TECHNICAL NOTE

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New Application for the Quantitation of Cocaine Base by Gas Chromatography

REFERENCE: Krueger, S. T., **"New Application for the Quantitation of Cocaine Base by Gas Chromatography,"** *Journal of Forensic Sciences*, JFSCA, Vol. 39, No. 1, January 1994, pp. 177–185.

ABSTRACT: The analysis of cocaine base is a major part of the forensic drug chemist's workload. Simplifying quantitative methodology can assist the drug chemist. Methods currently employed for quantitation of cocaine base utilize either an internal standard or a direct comparison. This gas chromatographic method for the quantitation of cocaine base employs a non-controlled reference standard and linear transformations. As a result, cocaine base can be quantitated by a single injection of a solution containing the analyte and a non-controlled reference standard of known concentration. Use of this methodology would minimize time expenditures, lower exposure to hazardous solvents and reduce expense of procuring difficult-to-obtain controlled reference standards while limiting detector response variations that can occur with multiple injections.

KEYWORDS: forensic science, quantitation, cocaine base, linear transformations, chromatographic analysis

The quantitation of cocaine base is an integral part of the work carried out in the Drug Enforcement Administration's Mid-Atlantic laboratory. Currently there are many different methodologies and instrumentation in use for the quantitation of controlled substances such as gas chromatography [*1*], high pressure liquid chromatography [*2–5*], gas chromatography-mass spectroscopy [*6–9*] and capillary gas chromatography [*10,11*]. In addition, other laboratories use other detectors interfaced with the gas chromatograph such as chemical ionization selected ion monitoring [*12,13*], nitrogen phosphorus [*14,15*], and flame ionization. The internal standard [*16*] or direct comparison methodologies are most commonly used for quantitation. The methodology discussed here is one of indirect comparison. A single injection is used to achieve the same purpose with accuracy equivalent to the methodologies mentioned above. This method was initially designed to give an estimate of the purity of cocaine base street samples. It has proven to be as accurate and reproducible as any method currently employed to quantitate cocaine base.

Received for publication 31 Dec. 1992; revised manuscript received 8 March 1993; accepted for publication 24 May 1993.

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A portion of this paper was given as an oral presentation at the 44th annual American Academy of Forensic Science meeting in New Orleans, LA, February 1992.

Materials and Methods

Apparatus

All gas chromatography was carried out on a Hewlett-Packard 5890 equipped with a flame ionization detector (FID) and a Hewlett-Packard 3393A model integrator. Cocaine eluted after docosane and preceded scopolamine and tetracosane for all columns used (HP-5 (5% phenyl methyl silicone), HP-1 (dimethyl silicone), and OV-1 [packed dimethyl silicone]) and all temperature ranges (205° to 250°C). The capillary columns were 6 M by 0.20 mm inside diameter, coiled capillary columns with 0.32 μm film thickness. The packed column was 6 feet in length with one-eighth inch diameter, solid support of chromosorb W-HP, mesh size 80/100 and 10% OV-1 liquid phase. The injector and manifold temperatures were 270°C and 280°C respectively. The carrier gas was helium, at a total flow rate of ca. 30 mL/min and a split ratio of 100:1.

Reagents

Standard cocaine base was supplied by the DEA Special Testing and Research Laboratory in McLean, Virginia, and verified to meet USP standards of 99% purity.

Stock Solutions

Tetracosane internal standard solution (0.5 mg/mL) was prepared by dissolving 2.00 g of tetracosane in 4 L of chloroform.

Docosane internal standard solution (0.3 mg/mL) was prepared by dissolving 300 mg of docosane in 1.00 L of chloroform.

Eicosane internal standard solution (0.4 mg/mL) was prepared by dissolving 400 mg of eicosane in 1.00 L of chloroform.

Cocaine standard solutions were prepared by dissolving 250 mg of cocaine base in 250 mL chloroform with subsequent dilutions. These solutions were used for the preparation of the calibration curves.

Preparation of Calibration Curves

The calibration curve was prepared with the x coordinate as concentration and the y coordinate as response. The response plotted is the ratio relative to an internal standard: response A/response B, where A is the compound of study and B is the internal standard of known concentration. Five standard solutions of A (concentration ca. 1.0, 0.8, 0.4, 0.2, and 0.1 mg/mL) were injected 5 to 10 times each (all with known concentration of B). The linearity of these species was not proven beyond 1.0 mg/mL, however, samples have been run at more than 3 mg/mL resulting in less than 5% error. The integrator provided a number of counts (response) for A and B, and the ratio was calculated (A/B). This ratio and the corresponding concentration of A is plotted as a straight line, the slope of which is obtained by linear regression of all five data points.

The linearity studies and corresponding calibration curves for cocaine base and tetracosane on a HP-5 capillary column are shown in Fig. 1. The slopes and regression data of these and other compounds studied are listed in Table 1 (eicosane, docosane and tetracosane were all used as internal standards, and found to be equally suitable). The response from each injection was calculated as a ratio relative to the internal standard of known concentration to obtain an xy data point. This ratio has proven to be stable over an extended period of time and does not change appreciably from one flame ionization detector to another or after preventive maintenance. The hydrogen flow does

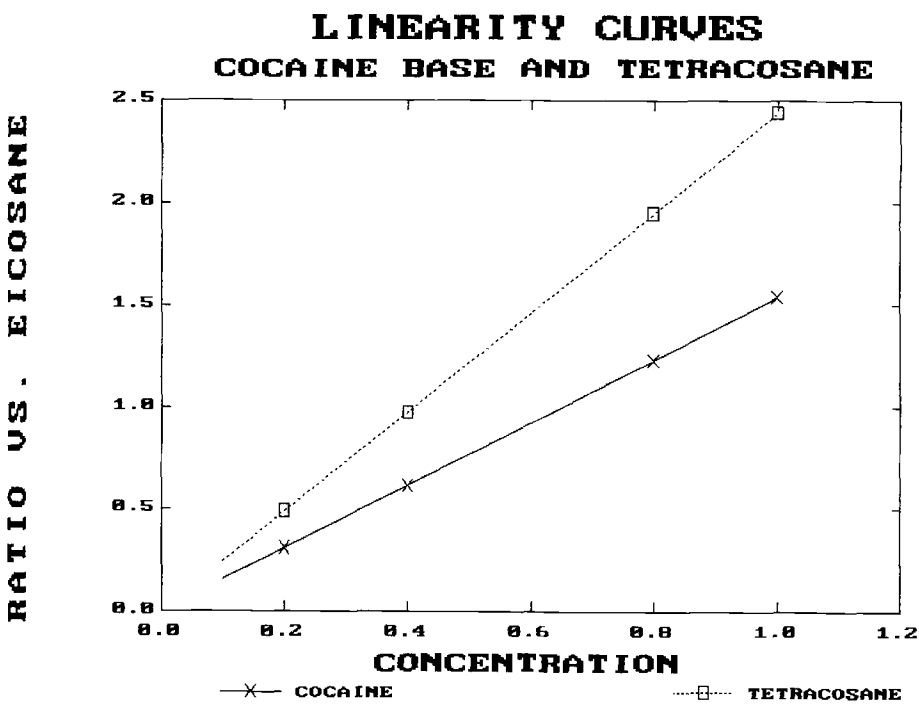

FIG. 1—*Calibration curves for cocaine base and tetracosane.*

TABLE 1—*Regression data from linearity studies.*

	Slope	Y-Int	Correlation	Equation
Docosane vs. Tetracosane (0.5 mg/mL)				
HP1	1.8336	+0.0097	0.9998	$Y = 1.8336X + 0.0097$
HP5	1.7858	+0.0026	0.9999	$Y = 1.7858X + 0.0026$
OV1	1.8009	+0.0071	0.9980	$Y = 1.8009X + 0.0071$
Tetracosane vs. Eicosane (0.4 mg/mL)				
HP1	2.438	+0.004	1.0000	$Y = 2.438X + 0.004$
Tetracosane vs. Docosane (0.4 mg/mL)				
HP1	2.3816	−0.0020	0.9999	$Y = 2.3816X - 0.0020$
HP5	2.5680	+0.0073	0.9999	$Y = 2.5680X + 0.0073$
Cocaine base vs. Eicosane (0.4 mg/mL)				
HP1	1.54	+0.02	1.0000	$Y = 1.54X + 0.02$
Cocaine base vs. Docosane (0.4 mg/mL)				
HP1	Cocaine and Docosane peaks overlap			
HP5	1.646	−0.0124	1.0000	$Y = 1.646X - 0.0124$
Cocaine base vs. Tetracosane (0.5 mg/mL)				
HP1	1.2047	−0.0026	0.9995	$Y = 1.2047X - 0.0026$
HP5	1.2031	−0.0159	0.9998	$Y = 1.2031X - 0.0159$
OV1	1.2392	−0.0203	0.9995	$Y = 1.2392X - 0.0203$
Scopolamine vs. Tetracosane (0.5 mg/mL)				
HP1	1.2955	−0.0033	0.9997	$Y = 1.2955X - 0.0033$
HP5	1.2492	−0.0482	0.9977	$Y = 1.2492X - 0.0482$
OV1	1.3649	−0.0281	0.9995	$Y = 1.3649X - 0.0281$

change the current in the detector, however, the ratio remains constant. If a need to verify the consistency of the ratios is demonstrated then a standard solution should be run. The inherent simplicity discovered in this method is that the parameters of the GC instrument (split ratio, oven temperature, gas flow rate, injection temperature, etc.) do not affect the ratios produced by the linearity curves. Therefore, response curves do not have to be developed repeatedly. They have proven to be stable for over two years in this laboratory.

Sample Procedure

The samples were homogenized by grinding in a mortar. A weighed portion (approximately 50 mg) of the powder was added to a 25 mL volumetric flask. To this flask a weighed amount of tetracosane (approximately 15 mg) was added and diluted to volume with chloroform. The solution was filtered, if insoluble materials were present, and approximately 3 μL were injected onto the GC column. The detectors response obtained from the chromatogram (showing no occurrence of overload under these conditions) corresponds to cocaine, tetracosane, and any other compounds present in the sample. From the response and the known concentrations, the quantitation of cocaine base can be obtained via the following formula:

$$\frac{y}{y'} = \frac{mX + b}{m'X' + b'}$$

where:

y and y' = response of cocaine and standard
m and m' = slopes of cocaine and standard
X and X' = concentration of cocaine and standard
b and b' = y-intercept of cocaine and standard

All data except for the concentration of cocaine (X) is available after the GC chromatogram is produced. The equation is simplified with the elimination of the y-intercepts. In theory, a concentration of zero should yield a response of zero. Although the y-intercepts (Table 1) were measured to be unequal to zero, in no case did the intercept exceed 1.3% of the 'm' value (well within the accuracy of the procedure). This leaves:

$$\frac{y}{y'} = \frac{mX}{m'X'}$$

Solved for X (concentration of cocaine):

$$X = \frac{ym'X'}{y'm}$$

Once X is calculated, the percent cocaine base can be obtained using:

$$\% = \frac{X(\text{dilution volume})}{(\text{sample weight})} 100$$

Further, if the sample is made up in one container, the dilution of sample and standard are equal and the concentration term X and X' (mg/mL) is simplified to mg.

Known Samples

A set of nine known cocaine base samples (ranging from 10 to 100%) were made containing various adulterants (benzocaine, procaine, and lidocaine). These were used to determine the accuracy of this method. Approximately 50 mg of sample was weighed and added to a 25 mL volumetric flask, along with approximately 15 mg of tetracosane. The sample was diluted to volume with chloroform, and an injection of 3 μL performed. Results of these analyses are shown in Table 2.

Results and Discussion

The use of a hydrocarbon (eicosane, docosane, and tetracosane) as an "internal" standard was justified with the following considerations: 1) these did not co-elute with any of the common adulterants seen in cocaine base street samples; 2) the pure standards are inexpensive and readily available through any chemical supply company; 3) they behave more uniformly in the FID detector, and 4) the hydrocarbon is relatively more stable in solution than most of the drugs or compounds chemically similar to cocaine.

Hydrocarbons such as tetracosane are not chemically similar to cocaine, and therefore would not behave identically or mimic the behavior of cocaine base in the FID. This drawback is minimized by linearity studies. Once a compound is proven to be linear (response from detector vs. concentration) within a specific concentration range it can be used as a standard against another compound whose response is linear over the same range, provided also that it does not break down in the injector or column, and does not react with the species being quantitated.

Scopolamine ($C_{17}H_{21}NO_4$ the same chemical formula as cocaine) was initially chosen as the "internal" standard due to its chemical similarity and similar response factors in the FID to cocaine base. Scopolamine salts (HBr and HCl) have limited solubility in such solvents as chloroform, so the base was isolated via basic extraction with chloroform. It was found to be difficult to insure 100% extraction of the scopolamine. Linearity studies were carried out with scopolamine and are shown in Table 1. A 9:1 solvent

TABLE 2—*Percent error data for nine known samples run on an HP-1 capillary column.*

Sample	Cocaine concentration	Experimental percentage	Actual percentage	Percent error
1	0.288	10.5	10.4	0.9
	0.114	10.3	10.4	0.9
2	0.500	20.9	20.5	1.9
	0.250	20.6	20.5	0.4
3	0.720	29.9	29.0	3.1
	0.360	29.6	29.0	2.0
4	0.800	38.8	39.8	2.5
	0.400	38.3	39.8	3.7
5	1.080	52.5	50.8	3.3
	0.540	51.6	50.8	1.5
6	1.300	57.9	56.9	1.7
	0.650	57.2	56.9	0.5
7	1.000	80.1	77.1	3.8
	0.500	78.6	77.1	1.9
8	1.600	93.8	91.3	2.7
	0.800	92.6	91.3	1.4
9	1.600	104.2	100.0	4.2
	0.800	102.3	100.0	2.3

AVERAGE PERCENT ERROR = 2.1

mixture of chloroform:methanol suitably dissolved the scopolamine salts but the addition of methanol caused some decomposition. Given these two problems, the focus shifted to the hydrocarbons as possible "internal" standards.

Slopes of the linearity curves for hydrocarbons are larger than for cocaine base. This is consistent with the literature [*17*], which states that the response per unit concentration of a sample in a FID decreases with an increase in the number of nitrogen and oxygen atoms and increases with an increase in the number of carbon atoms.

Data from the preceding experiments shows good correlation between response and concentration for the capillary column (double concentration, response exactly doubles). This is not the case for the packed columns, where the response and concentration are not correlated (doubled concentration does not equal a doubled response). The absorbent material in the packed column likely holds back a certain amount of sample for each injection and upon doubling the concentration of the injection, the column would hold back the same amount of sample, yielding an inexact correlation and a larger response than expected. When examining the *y*-intercept data from Table 1, some conclusions can be drawn concerning this situation. The *y*-intercept (a small and negligible figure) gives a relative value to the positive *x*-intercept, which represents the amount of sample that can not be detected or that is held back by the column. The HP-5 and OV-1 columns consistently have larger *y*-intercepts (more sample is held back). A possible explanation is the OV-1 packed column holds back sample owing to an increased number of active sites, and the HP-5 capillary column holds back sample due to its increased polarity over the HP-1 capillary column.

This is illustrated graphically in Figs. 2 and 3. The experimental percentage is plotted against the actual percentage of the nine known samples for a HP-1 column and a HP-5 column. Note that at the higher percentages, the HP-1 column gives higher values than

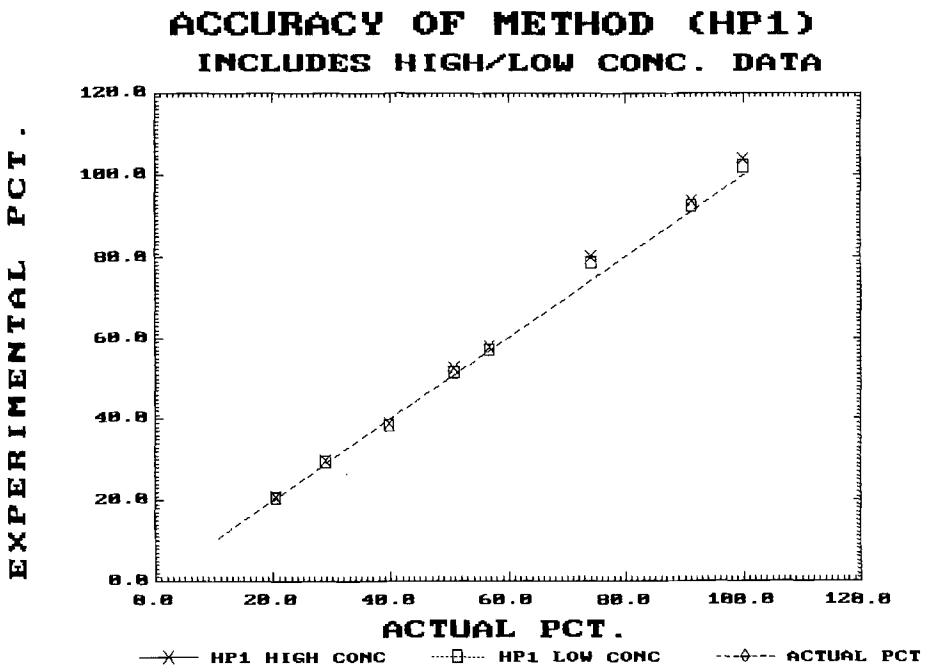

FIG. 2—*A plot of experimental percentage vs. actual percentage for the nine known samples using an HP-1 column.*

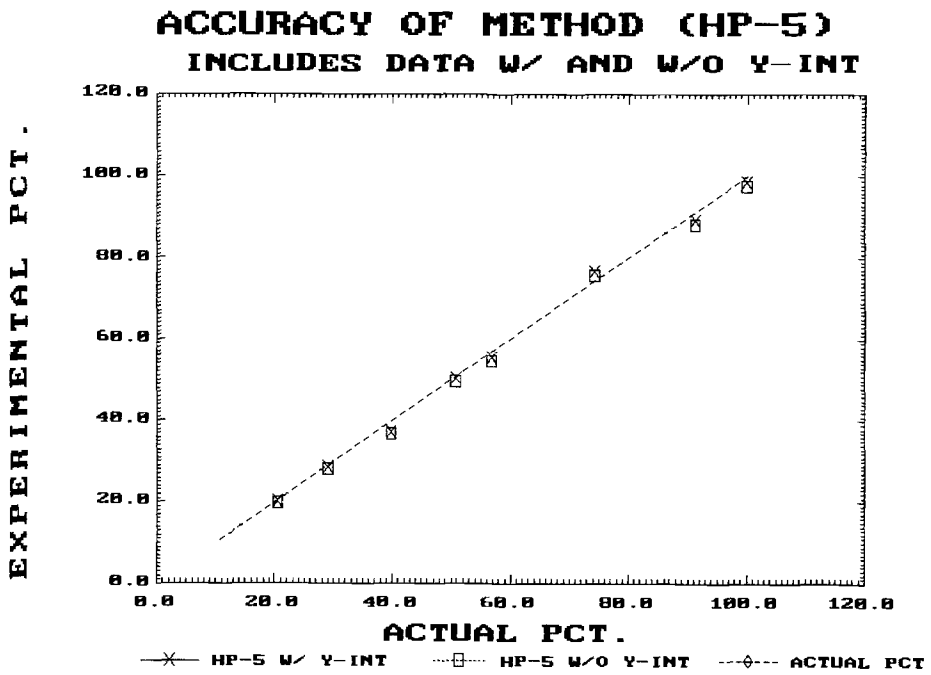

FIG. 3—*A plot of experimental percentage vs. actual percentage for the nine known samples using an HP-5 column.*

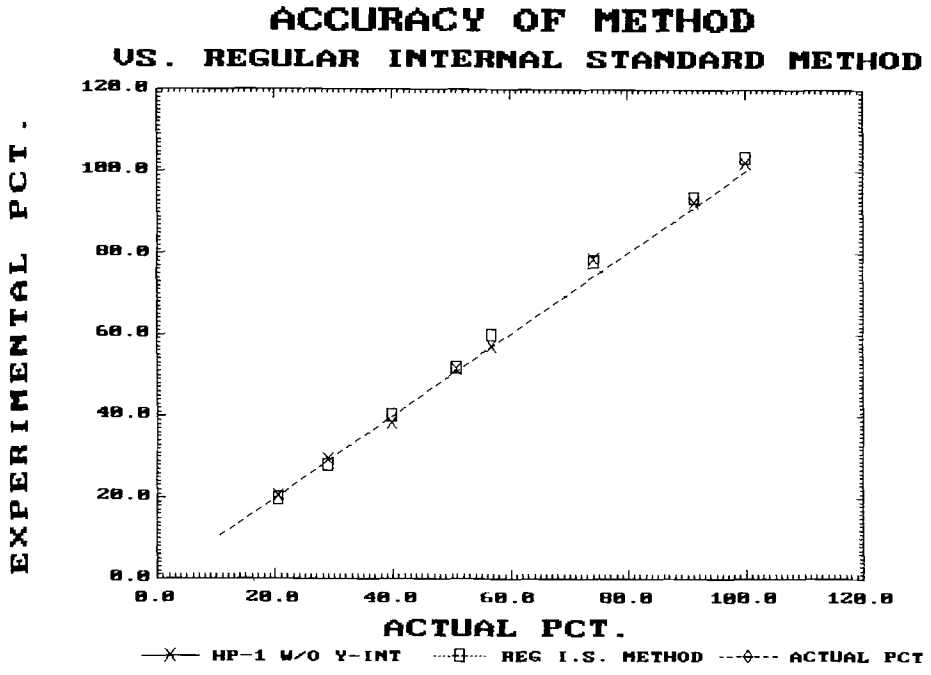

FIG. 4—*A plot of experimental percentage vs. actual percentage for the nine known samples using an HP-1 column and calculating the percent using the regular internal standard method.*

TABLE 3—*Percent error data for nine known samples run on an HP-5 capillary column.*

Sample	Cocaine concentration	Experimental percentage	Actual percentage	Percent error
1	0.100	10.0	10.4	3.8
2	0.230	19.8	20.5	3.4
3	0.350	28.1	29.0	3.1
4	0.400	37.2	39.8	6.5
5	0.520	50.3	50.8	1.0
6	0.630	55.4	56.9	2.6
7	0.500	76.6	77.1	0.6
8	0.780	89.1	91.3	2.4
9	0.800	98.8	100.0	1.2

AVERAGE PERCENT ERROR = 2.7

the actual, and the HP-5 column gives lower values (the HP-5 holding back more sample). Figure 4 shows the relationship of the actual percentage to the percentage obtained via the internal standard method on the HP-1 column of the nine known samples. Note also that the first of nine data points is not represented but corresponds to the left end of the straight line in Figs. 2, 3 and 4. These two graphs illustrate the validity and applicability of this new method of quantitating cocaine base.

Ideal Conditions

Table 3 shows the data of the nine known samples run on the HP-5 column. When comparing the percent errors and the concentrations from Tables 2 and 3, the HP-1 gives a 1.7% error when the concentration is kept below 0.800 mg/mL, in contrast to the HP-5 where the average percent error is 2.5%. However, when the concentration is higher than 0.800 mg/mL the percent error for the HP-1 column increases to 3.14. Two observations concerning optimal conditions are: 1) the concentration of the sample should be below 0.800 mg/mL and 2) a HP-1 capillary column is preferred to either a HP-5 capillary column or a OV-1 packed column.

Summary

Cocaine base currently is quantitated at the Mid-Atlantic Laboratory by a single level calibration using an internal standard method. This method employs the injection of a standard cocaine solution of a known concentration. The response and the known concentration provide a single data point and a calibration curve is drawn from the origin to that point. The resultant line is the single level calibration. This method assumes two things: 1) the calibration curve (line) passes through the origin; and 2) the slope of the line created by the single data point and the origin is the same as that of a true linearity study. In reality these calibration curves do not pass through the origin giving rise to inaccuracies in their slopes. If, however, the sample concentration approaches that of the standard this error approaches zero. The method described here does not make these assumptions, and has been demonstrated to be as accurate as any other method currently used in the quantitation of cocaine base.

The methodology described here was originally designed to enable the chemist to give a quick estimate as to the quantitation of cocaine base in a street sample. Results presented here show that under optimized conditions this method gives as accurate an answer as any other direct comparison or internal standard method. Therefore, the pos-

sibility now exists for the quantitation of cocaine base without the use of cocaine base standard, without the use of an internal standard, and with a single injection.

Acknowledgment

I would like to thank Henry F. Blum for the hours of helpful insight he has put into this project and to Earl Parrish, Norman Mausolf, Richard Karasiewski, and Roger Canaff for their helpful input.

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