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Comparison of Illicit Cocaine by Determination of Minor Components

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ABSTRACT: High-performance liquid chromatography (HPLC) and gas chromatography (GC) combined with both a flame ionization detector (FID) and a mass spectrometer (MS) were used to determine the minor components in illicit uncut cocaine. Two HPLC systems were used to determine, in cocaine exhibits, the relative amounts of the main coca alkaloids: cocaine, cis-cinnamoylmethylecgonine (CIS), and trans-cinnamoyl-methylecgonine (TRANS). GC/FID and GC/MS were used to determine only the acidic and neutral components of cocaine exhibits. The significance of the presence of these minor components and their use to the forensic chemist for the matching and comparison of exhibits are discussed.

KEYWORDS: toxicology, cocaine, chemical analysis, minor components, impurities, other alkaloids, sample matching, sample linking

The presence of minor components in illicit cocaine has been recognized for a number of years. The identification and determination of alkaloidal components have been reported [*1,2*]. More recently, the isolation and identification of the truxillines [*3*] and the detection of the truxinic and truxillic acids [*4*] have been described. The solvents used in the production of the hydrochloride salt have also received attention.[2,3,4]

The presence of these minor components is of fundamental interest to those involved in coca research, the assessment of health risks associated with the use of cocaine, and the generation of forensic science evidence. Enforcement agencies in Canada are becoming aware, through discussions with Health Protection Branch staff, that the sophistication of scientific capability in the examination of cocaine has improved dramatically in the last few years. The availability of more moderately priced mass spectrometers and their routine application to illicit-drug work has had a major impact on the forensic drug laboratory [*1*]. Forensic science evidence with regard to the similarity of cocaine exhibits can be useful in a number of ways to the successful conclusion of enforcement activities. First, enforcement operations involving the undercover purchase of drugs can be more effectively directed with knowledge of the similarities among and sources of the materials purchased. Second, the distribution patterns within a limited geographic area may be determined through the similarities among cocaine exhibits seized or purchased. Finally,

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[2]Clark, Charles C., 1981, personal communication.

[3]Kiser, Wilmer O., 1986, personal communication.

[4]Churchill, Katherine T., 1985, personal communication.

forensic science evidence about the similarities of cocaine samples can support criminal charges such as conspiracy, trafficking, and possession for the purpose of trafficking.

The need for the development of useful information for these purposes led us to conduct studies on both randomly sampled cocaine and cocaine from a known source. Samples randomly selected and submitted from across Canada were examined for the relative amounts of cocaine, cis-cinnamoylmethylecgonine (CIS), and trans-cinnamoyl-methylecgonine (TRANS). Samples from a large importation (500 kg) of cocaine known to have been produced by two laboratories were also examined. The chromatographic profiles of the acidic and neutral components of many of these samples were also determined. The study was restricted to the analysis of "uncut" cocaine.

Experimental Procedure

All chemical standards were available from our house collection and had been either obtained from manufacturers or synthesized in our laboratories. All solvents were high-performance liquid chromatography (HPLC) grade. Water was obtained from a Barnstead Nanopure II system.

High-Performance Liquid Chromatography

The two liquid chromatographs used were similar to those reported previously [*1*].

System 1 consisted of a Spectra Physics Model 8800 pump, an 8780 autosampler, an 8480 XR variable wavelength detector, and a 4270 integrator. The column used was an LC-8, 5-μm reversed-phase column, 250 by 4.6 mm inside diameter (Supelco Inc., Bellefonte, Pennsylvania). The detector was operated at 280 nm. The mobile phase which was mixed by the pump consisted of acetonitrile-tetrahydrofuran-0.1% volume/volume (v/v) aqueous triethylamine (35:10:55, v/v/v).

System 2 consisted of a Spectra Physics Model 8100 pump-autosampler combination, a Hewlett-Packard Model 1040-*M* diode array detector, and a Hewlett-Packard Model 7957B integrator and data system. The column used was an RP-8, 10-μm reversed-phase column, 250 by 4.6 mm inside diameter (Brownlee, Santa Clara, California). Both systems were operated with 10-μL injection loops at a flow rate of 1.0 mL/min. Although detector output was collected over the range of 210 to 400 nm, the signal at 280 nm (4-nm bandwidth) was used to quantify peaks; the reference wavelength was 550 nm (100-nm bandwidth). The mobile phase was acetonitrile-0.05-*M* potassium phosphate monobasic (KH_2PO_4), pH 5.0 (33:67, v/v). This buffer was prepared by the addition of a few drops of about 12 *N*-potassium hydroxide solution to a 0.05-*M* potassium hydrogen phosphate solution until a pH of 5.0 was obtained.

Linear Solution Preparation—For the determination of the linearity of detection of cocaine hydrochloride, a stock solution of 10 mg/mL was diluted to give final concentrations of 0.5, 1.0, and 2.0 mg/mL in methanol. For the determination of the linearity of response of CIS and TRANS, stock solutions of 0.4 mg/mL in methanol of each of the alkaloids were prepared. Six aliquots (0.125 to 2.5 mL) of each of these solutions were added to each of six 10-mL volumetric flasks containing 20 mg of cocaine hydrochloride; the flasks were made to volume with methanol. This resulted in a range of relative amounts of the two co-alkaloids of 0.25 to 5.0% with respect to cocaine.

Sample Preparation—Samples were prepared for HPLC analysis by the addition to a suitable volumetric flask sufficient material to yield a final concentration of 2 mg/mL. After an amount of methanol representing about one half the volume of the flask was added, the flask was swirled to dissolve the sample. The flask was then made to volume with methanol and mixed thoroughly. An aliquot of the solution was analyzed on the same day, using each of the two HPLC systems.

Gas Chromatography/Mass Spectrometry (GC/MS) and Flame Ionization Detection (FID)

GC/MS—The gas chromatograph was a Carlo Erba Vega Series 6000 equipped with a Grob-type split/splitless injector and a 15-m by 0.25-mm DB-5 column, 0.25-μm film thickness (J & W Scientific Inc., Rancho Cordova, California). The GC was operated at a head pressure of 40 kPa of helium (linear velocity at 160°C = 55 cm/s). The injector was maintained at 275°C. The temperature program consisted of injection at 160°C with a 3-min hold time, followed by a ramp rate of 3°C/min to 255°C with a 1-min hold time, followed by a ramp of 20°C/min to a maximum of 295°C with a 1-min hold time. One-microlitre injections were made at a split ratio of approximately 25:1. The mass spectrometer was a Finnigan Mat Model 800 Ion Trap Detector. The gas chromatograph's column effluent was introduced to the ion trap by direct attachment of the column. No splitting occurred at the GC/MS interface. The ion trap was operated in electron impact mode and set to acquire data at 1 scan/s over a mass range of 40 to 500 amu.

GC/FID—The gas chromatograph was a Hewlett-Packard Model 5890 equipped with a flame ionization detector (FID), split/splitless injector and a Model 5895A ChemStation data system/system controller. The column was the same as that used in the GC/MS. The GC was operated at a head pressure of 85 kPa (12 psi) (linear velocity at 160°C = 40 cm/s) of helium. The injector was maintained at 275°C. The temperature program was the same as that used with the GC/MS. The temperature of the FID detector was maintained at 275°C with a mixture of air at 400 mL/min and hydrogen at 30 mL/min. Nitrogen was used as makeup gas at a flow of 30 to 40 mL/min. One-microlitre injections were made at a split ratio of approximately 25:1, as with GC/MS.

Sample Preparation—Exhibits were prepared for GC/MS and GC/FID examination using an extraction procedure similar to that described for heroin [5]. This extraction of an acidic solution of the exhibits results in complete removal of free alkaloid components, leaving only the acidic and neutral compounds. Approximately 400 mg of the sample were dissolved in 5 mL of 0.5-*M* sulfuric acid (H_2SO_4). The solution was extracted with one 5-mL aliquot of toluene by shaking for about 2 min in a disposable, screw-capped glass centrifuge tube. The tube was centrifuged briefly to aid in the separation of the layers. As much as possible of the toluene was withdrawn and extracted again with a fresh 5-mL aliquot of the sulphuric acid solution. After the solution was centrifuged, the toluene layer was again withdrawn to a culture tube (70 by 10 mm), and most of the solvent was removed by evaporation under a stream of nitrogen at 40°C. The samples could then be stored in a refrigerator (5°C), if necessary, for future analysis. Immediately before the sample was injected into the chromatograph, the remaining toluene was removed by evaporation and the residue immediately dissolved in 50 μL of ethyl acetate. Duplication 1-μL aliquots of the ethyl acetate solution were analyzed (manual injection) by both GC/MS and GC/FID on the same day.

Stability Chamber

A GCA/Precision Scientific oven, Model 6M, was used to maintain the temperature of the humidity chambers to ± 1°C. Humidity control was achieved by exposing the drug in open glass vials to the equilibrated vapor phase over saturated salt solutions in a desiccator. The salts and the resulting relative humidities of their saturated solutions were sodium bromide (50 to 57%) and sodium chloride (75%) [6].

The Cocaine Samples

The samples were derived from two sources. Some of the samples were submitted by Royal Canadian Mounted Police detachments from across Canada and were taken from

seizures of more than one kg of cocaine. Approximately 1 g of these samples was submitted for analysis. A larger number of samples was obtained from one major seizure of cocaine. In 1989, 500 kg of cocaine were imported into Canada by plane. The cocaine had been shipped directly from South America and, when seized, was contained in its original packaging. The shipment consisted of 25 bundles of 20 kg. Each of the bundles consisted of a Fiberglas bag, in which were found packages of two cocaine bricks; each brick weighed approximately 0.5 kg. Each exterior bag was labeled with an identifying mark which appeared to be a family name: 17 bundles carried a mark which we will describe here as "MFR A"; 8 bundles carried an identifying mark which we will describe here as "MFR B." The entire seizure had been stored at ambient temperature for about six months prior to sampling. Samples were taken by cutting the plastic wrapping immediately covering the cocaine and using a corkborer to withdraw a sample (2 g) of the drug. In addition, a 10-g sample was taken from one of the "MFR A" bricks. The cocaine was placed and stored in capped glass scintillation vials. In some cases two samples were taken from the center and corner of the same brick. Two samples from different bricks within the same bundle were also taken. At least one sample was taken from each of the bundles. Within 48 h of collection, the samples were placed under refrigeration (10°C) until analysis.

Results and Discussion

In our previous article [*1*], we reported that about 30% of the cocaine exhibits examined contained detectable quantities of the other coca alkaloids, CIS and TRANS. A review of recent analytical data from the laboratory indicates the percentage of samples now containing these alkaloids to be about 95%; it is unusual today to find samples with no detectable related alkaloids. This is significant because it provides greater assurance to the analyst that measurable co-alkaloid ratios will be available from samples. This finding may also be an indication that potassium permanganate, the traditional oxidizing agent used to remove the cinnamoyl alkaloids from crude coca extracts, is less available in producing areas. It is noteworthy that the samples obtained from "MFR B" had probably been subjected to potassium permanganate treatment. Although the other alkaloids were detectable, the amounts present were too small to be of quantitative use. It should be recognized that the data for the determination of the alkaloid ratios in cocaine do not involve, under most circumstances, considerable additional resource demands on the chemist. Both HPLC systems used for the development of these data were primarily designed for the quantification of the cocaine in exhibits. Most large seizures of cocaine made in Canada are routinely quantified at the request of the enforcement agency for court purposes.

As mentioned previously [*1*], HPLC System 1 is, from our experience, more durable and better suited to routine analysis. HPLC System 2, on the other hand, provides greater capabilities in data acquisition. The solvent system used with this latter system allows for the generation of ultraviolet spectra over the full range. The solvent system used in HPLC System 1 prohibits the acquisition of spectral information below about 240 nm due to absorption. The use of the diode array detector in HPLC System 2 further enhances the capabilities of the system by permitting data manipulation characteristic of this type of detector. However, the extended capabilities of this detector were not essential to the acquisition of data for this study. The detection wavelength of 280 nm was chosen as a result of the greater relative absorptivities of the CIS and TRANS at this wavelength in comparison with cocaine. CIS and TRANS show approximately 11 and 28 times greater response factors than cocaine at 280 nm, respectively. We calculate the co-alkaloid ratios cocaine/CIS (R_{CIS}) and cocaine/TRANS (R_{TRANS}) using the absolute integration values obtained from the chromatographic data. This precludes the necessity of having available authentic standards of the isomeric cinnamoyl alkaloids.

The performance of the two HPLC systems was evaluated to determine intra-sample and daily variation of the R_{CIS} and R_{TRANS} ratios. The correlation coefficients of the linear curves for known amounts of cocaine, CIS, and TRANS are given in Table 1. The reproducibility (ten determinations) of the absolute amount of cocaine present in a single standard mixture and of the CIS and TRANS ratios is also given in Table 1. The minimum detectabilities (3 × baseline) as a percentage of cocaine at 2 mg/mL of CIS and TRANS, respectively, were 0.01 and 0.005% using System 1 and 0.03 and 0.02% using System 2. The variation in the data generated by the two HPLC systems was determined by the analysis on the same day of ten weighed aliquots of the same sample. Variation from day to day was determined by analysis of ten aliquots of this same sample during the course of the stability study described below. The results for both HPLC systems are given in Table 2. Chromatograms of the same sample obtained on the two systems are shown in Figs. 1 and 2.

The utility of the relative amounts of the other two alkaloids to the forensic chemist for the comparison of cocaine exhibits depends on two factors: the homogeneity of the amounts of CIS and TRANS throughout bricks and production lots and the relative stability of these alkaloids in relation to cocaine during storage and distribution.

The homogeneity of the bricks of cocaine was verified by the analysis of duplicate samples taken from eight "MFR A" bricks. The results presented in Table 3 indicate that the bricks were homogeneous in both cocaine content and the relative amounts of the three alkaloids. The determination of the alkaloid ratios of two samples taken from different places in the same brick indicated less than 2.5% variation in the ratios. However, it is apparent from the HPLC results presented in Table 3 that some of the "MFR A" samples are different. For example, both alkaloid ratios for Bricks 1 and 2 are significantly different. The approximately 8% difference in the R_{CIS} values of Bricks 1 and 3 also appears to indicate a difference between samples. These small differences may be due to preparation of the cocaine in lots. The "MFR A" bricks must have been derived from distinct lots which differed at some point in their preparation.

These differences are also revealed in the results from the analysis of bricks from the same bundle. Table 4 summarizes these results. It can be seen that the two bricks analyzed from each of bundles 16 and 25 contained different values for both R_{CIS} and R_{TRANS}. In the case of the two bricks derived from bundle 19, the differences between the bricks is reflected only in the different R_{CIS} values.

TABLE 1—*HPLC system validation data.*

Substance	Concentration,[a] mg/mL	Correlation Coefficient[b]		% RSD[c]	
		System 1	System 2	System 1	System 2
		LINEARITY			
Cocaine	0.5–2	0.9999	0.9999	. . .	. . .
Cis	0.005–0.1	0.9995	0.9998	. . .	. . .
Trans	0.005–0.1	0.9993	0.9999	. . .	. . .
		REPRODUCIBILITY			
Cocaine	1.7	. . .	. . .	0.70	0.15
R_{CIS}	0.066	. . .	. . .	0.43	0.42
R_{TRANS}	0.042	. . .	. . .	0.68	0.62

[a]Linearity expressed in range.
[b]Determined for linearity.
[c]RSD = relative standard deviation, which is determined for reproducibility.

TABLE 2—*System performance characteristics.*

	System 1				System 2			
	High	Low	Mean	%RSD[a]	High	Low	Mean	%RSD
				SAME DAY				
Cocaine, %	84.8	83.4	84.2	0.5	84.7	82.0	83.7	1.0
R_{CIS}	2.41	2.35	2.38	0.8	2.44	2.36	2.38	0.8
R_{TRANS}	1.46	1.44	1.45	0.7	1.62	1.60	1.61	0.6
				DIFFERENT DAYS				
Cocaine, %	86.5	81.5	84.5	2.0	85.2	81.5	83.7	1.4
R_{CIS}	2.37	2.32	2.35	0.8	2.40	2.34	2.38	0.8
R_{TRANS}	1.46	1.42	1.44	0.7	1.62	1.60	1.61	0.6

[a]% RSD = relative standard deviation.

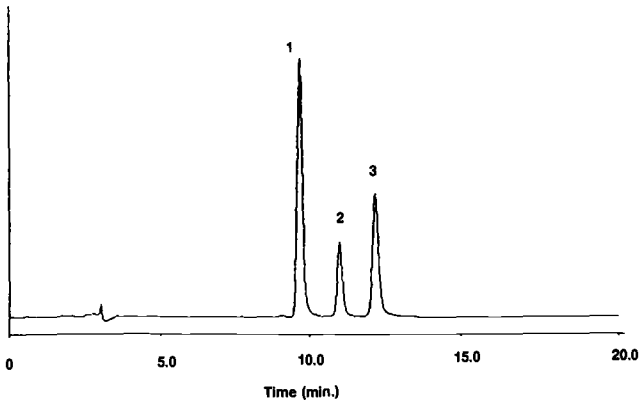

FIG. 1—*HPLC chromatogram of a cocaine exhibit using HPLC System 1. Instrumental parameters are given in the text. Peak identification:* (1) *cocaine,* (2) *CIS,* (3) *TRANS.*

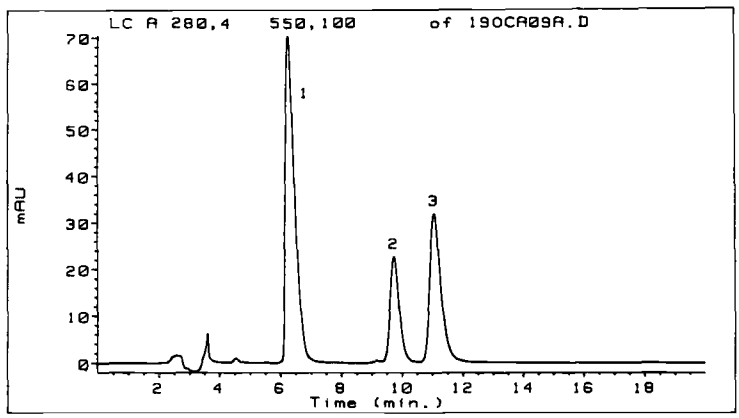

FIG. 2—*HPLC chromatogram of the same exhibit as in Fig. 1 using HPLC System 2. Instrumental parameters are given in the text. Peak identification:* (1) *cocaine,* (2) *CIS,* (3) *TRANS.*

Stability of Alkaloid Ratios

The use of the relative amounts of the co-alkaloids for matching samples is dependent on the relative stabilities of the components. Cocaine can be expected to be subjected to rather different storage conditions during distribution. A short stability study was conducted to establish the continued integrity of the ratios even under drastic conditions. The 10-g sample taken from one of the "MFR A" bricks was ground to a fine powder. This sample was chosen because there was sufficient material for the study and the relative amounts of the other coca alkaloids were quite high. For the determination of the stability of the alkaloid ratios, approximately 20-mg samples of this material were accurately weighed into scintillation vials and the vials were placed into one of two temperature-controlled humidity chambers at 60°C. The remainder of the sample was stored at ambient temperature and analyzed on different days to determine the daily variation in the results from the two HPLC systems. The humidity of the chambers was maintained at approximately 50 and 75%. One vial was withdrawn from each of the chambers on the day of analysis. On the same day, a weighed aliquot of the unheated sample was analyzed as a daily control.

TABLE 3—*Analysis of duplicate samples from the same brick determined using HPLC System 1 and (System 2).*

Brick	Cocaine, %	Alkaloid Ratios	
		R_{CIS}	R_{TRANS}
1	87.5 (86.2)	2.36 (2.37)	1.36 (1.53)
1	86.7 (86.5)	2.39 (2.37)	1.37 (1.53)
2	88.8 (87.5)	3.18 (3.22)	1.71 (1.85)
2	89.0 (88.6)	3.11 (3.14)	1.68 (1.83)
3	85.3 (82.7)	2.58 (2.59)	1.35 (1.50)
3	86.2 (84.9)	2.59 (2.61)	1.34 (1.49)
4	85.9 (86.4)	2.46 (2.47)	1.37 (1.52)
4	85.1 (85.7)	2.48 (2.50)	1.37 (1.53)
5	84.8 (84.0)	2.35 (2.38)	1.36 (1.53)
5	85.3 (85.4)	2.40 (2.42)	1.35 (1.51)
6	87.4 (88.4)	2.22 (2.21)	1.45 (1.62)
6	86.4 (86.5)	2.27 (2.31)	1.44 (1.63)
7	86.4 (86.4)	2.58 (2.62)	1.45 (1.60)
7	87.1 (88.4)	2.59 (2.62)	1.43 (1.60)
8	85.7 (85.6)	2.70 (2.74)	1.40 (1.58)
8	88.3 (85.6)	2.66 (2.65)	1.42 (1.56)

TABLE 4—*Analysis of different bricks from the same bundle determined using HPLC System 1 and (System 2).*

Bundle	Cocaine, %	Alkaloid Ratios	
		R_{CIS}	R_{TRANS}
16	85.7 (86.1)	2.45 (2.46)	1.43 (1.58)
16	85.2 (85.2)	2.62 (2.63)	1.36 (1.50)
18	85.6 (86.6)	2.52 (2.51)	1.37 (1.52)
18	85.7 (85.6)	2.54 (2.56)	1.38 (1.53)
19	86.7 (86.9)	2.27 (2.27)	1.45 (1.62)
19	86.0 (86.2)	2.50 (2.53)	1.41 (1.57)
20	85.6 (87.6)	2.39 (2.40)	1.39 (1.55)
20	85.1 (86.0)	2.43 (2.46)	1.41 (1.57)
21	87.1 (87.4)	2.25 (2.25)	1.45 (1.61)
21	87.0 (87.1)	2.22 (2.24)	1.44 (1.62)
25	87.4 (88.2)	3.23 (3.29)	1.72 (1.87)
25	88.2 (87.6)	2.70 (2.70)	1.44 (1.58)

After one day in the stability chambers, the samples had liquefied. The liquefaction of cocaine hydrochloride under conditions of high humidity has been observed previously.[5] Since the samples had been accurately weighed prior to introduction to the chambers, sample preparation involved only the dissolution of the sample to a standard volume with methanol and injection of the solution. The results of the analysis using both the HPLC systems are given in Tables 5 and 6. After 13 days, only one other significant peak was detected in the chromatograms of the samples. This peak was shown to be due to methyl ester hydrolysis by injection of a sample of authentic benzoylecgonine. No

[5]See footnote 3.

TABLE 5—*Stability of cocaine and CIS and TRANS ratios HPLC System 1.*

	Humidity, %					
	Cocaine		R_{CIS}		R_{TRANS}	
Day	75	50	75	50	75	50
1	84.2	84.1	2.36	2.35	1.43	1.43
2	85.2	85.4	2.33	2.32	1.43	1.43
5	86.4	86.1	2.34	2.35	1.42	1.42
7	83.5	82.2	2.34	2.34	1.42	1.42
9	81.0	78.9	2.34	2.32	1.45	1.44
13	80.9	76.2	2.32	2.30	1.44	1.43
		Mean	2.34	2.33	1.43	1.43
		%RSD	0.51	0.77	0.70	0.49

TABLE 6—*Stability of cocaine and CIS and TRANS ratios HPLC System 2.*

	Humidity, %					
	Cocaine		R_{CIS}		R_{TRANS}	
Day	75	50	75	50	75	50
1	82.9	83.0	2.34	2.36	1.60	1.60
2	83.7	84.3	2.39	2.37	1.60	1.60
5	83.7	82.3	2.35	2.36	1.59	1.60
7	82.3	81.3	2.35	2.34	1.59	1.59
9	81.5	78.7	2.35	2.35	1.59	1.59
13	80.6	75.5	2.38	2.43	1.62	1.61
		Mean	2.36	2.37	1.60	1.60
		%RSD	0.76	1.26	0.69	0.44

peaks corresponding to cis- and trans-cinnamoylecgonine were detected. The percentage of cocaine found in the samples reflects this degradation. However, the limited changes to the CIS and TRANS ratios indicate that comparable rates of hydrolysis occur with all three alkaloids in this sample.

Gas Chromatography/Mass Spectrometry

After evaporation, the toluene extracts of the exhibits were redissolved in the more polar ethyl acetate to effect more complete dissolution of the residues. During initial studies, the toluene was completely evaporated from some duplicate samples and the residue was redissolved in ethyl acetate. The GC profiles of these samples differed from one another particularly in the total amount of detected signal by both GC/MS and GC/FID. This may have been due to adsorption of the components of the extract onto the glass surface of the culture tube which was untreated. Leaving about 250 μL of the toluene in the culture tube until final evaporation and immediate re-dissolution in ethyl acetate eliminated this phenomenon. The GC ramp conditions used for the study were chosen to eliminate, during the initial filament-off time (60 s) of the MS, the detection of large amounts of volatile components that may be present in the sample. Excluded from the chromatogram, starting a this temperature, are substances such as benzoic acid, methyl benzoate, and the methyl cinnamates which are common in many exhibits. Their routine detection in samples would be unremarkable. We considered the mass spectrom-

eter essential in these initial studies in order to assess the relevance of the detected peaks in the chromatograms. After 36 different samples (the "MFR A" and "MFR B" are counted as only 3) were analyzed, more than 100 different peaks have been detected. None has been conclusively identified. These substances may be derived from the coca plant, the extraction process, the method of production of coca paste, or the preparation of the hydrochloride salt. However, the mass spectrometer has been able to suggest, through the spectra generated, the chemical classification, the identity, and the relationship of a number of substances. The detection and use of irrelevant peaks are diminished when the mass spectra of substances are available. The availability of the mass spectra of detected peaks also greatly assists in the correlation of chromatograms by peak identification. The evaluation of peak relevance was made on the basis of suspected molecular structure and mass spectral similarity to known substances. For example, the presence in a spectrum of ion fragments 68, 82, 105, 168, 182 [*1,2*] was considered to indicate that the substance was cocaine-like. Some substances, due to the presence in their spectra of these and other characteristic ions [*3*], could be recognized as derived from the coca plant. Many other substances could be classified only as hydrocarbon-like due to their characteristic mass spectra. Hydrocarbons as well as phthalates were excluded from consideration. The former may have relevance but are usually present in small amounts. The latter are frequently present in larger amounts but they may be derived from plastic packaging in which many samples are submitted to the laboratory.

An example of the total ion chromatogram of an exhibit is shown in Fig. 3. Due to the extended acquisition time, the *X*-scale is very compressed. Figure 4 shows the chromatogram in about 10-min segments of the acquisition expanded to a full page of display. This format demonstrates the number and variety of substances detected; it also permits an easier visual comparison of the results of the analysis of samples by page-by-page examination.

Gas Chromatography/Flame Ionization Detection

The establishment of relevant peaks in the chromatograms by GC/MS permits the use of GC/FID data for the comparison of samples. The chromatograms obtained from the two systems which use the same column and ramp profile are comparable. The relative intensities of the detected peaks differ due to the differences in sensitivities of the detected materials by MS and flame ionization. Generally, the GC/FID system gives better sensitivity and peak resolution than the GC/MS system. Figure 5 is the complete GC/FID chromatogram of the same sample as that shown in Fig. 3. Figure 6 shows expanded segments of similar chromatographic time windows as used for expansion of the total ion

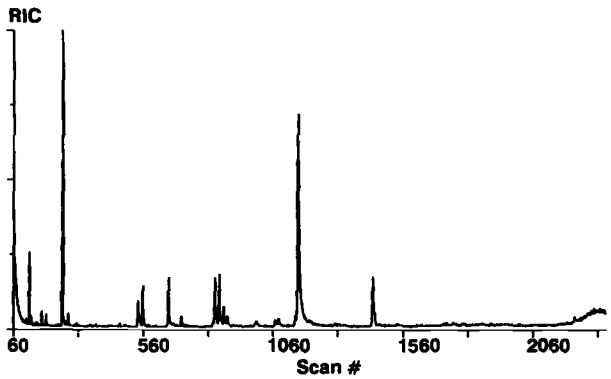

FIG. 3—*Complete total ion chromatographic profile of a cocaine exhibit.*

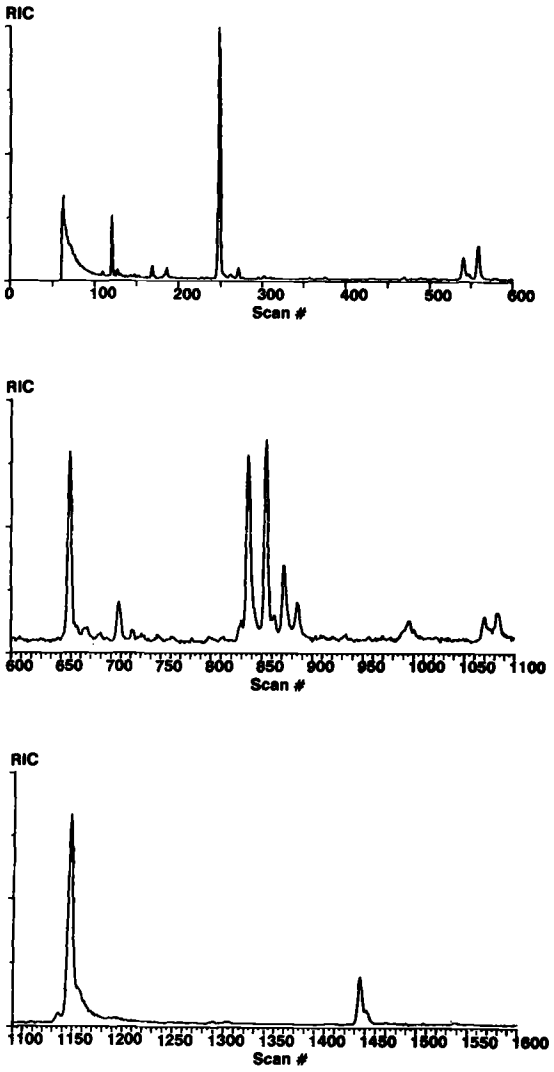

FIG. 4—*Expanded segments: 61–600 s; 600–1100 s; 1100–1600 s of chromatogram shown in Fig. 3.*

chromatogram (Fig. 4). The amount of data acquired over the long chromatogram does not easily permit the comparison of exhibits. Therefore, the screening of samples is best accomplished by reviewing only one chromatographic time window. A time frame of approximately 10 to 28 min was found to be more appropriate since this window contains peaks readily identified as derived from the coca plant. The use of the chromatographic profile for sample matching relies on the method's ability to detect sufficient relevant substances to be able to differentiate samples. Figures 7 and 8 are examples of six profiles obtained on ostensibly unrelated exhibits; the figures clearly demonstrate the differences in chromatographic peaks obtained on these samples.

As for the co-alkaloid ratios, in order for the GC/MS and GC/FID data to be useful, the chromatographic profiles had to be reproducible. Sixteen duplicate samples were analyzed on different days. The chromatographic profiles were always equivalent. Slight

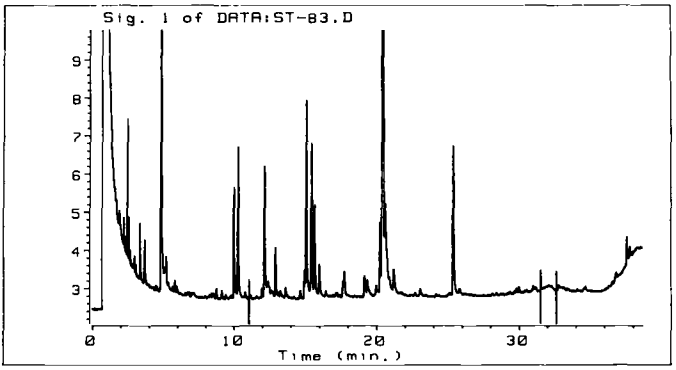

FIG. 5—*Complete FID chromatogram of the profile of the cocaine exhibit whose total ion chromatogram is shown in Fig. 3.*

differences were detected in the relative intensities of some of the smaller peaks, but the predominant peaks remained the same. For example, Figs. 9 and 10 are the results obtained by GC/FID analysis on two different samples on different days.

The use of the GC profile to compare exhibits that may have been stored under different conditions requires the demonstration of the preservation of the integrity of the profile. For the determination of the stability of the chromatographic profiles, duplicate 400-mg aliquots of "MFR A" and "MFR B" samples were stored in the temperature-controlled humidity chambers described previously. Two of these were withdrawn and analyzed on the same day. Analysis of the profiles of the samples kept in the humidity chambers for up to 18 days indicated the appearance of only one additional chromatographic peak at 1425 s (GC/MS), in comparison with the original profile. The samples stored at 50% humidity exhibited the greater, albeit small, amount of this unidentified material. Only small differences in other peak intensities were apparent.

The GC profiles of samples from "MFR A" which exhibited different alkaloid ratios were essentially the same. For example, the GC profile analysis of the two samples taken from bundle 16, which were decidedly different by HPLC (Table 4), were the same. In fact, all of the profiles derived from "MFR A" cocaine are essentially the same and all of the profiles from the "MFR B" cocaine are essentially the same. This may be due to the coca plant material or to the manufacturing process, which is specific to each of the "manufacturers."

The storage and manipulation of the data obtained from both the HPLC systems and the GC/MS and GC/FID require a comment. At present, because the number of samples examined is relatively limited, the HPLC results are manipulated manually. Each of the R_{CIS} and R_{TRANS} values are calculated manually from the absolute integration values of the cocaine, CIS, and TRANS peaks. The nature of these values allows for ready storage and manipulation in spreadsheet or database programs. We have used the latter of these to store and manipulate data from the analysis of samples derived from large, continuing "buy" projects and samples submitted on a random basis. The application is reasonably simple.

On the other hand, data from the GC/MS and GC/FID are not as amenable as HPLC data to computer storage and manipulation. The relatively small amount of data generated to date has not warranted the development of a computer-based profile matching program. However, after the analysis of approximately 100 different samples, it became necessary to devise a system for the storage, retrieval, and comparison of profiles. We have developed a catalog system based on the number and nature of detected significant substances in the GC/MS profile. The system results in the assignment of alphanumeric

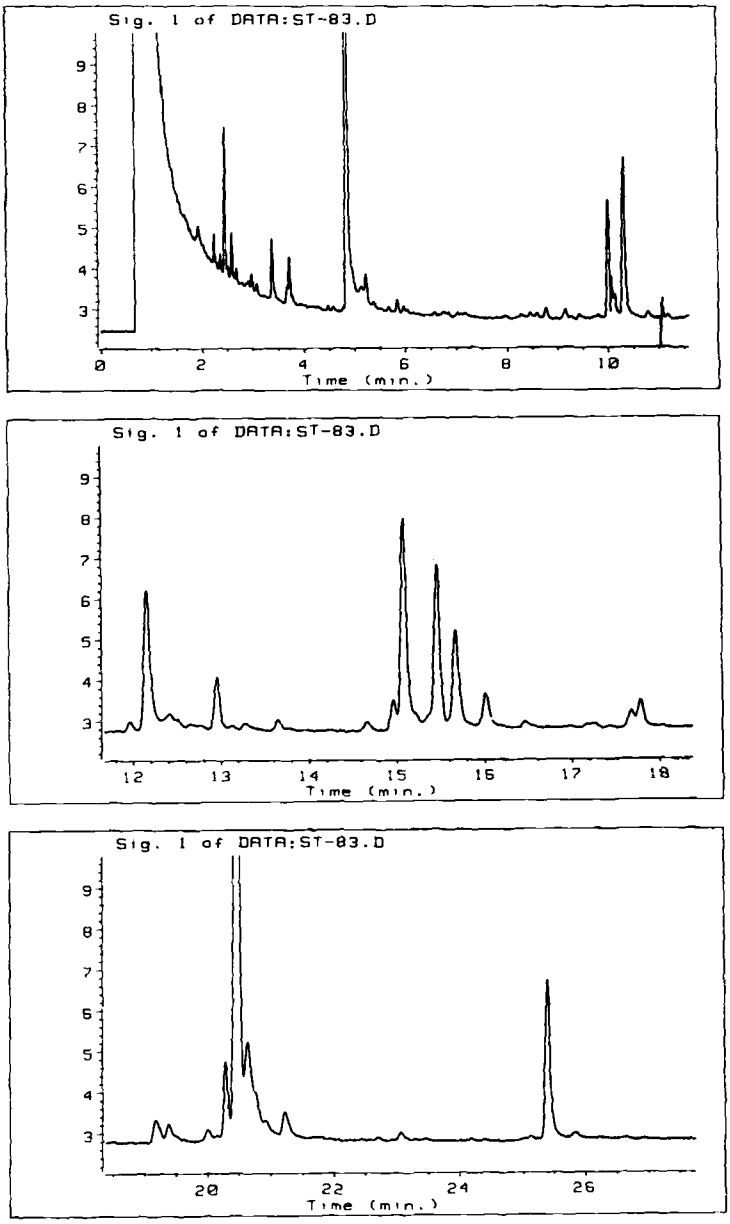

FIG. 6—*Expanded segments: 0–11.25 min; 11.40–18.40 min; 18.50–27.00 min of chromatogram shown in Fig. 5.*

codes to each chromatogram. First, the normalized total ion chromatogram over the time range 600 to 2000 s is examined for peaks of 50% or greater intensity in relation to the highest peak. The background subtracted mass spectra of only these peaks are recorded and matched, using the instrument's library, to peaks previously encountered. Each of these previously encountered entities had been assigned a letter of the alphabet based on its increasing elution from the column and its mass spectra recorded in a small library. These same letters were then applied to the particular peaks found in the chromatogram

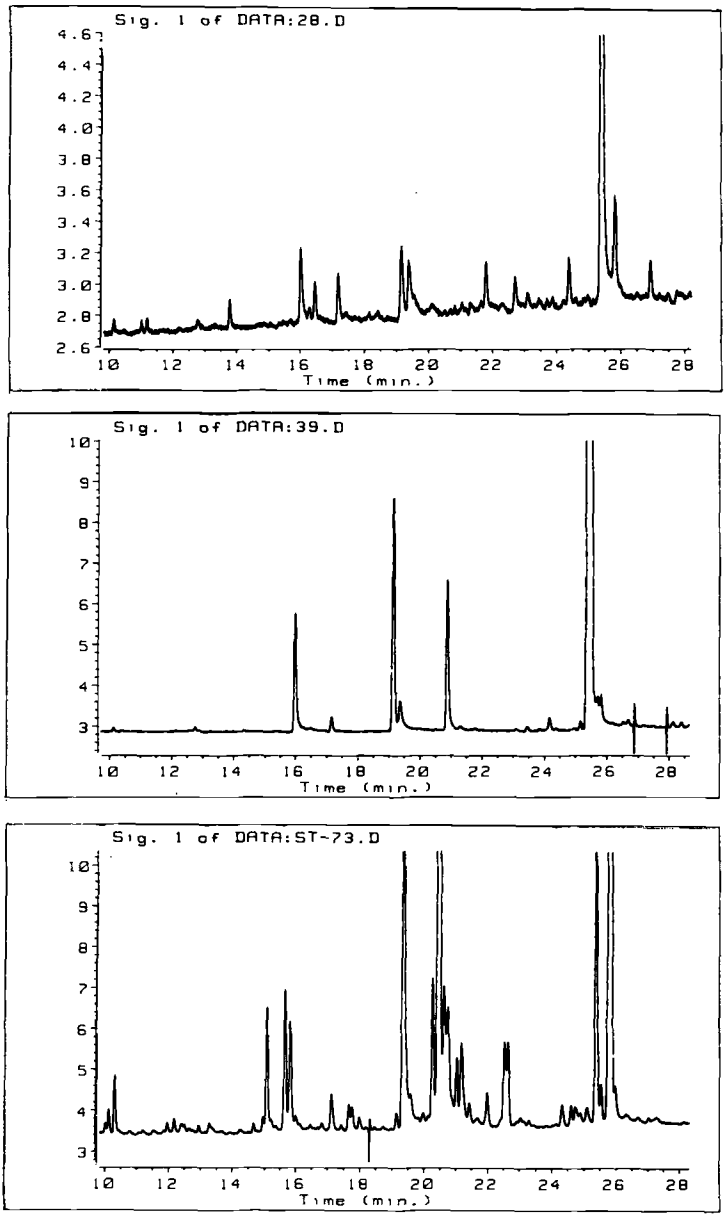

FIG. 7—*FID chromatographic profile (10–28 min) of three different exhibits.*

of the sample. These letters are the alphabetical parts of the code assigned to the sample. To date, only 15 different significant peaks have been encountered. Next, the normalized chromatogram is vertically expanded four times and the number of peaks greater than baseline +20% are counted. These peaks do not necessarily correspond to the 15 that have been previously encountered, but in practice many do. This number is the numeric part of the code assigned to the sample. The numeric designation will always equal or exceed the number of alphabetical designations. This coding system results in designations

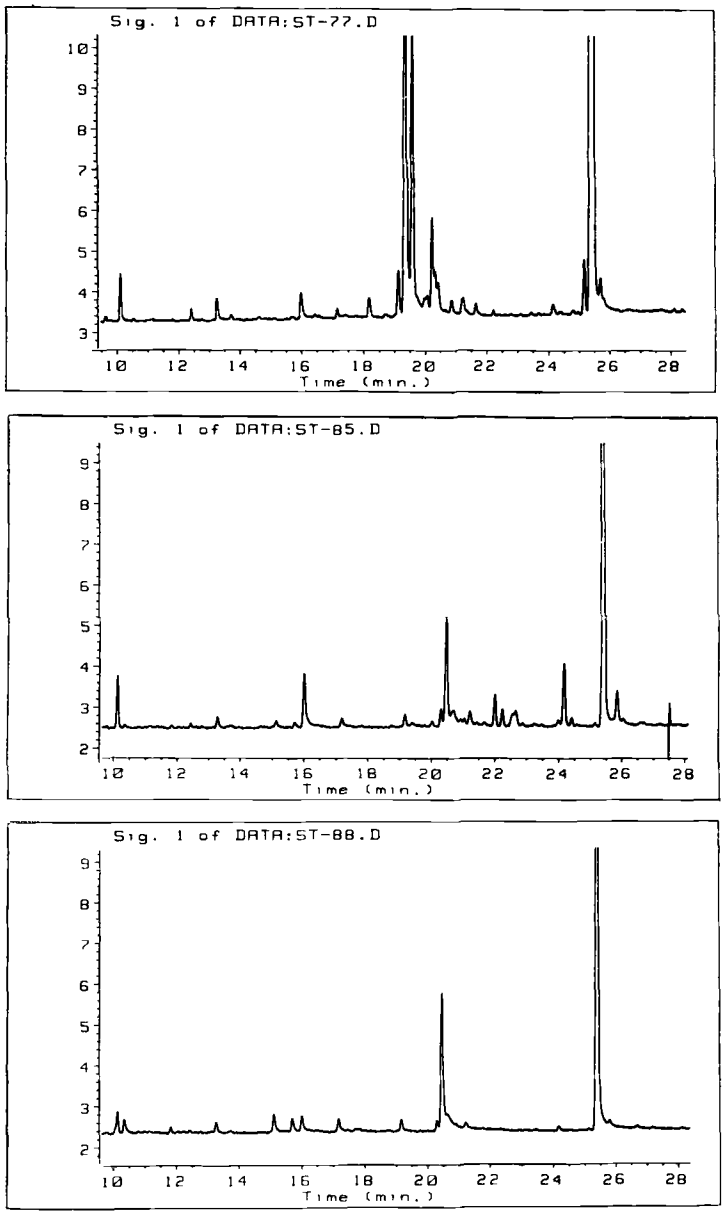

FIG. 8—*FID chromatographic profile (10–28 min) of three different exhibits.*

such as 4AC, 1B, etc. So far, the highest numeric designation was nine and the highest number of peaks used for alphabetical designation was three. The limitation of this system is recognized. The major drawback is in the numerical designation since some peaks may be marginally more or less than the 20% cutoff. This could result in similar or identical samples having designations of 4BC and 3BC. However, because the entire chromatogram would need examination before sample matches are made, the system can be expected to eliminate many samples from consideration.

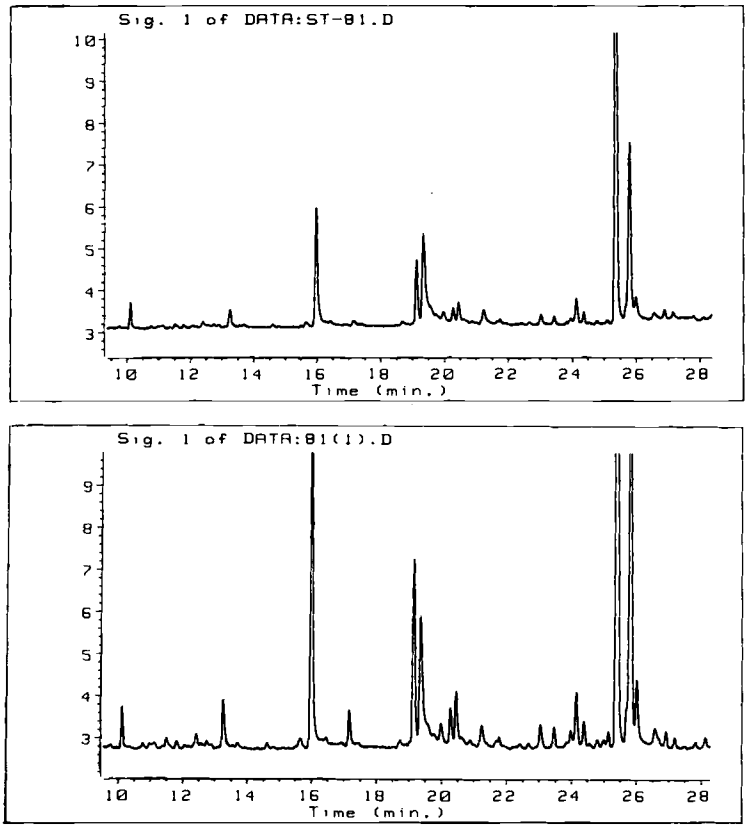

FIG. 9—*FID chromatographic profiles (10–28 min) of an exhibit obtained on two different days.*

Conclusions

Over the past few years, our laboratory has used the relative amounts of the other coca alkaloids in cocaine exhibits to provide intelligence information to enforcement agencies. This has been found particularly useful by our clients in investigations involving multiple "buys." Until recently, our opinion regarding the relationships of exhibits has been founded solely on the comparison of the presence of the relative amounts of the three main coca alkaloids.

The validity of the usefulness of these co-alkaloid ratios for comparing exhibits is supported by the results of this study. The data presented in Table 2 indicate that the day-to-day variation in the CIS and TRANS ratios determined on both HPLC systems is less than 1%. A review of the data from Table 3 indicates that within a block of cocaine the variation in the CIS and TRANS ratios varies up to 2.2% (CIS ratio, Brick 2). This particular brick appears to have been the most heterogeneous. The combination of excellent system performance and good sample homogeneity allows for the use of a narrow window in analytical data to determine differences in samples based on different alkaloid ratios. From these data, we consider variations of more than 3% in either of the CIS or TRANS ratios sufficient for the samples to be declared different. This does not mean that they are unrelated, but rather that they did not originate from the same "brick" of cocaine. An examination of the data in Table 4 provides, for illustration, examples of ratios generated on related samples. It can easily be seen that the two samples

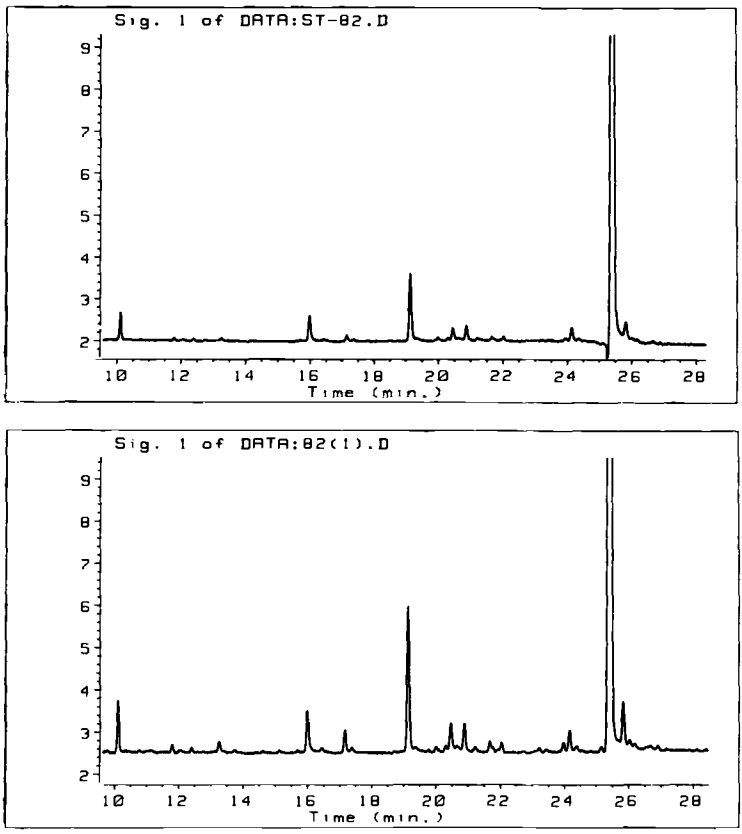

FIG. 10—*FID chromatographic profiles (10–28 min) of an exhibit obtained on two different days.*

taken from each of bundles 18, 20, and 21 are the same—the variations of both the CIS and TRANS ratios are within experimental differences. Similarly, the two samples from bundle 25 can be seen to be different because the CIS and TRANS ratios differ by more than 17%. However, the situation for the samples taken from bundles 16 and 19 is not as clear. The CIS and TRANS ratios of the two samples taken from bundle 16 differ by 6 and 5%, respectively. These samples we conclude originated from different lots. Similarly, the two samples derived from bundle 19 differ in their CIS and TRANS ratios by 9 and 2.8%. Again, we conclude the two samples were derived from different lots of cocaine. For the comparison of large sample populations, the application of recognized statistical methods could be used. However, in practical use in our laboratory, the number of samples to be compared is most frequently small (six or less) and the application of the empirical window mentioned above is adequate for data comparison.

During this same time, we also examined some exhibits to determine the solvent residues present in cocaine. In two exhibits, the relative amounts of the three major alkaloids were found to be the same, but the solvent residues in the exhibits were found to be different. The exhibits could not have been derived from the same cocaine if the solvents used in preparing the hydrochloride salt were different. This indicated that comparisons of exhibits based solely on the alkaloid contents could lead to accidental coincidences and erroneous conclusions. This was particularly surprising considering the wide range of the relative amounts of each of the other two alkaloids, each of which is independent of the other. A random sampling of 41 exhibits indicated the ratios of R_{CIS}

and R_{TRANS} using System 1 varied from infinity—there were no detectable amounts of either of the other two alkaloids—to 1.54 (R_{CIS}) and 0.65 (R_{TRANS}).

It was clear that more comprehensive analytical data on cocaine exhibits were necessary for more reliable comparisons. We had proposed [2] that another alkaloid, norcocaine, present in some cocaine exhibits might provide additional information for comparison purposes. However, the rare presence and low amounts of this alkaloid in exhibits limit its general utility for comparing different exhibits. It was for this reason that we turned to the examination of acidic extracts of cocaine exhibits. These extracts, which are devoid of the major coca alkaloids, provide information on the presence of an entirely different collection of substances.

The use of a mass spectrometer was invaluable for initial studies in order to determine the relevance of detected peaks. The comparison of mass spectra of the peaks in the profiles is still an essential component of the entire comparison process in our laboratory. However, the excellent correspondence between the GC/MS and GC/FID profiles indicates that the GC/FID also has a role in the screening of large numbers of samples.

In previous studies of the comparison of exhibits for enforcement agencies, we relied upon the co-alkaloid ratios to provide the first "close match" of samples and upon the profiles to provide additional confirmation of the similarities of samples. However, the results indicate that this approach permits only the linking of samples derived from the same or closely related batches; samples from the same processing facility with significantly different alkaloid ratios would not be identified as being related. Judging from the results of the profiles of the samples analyzed during this study, the profiling alone is able to distinguish large production batches or manufacturers. The similarity of the profiles from all of the "MFR A" and "MFR B" samples supports this. Therefore, the profiles may be more useful in determining distribution patterns over a wide area. However, further studies are needed to determine the profiles of more cocaine exhibits that have been seized as the result of a single police investigation.

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