John F. Casale,[1] *B.S. and Richard W. Waggoner, Jr.,*[1] *Ph.D.*

A Chromatographic Impurity Signature Profile Analysis for Cocaine Using Capillary Gas Chromatography

REFERENCE: Casale, J. F. and Waggoner, R. W., Jr., **"A Chromatographic Impurity Signature Profile Analysis for Cocaine Using Capillary Gas Chromatography,"** *Journal of Forensic Sciences*, JFSCA, Vol. 36, No. 5, Sept. 1991, pp. 1312–1330.

ABSTRACT: The signature patterns of cocaine samples were examined by capillary gas chromatography using 14 impurities commonly found in illicit cocaine samples. The procedure is based on acidic, basic, and neutral impurities introduced from the coca plant and from the processing of cocaine in clandestine laboratories. Impurities containing either alcoholic, *N*-nor, or carboxylic acid functional groups were analyzed as their silyl derivatives. The reported procedure provides a simple one-step assay for obtaining chromatographic impurity signature profile analyses (CISPA) of illicit cocaine samples using flame ionization detection (FID).

KEYWORDS: toxicology, cocaine, chromatographic analyses, cocaine impurities, signature profile, gas chromatography, CISPA

Currently, the abuse of cocaine has become epidemic in all segments of society, and enforcement strategies are now being directed more toward conspiracy cases. Cocaine conspiracy cases are some of the most difficult criminal cases to prove. Suspects are usually linked together through telephone toll records, hotel receipts, surveillance of suspects, and co-conspirator testimony. One of the nagging problems in drug enforcement has been the lack of a technique to match cocaine exhibits believed to originate from the same source or batch for such cases. The value of a chromatographic impurity signature profile analysis (CISPA) with pattern matching for intelligence and evidential purposes has become evident from numerous requests by law enforcement investigators and other forensic laboratories. A CISPA procedure determining if two or more exhibits of cocaine came from the "same batch" would be a tremendous benefit to law enforcement as an investigative tool and can ultimately be used in judicial proceedings.

The theory and background of drug signature profiling has been discussed at length by Neumann [*1*]. Several researchers have developed methodologies to obtain signature patterns for amphetamine [*2–10*], methamphetamine [*11–15*], opium alkaloids [*16–28*], and cannabis [*29–35*]. It was first shown by Moore [*36*] that gas chromatography was the method of choice for analysis of illicit cocaine and related coca alkaloids. Others have recently begun developmental work on signature profiling of cocaine [*37–48*]. These methods are based on impurities present as natural products or by-products of chemical

This work was supported by both the 1988 Federal Drug Grant Fund and the North Carolina Department of Crime Control and Public Safety under Grant No. 170-188-E6-D009. Presented in part at the International Symposium on the Forensic Aspects of Trace Evidence, Quantico, VA, 24–28 June 1991. Received for publication 13 Dec. 1990; revised manuscript received 5 Feb. 1991; accepted for publication 8 Feb. 1991.

[1]Senior research chemist and forensic chemist, respectively, Drug Chemistry Laboratory, State Bureau of Investigation, Raleigh, NC.

manipulation. Virtually all cocaine samples contain alkaloid impurities at flame ionization detection (FID) levels. Impurities can be introduced from several sources. These include co-extracted alkaloids from the coca plant, processing chemicals, solvents, packaging materials, and inorganic insolubles (sulfates, carbonates, and so forth). Impurities may also be introduced from the chemical modification of cocaine or other coca alkaloids via chemical processing, extreme environmental conditions after packaging (heat and humidity), and the relative instability of cocaine hydrochloride itself. Impurities (Fig. 1) which are examined by this method are benzoic acid (*1*), anhydroecgonine methyl ester (*2*), anhydroecgonine (*3*), *trans*-cinnamic acid (*4*), ecgonine methyl ester (*5*), ecgonine (*6*), tropacocaine (*8*), benzoylecgonine (*11*), norcocaine (*12*), beta-truxinic acid (*13*), alpha-truxillic acid (*14*), *cis*-cinnamoyl ecgonine methyl ester (*15*), *trans*-cinnamoyl ecgonine methyl ester (*16*), and *N*-formyl cocaine (*17*). These impurities can be examined

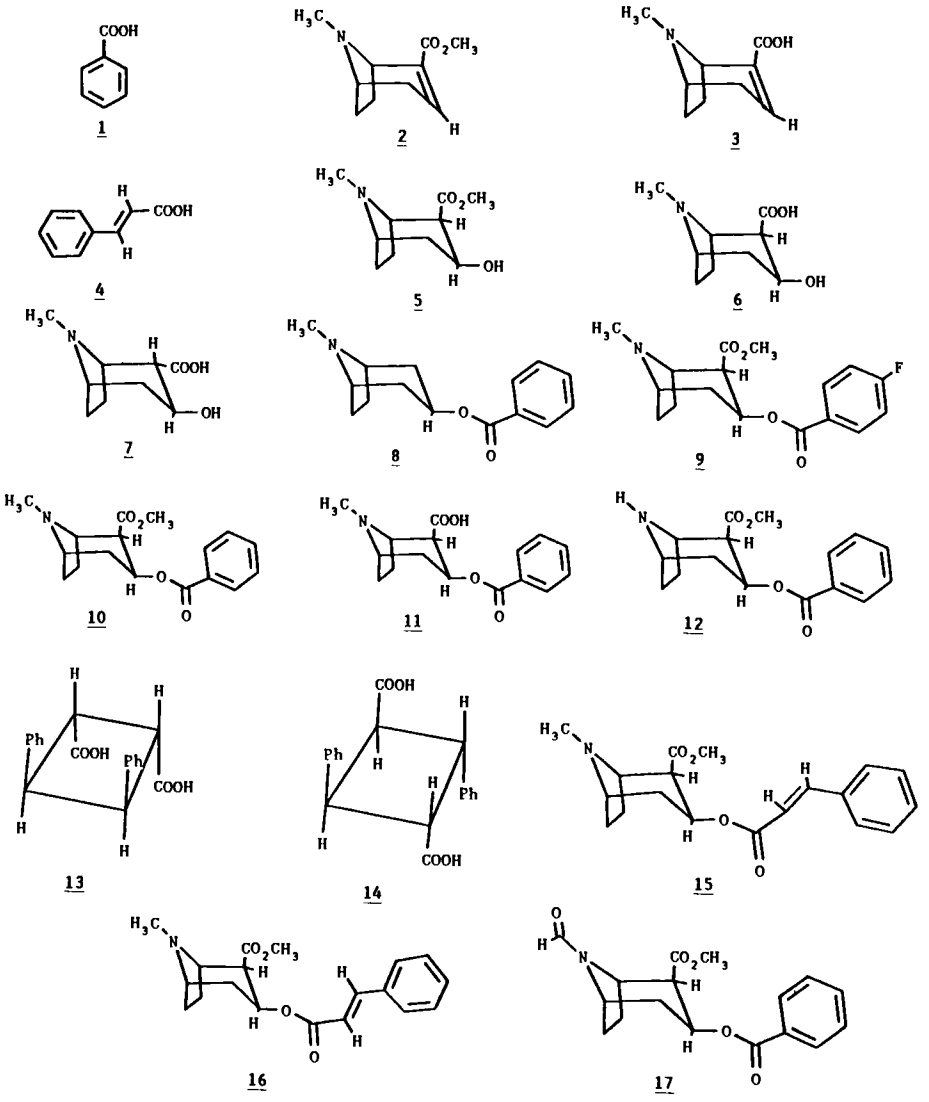

FIG. 1—*Structural formulas of compounds.*

together as a multipeak chromatogram obtained by FID capillary gas chromatography for comparison of two or more exhibits. Comparison of chromatograms containing trace impurities for signature profiling can ultimately be used for intelligence information and investigative leads, and as corroborative evidence to show a relationship or linkage between exhibits in conspiracy cases. Several procedures have previously been reported for cocaine impurity analysis, but these only examined a small or select group of impurities or used complicated and time-consuming steps. This paper presents a rapid, one-step derivatization to obtain FID capillary gas chromatographic signature profile patterns for combined acidic, basic, and neutral impurities commonly found in illicit cocaine samples.

Experimental Materials and Methods

A Hewlett-Packard Model 5890 gas chromatograph was used to generate all standard and sample chromatograms. A 30-m, 0.25-mm inside diameter, fused silica capillary column coated with DB-1701 (J&W Scientific) at a film thickness of 0.25 μm was employed. Helium (99.999 ultrahigh purity) was the carrier gas at a flow rate of 30 cm/s. The injection port was maintained at 230°C, and samples were injected in the split mode (50:1) by a Hewlett-Packard Model 7673A auto injector. The oven temperature was multilevel programed as follows: Level 1, initial temperature, 180°C; initial hold, 1.0 min; temperature program rate, 4°C/min; final temperature, 200°C; final hold, 0 min; Level 2, temperature program rate, 6°C/min; final temperature, 275°C; final hold, 11.5 min. Nitrogen was used as the makeup gas at a flow rate of 30 mL/min. Detection was flame-ionization operated at 280°C and interfaced with a Hewlett-Packard Pascal Chem Station (Version 4.0) for data processing. All chromatograms were recorded at an attenuation of 2^4 and a chart speed of 0.66 cm/min.

Mass spectra of all cocaine impurities were obtained on a Hewlett-Packard Model 5971A mass selective detector (MSD) operated under electron ionization (EI) conditions at 70 eV and in full scan mode.

Statistical computations were obtained from SAS Institute [*49*] using BASE SAS, SAS/STAT, and SAS/ASSIST software (Version 6.04).

Reagents and Standards

The *N,O*-bis (trimethylsilyl) acetamide (BSA) was obtained from Pierce Chemical. All other reagents were of reagent grade quality. Natural cocaine (*10*) was obtained from an authentic reference collection of this laboratory. Benzoic acid, tropacocaine, and *trans*-cinnamic acid were obtained from Aldrich Chemical, Sigma Chemical, and Mallinckrodt Chemical, respectively. Anhydroecgonine methyl ester was prepared according to Clark et al. [*50*]. Ecgonidine was prepared via acid hydrolysis of anhydroecgonine methyl ester. Ecgonine, ecgonine methyl ester, and *para*-fluorococaine (*9*) (ISTD) were prepared according to Casale [*42*]. Norcocaine, benzoylecgonine, *N*-formyl cocaine, *trans*-cinnamoylecgonine methyl ester, and the dextrorotatory enantiomer of cocaine were prepared according to Baldwin [*51*], Findlay [*52*], Brewer and Allen [*43*], Moore [*36*], and Casale [*53*], respectively. Samples of alpha-truxillic acid and beta-truxinic acid were provided by the U.S. Drug Enforcement Administration's Special Testing and Research Laboratory.

Preparation and Derivatization of Standards

Individual chloroform solutions of *trans*-cinnamoylecgonine methyl ester (560.0, 280.0, 112.0, and 56.0 μg/mL), *N*-formyl cocaine (85.0, 42.5, 17.0, and 8.5 μg/mL), anhydroecgonine methyl ester (310.0, 155.0, 62.0, and 31.0 μg/mL), and tropacocaine (200.0, 100.0,

40.0, and 20.0 μg/mL) were prepared containing 87.5 μg/mL of the ISTD. Individual derivatized chloroform solutions of ecgonidine (103.0, 51.5, 20.6, and 10.3 μg/mL), benzoic acid (85.0, 42.5, 17.0, and 8.5 μg/mL), ecgonine (367.0, 183.5, 73.4, and 36.7 μg/mL), ecgonine methyl ester (161.0, 80.5, 32.2, and 16.1 μg/mL), norcocaine (169.0, 84.5, 33.8, and 16.9 μg/mL), benzoylecgonine (328.0, 164.0, 65.6, and 32.8 μg/mL), alpha-truxillic acid (157.0, 78.5, 32.0, and 16.0 μg/mL), beta-truxinic acid (53.0, 26.5, 10.0, and 5.0 μg/mL), and *trans*-cinnamic acid (90.0, 45.0, 18.0, and 9.0 μg/mL) were prepared by first derivatizing the accuracy weighed standard with BSA for 15 min at 80°C, cooling to room temperature, and diluting to the appropriate volume with chloroform. A chromatogram from a mixed standard solution is illustrated in Fig. 2.

Sample Analysis

A modification of the procedures of Moore [*47*] and Casale [*42*] was used and is described as follows: A 4 to 5-mg sample of either unadulterated cocaine base or hy-

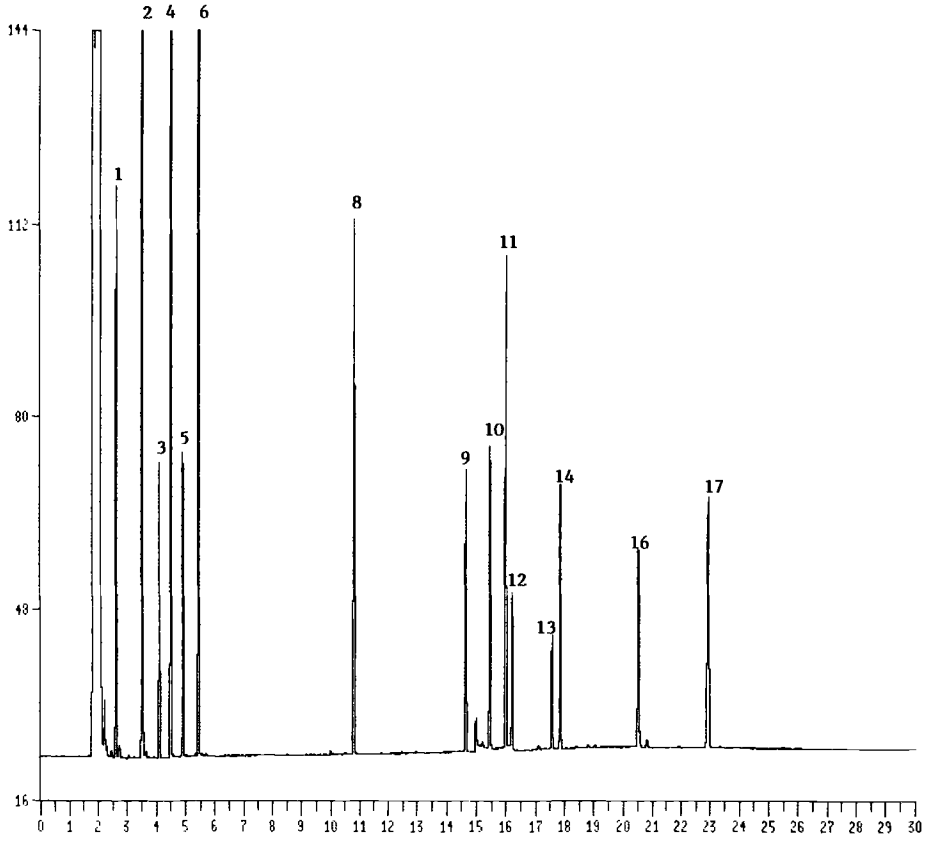

FIG. 2—*Capillary GC-FID of mixed standards (on column concentrations). Peaks:* 1 = *benzoic acid-*O*-TMS derivative, 8.4 ng;* 2 = *anhydroecgonine methyl ester, 29.2 ng;* 3 = *anhydroecgonine-*O*-TMS derivative, 5.3 ng;* 4 = trans*-cinnamic acid-*O*-TMS derivative, 18.8 ng;* 5 = *ecgonine methyl ester-*O*-TMS derivative, 5.9 ng;* 6 = *ecgonine-di-*O*-TMS derivative, 14.5 ng;* 8 = *tropacocaine, 12.2 ng;* 9 = para*-fluorococaine (ISTD), 8.7 ng;* 10 = *cocaine, 9.0 ng;* 11 = *benzoylecgonine-*O*-TMS derivative, 13.3 ng;* 12 = *norcocaine-*N*-TMS derivative, 5.1 ng;* 13 = *beta-truxinic acid-di-*O*-TMS derivative, 4.0 ng;* 14 = *alpha-truxillic acid-di-*O*-TMS derivative, 5.5 ng;* 16 = trans*-cinnamoylecgonine methyl ester, 7.1 ng; and* 17 = N*-formyl cocaine, 19.7 ng.* Cis*-cinnamoylecgonine methyl ester retention time = 18.47 min.*

drochloride was accurately weighed into a 1.5-mL septa top vial. To this vial was added 200 μL of 175 μg/mL *p*-fluorococaine (ISTD) in chloroform (25°C) and 200 μL of BSA (25°C). The vial was capped and heated at 80°C for 15 min to complete derivatization. The vial was allowed to cool to room temperature for 30 min, and 5 μL of the solution was injected into the gas chromatograph. Multiple samples for comparative purposes were weighed, diluted, and derivatized at the same time. Sample chromatograms are illustrated in Fig. 3.

Results and Discussion

Derivatization and Chromatography

Each standard containing an alcoholic, *N*-nor, or carboxylic acid functional group was completely derivatized after 15 min at 80°C. Cocaine, tropacocaine, anhydroecgonine methyl ester, cinnamoylecgonine methyl esters, and *N*-formyl cocaine will not form a silyl derivative with BSA. The retention time for each component is listed in Table 1. The EI-MS of all derivatized and underivatized standards (Table 2) confirmed correct chemical designations and each chromatographed as a single peak. All standards were stable in BSA once derivatized and exhibited no decomposition up to 24 h. Since an

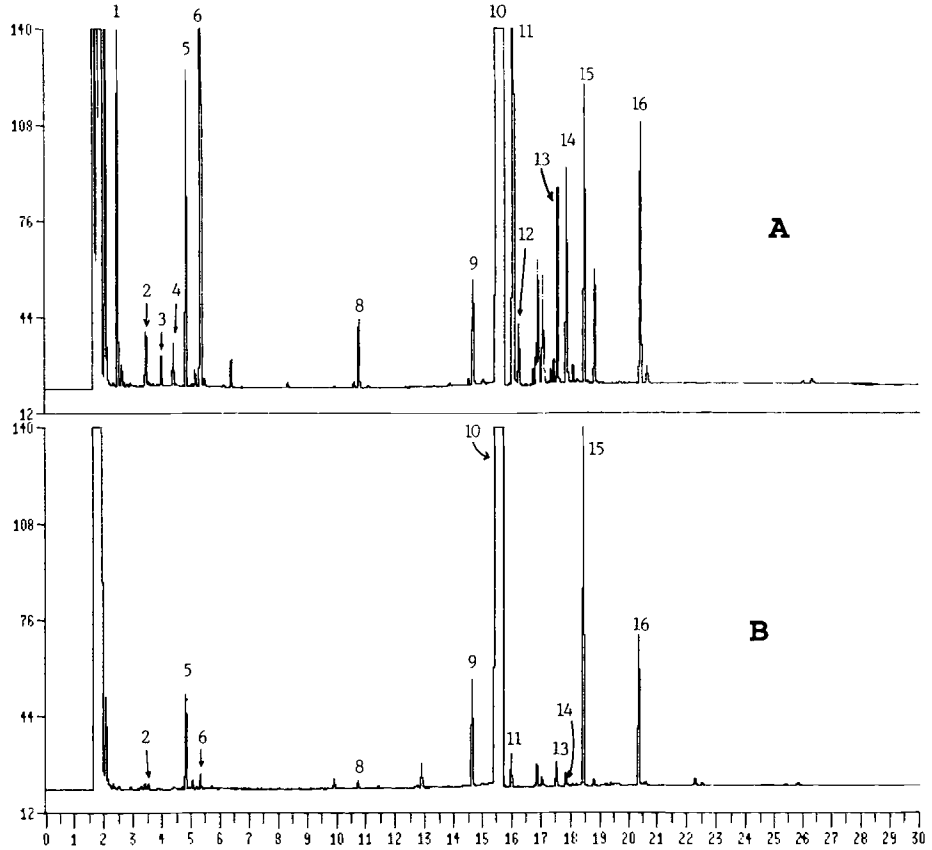

FIG. 3—*Capillary GC-FID of* (A) *cocaine HCl sample and* (B) *cocaine base sample. For peaks, see Table 1.*

TABLE 1—*Retention times for cocaine impurities.*

Compound	Retention Time, min
Benzoic acid (*1*)[a]	2.60
Anhydroecgonine methyl ester (*2*)	3.52
Anhydroecgonine (*3*)[a]	4.10
Trans-cinnamic acid (*4*)[a]	4.36
Ecgonine methyl ester (*5*)[a]	4.94
Ecgonine (*6*)[b]	5.45
Tropacocaine (*8*)	10.81
Para-fluorococaine (*9*)[c]	14.68
Cocaine (*10*)	15.45
Benzoylecgonine (*11*)[a]	16.04
Norcocaine (*12*)[d]	16.26
Beta-truxinic acid (*13*)[b]	17.61
Alpha-truxillic acid (*14*)[b]	17.89
Cis-cinnamoylecgonine methyl ester (*15*)	18.47
Trans-cinnamoylecgonine methyl ester (*16*)	20.55
N-formyl cocaine (*17*)	22.93

[a]Chromatographed as the *O*-TMS derivative.
[b]Chromatographed as the di-*O*-TMS derivative.
[c]Internal standard (ISTD).
[d]Chromatographed as the *N*-TMS derivative.

authentic sample of *cis*-cinnamoylecgonine methyl ester was not available, the FID response was assumed to have an equal molar response to the trans isomer. The linearity of the system was confirmed over the concentration ranges (as listed in the Experimental Materials and Methods section) for all standards and the coefficient of correlation exceeded 0.998 for each. All quantitative results are expressed as the free base and are in percentages of the total content of the cocaine sample (Table 3).

Para-fluorococaine was selected as the internal standard for several reasons. First, it is structurally similar to a majority of the alkaloids quantitated in this study and would naturally afford a similar FID molar response. Second, *p*-fluorococaine has excellent chromatographic properties, being a fluoro derivative, and does not interfere with coca alkaloids or any commonly found diluents. Finally, this internal standard was found to be very stable. A stock solution kept for over one year at 7°C in chloroform yielded no detectable hydrolysis or decomposition products when derivatized with BSA and consistently produced the same number of integrated counts for *p*-fluorococaine over that period.

Resolution of alkaloids and related impurities was best achieved on a DB-1701 column. However, disilyl-ecgonine and disilyl-pseudoecgonine coeluted under the conditions used. It should be noted that pseudoecgonine (*7*) has been reported as an impurity in only a small percentage of cocaine samples [*42*]. The DB-1701 column showed little degradation or column bleed after over 1000 injections. The cross-linked cyanopropylphenyl/methyl phase appears highly resistant to BSA at temperatures up to 280°C. No evidence of column overloading or peak tailing was observed with the high concentrations of cocaine used (12.5 mg/mL). Methanol injections between sample analyses gave no indication of sample carryover or flashback. Over 100 samples per week could be routinely analyzed using this procedure.

Impurity Formation

Many cocaine impurities are the result of chemical alteration of coca constituents, whether deliberate or not. The several possible routes to many of the impurities found

TABLE 2—*An eight-peak index for the electron ionization mass spectra of cocaine impurities.*

Compound	MW	Masses						Intensities								
1[a]	194	179	77	105	135	51	45	180	194	100	80	77	62	23	21	16	7
2	181	152	42	181	82	122	153	120	138	100	25	17	16	13	13	12	11
3[a]	239	210	42	122	82	75	239	224	183	100	33	27	24	21	20	18	6
4[a]	220	131	205	103	77	161	145	220	135	100	87	85	84	63	32	20	17
5[a]	271	82	96	94	271	182	240	155	212	100	82	41	32	28	27	20	12
6[b]	329	82	73	96	97	147	212	314	329	100	56	50	35	10	5	5	3
8	245	124	82	94	77	83	105	245	140	100	51	50	37	24	19	12	5
9	321	82	182	123	94	96	321	198	290	100	91	51	47	29	16	8	6
10	303	82	182	77	94	105	96	303	122	100	80	58	55	42	23	11	9
11[a]	361	82	105	240	94	77	361	122	256	100	44	36	35	34	14	10	5
12[c]	361	73	105	240	140	179	152	346	361	100	76	72	63	34	28	22	10
13[b]	440	205	73	131	220	103	161	322	440	100	48	41	34	26	19	6	0.5
14[b]	440	205	131	73	103	220	161	322	440	100	50	37	34	26	18	6	0.8
15/16	329	82	182	96	94	103	131	329	238	100	68	54	53	37	32	17	13
17	317	105	168	77	68	136	289	195	80	100	95	83	57	48	41	39	33

[a]Chromatographed as the *O*-TMS derivative.
[b]Chromatographed as the di-*O*-TMS derivative.
[c]Chromatographed as the *N*-TMS derivative.

in cocaine are illustrated in Fig. 4; many of these are a direct result from acid or base hydrolysis of cocaine. Others are from chemical manipulation of crude coca paste via permanganate oxidation [*43*]. The bulk of cocaine impurities are the consequence of a manufacturing process or storage or both. Each time cocaine is manipulated, the presence or concentrations of the impurities change, thus giving a new or different chromatographic profile.

Benzoic acid (*1*) is principally an impurity by-product from the hydrolysis of cocaine to ecgonine or ecgonine methyl ester. The formation of benzoic acid would arise particularly after hydrochloride conversion of cocaine base. Benzoic acid would not be carried over in substantial quantity as a co-extractant from coca paste to cocaine base because of its solubility in bicarbonate. It is unlikely that much of this carboxylic acid would be originally present in cocaine hydrochloride samples of clandestine manufacture because of its high solubility in diethyl ether/acetone utilized in the conversion of cocaine base to cocaine hydrochloride. Some benzoic acid will also be produced from the hydrolysis of benzoylecgonine, but to a much lesser degree because of the relatively low concentrations of benzoylecgonine as compared with cocaine. Hydrolysis of *N*-formyl cocaine, tropacocaine, and norcocaine would yield a negligible amount of benzoic acid since these three compounds are only present in trace amounts. Any benzoic acid present in coca leaves would not survive clandestine extraction procedures in appreciable amounts.

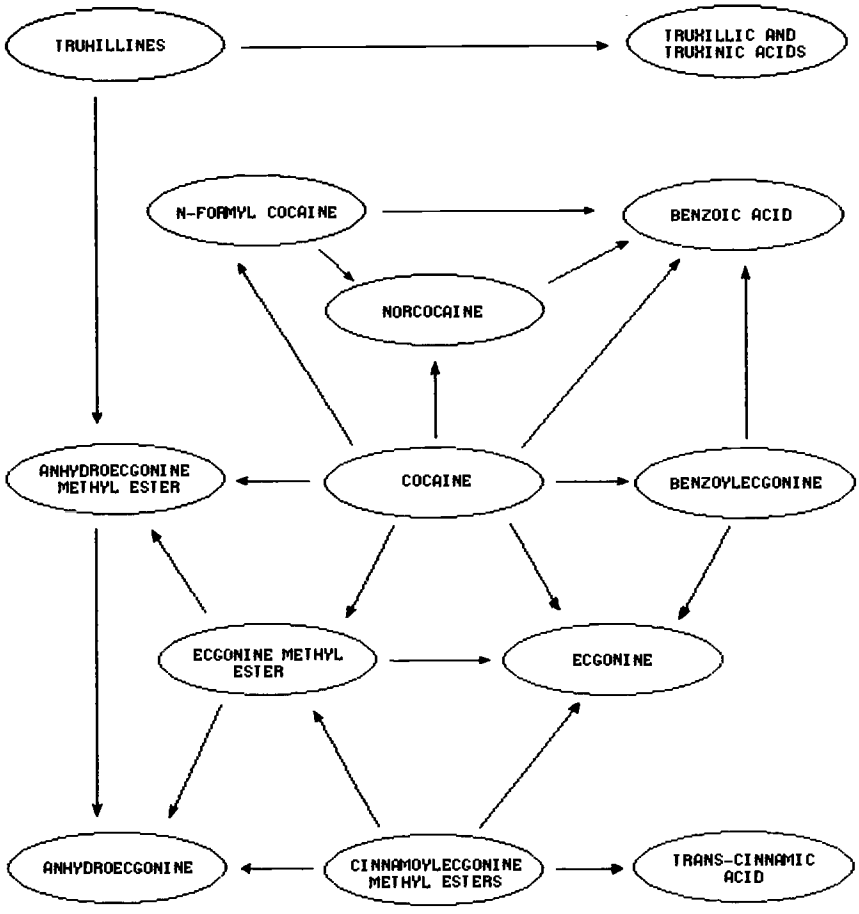

FIG. 4—*Impurity and decomposition pathways.*

Anhydroecgonine methyl ester (*2*), a basic trace impurity, is a hydrolysis by-product of cocaine produced upon elimination of one mole of water after removal of benzoic acid through acid hydrolysis. Much harsher conditions are required to produce No. *2*, and its presence is more pronounced in exhibits that have undergone substantial hydrolysis. Another source of this ester is the hydrolysis of the eleven intact truxillines, and smaller amounts of anhydroecgonine methyl ester will be generated from ecgonine methyl ester. Hydrolysis of truxillines will yield their respective truxillic/truxinic acids and No. *2*. Anhydroecgonine methyl ester, as a basic compound, will co-extract with cocaine through clandestine work-up procedures. An exception to this would be the potassium permanganate oxidation procedure [*43*] used as a purification step in many clandestine operations to remove alkaloids possessing double bonds. Exhibits that appear to have been through an oxidative clean-up procedure have negligible levels of No. *2*, because we have observed that low concentrations of cinnamoyl esters coincide with negligible levels of No. *2*. Concentrations of No. *2* found in this study were small when compared with other impurity alkaloids except for exhibits where excessive hydrolysis was evident.

Anhydroecgonine (*3*), an amphoteric trace impurity, is also produced from acid hydrolysis and elimination of water. Decomposition of several alkaloids (cocaine, benzoylecgonine, ecgonine, anhydroecgonine methyl ester, and ecgonine methyl ester) will lead to anhydroecgonine. It will not co-extract along with cocaine because of its acidity and solubility in base. Anhydroecgonine will not survive permanganate oxidation; accordingly, its presence in hydrochloride samples would be a product of hydrolysis after salt conversion. The concentrations of anhydroecgonine found in cocaine exhibits were found to be proportional to total sample hydrolysis and decomposition.

Trans-cinnamic acid (*4*) is a product from the hydrolysis of *trans*-cinnamoylecgonine methyl ester and *cis*-cinnamoylecgonine methyl ester. The hydrolysis of *cis*-cinnamoylecgonine methyl ester will yield *cis*-cinnamic acid, but the *cis*-acid will isomerize to the *trans*-acid because of its relative instability in acid conditions [*54*] and its tendency to isomerize to the *trans*-acid in the presence of light, as shown by Hocking [*55*]. The formation and presence of *trans*-cinnamic acid in cocaine samples is analogous to the formation of benzoic acid from cocaine. The relative concentration of *trans*-cinnamic acid usually correlates to the amounts of parent compounds present (that is, low cinnamoylecgonine methyl esters and high cinnamic acid, high cinnamoylecgonine methyl esters and low or undetected cinnamic acid).

Ecgonine methyl ester (*5*) is present as an impurity from two separate sources: it is found as an alkaloid in coca leaves [*56*] and is also a product from the hydrolysis of cocaine. Lesser amounts are derived from oxidative procedures. Ecgonine methyl ester will co-extract with cocaine from the coca leaf because of its basic properties; however, it would not be expected to survive substantially in clandestine oxidation procedures. Ecgonine methyl ester is also easily saponified as the free base [*53*] to ecgonine. The primary source of ecgonine methyl ester appears to be from hydrolysis of cocaine, and its presence is due primarily to decomposition after salt conversion. The concentration of ecgonine methyl ester will not necessarily correlate with the concentration of benzoic acid.

Ecgonine (*6*) has also been reported in coca leaves [*56*]. It is a carboxylic acid that is highly soluble in water and is not easily extracted from aqueous solutions. It is unlikely that much ecgonine would survive up to the salt conversion process because of its solubility in aqueous solutions and reactivity in oxidative procedures. The bulk of ecgonine in cocaine samples will be derived from acid hydrolysis of cocaine, with lesser amounts from permanganate oxidation of cinnamoyl ecgonine methyl esters and hydrolysis of ecgonine methyl ester. Ecgonine is one of the predominante impurities found in illicit cocaine exhibits and usually correlates to the amount of benzoic acid found. The presence of

ecgonine and its relative concentration are good indicators in subclassifying exhibits thought to be of common origin.

Tropacocaine (*8*) is a naturally occurring alkaloid found in coca leaves [*57*] and is not a product from the decomposition of cocaine or coca-related alkaloids. The detection of tropacocaine is a very useful indicator in subclassifying exhibits thought to be of common origin. Tropacocaine is a commercially available local anesthetic, but is rarely found as a diluent in cocaine samples. Concentrations exceeding 1% tropacocaine should be treated as highly suspicious with regard to the origin of the tropacocaine.

Cis- and *trans*-cinnamoylecgonine methyl esters (*15* and *16*), commonly referred to as cinnamoylcocaines, are also naturally occurring alkaloids found in the coca leaf. Neither is a product from decomposition of coca alkaloids. Each ester will be carried through solvent extraction procedures along with cocaine. As a general rule, the concentration of *cis*-cinnamoylecgonine methyl ester is higher than the trans isomer in illicit cocaine samples. Exhibits containing a high *trans*-to-*cis* ratio of these esters are unique in subclassifying cocaine comparisons. Labell et al. [*48*] stated that the relationship of cocaine/*cis*-ester and cocaine/*trans*-ester ratios was used as a basis for linking samples. We have found that those ratios and relationships are useful in a preliminary examination to rule out linkages but are not conclusive in themselves as to same source exhibits. Some or all of these alkaloids are removed during the clandestine potassium permanganate oxidation procedures.

Benzoylecgonine (*11*) has been reported as present in coca leaves [*58*] but is also a product of cocaine hydrolysis. It will be produced to a lesser extent from permanganate oxidation procedures. Benzoylecgonine will be produced predominantly as an impurity from acid hydrolysis of cocaine. It will not co-extract with cocaine in clandestine work-up procedures because of its high solubility in weak base and insolubility in diethyl ether.

N-formyl cocaine (*17*) is a neutral processing by-product from the permanganate oxidation of cocaine. Its presence has not been reported in coca leaves. Its formation as an impurity in illicit cocaine samples has been discussed at length by Brewer and Allen [*43*]. The concentration of *N*-formyl cocaine in cocaine samples is proportional to permanganate oxidation reaction length. *N*-formyl cocaine will be minimally co-extracted with cocaine during clandestine work-up procedures to appear in the final product.

Norcocaine (*12*) is a basic manufacturing by-product from permanganate oxidation. Brewer and Allen indicate that norcocaine is a hydrolysis product from a Schiff's base intermediate during permanganate oxidation [*43*]. Norcocaine can also be produced from the demethylation of cocaine in diethyl ether containing elevated levels of peroxides.[2] This would be especially prevalent in clandestine operations which recycle diethyl ether for conversion of cocaine base to cocaine hydrochloride. Norcocaine will co-extract with cocaine.

There are eleven stereoisomers of the truxillic/truxinic acids. Each is a dicarboxylic acid from the acid hydrolysis of their respective truxilline. It has been shown by Moore et al. [*44*] that "free isomeric" truxillic and truxinic acids were present in cocaine samples and were probably the result of truxilline hydrolysis. Truxillic/truxinic acids are soluble in weak base and would not survive in substantial quantity in clandestine work-up procedures. Therefore, the bulk of truxillic/truxinic acids found in cocaine samples would be an effect of truxilline decomposition. A lack of authentic standards for the remaining nine truxillic/truxinic acids prevented their quantitation in this study. However, several of these dicarboxylic acids were evident and could be tentatively identified as their disilyl derivatives chromatographing in the region of 16.5 to 19.0 min (Fig. 3*a*).

No pseudoecgonine methyl ester was detected in any of the samples analyzed. This ester could only be present from anhydrous base hydrolysis of cocaine, benzoylecgonine,

[2]Moore, J. M., DEA Special Testing Laboratory, personal communication, 1990.

or ecgonine methyl ester in the presence of methanol. However, pseudoecgonine methyl ester could be present as an impurity from the clandestine total synthesis of cocaine [*53*]. The commonly employed clandestine total synthetic route yields both ecgonine methyl ester and its C-2 epimer, pseudoecgonine methyl ester, as intermediates. In addition, traces of pseudococaine would also be present along with total synthesis impurities as described by Cooper and Allen [*59*].

Sample Comparisons

When cocaine hydrochloride (Fig. 3*a*) is converted to cocaine base via the conventional bicarbonate "crack process," many of the impurities containing a carboxylic acid moiety are reduced substantially (Fig. 3*b*). If cocaine "free base" is made from an ether/bicarbonate extraction, virtually all the carboxylic acids are removed because of their solubility in bicarbonate. The salt and base forms of cocaine can easily be distinguished via solubility tests or infrared analysis. Cocaine base derived from the bicarbonate/water "crack" process and from the diethyl ether/bicarbonate "free base" process can usually be differentiated by the presence of sodium bicarbonate in "crack" cocaine. However, the absence of sodium bicarbonate cannot rule out a "crack" process. Chromatographic profiling of impurity signature patterns has a potential use in differentiating "crack" from "free-base" cocaine. Free base exhibits will have a marked decrease in impurities possessing carboxylic acid functional groups (that is, ecgonine, benzoic acid, benzoylecgonine, and so forth) because of their higher solubility in bicarbonate solutions. In addition, benzoylecgonine is insoluble in diethyl ether. Cocaine exhibits derived from a "free base" process give few peaks in a chromatographic profile and are not useful in determining same-batch origins.

Each time cocaine is manipulated (such as by extraction, grinding, crystallization, storage at excessive heat, etc.), the composition of impurities within that sample is changed. Hundreds of different batches of cocaine seized over a five-year period were analyzed, and no two batches produced analogous impurity signature patterns. Chromatographic profiles were found to be unique for separate batches. It should be noted that the chromatographic patterns were not judged solely on the 14 impurities quantitated, but on the entire chromatographic pattern, which includes many unidentified components that chromatograph.

Chromatographic subtractions of exhibits were possible using Hewlett-Packard's Pascal Version 4.0 software. Chromatographic patterns (Figs. 5 and 6) could be subtracted from each other ($x - y$) to produce a chromatographic difference (Fig. 7), thus aiding the visual inspection of two chromatograms believed to be of common origin. Hypothetically, if two exhibits are from the same batch, they would yield a straight line after chromatographic subtraction. However, because of large peaks (solvent and cocaine) and small differences in retention times, a straight line could not be achieved because of software limitations. Thus, two exhibits from the same batch do give rise to some peaks in the subtracted difference of two chromatograms.

This method is advantageous in that only a small representative sample of unadulterated cocaine hydrochloride or cocaine base is required. The procedure can be used on adulterated cocaine hydrochloride samples provided a "flake" or "rock" can be removed and a clean sample of approximately 5 mg can be isolated. All adulterated "crack" cocaine exhibits were found to be uniform and presented no analytical problems. This technique is unlike that of Labell [*48*] because only coca-related impurities are examined instead of diluents, adulterants, and a few coca-related compounds. Any technique which attempts to quantify cocaine impurities in exhibits that have been diluted or adulterated after clandestine chemical/manufacturing processes is doomed to large errors in sampling [*60*].

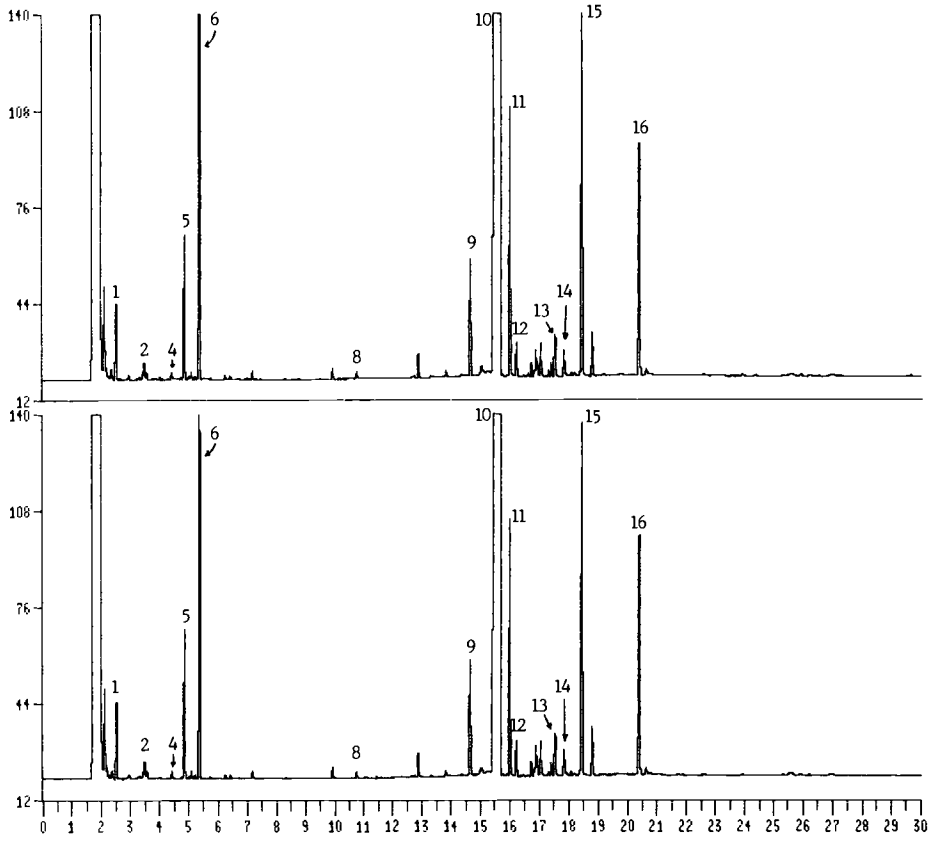

FIG. 5—*Two capillary GC-FID chromatograms of cocaine (same batch). For peaks, see Table 1.*

GC Artifacts

The possibility of artifacts produced by gas chromatography (GC) or sample preparation must always be considered when natural products are identified. All the compounds quantitated in this study, except tropacocaine and the cinnamoyl esters, could be produced as an analytical artifact. Artifacts can be introduced from several sources prior to derivatization. Extractions may inadvertently enhance or eliminate certain impurities because of their physical and chemical properties. Extractions may also promote impurity formation as artifacts (such as demethylation of cocaine to norcocaine in peroxide-enriched diethyl ether). Mixing and grinding of cocaine hydrochloride samples will subject the sample to excessive hydration, promoting decomposition of cocaine and related alkaloids, and also introduce sampling errors [*60*]. The cocaine samples in this study were derivatized directly after addition of the internal standard, thus eliminating possible artifact formation prior to derivatization.

Artifacts may also be produced from derivatization techniques and chromatography. No chromatographic or derivatization-induced artifacts were detected from individually derivatized solutions of the standards. In addition, no artifacts were detected from analysis of unnatural cocaine (dextrorotatory enantiomer) derivatized at a concentration of 12.5 mg/mL. Anhydroecgonine methyl ester can be generated as a major artifact in cocaine samples from thermal elimination of benzoic acid from cocaine in a GC injection port [*46*]. Lukaszewski and Jeffery found that anhydroecgonine methyl ester would be formed

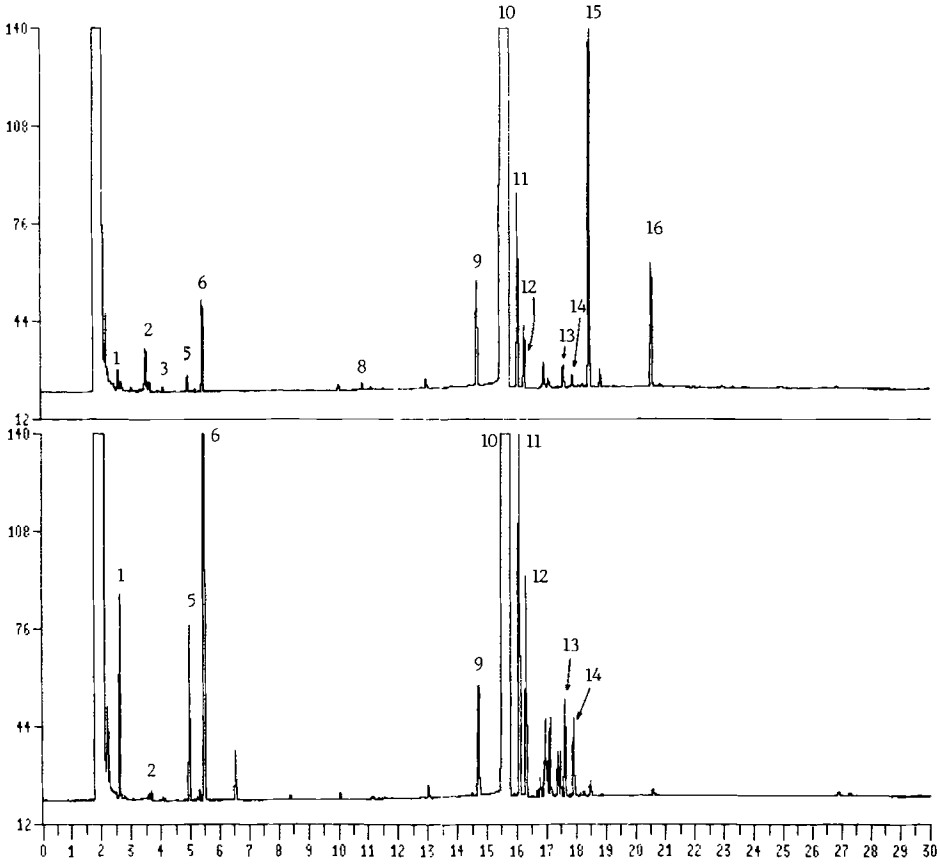

FIG. 6—*Two capillary GC-FID chromatograms of cocaine (different batches). For peaks, see Table 1.*

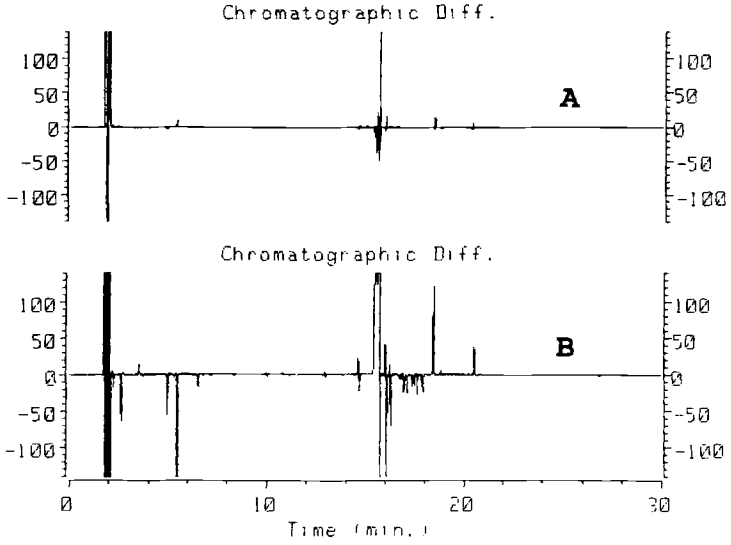

FIG. 7—*Chromatographic difference of* (A) *Fig. 5 "same batch" and* (B) *Fig. 6 "different batches."*

at an injection port temperature of 250°C. Moore et al. [*44*] have shown that thermal decomposition of truxillines can yield their respective truxillic/truxinic acids and anhydroecgonine methyl ester. In our study, no anhydroecgonine methyl ester was produced or detected at an injector temperature of 230°C when any of the standards containing the tropane moiety were introduced.

Nonuniform Batches

A small percentage of kilograms examined by multiple sampling showed a substantial variation of impurities. The visual appearance of differing color striations within a kilogram block of cocaine were indications of nonuniformity. Impurity variations within a kilogram seizure are most probably the result of differing final crops of crystallization being combined or extreme environmental conditions after packaging. If a kilogram package is placed on a heat source, the portion closest to that source will decompose and produce impurities at a greater rate than the portion furthermost away from that source.

Statistical Analysis

Elementary statistical information was computed by the PROC PRINCOMP in the SAS/STAT software package [*49*]. This procedure computed the averages and standard deviations (Table 3) of the concentration measurements from the 13 impurity variables obtained from 368 randomly chosen separate batches. One variable (*N*-formyl cocaine) was not considered in the statistical analysis because few samples had any appreciable amounts of *N*-formyl cocaine present. As can be seen from Table 3, 6 of the impurity variables have significantly higher standard deviations than the other variables. These high standard deviations show that the concentrations of the variables (impurities) have large variations between samples. Therefore, these impurities are the most useful in determining whether exhibits were "same batch samples."

A correlation matrix was obtained from the 368 observations used in calculating Table 4. The correlation matrix is a square matrix which shows the linear relationship between the variables as measured by their correlation coefficient. If two variables are highly correlated with each other, a redundancy may occur. The correlation analysis (Table 4) showed a marginally high correlation between *trans*-cinnamoylecgonine methyl ester and *cis*-cinnamoylecgonine methyl ester and between benzoic acid and ecgonine (each with a correlation coefficient of 0.90). The presence and formation of benzoic acid correlates highly to ecgonine, as expected. The correlation between benzoic acid and ecgonine is logical because both are the principal products from acid hydrolysis of cocaine. Benzoic acid also shows a moderate correlation to other carboxylic acids (that is, ecgonidine, *trans*-cinnamic acid, and benzoylecgonine). Interestingly, the truxillic/truxinic acids show low correlations to benzoic acid. This could be due to the relatively low stability of the truxillines in compared with cocaine in acidic conditions. Anhydroecgonine methyl ester does not correlate well with any of the other impurities studied in this method, and its formation is assumed to be independent of the other alkaloids. Anhydroecgonine and *trans*-cinnamic acid give only moderate correlations to benzoic acid and ecgonine. *Trans*-cinnamic acid did not correlate directly with its parent compounds, *cis*-cinnamoylecgonine methyl ester and *trans*-cinnamoylecgonine methyl ester, perhaps because of the isomerization of *cis*-cinnamic acid to *trans*-cinnamic acid. Ecgonine methyl ester showed a moderate correlation to the truxillic/truxinic acids as compared with other alkaloids and is most likely a function of truxilline hydrolysis. Ecgonine shows a moderate correlation to all other carboxylic acids studied (high correlation to benzoic acid of 0.90), as expected. Tropacocaine does not correlate well with any impurity alkaloids since it is a naturally

TABLE 3—*The averages, standard deviations, and ranges obtained from the 13 impurity variables.*[a]

Compound	1	2	3	4	5	6	8	11	12	13	14	15	16
Average	0.10	0.05	0.01	0.01	0.11	0.46	0.05	0.51	0.12	0.18	0.09	1.36	0.94
SD	0.16	0.08	0.02	0.03	0.21	0.54	0.12	0.60	0.24	0.14	0.08	0.86	0.61
Range	0.01–1.34	0.01–0.56	0.01–0.23	0.01–0.28	0.01–1.36	0.01–4.78	0.01–0.91	0.01–5.37	0.01–2.40	0.01–1.01	0.01–0.79	0.01–3.75	0.01–2.54

[a]The data are derived from 368 separate batch exhibits.

TABLE 4—*The correlation matrix of the 13 impurity variables.*[a]

Compound	1	2	3	4	5	6	8	11	12	13	14	15	16
1	1.00	0.16	0.61	0.47	0.18	0.90	0.04	0.50	−0.07	0.27	0.39	−0.10	−0.04
2	0.16	1.00	0.20	0.10	−0.11	0.08	0.23	0.34	−0.08	−0.10	0.08	0.02	0.09
3	0.61	0.20	1.00	0.23	−0.01	0.48	0.02	0.20	−0.07	0.04	0.13	−0.04	−0.02
4	0.47	0.10	0.23	1.00	−0.02	0.49	−0.02	0.17	−0.02	−0.01	0.16	−0.01	0.04
5	0.18	−0.11	−0.01	−0.02	1.00	0.37	0.02	0.38	−0.07	0.49	0.38	0.07	0.07
6	0.90	0.08	0.48	0.49	0.37	1.00	0.03	0.58	−0.10	0.42	0.51	−0.08	−0.01
8	0.04	0.23	0.02	−0.02	0.02	0.03	1.00	0.03	−0.08	−0.12	0.18	−0.01	0.17
11	0.50	0.34	0.20	0.17	0.38	0.58	0.03	1.00	−0.15	0.49	0.55	−0.06	−0.01
12	−0.07	−0.08	−0.07	−0.02	−0.07	−0.10	−0.08	−0.15	1.00	−0.11	−0.01	−0.31	−0.31
13	0.27	−0.10	0.04	−0.01	0.49	0.42	−0.12	0.49	−0.11	1.00	0.47	−0.08	−0.08
14	0.39	0.08	0.13	0.16	0.38	0.51	0.18	0.55	−0.01	0.47	1.00	−0.08	−0.02
15	−0.10	0.02	−0.04	−0.01	0.07	−0.08	−0.01	−0.06	−0.31	−0.08	−0.08	1.00	0.90
16	−0.04	0.09	−0.02	0.04	0.07	−0.01	0.17	−0.01	−0.31	−0.08	−0.02	0.90	1.00

[a]The data are derived from 368 separate batch exhibits.

occurring alkaloid in the plant. Norcocaine does not correlate well to other alkaloids either, except for correlation coefficients of −0.31 to the cinnamoylecgonine methyl esters. The mechanisms for norcocaine formation (demethylation of cocaine and hydrolysis of *N*-formyl cocaine) are in agreement with these data. Benzoylecgonine shows moderate correlations to other carboxylic acids, as expected. The truxillic/truxinic acids show moderate correlations to each other and with ecgonine and ecgonine methyl ester. Interestingly, the truxillic/truxinic acids show little correlation to anhydroecgonine methyl ester, thus lending more evidence to the truxillic/truxinic acids being true impurities of an exhibit and not as artifacts of the analysis. The cinnamoylecgonine methyl esters correlate highly with each other and poorly with other impurity alkaloids. Good correlations of the cinnamoylecgonine methyl esters to each other are expected since both are present in coca leaves and each will be carried through solvent extraction procedures along with cocaine. No significant correlation was observed between any of the other variables.

Principal Component Analysis

In addition, a principal component analysis was performed on these data. The principal components are linear combinations of the original variables, which explain the variance structure. This analysis is useful for data reduction. It is necessary to have all components to reproduce the total system variability. If much of this variability can be explained by a small number of the principal components (k), then there is almost as much information in the k components as in the original variables. Therefore, these k components can replace the initial variables with little loss of information. These principal components can be computed from either the correlation matrix or the covariance matrix. The covariance matrix measures how the variables vary with respect to one another. The eigenvalues obtained from these matrices are useful in judging the relative data reduction effectiveness of each principal component. The eigenvalues (E), which can be thought of as the variance of a particular principal component, define the proportion of total variance as seen in the following relationship:

$$\text{Proportion of total variance due to } i^{\text{th}} \text{ principal component} = \frac{E_i}{E_1 + E_2 + E_3 + \ldots E_p}$$

where $i = 1, 2, 3, \ldots p$ and $p =$ the number of variables. Normally, the correlation matrix is chosen because the data in this matrix have been standardized. A nonstandardized covariance matrix may contain data from variables which have different units or data which have differing degrees of variation. If this occurs, then variables with large variances are more strongly linked with components with large eigenvalues and variables with small variances are more strongly linked with components with small eigenvalues. The observations in this study are all expressed as percentages, so no problems of this nature were expected. The application of principal component analysis on the correlation matrix yielded little data reduction capacity. Eight principal components were necessary to obtain over 90% of the total variance. However, when the principal component analysis was performed on the covariance matrix, only three components were necessary to explain over 90% of the total variance. Therefore, the principal components taken from the covariance matrix can better replace variables than principal components taken from the correlation matrix. We are presently undecided as to whether we should pursue this type of analysis further.

Method and Assay Reproducibility

The reproducibility of the method was tested by determining the precision of replicate injections of a randomly selected exhibit and also from replicate injections of multiple samples of that exhibit. Five replicate injections were performed on 5 random samples of a uniform exhibit, thus producing 25 chromatograms (5 chromatograms per sample). The standard deviations of impurity concentrations were measured for both the replicate injections of the same sample and the corresponding injections from the 5 separate random samples. The standard deviations for multiple sample reproducibility of impurities ranged from 0.00055 to 0.0207. The standard deviations obtained from replicate injections from all 5 samples were roughly an order of magnitude less (0.00032 to 0.0031) than those for multiple sampling. This strongly suggests that the predominant portion of error is due to the weighing and dilution of samples. The overall absolute error of the assay was determined from the above test to be less than 1%.

Pattern Recognition of Chromatographic Profiles

The ultimate goal of this type of analysis is to build a computerized database of samples to be searched against new exhibits for possible matches. Manual searching of new chromatographic profiles against data from hundreds of chromatographic profiles would be a monumental task if not impractical. A database and computerized search capability would speed exhibit correlations in narrowing a broad field of possible matches for the analyst, thus enhancing a timely output of intelligence information. Older chromatographic profiles could be purged periodically from the database as it is built and utilized to speed correlations, since the probability is remote that two exhibits seized months apart would have originated from the same batch. We are in the process of developing such a software tool to identify chromatograms of cocaine impurities derived from the same batch. Statistical programs and neural networks (artificial intelligence) are two types of software that have been evaluated as comparison tools. Several commercial statistical software packages were evaluated for their ability to do pattern recognition. These techniques were based on the relative concentration of each impurity found in an exhibit in addition to elementary statistical information obtained from SAS [*49*] and used in principal component analyses. Very limited success was obtained using those techniques which involved clustering-type statistical computations. The conventional statistical software programs were inherently slow and produced an unacceptably high number of false positive and false negative results. We have had good preliminary success with an experimental neural network pattern recognition program and further work is planned with its development. Neural network pattern recognition appears to be a reliable means of establishing a common source identity for each batch of cocaine.

Conclusions

Presented here is a simple, one-step derivatization procedure obtaining chromatographic impurity signature profile analyses (CISPA) of cocaine to determine if two or more exhibits could have originated from the same batch. Eliminating sample pre-preparation (extractions or chemical modifications) is advantageous in limiting artifacts or impurities from being introduced from operator manipulation of the exhibit. Statistical analysis showed only two sets of impurities with high correlations, thus exhibiting a good degree of randomness between impurities of differing batches of cocaine. High-resolution capillary gas chromatography of derivatized coca alkaloids is the method of choice for CISPA. The presented procedure was sufficiently sensitive using FID for determining same batch exhibits. Exhibits of common batch origin could be determined and gave virtually identical chromatographic profiles, while profiles were found to be unique for

separate batches. The implementation of a reliable computerized pattern recognition program would greatly enhance this methodology, and further work using more sensitive methodology such as gas chromatography-electron capture detection could be pursued in determining country of origin. The forensic chemist utilizing CISPA methodology for cocaine should be well versed in the chemistry of cocaine and have analyzed hundreds of differing batches of cocaine to become familiar with batch-to-batch differences.

Acknowledgments

We thank Dr. Jim Watterson of the Research Triangle Institute for his assistance in developing the neural network pattern recognition program.

References

[1] Neumann, H., *Proceedings of the International Symposium on the Forensic Aspects of Controlled Substances*, March, 1988, pp. 121–129.
[2] Kram, T. C., *Journal of Forensic Sciences*, Vol. 24, No. 3, 1979, pp. 596–599.
[3] Van Der Ark, A. M., Theeuwen, A. B. E., and Verweij, A. M. A., *Pharmaceutisch Weekblad*, Vol. 112, 1977, pp. 977–979.
[4] Van Der Ark, A. M., Sinnema, A., Van Der Toorn, J. M., and Verweij, A. M. A., *Pharmaceutisch Weekblad*, Vol. 113, 1978, pp. 341–343.
[5] Van Der Ark, A. M., Sinnema, I., Theeuwen, A. B. E., Van Der Toorn, J. M., and Verwey, A. M. A., *Pharmaceutisch Weekblad*, Vol. 113, 1978, pp. 41–45.
[6] Van Der Ark, A. M., Sinnema, I., Van Der Toorn, J. M., and Verweij, A. M. A., *Pharmaceutisch Weekblad*, Vol. 112, 1977, pp. 980–982.
[7] Sinnema, A. and Verweij, A. M. A., *Bulletin on Narcotics*, Vol. 33, No. 3, 1981, pp. 38–54.
[8] Sanger, D. G., Humphreys, I. J., Patel, A. C., Japp, M., and Osborne, R. G. L., *Forensic Science International*, Vol. 28, 1985, pp. 7–17.
[9] Van Der Ark, A. M., Verweij, A. M. A., and Sinnema, I., *Journal of Forensic Sciences*, Vol. 23, No. 4, 1978, pp. 693–700.
[10] Lomonte, J. N., Lowry, W. T., and Stone, I. C., *Journal of Forensic Sciences*, Vol. 21, No. 3, 1976, pp. 575–582.
[11] Bailey, K., Boulanger, J. G., Legualt, D., and Taillefer, S. L., *Journal of Pharmaceutical Sciences*, Vol. 63, No. 10, 1974, pp. 1575–1578.
[12] LeBelle, M., Sileika, M., and Romach, M., *Journal of Pharmaceutical Sciences*, Vol. 62, 1973, p. 862.
[13] Barron, R. P., Kruegel, A. V., Moore, J. M., and Kram, T. C., *Journal of the Association of Official Analytical Chemists*, Vol. 57, No. 5, 1974, pp. 1147–1158.
[14] Allen, A. C. and Kiser, W. O., *Journal of Forensic Sciences*, Vol. 32, No. 4, 1987, pp. 953–962.
[15] Allen, A. C. and Cantrell, T. S., *Forensic Science International*, Vol. 42, 1989, pp. 183–199.
[16] Allen, A. C., Moore, J. M., and Cooper, D. A., *Journal of Organic Chemistry*, Vol. 56, 1983, pp. 3951–3954.
[17] Moore, J. M., Allen, A. C., and Cooper, D. A., *Analytical Chemistry*, Vol. 56, 1984, pp. 642–646.
[18] Schwartz, R. S. and David, K. O., *Analytical Chemistry*, Vol. 57, 1985, pp. 1362–1366.
[19] Allen, A. C., Cooper, D. A., Moore, J. M., and Teer, C. B., *Journal of Organic Chemistry*, Vol. 49, 1984, pp. 3462–3465.
[20] Allen, A. C., Cooper, D. A., Moore, J. M., Gloger, M., and Neumann, H., *Analytical Chemistry*, Vol. 56, 1984, pp. 2940–2947.
[21] Lurie, I. S. and Allen, A. C., *Journal of Chromatography*, Vol. 317, 1984, pp. 427–442.
[22] Moore, J. M., *Journal of Chromatography*, Vol. 281, 1983, pp. 355–361.
[23] Moore, J. M., *Journal of Chromatography*, Vol. 147, 1978, pp. 327–336.
[24] Moore, J. M. and Klein, M., *Journal of Chromatography*, Vol. 154, 1978, pp. 76–83.
[25] Narayanaswami, K., Golani, H. C., and Dua, R. D., *Forensic Science International*, Vol. 14, 1979, pp. 181–190.
[26] Moore, J. M., Allen, A. C., and Cooper, D. A., *Analytical Chemistry*, Vol. 56, 1984, pp. 642–646.
[27] Huizer, H., *Journal of Forensic Sciences*, Vol. 28, No. 1, 1983, pp. 40–48.
[28] Lurie, I. S., Sottolano, S. M., and Blasof, S., *Journal of Forensic Sciences*, Vol. 27, No. 3, 1982, pp. 519–526.

[29] Brenneisen, R. and ElSohly, M. A., *Journal of Forensic Sciences*, Vol. 33, No. 6, 1988, pp. 1385–1404.
[30] Turner, C. E. and Hadley, K. W., *Journal of Pharmaceutical Sciences*, Vol. 62, 1973, pp. 251–255.
[31] Turner, C. E., Hadley, K. W., and Fetterman, P. S., *Journal of Pharmaceutical Sciences*, Vol. 62, 1973, pp. 1739–1741.
[32] Stromberg, L., *Journal of Chromatography*, Vol. 68, 1972, pp. 253–258.
[33] Holley, J. H., Hadley, K. W., and Turner, C. E., *Journal of Pharmaceutical Sciences*, Vol. 64, 1975, pp. 892–894.
[34] DeZeeuw, R. A., Wijsbeek, J., and Malingre, T. M., *Journal of Pharmacy and Pharmacology*, Vol. 25, 1973, pp. 21–26.
[35] Novotny, M., Lee, M. L., Low, C. E., and Raymond, A., *Analytical Chemistry*, Vol. 48, 1976, pp. 24–29.
[36] Moore, J. M., *Journal of the Association of Official Analytical Chemists*, Vol. 56, No. 5, 1973, pp. 1199–1205.
[37] Noggle, F. T. and Clarke, C. R., *Journal of the Association of Official Analytical Chemists*, Vol. 65, No. 3, 1982, pp. 756–761.
[38] Gill, R., Abbott, R. W., and Moffat, A. C., *Journal of Chromatography*, Vol. 301, 1984, pp. 155–163.
[39] Majlat, P. and Bayer, I., *Journal of Chromatography*, Vol. 20, 1965, p. 187.
[40] Jane, I., Scott, I. J. A., Sharpe, R. W. L., and White, P. C., *Journal of Chromatography*, Vol. 214, 1981, pp. 243–248.
[41] Lurie, I. S., Moore, J. M., and Kram, T. C., *Journal of Chromatography*, Vol. 405, 1987, pp. 273–281.
[42] Casale, J. F., *Forensic Science International*, Vol. 47, 1990, pp. 277–287.
[43] Brewer, L. and Allen, A. C., *Journal of Forensic Sciences*, Vol. 36, No. 3, May 1991, pp. 697–707.
[44] Moore, J. M., Cooper, D. A., Lurie, I. S., Kram, T. C., Carr, S., Harper, C., and Yeh, J., *Journal of Chromatography*, Vol. 410, 1987, pp. 297–318.
[45] Lurie, I. S., Moore, J. M., Kram, T. C., and Cooper, D. A., *Journal of Chromatography*, Vol. 504, 1990, pp. 391–401.
[46] Lukaszewski, T. and Jeffery, W. K., *Journal of Forensic Sciences*, Vol. 25, No. 3, 1980, pp. 499–507.
[47] Moore, J. M., *Journal of Chromatography*, Vol. 101, 1974, pp. 215–218.
[48] LaBelle, M., Lauriault, G., Callahan, S., Latham, D., Chiarelli, C., and Beckstead, H., *Journal of Forensic Sciences*, Vol. 33, No. 3, 1988, pp. 662–675.
[49] SAS Institute, Inc., Cary, NC, 1990.
[50] Clarke, D. L., Daum, S. J., Gambino, A. J., Aceto, M. D., Pearl, J., Levitt, M., Cumiskey, W. R., and Bogado, E. F., *Journal of Medicinal Chemistry*, Vol. 16, No. 11, 1973, pp. 1260–1267.
[51] Baldwin, S. W., Jeffs, P. W., and Natarajan, S., *Synthetic Communications*, Vol. 7, 1977, p. 79.
[52] Findlay, S. P., *Journal of the American Chemical Society*, Vol. 76, No. 11, 1954, pp. 2855–2862.
[53] Casale, J. F., *Forensic Science International*, Vol. 33, 1987, pp. 275–298.
[54] Noyce, D. S., King, P. A., Kirby, F. B., and Reed, W. L., *Journal of the American Chemical Society*, Vol. 84, 1962, pp. 1632–1638.
[55] Hocking, M. B., *Canadian Journal of Chemistry*, Vol. 47, 1969, pp. 4567–4576.
[56] DeJong, A. W. K., *Recueil des Travaux Chimiques des Pays-Bas*, Vol. 59, 1940, pp. 687–693.
[57] Pelletier, S. W., *Chemistry of the Alkaloids*, Van Nostrand Reinhold Corporation, New York, 1970, Chapter 15.
[58] Novak, M., Salemink, C. A., and Khan, I., *Journal of Ethnopharmacology*, Vol. 10, No. 3, 1984, pp. 261–274.
[59] Cooper, D. A. and Allen, A. C., *Journal of Forensic Sciences*, Vol. 29, No. 4, 1984, pp. 1045–1055.
[60] Kratochuil, B. and Brown, B., *Journal of Forensic Sciences*, Vol. 29, No. 2, 1984, pp. 493–499.

Address requests for reprints or additional information to
John F. Casale
Drug Chemistry Laboratory
State Bureau of Investigation
3320 Old Garner Road
P. O. Box 29500
Raleigh, NC 27626-0500