, v/v) delivered isocratically at 1.2 ml/min; and a C-18 reversed-phase column prepared

identically to the C-8 column described above and a mobile phase of acetonitrile:tetrahydrofuran:0.1% v/v triethylamine in water (110:15:75, v/v/v) delivered isocratically at 1 ml/min. Calibration curve was determined with external standards of cocaine varying in concentrations from 25 to 200 μg/ml.

Statistical Analysis: HPLC and GC data were analyzed by the Axum statistical program (TriMetrix, Seattle, WA) to determine the 95% confidence intervals. Standard linear regression was used to determine the slope and intercept from a minimum of six replicates per data point.

RESULTS AND DISCUSSION

GC Analysis: Figure 1 shows the GC chromatograms of (a) 250 ng cocaine standard, (b) cleanup leaf extract and (c) crude leaf extract. Cocaine and related alkaloids in the extracts were resolved well on this DB-5 capillary column. The retention time for cocaine was about 7.0 min. Although cocaine and many of the tropane alkaloids in *E. coca* leaf extracts were isolated and identified in earlier investigations (1,2,3), confirmations of cocaine and several alkaloids were made by GC/MS spectral analysis during the present investigation (7). Some additional tropane alkaloids were detected in the extracts and they will be reported in a later article. A comparison of the chromatograms for crude and cleanup extracts reveals that there was no observable difference in the separation of cocaine in the two analyses. These results are in agreement with the capillary GC study of cocaine by Lydon, et al. (5) and the GC/MS study by Rivier (3), who first demonstrated that cocaine in crude leaf extracts could be resolved on capillary column without further purification. Analysis of crude leaf extracts was further validated in the present study by

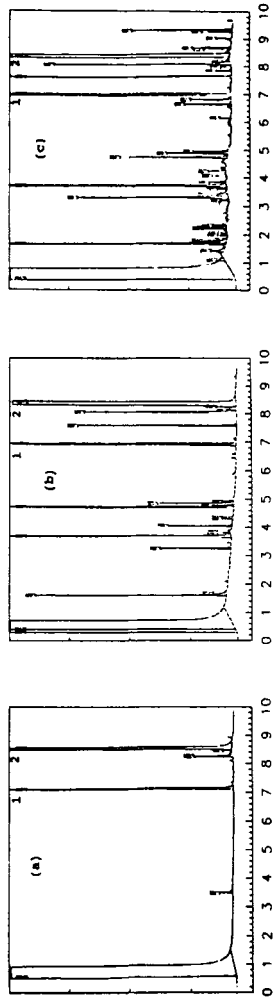

Figure 1. GC chromatograms of cocaine: (a) 250 ng cocaine standard; (b) a cleanup E. coca leaf extract; (c) a crude E. coca leaf extract. Peak 1 is cocaine. Peak 2 is 4-androstene-3,17-dione.

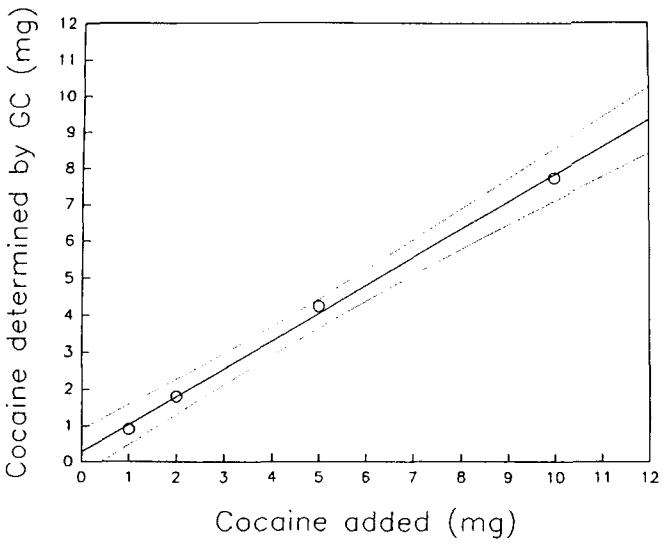

Figure 2. Standard cocaine added to E. coca leaf extracts at 1, 2, 5 and 10 mg, respectively, and determined by capillary GC. The amount of cocaine in the 1-ml aliquot of the crude leaf extract was subtracted from the final cocaine content of the fortified sample. The dotted lines indicate a 95% confidence interval about the regression line. Regression statistics: slope = 0.755, y-intercept = 0.283, correlation coefficient (r) = 0.999.

fortifying ethanolic extracts with standard cocaine ranging from 1.0 to 10 mg and then analyzing the extracts by capillary GC. The results in Figure 2 show that linearity was obtained within this experimental range and that the peak of interest was cocaine.

The calibration curve for cocaine was run in a similar manner using the internal standard, 4-androstene-3,17-dione, as reported earlier (1). The response factor (slope b) and the correlation coefficient (r) were 0.66 and 0.99, respectively. The minimum detection limit was

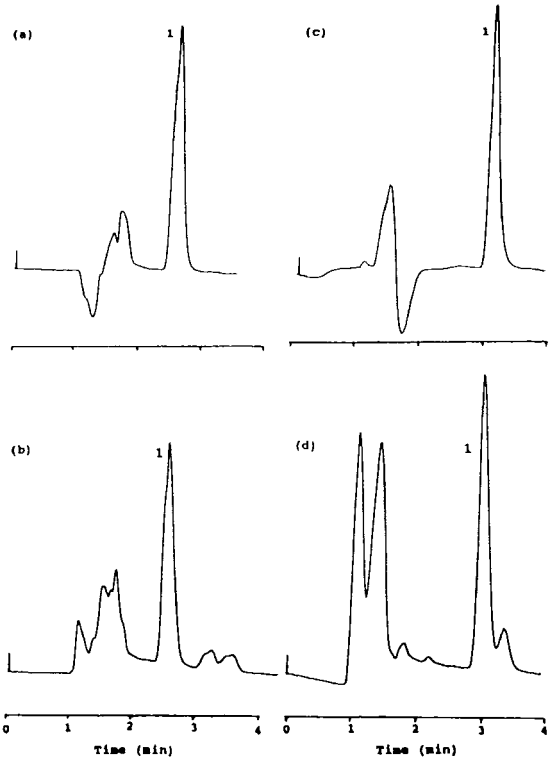

Figure 3. HPLC chromatograms of (a,c) standard cocaine (250 ng) and (b,d) E. coca crude leaf extracts. Chromatograms (a,b) were run on a 12.5 cm x 4.6 mm C-8 column with a mobile phase of acetonitrile: 1.0% triethylamine pH = 4.0 (40:60; v/v) delivered at 1.2 ml/min and chromatograms (c,d) were run on a 12.5 cm x 4.6 mm C-18 column with a mobile phase of acetonitrile: tetrahydrofuran: 0.1% triethylamine (110:15:75; v/v/v) delivered at 1.0 ml/min. Peak 1 is cocaine.

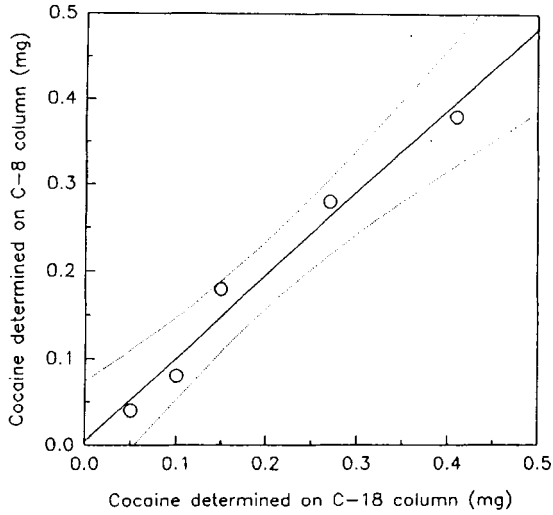

Figure 4. Equivalency of C-8 and C-18 columns used for the HPLC determination of cocaine in E. coca leaf extracts fortified with 0.05, 0.10, 0.20, 0.30 and 0.40 mg cocaine. The dotted lines indicate a 95% confidence interval about the regression line. Regression statistics: slope = 1.020, y-intercept = 0.001, correlation coefficient (r) = 0.973.

2.0 ng of cocaine (S/N>3). Turner et al. (1) reported 10 ng as the detectable limit of cocaine using FID and a packed GC column.

HPLC Analysis: Chromatograms in Figure 3 show the isocratic separations of 500 ng of a cocaine standard (a) on C-8 column and (b) on C-18 column. The graph in Figure 4 shows that equivalent results were obtained from these two columns as revealed by the slope and intercept not being statistically different from 1 and 0, respectively. The response of the UV detector (240 nm) was linear over the 25-200 ug/ml cocaine concentration range. The minimum detection limit was 5.0 ng of cocaine

Table I. HPLC and GC analyses of cocaine in E. coca leaves.

Avg. Dry Wt.	Nature of Extract	Cocaine Content (%)[a] HPLC[b] Mean ± S.D. (C.V.%)	GC[c] Mean ± S.D. (C.V.%)
0.05 g	Crude	0.53 ± 0.110 (20.75%)	0.61 ± 0.110 (18.03%)
	Cleanup	0.41 ± 0.120 (29.27%)	0.47 ± 0.100 (21.28%)
0.10 g	Crude	0.59 ± 0.100 (16.95%)	0.55 ± 0.104 (18.91%)
	Cleanup	0.51 ± 0.080 (15.69%)	0.49 ± 0.090 (18.37%)
0.50 g	Crude	0.52 ± 0.052 (10.00%)	0.52 ± 0.054 (10.40%)
	Cleanup	0.42 ± 0.060 (14.29%)	0.40 ± 0.049 (12.19%)
1.00 g	Crude	0.56 ± 0.037 (6.61%)	0.54 ± 0.027 (5.00%)
	Cleanup	0.35 ± 0.048 (13.71%)	0.35 ± 0.044 (12.57%)
2.00 g	Crude	0.50 ± 0.033 (6.60%)	0.55 ± 0.059 (10.73%)
	Cleanup	0.41 ± 0.028 (6.83%)	0.38 ± 0.028 (7.78%)
3.00 g	Crude	0.53 ± 0.008 (1.51%)	0.56 ± 0.030 (5.36%)
	Cleanup	0.50 ± 0.047 (9.40%)	0.39 ± 0.030 (7.69%)
4.00 g	Crude	0.61 ± 0.024 (3.93%)	0.59 ± 0.048 (8.14%)
	Cleanup	0.50 ± 0.058 (11.60%)	0.48 ± 0.029 (6.04%)

[a]The results are the mean of six or more replicates ± standard deviation (coefficient of variation,%). [b]C-8 reversed-phase column. [c]DB-5 capillary column.

(S/N>3). A comparison between HPLC chromatogram (2c) and GC chromatogram (1b) reveals that fewer alkaloids were detected by HPLC and that the resolution of cocaine on HPLC columns was poorer than on capillary GC column.

Analyses of Leaves: Table I gives the cocaine content (%) of air-dried E. coca leaves (0.05- 4.0 g) measured by GC and HPLC methods in crude and cleanup leaf extracts. The cocaine (0.50 to 0.61 %) in the crude leaf extracts was in fairly close agreement for the GC and HPLC analyses. Turner et al. (1) reported 0.60% cocaine content (mean) for three replicated E. coca leaf extracts that were taken through the cleanup procedure. Cocaine

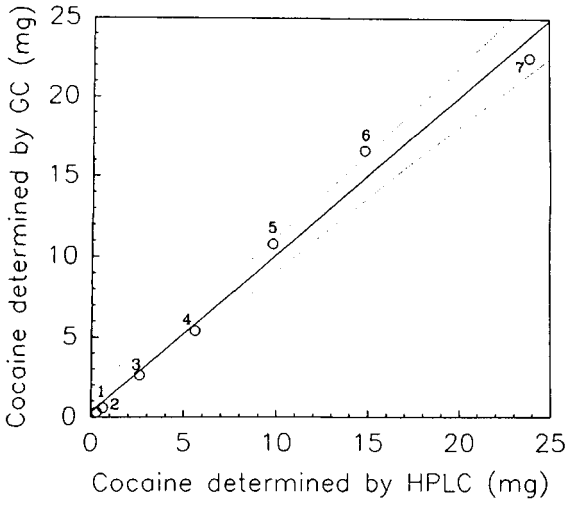

Figure 5. Comparison of GC and HPLC methods for determining the amounts of cocaine in E. coca leaf extracts. The amounts of cocaine were calculated from the cocaine contents for samples 1-7 in Table I. The dotted lines indicate a 95% confidence interval about the regression line. Regression statistics: slope = 1.005, y-intercept = -0.209, correlation coefficient (r) = 0.993.

content in the cleanup extracts in the present study was nearly 20% lower than that found in the crude extracts, presumably due to losses in the liquid-liquid partitioning of cocaine. The results in Table I also show that the precision of the analyses decreases with the smaller sample size as revealed by the larger coefficients of variation (%). However, linearity was obtained as shown in Figure 5 where the amounts of cocaine measured by the GC and HPLC methods were plotted against each other for the seven determinations (Table I).

Table II. Summary of HPLC and GC analyses of cocaine.

	HPLC[a]	GC[b]
1. Analysis Time (min)		
Sample preparation (crude)	25	25
" " (cleanup)	55	55
Instrument run time	5	15
Total analysis time (crude)	30	40
" " " (cleanup)	60	70
2. Detection Limit of Cocaine (ng)	5	2
3. Retention Time (min.)	2.5	7
4. Precision (C.V.%)		
1.0-g Sample (crude)	6.61	5.00
1.0-g " (cleanup)	13.71	12.75
0.05-g " (crude)	20.75	18.00
0.05-g " (cleanup)	29.27	21.28
5. Resolution (Cocaine)	Fair	Good
6. Resolution (Related Alkaloids)	Poor	Good

[a]C-8 reversed-phase column. [b]DB-5 capillary column.

A summary of the overall performance of the HPLC and GC methods is given in Table II.

CONCLUSIONS

Although capillary GC and HPLC provide fairly equivalent determinations of cocaine in coca leaves, HPLC is our method of choice because of the advantages of being simpler for sample preparation and faster in run time. Also, analysis of cocaine in crude leaf extracts in comparison with cleanup extracts for cocaine was faster and more efficient and the extracts did not affect the life and efficiency of the columns after hundreds of determinations.

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