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Comparison Analysis of Illicit Cocaine Samples

REFERENCE: Janzen, K. E., Walter, L., and Fernando, A. R., **"Comparison Analysis of Illicit Cocaine Samples,"** *Journal of Forensic Sciences*, JFSCA, Vol. 37, No. 2, March 1992, pp. 436–445.

ABSTRACT: A rapid method for comparison analysis of illicit cocaine samples has been developed. The raw data are obtained by capillary gas chromatography using a nitrogen-phosphorus detector. The area ratios of four alkaloids (tropacocaine, norcocaine, *cis*-cinnamoylcocaine, and *trans*-cinnamoylcocaine) to cocaine are calculated for each sample. These ratios are compiled in a computer database which allows easy comparison of samples and makes possible reliable conclusions regarding their commonality of origin.

KEYWORDS: toxicology, cocaine, chromatographic analysis, computers, capillary gas chromatography

The ability to establish a common origin for illicit drug samples is valuable in providing both investigative information and evidence in proving drug-related conspiracies in court. Previous successful comparisons of cocaine samples adulterated by numerous other drugs and sugars [*1*] have resulted in requests by drug enforcement personnel for comparisons of cocaine samples that are relatively pure and unadulterated. Accordingly, a method was developed for a detailed analysis of illicit cocaine samples to study the differences and similarities among them.

A variety of approaches to sample differentiation have been used by other researchers [*2,3*]. The most common method involves the generation of peak-enriched chromatograms using gas chromatography (GC) or high-performance liquid chromatography (HPLC), usually coupled with selective extraction or derivatization of the drug sample [*4–11*]. The resulting complex chromatogram or "fingerprint" is used as a basis for manual comparison of samples. The main disadvantages to this approach are the high level of technical expertise required to produce detailed chromatograms without introducing artifacts and the difficulty of visually comparing large numbers of intricate chromatograms.

Preliminary results have shown the presence of four minor cocaine-related alkaloids in most illicit cocaine samples: these are tropacocaine, norcocaine, and the isomers *cis*-cinnamoylcocaine and *trans*-cinnamoylcocaine. The variations in the relative amounts of these alkaloids suggest that they might be useful for comparison purposes. Several authors have suggested the possible use of the relative concentrations of one or another of these

Received for publication 14 March 1991; revised manuscript received 8 July 1991; accepted for publication 26 July 1991.

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alkaloids in subspecies distinction [*12–14*], in determination of the geographical origin of the coca leaves [*13,14*], or as a means of cocaine sample comparison [*15*].

Gas chromatography analysis of concentrated cocaine samples showed the reproducibility of the area ratios of tropacocaine, norcocaine, and *cis*- and *trans*-cinnamoylcocaine to cocaine within subsamples of large lots of illicit cocaine. These intrasample variations were much smaller than the variations among a random sample of cocaine exhibit materials submitted for routine analysis and quantitation. To draw meaningful conclusions regarding the similarity of two cocaine samples, data must also be available to show their dissimilarity to most of the unrelated cocaine samples. A large database of alkaloid/cocaine area ratios can quickly be searched by computer for any possible matches. The combination of four ratios for each cocaine sample greatly increases the selectivity of the comparison. Positive matches can then be further checked by visual comparison of chromatograms. Comparing cocaine samples by this approach offers several advantages: the technique is rapid and simple, it uses common laboratory equipment, and it produces numerical data that can be compiled in a computer database to facilitate the comparison of large numbers of samples.

Materials and Methods

Sampling

The cocaine exhibits were mixed by hand using a mortar and pestle [*16*] or by a mechanized "Retsch" mortar grinder, Type RM O, to achieve relatively homogeneous samples. Analytical samples of approximately 0.5 g of white powder were removed from each exhibit by taking portions of material from various surface positions in the mortar. Kilogram exhibits were subdivided manually into six subsamples before any mixing. Each subsample was then homogenized as described.

Analysis

The cocaine content of each sample was quantitated isothermally at 230°C using a Hewlett-Packard 5730A gas chromatograph equipped with a 12.5-m DB-1 methyl silicone capillary column (0.25-μm film thickness, 0.32-mm internal diameter) and a flame-ionization detector. Bupivacaine was used as an internal standard, and the results were calculated as the percentage of cocaine base by weight. The cocaine content of larger exhibits was reported as an average of the analyzed subsamples.

Sample profiles were generated using a Hewlett-Packard 5890 gas chromatograph equipped with a 12.5-m HP-1 cross-linked methyl silicone capillary column (0.5-μm film thickness, 0.2-mm internal diameter, 50:1 split ratio), nitrogen-phosphorus detector, and HP-7673A automatic sampler. Helium was used as the carrier gas at a flow rate of 1.5 mL/min. The helium makeup gas, air, and hydrogen were set at 30, 80, and 3 mL/min, respectively. The gas chromatography retention times and peak areas were calculated using an HP-1000 laboratory distributed system. The gas chromatograph was programed for an initial temperature of 120°C for 2 min, followed by a 6°C/min increase to the final temperature of 320°C, which was held for an additional 5 min. The injection port temperature was optimized at 215°C and the detector temperature was set at 325°C.

The samples were weighed to contain the equivalent of 30 mg of cocaine at 80% (by weight of the base) and dissolved in 2 mL of absolute ethanol. One microlitre of this solution was injected into the gas chromatograph. The resulting peak area ratios for tropacocaine, norcocaine, and *cis*- and *trans*-cinnamoylcocaine relative to cocaine were calculated and entered in a database. The samples were compared on the basis of these peak area ratios as well as the gas chromatography traces.

The retention times of tropacocaine, norcocaine, and *cis*- and *trans*-cinnamoylcocaine and cocaine were established using standards obtained through the Royal Canadian Mounted Police licensed dealer in Ottawa, Canada. The standards were confirmed by analysis using a Hewlett-Packard 5890 gas chromatograph, with a DB-1 capillary column, coupled to a Finnigan Incos-50 mass spectrometer.

Results and Discussion

Gas chromatography profiles using a nitrogen-phosphorus detector (GC-NPD) were generated for numerous samples. The peaks due to methylecgonidine, methylecgonine, and benzoylecgonine [*17*] were unsuitable for comparison purposes because all can arise from the decomposition of cocaine. However, the relative amounts of tropacocaine, norcocaine, and *cis*- and *trans*-cinnamoylcocaine were consistently reproducible for matched samples (Fig. 1). Moreover, these same four alkaloids seemed to vary widely in unrelated samples (Fig. 2).

As it is both tedious and inaccurate to compare a large number of chromatograms visually, the data were converted to a numerical form by calculating the peak area ratios for tropacocaine, norcocaine, and *cis*- and *trans*-cinnamoylcocaine relative to cocaine. This allows computerized screening of cocaine samples, followed by careful visual scrutiny of the GC profiles of potential matches. Table 1 illustrates a representative set of data for ten unrelated cocaine samples: it is typical of the variation in area ratios observed.

To test sample stability, replicate cocaine samples were subjected to 60°C temperatures or ultraviolet light at 254 nm for 24 h. No significant changes in the area ratios were observed after these treatments in comparison with the area ratios of untreated control samples. Two other sets of samples were reanalyzed after a period of six months and produced results consistent with the original data. Another set of samples was mixed with mannitol, and the ratios were unaffected. These results suggest that the area ratios of norcocaine, tropacocaine, and *cis*- and *trans*-cinnamoylcocaine to cocaine are stable and relatively unaffected by storage conditions or by the addition of diluents.

In contrast to Table 1, in which the wide variation of area ratios observed in the unrelated cocaine samples is shown, Table 2 shows results from an actual case, in which the area ratios are strikingly similar. Two accused cocaine traffickers were found to have cocaine samples on their persons when arrested. Information from a wiretap led police to a large cache of cocaine at a remote location. A request was made to help connect the samples found on the accused to the bulk of the cocaine by comparison analysis. The samples seized from both accused individuals and a large number of 28-g cocaine samples from the cache had all been previously mixed with lidocaine. A larger sample weighing approximately 1 kg consisted of purer cocaine (Cache 1). The area ratios for all the exhibits were very similar and were apparently independent of the presence of lidocaine. Portions of the large cocaine sample were presumably mixed with lidocaine and packaged for street sale, resulting in a set of exhibits with area ratios indicative of a common cocaine origin.

The alkaloidal impurities in illicit cocaine samples arise as biosynthetic constituents of the coca plant and vary depending on the species of plant, its age, and the environment in which the plant was grown [*12–14,18*]. Alkaloids are coextracted with cocaine, and some can be altered during the manufacturing process. For example, the use of the oxidizing agent potassium permanganate by some clandestine cocaine laboratories will remove some or all of the *cis*- and *trans*-cinnamoylcocaines, depending upon how long the reaction is allowed to continue. The coca leaves are collected from a large area and refined to cocaine in these South American clandestine operations. Any given batch will have its own chemical makeup, which remains reasonably constant after it is moved to Canada for distribution, even when mixed with various adulterants. Such adulterants are

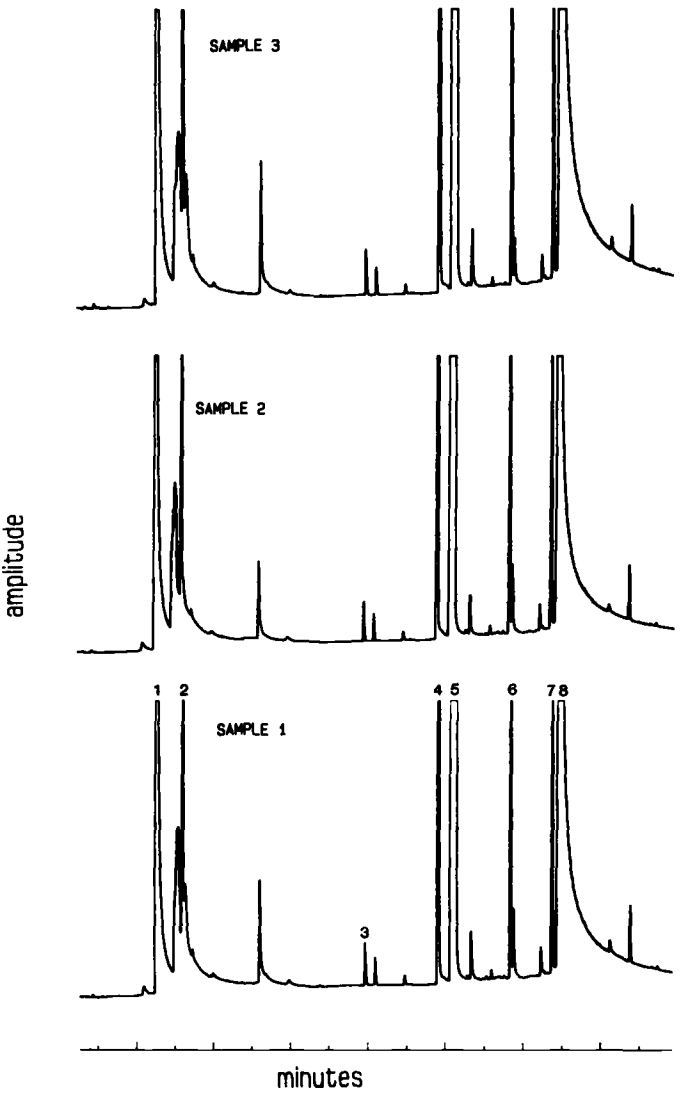

FIG. 1—*GC-NPD profiles of matching samples. Peak identification: 1 = methylecgonidine; 2 = methylecgonine; 3 = tropacocaine; 4 = norcocaine; 5 = cocaine; 6 =* cis-*cinnamoylcocaine; 7 =* trans-*cinnamoylcocaine; 8 = benzoylecgonine.*

not likely to interfere with the comparison analysis proposed in this paper, as they rarely have GC retention times that are the same as tropacocaine, norcocaine, *cis-* or *trans-*cinnamoylcocaine, or cocaine. This makes possible the exciting possibility of "tracing" an illicit sample through various levels of the cocaine trade, even when the sample is mixed with other drugs or sugars en route.

Meaningful conclusions regarding the similarities or dissimilarities among cocaine exhibits require an examination of sample variability. Intrasample variability was studied using cocaine samples that had been submitted as single kilogram quantities. The kilogram exhibits were subdivided into six subsamples each before any mixing. The resulting

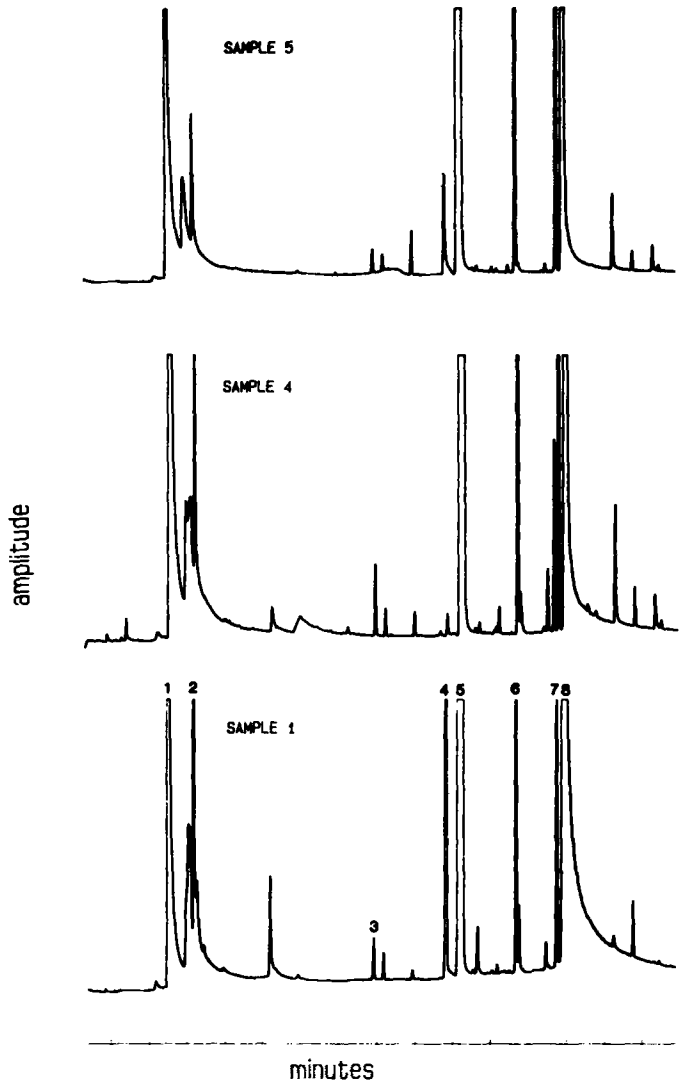

FIG. 2—*GC-NPD profiles of random cases. Sample 1 and the peak identification are as in Fig. 1.*

subsamples were then mixed and analyzed as separate exhibits. These provided profiles and area ratios that were assumed to be representative of those that might be obtained if a larger quantity of cocaine was divided for sale on the street, seized from various customers, and subsequently submitted for comparison analysis.

The mean values of the area ratios for each set of six subsamples, standard deviations, and coefficients of variation are summarized in Table 3. Although the coefficients of variation for the kilogram exhibits range from 1 to 13%, which reflects the various degrees of homogeneity within larger samples, the variability seen within these samples is small when compared with the variation seen in the general population. The analytical variability was on the same order of magnitude as that seen in the most homogenous of the kilogram samples. The coefficients of variation for the four area ratios of the actual case

TABLE 1—*Random case profiles.*[a]

Case	COC %	Alkaloid Area Ratios[b]			
		TROP/COC	NOR/COC	CIS/COC	TRANS/COC
1	72	0.645	1.20	7.40	4.18
2	47	13.58	1.37	3.76	3.60
3	77	2.18	24.23	7.43	6.73
4	85	0.292	2.22	0.826	0.567
5	84	0.755	25.95	19.67	9.70
6	83	3.12	28.22	2.07	1.12
7	55	0.474	3.49	30.70	29.29
8	49	0.240	4.74	9.59	5.23
9	82	4.94	0.0	2.04	0.946
10	61	15.32	1.10	16.42	15.10

[a]The abbreviations are as follows: COC = cocaine; TROP = tropacocaine; NOR = norcocaine; CIS = *cis*-cinnamoylcocaine; TRANS = *trans*-cinnamoylcocaine.
[b]The ratios have been multiplied by a factor of 1000, and the percentages are calculated as weight of the cocaine base.

TABLE 2—*Actual case with matching profiles.*[a]

Exhibit	COC %	L %	Alkaloid Area Ratios[b]			
			TROP/COC	NOR/COC	CIS/COC	TRANS/COC
Cache 1	72	. . .	0.645	1.20	7.40	4.18
Cache 2	65	5	0.753	1.27	7.40	3.94
Cache 3	60	6	0.760	1.32	7.47	3.84
Cache 4	60	5	0.729	1.28	7.18	3.98
Cache 5	60	5	0.684	1.23	7.26	3.96
Cache 6	60	4	0.696	1.30	7.99	4.45
Cache 7	58	6	0.691	1.33	7.77	4.30
Accused A1	60	5	0.694	1.25	7.34	3.88
Accused A2	62	5	0.657	1.28	7.33	4.04
Accused B1	63	5	0.624	1.20	7.32	3.97
Accused B2	60	6	0.697	1.34	7.55	4.08

[a]The abbreviations are as in Table 1. L = lidocaine.
[b]The ratios have been multiplied by a factor of 1000 and the percentages are calculated as weight of the cocaine and lidocaine bases.

illustrated in Table 2 are comparable to those seen in the intrasample variability study, which supports the conclusion that these samples came from a larger common cocaine sample.

As a part of this study, a database consisting of 76 unrelated unique exhibits was compiled. To search the database for potentially matching samples, a pattern recognition approach was proposed [*19–21*]. When more than two parameters are measured, each sample can be represented by a point in multidimensional space. The euclidean distance between two points in n-dimensional space with coordinates $(x_1, x_2, \ldots x_n)$ and $(y_1, y_2, \ldots y_n)$ can be calculated using the expression

$$d^2 = \sum_{i=1}^{n} (x_i - y_i)^2$$

For a given set of samples, the magnitude of d, the euclidian distance, can then be used as a basis for separating the samples into clusters. For each sample, a unique location

TABLE 3—*Intrasample variability* (n = 6).[a]

Sample	TROP/COC			NOR/COC			CIS/COC			TRANS/COC		
	$\bar{X}$	SD	CV%	$\bar{X}$	SD	CV%	$\bar{X}$	SD	CV%	$\bar{X}$	SD	CV%
1	0.25	0.0070	2.80	4.74	0.19	4.01	9.59	0.35	3.65	5.23	0.28	5.35
2	0.46	0.014	3.04	0.0	. . .	. . .	7.31	0.41	5.61	2.97	0.14	4.71
3	1.90	0.036	1.89	0.29	0.028	9.66	9.68	0.11	1.14	6.11	0.079	1.29
4	4.88	0.22	4.51	0.0	. . .	. . .	1.97	0.073	3.71	0.97	0.068	7.01
5	3.34	0.43	12.87	0.0	. . .	. . .	7.01	0.63	8.99	4.42	0.50	11.31
6	13.58	0.78	5.74	1.37	0.050	3.65	3.76	0.24	6.38	3.60	0.22	6.11
7	2.18	0.12	5.50	24.23	0.34	1.40	7.43	0.23	3.09	6.73	0.22	3.27
8	0.60	0.070	11.67	1.23	0.019	1.54	7.72	0.10	1.29	4.25	0.070	1.65
9[b]	0.694	0.042	6.05	1.27	0.050	3.94	7.46	0.24	3.22	4.06	0.18	4.43

[a]The abbreviations are as in Table 1. $\bar{X}$ = mean; SD = standard deviation; CV = coefficient of variation.
[b]Actual case from Table 2 (n = 11).

was defined in a four-dimensional space using the four alkaloid/cocaine ratio values. The euclidian distance representing two samples was used as a measure of the similarity between the samples.

A computer program was written in BASIC on an Apple MacIntosh microcomputer to evaluate the euclidian distances between a test sample and those contained in the library and to locate the library samples that were closest to the test sample. The test cases showed that the related samples tend to form a cluster of points in the four-

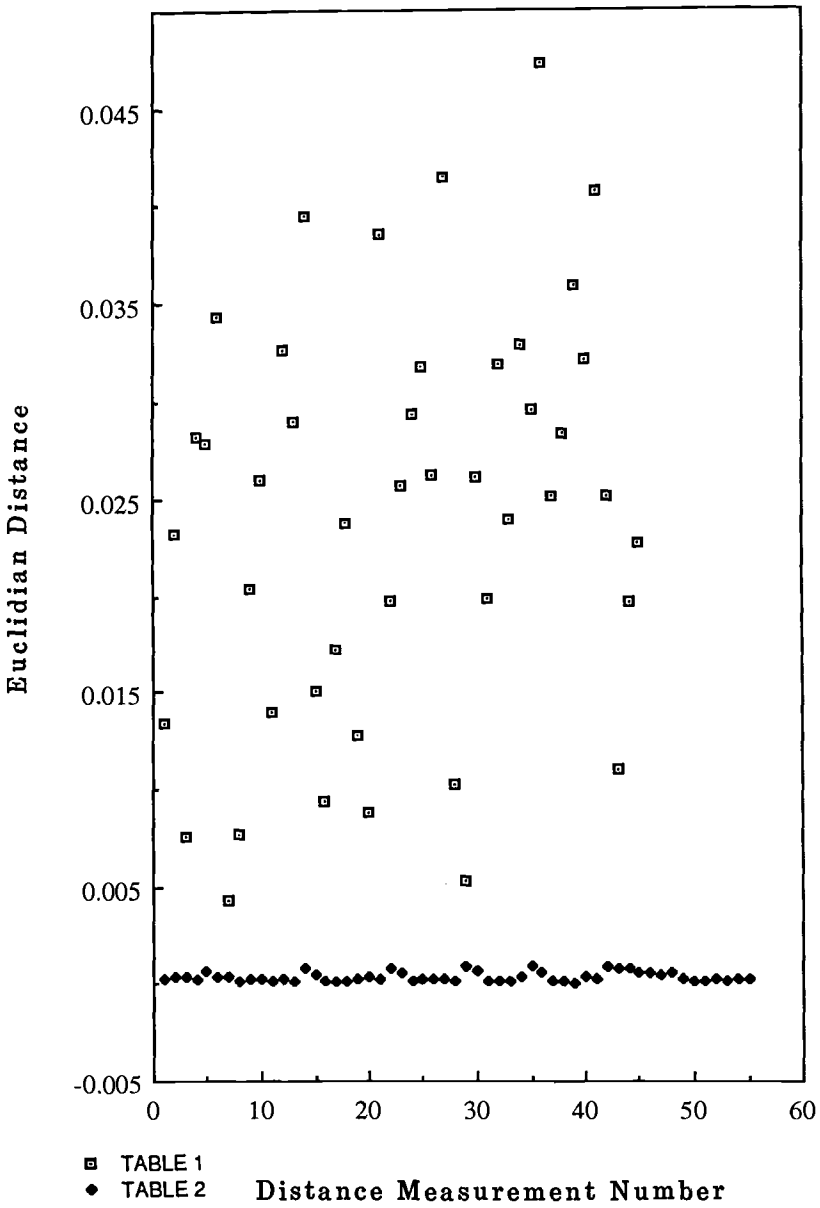

FIG. 3—*Euclidian distances for the data in Tables 1 and 2.*

dimensional space. The euclidian distance measurements between these points are smaller than those between points in the general database.

As an illustration, euclidian distance values were calculated for the data in Table 1 (random case profiles) and Table 2 (matching profiles). Table 1 has 10 data sets resulting in 45 distance measurements; Table 2 has 11 data sets resulting in 55 distance measurements. These euclidian distance measurements are plotted in Fig. 3. The data of related samples presented in Table 2 form a cluster in four-dimensional space with distance measurements ranging from 0.000 091 to 0.000 94. In contrast, the distances for the data of unrelated samples from Table 1 are scattered from 0.0043 to 0.047.

After possibly related samples are identified in this way, the original data for individual ratio values must be compared. Final confirmation is obtained by visual comparison of the actual gas chromatography profiles.

Conclusions

A new approach to comparison analysis of illicit cocaine samples is presented. It is rapid and simple enough to be practiced by most qualified laboratories on a routine basis, providing data that can be used to draw reliable conclusions regarding the commonality of origin of illicit cocaine samples. The strength of the conclusions depends on the size of the database developed by a given laboratory and on the statistical significance that can be derived for any given case. If the appropriate background work is completed, comparison analysis of illicit cocaine samples using alkaloid/cocaine ratios can provide strong evidence in a court of law and reliable investigative information in drug enforcement.

Acknowledgments

We gratefully acknowledge the advice of Dr. B. Kratochvil, professor of analytical chemistry and chairman of the Department of Chemistry, at the University of Alberta, in the preparation of this paper, particularly in the area of analytical statistics. We thank R. Percy and R. Grant of the RCMP Forensic Laboratory in Edmonton for their computer assistance in this research project. The assistance of S. Backs (M.Sc. in analytical chemistry) in proofreading the manuscript and the cooperation of Alberta Drug Enforcement personnel were greatly appreciated.

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