# Cocaine Profiling Methodology — Recent Advances

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Cocaine Profiling Methodology — Recent Advances


ABSTRACT: The rationale for developing cocaine profiling methodology is described. Current cocaine signature procedures in use at the U.S. Drug Enforcement Administration's Special Testing and Research Laboratory are reviewed. Newer selective and sensitive methodology, recently developed, is described. That methodology detects more alkaloidal impurities in refined illicit cocaine than heretofore reported. The alkaloidal impurities were isolated from the bulk cocaine matrix by alumina column chromatography and detected using capillary gas chromatography-mass selective detection in the selected ion mode. Fifty-one refined illicit cocaine samples were subjected to this methodology for the determination of 15 selected alkaloids. Reproducibility data are reported. Methodology for the isolation, detection, and characterization of coca alkaloids in South American coca leaf, a commercial coca-leaf extract, and a large seizure of refined illicit cocaine is reviewed.

KEY WORDS: Cocaine, Gas chromatography/Mass spectrometry, profiling, signature.

INTRODUCTION

This review focuses upon new cocaine profiling methodology introduced recently by our laboratory, the U.S. Drug Enforcement Administration’s (DEA) Special Testing and Research Laboratory (STRL). This methodology detects many more coca alkaloidal impurities in refined illicit cocaine than previously reported, providing valuable impurity signature profiles. It is believed to be the most specific and sensitive method to date for many of the alkaloidal impurities in that matrix. This procedure was developed primarily to evaluate its utility as a geographic determinator. Secondly, its use in cocaine sample comparison cases is addressed. This method was also subjected to rigorous reproducibility studies, and selected alkaloids were comparatively determined for 51 unadulterated and undiluted (UAU) refined illicit cocaine samples. Unless otherwise specified, the term “cocaine” will mean cocaine hydrochloride.

Also reviewed are recently published and unpublished studies that describe the detection and structural characterization of new trace-level coca alkaloids in South American coca leaf, in a commercial extract of Peruvian/Bolivian coca leaves, and in a large seizure of a UAU refined illicit cocaine sample. This work was a necessary precursor to the development of the new cocaine signature methodology.

Rationale for Developing Drug Profiling Methodology

The development of illicit drug impurity signature procedures uses primarily chromatographic methodology, the most important being capillary gas chromatography (cGC). This is usually interfaced with detectors that include flame-ionization detection (FID), electron-capture detection (ECD), and mass selective detection (MSD) [12,16]. High-performance liquid chromatography (HPLC) and capillary electrophoresis (CE) in its various forms can also be useful in drug signature methodology; ultraviolet (UV) and fluorescence (FLR) detection are mostly used for HPLC determinations [12,16]. Presently, chromatographic profiling of illicit drugs has been, or is being, developed primarily for cocaine, heroin, the amphetamines, and cannabis.

The use of illicit drug impurity signature profiles is applicable in:

1. the chromatographic comparison of illicit drug manufacturing impurities and byproducts of multiple seizures of the same drug to determine if they were derived from a common source, e.g., from the same drug “batch” or clandestine laboratory. (A positive correlation between samples could indicate a criminal conspiracy, perhaps supporting an eventual criminal prosecution [21].)
2. The determination of geographic region or country of origin for the contraband. This allows enforcement officials to focus their activities in those areas responsible for production of the illicit drug. (For drugs of botanical origin, such as heroin and cocaine, the extraction of the drug or its precursor from the plant, and its subsequent refinement, can occur in more than one location, complicating origin determination. For this reason, it is useful to examine both alkaloidal impurities and manufacturing byproducts using multiple chromatographic profiling methods.)
3. The chromatographic examination and determination of manufacturing byproducts and solvent residues in the illicit drug. (This information is important in that it allows enforcement personnel to monitor solvents and chemicals used in the process; this could lead to more stringent controls of such material.)
4. The differentiation of illicit drugs from their commercially produced and synthetic counterparts.
I. COCAINE PROFILING METHODOLOGY

A. Cocaine Profiling Methodology and Illicit Cocaine Manufacture

Cocaine profiling methodology has become increasingly more sophisticated and in greater use during the past 5–10 years. Most of those methods, developed by a number of international forensic laboratories, utilized cGC chromatographic profiling with a multiplicity of detection systems. To a lesser extent HPLC, using UV detection, has also been applied in cocaine profiling. These methods have been described in recent reviews [12,16].

The U.S. DEA’s STRL has had an ongoing interest in continuing the enhancement of cocaine profiling methodology in terms of reproducibility, quantitative accuracy, sensitivity, and especially, specificity. Given the hundreds of thousands of “batches” of refined illicit cocaine produced annually from South American coca, the importance of high signature selectivity cannot be overemphasized. This is especially so in cocaine comparison cases in which criminal prosecution and expert witness testimony are a likelihood. (During 1997 and 1998, the STRL received, for the first time, several cocaine comparison cases involving homicides; in these cases, some cocaine exhibits were found in the possession of the alleged perpetrator and others associated with the victim(s); presumably, in each case, a positive chemical correlation between different exhibits could support a linkage between murder suspects and victims.)

In a somewhat analogous comparative forensic application, there has been over recent years a marked improvement in the sensitivity and specificity of human DNA profiling. Of course, the uniqueness of human fingerprints has long been established. It is our aim that the chromatographic profiling methods described below for cocaine can achieve a comparable specificity as that for human fingerprinting and DNA profiling techniques.

Geographic origin determination, as mentioned above, is another legitimate pursuit in the development of drug impurity signature profiles. Unlike heroin, which is produced from opium in disparate areas of the world, cocaine is derived from coca leaf cultivated almost exclusively in the South American Andean countries of Colombia, Ecuador, Peru, and Bolivia. These being contiguous countries poses a unique challenge in determining country and/or geographic origin of refined illicit cocaine. Ideally, a determination of where the coca leaf was cultivated and cocaine extracted, versus where the cocaine was refined and converted to the hydrochloride, are necessary goals.

All of the above countries cultivate the coca leaf Erythroxylum coca v. coca (ECVC), the major source of cocaine [12,16]. Additionally, in Colombia another cocaine-bearing cultivar is found, namely, Erythroxylum novogranatense v. novogranatense (ENVN) [12,16]. It is also suspected that there could be hybrids of the ECVC cultivar.

Refined illicit cocaine is known to be produced entirely within each of the above countries from native leaf. It is also recognized that refined cocaine base is sometimes produced from Bolivian and Peruvian leaf in those countries; the base later is transported to Colombia for further refinement and conversion to cocaine hydrochloride. The clandestine manufacture of refined illicit cocaine is mostly an unsophisticated process, involving multiple extractions and precipitations, an oxidation step and then conversion of the refined cocaine base to its hydrochloride [2]. During the manufacturing process, numerous lesser alkaloids of the coca leaf are co-extracted with cocaine and are “carried through” to the final hydrochloride product. Also present in the cocaine hydrochloride sample are manufacturing byproducts, including hydrolysis and oxidation products, the latter being produced during the potassium permanganate step.

The chromatographic signature methodology for cocaine currently in use at the DEA’s STRL is briefly reviewed below.

B. Current Cocaine Profiling Methodology at DEA-STRL

Presently, the STRL employs six independent cocaine profiling methods for sample comparison analyses, geographic origin studies, and solvent determinations. These methods are utilized for the detection and comparative determination of alkaloidal impurities, manufacturing byproducts, and solvent residues. These methods are briefly described below.

1. Chromatographic Impurity Signature Profile Analysis (CISPA) Method

This method is based on the determinations of the content of the alkaloidal impurities cis- and trans-cinnamoylcocaine, along with tropacocaine. Also determined are the hydrolysis and oxidation manufacturing byproducts of cocaine, along with hydrolysis products of lesser coca-leaf alkaloids. The UAU cocaine sample is subjected directly to trimethylsilylation, followed by cGC-FID determination using a moderately polar capillary column [8].

2. Trimethoxy Method

The trimethoxy method determines the trimethoxy-substituted analogs of cocaine, cis/trans-cinnamoylcocaine and tropacocaine, as well as miscellaneous 3-oxo-substituted tropane esters [3,5]. The UAU sample is subjected to ion-pairing (NaCl/dil HCl) Celite column chromatography, followed by cGC-FID determination using a non-polar capillary column [5].
3. Base Extraction-Alumina Column-Recrystallization (BAR) Method
This method determines 3-oxo-heteroaryl and 3-oxo-hydrocarbon-substituted-2-carbomethoxytropane alkaloids. The UAU sample is converted to the base and then subjected to alumina column chromatography followed by a double recrystallization step and examination of the mother liquor. The determinative step uses cGC-MSD in the selected ion monitoring (SIM) mode and a non-polar capillary column [4,7].

4. Rapid Truxillines Method
This method determines 10 of the 11 isomeric truxillines. The UAU sample is reduced directly with lithium aluminum hydride in ethyl ether, followed by extraction of the resultant diols and derivatization with heptafluorobutyric anhydride. cGC-ECD is used in the determinative step, with a moderately polar capillary column. This is a recently modified method [1] of already-published truxilline methodology [11,15,19].

5. N-Norcocaine (N-NOR) Method
In this method, the levels of alkaloidal 6- and 7-exo, as well as 6- and 7-exo-hydroxycocaines along with the manufacturing byproducts N-norcocaine and N-benzoylnorecgonine methyl ester are determined. Also detected in the UAU cocaine samples are unknown coca alkaloids and numerous N-nortropane-type manufacturing byproducts. The determinative step uses cGC-ECD with a moderately polar capillary column [18].

6. Solvent Method
This method determines the residual solvent composition in UAU cocaine samples using headspace sampling and analysis by cGC-MSD-SIM. A slightly polar capillary column [22] is used for this purpose. The most commonly encountered cocaine processing solvents include petroleum ether, methyl ethyl ketone, ethyl acetate, and methylene chloride, among others. Methods 1–6 from above report comparative quantitative values relative to an accurately weighed amount of cocaine (see Figure 1). Furthermore, Methods 1–5 utilize structurally related internal standards, introduced subsequently to the sample weighing. Method 6 for solvent residues employs deuterated internal standards. The use of structurally related and isotopic internal standards in these methods assured good reproducibility and accurate quantitative data (relative to cocaine). Relative standard deviation results for the six methods was typically below ±3%. Furthermore, the large number of target alkaloidal impurities, manufacturing byproducts, and solvent residues resulted in very high method specificity.

C. Cocaine Profile Data Bases and Search Routine

All UAU samples received by the STRL for routine analyses are also subjected to the CISPA and Solvent Method. As of January 1998, the STRL had compiled a substantial signature database for UAU cocaine samples using the above two profiling methods. This included in excess of 8,000 exhibits using the CISPA Method and more than 4,000 samples using the Solvent Method. The 8,000-plus sample CISPA Method database was unique in that it could be accessed by a computer software program referred to as “SNIFTER” [9]. This program does a sample-to-sample search of the CISPA Method database to find cocaine exhibits with markedly similar CISPA chromatographic impurity profiles. Those samples that exhibited a positive correlation using the CISPA Method are then subjected to further comparative signature analyses using Methods 2–6 to determine if those samples were, indeed, chemically related. If a positive correlation is obtained using the other methods, then the submitting office/agency could be contacted and notified as to the results.

Whereas all UAU cocaine samples received at STRL for solvent residue analyses (Solvent Method) are also subjected to the CISPA Method, only about 20% of these are routinely subjected to Methods 2–5. Presently, a data base of about 500 samples for these latter methods has been accumulated. If cocaine exhibits are submitted to STRL for the expressed purpose of comparative analyses, they are first “screened” using the CISPA Method. If a positive correlation between samples is realized, then the other five methods can be applied.

In order to enhance the ability to determine the geographic origin of refined illicit cocaine samples, additional methodology has been developed recently. This method, which also appears promising for use in cocaine comparison cases, is described in the next section.

II. NEW COCAINE PROFILING METHODOLOGY

Of the alkaloids determined in the cocaine profiling Methods 1–5 cited above, many were first isolated and structurally characterized in refined illicit cocaine and then, subsequently, characterized in South American coca and its commercial extracts. Conversely, some of the alkaloids were first characterized in coca leaf/extracts and then their presence confirmed in refined cocaine. The
manufacturing byproducts (hydrolysis and oxidation compounds) were, of course, determined only in refined cocaine (CISPA Method).

The new cocaine profiling procedure described in this section is a modification of the recently published methodology, that is based on the detection and/or structural characterization of many new alkaloids in South American coca leaf and in commercial coca leaf extracts (E. Harz) [13]. The presence of most of these alkaloids was subsequently confirmed in a large seizure of refined illicit cocaine. These new alkaloids totaled 100–150 in number, many of which could be comparatively determined using the new cocaine signature methodology.

In addition to the detection and comparative determination of the newest coca leaf alkaloids recently reported [13], the new cocaine signature method also incorporated most of the alkaloids determined in five of the current cocaine signature profile methods described previously. This is accomplished using a single sample weight, as compared with five individual sample weights currently used. Furthermore, in the new method most of the bulk cocaine matrix (500 mg) is removed via alumina column chromatography, thereby enhancing the detection for most alkaloids. Sensitivity enhancement is also realized for many alkaloids by using mass selective detection in the SIM mode. This is especially so, e.g., for the hydroxy-cocaines, in which about 50% of the ion current resides in the fragment ion m/z 94. Of course, the use of the MSD in the SIM mode significantly enhances the specificity of this method. Finally, the new signature procedure can more readily accommodate adulterated and diluted ("cut") samples, compared with current methods.

A. Overall Scheme

A schematic representing the alumina column chromatography separation of cocaine alkaloidal impurities is shown in Figure 2 and described as follows:

1. An amount of "uncut" sample equivalent to about 500 mg of cocaine hydrochloride is accurately weighed into a 15-mL centrifuge tube and dissolved in 7.0 mL of water.
2. To the solution is added 0.5 g of sodium bicarbonate; the tube is capped tightly and then vortexed until precipitation of cocaine base is complete.
3. To the tube is added 5.0 mL of chloroform, containing mixed internal standards, and the resultant phases are mixed vigorously, followed by centrifugation. (The names and structures of internal standards are shown in Figure 3. The amounts of these compounds in 5 mL chloroform are: 2'-furanyllagomine ethyl ester, 61.0 µg; p-fluorotropacocaine, 206.6 µg; tropylecgonine ethyl ester, 77.9 µg; hydrocinnamoyltropine, 503.0 µg; hydrocinnamoyltropacocaine, 19.5 µg; apotropoylaleatine ethyl ester, 40.4 µg; acetyllectogine ethyl ester, 101.5 µg; trimethoxyxoxycocaine, 1,378.5 µg; atropine, 4.0 µg; p-fluorobenzoyltropine, 10.1 µg.)
4. The chloroform layer (sans water layer) is carefully transferred to a funnel holding 10 g of anhydrous sodium sulfate and the filtrate collected in a 50-mL graduated conical glass tube.
5. Steps 3 and 4 are repeated (but using chloroform without mixed internal standards), and then the sodium sulfate is rinsed with additional chloroform until a total of 12–13 mL is collected in the tube.
6. The combined chloroform extracts are evaporated just to residue in a heated water bath (60–80 °C) with the aid of a nitrogen stream.
7. Prepare a glass chromatographic column (Kontes: 380-mm x 20-mm ID with frit and stopcock) packed with: lower layer, 5 g sand; middle layer, 10.0 g basic aluminum oxide (Aldrich, Brockmann I, ca 150 mesh, 58 Ångstrom) containing 4.0% water; upper layer, 5 g sand. The column is placed over a 50-mL graduated conical glass tube.
8. The residue in the tube from Step 6 is dissolved in 1.5–2.0 mL of chloroform/petroleum ether (8:2) and the solution transferred quantitatively to the prepared column in Step 7.
9. The tube is washed with an additional 10 mL of chloroform/petroleum ether (8:2) in 2-mL aliquots, each wash being transferred to the column.
10. After adjusting the stopcock to provide a flow rate of ca 3 mL/min, 30 mL of chloroform/petroleum ether (8:2) are added to the column, the eluate being collected in the 50-mL tube. This fraction is identified as FRAC A. (Further treatment and analysis of this fraction are discussed in a later section.)
11. To the column is then added 30 mL of chloroform/acetone (8:2) and collected in another 50-mL tube. This fraction is identified as FRAC B. (Further treatment and analysis of this fraction are discussed in a later section.)
12. To the column is then added 35 mL of acetone/methanol (97:3). (If reduction in flow rate is observed, the stopcock is readjusted to allow a flow rate of ca 3 mL/min.) This fraction is identified as FRAC C. (Further treatment and analysis of this fraction are discussed in a later section.)
13. The final eluant added to the column is 30 mL of acetone/methanol (1:1). This fraction, collected in a fourth tube, is identified as FRAC D. (Further treatment and analysis of this fraction are discussed in a later section.)
14. The eluates in the four tubes are evaporated to residue on a warm water bath (60–80 °C) with the aid of a nitrogen stream.

The new cocaine profiling method was applied to 51 UAU, refined illicit cocaine samples that had been submitted to STRL during 1997. These survey analyses also included comparative determinations for a selected number of alkaloids. A different UAU cocaine sample, available in a greater amount than typical samples, was selected for repeated analysis to assess reproducibility of the method. Furthermore, this new procedure was applied for the first time in a cocaine comparison case involving a homicide.

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In that case, a sample of cocaine was retrieved from the suspected perpetrator and another cocaine exhibit was found at the murder site. Although a positive alkaloidal profile correlation between these two exhibits could not be established, comparative chromatograms from that case are illustrated in this discussion; this is to support the sensitivity and selectivity of this method.

Examination of the four alumina column eluates (FRAC's A–D) by cGC-MSD-SIM for the 51 cocaine samples revealed that all fractions yielded reconstructed capillary gas chromatograms that were peak-rich. The same was also the case for the reproducibility-test sample and the comparison case exhibits. It had also been determined previously (see Section D below) that FRAC A and FRAC B held virtually no alkaloids with derivatizable hydroxy groups, nor derivatizable primary or secondary amino functions. Conversely, most of the structurally characterized alkaloids found in FRAC C and FRAC D possessed a single hydroxy group that was readily derivatized with MSTFA. When the derivatization step was omitted, the chromatographic behavior and sensitivity degraded to an unacceptable level. It is also important to note that of the four alumina column fractions, FRAC C appeared to provide the most analytically useful data for all cocaine samples examined. For that reason, we will begin our discussion with that fraction. (Note: cGC-MSD-SIM parameters: 30-M x 0.25-mm ID fused-silica capillary column (J & W Scientific) coated with DB-1701 (film thickness: 0.25 mm). Oven temperature program: initial temp, 100 °C; initial hold, 5.0 min; program rate, 3.0 °C/min; final hold, 20 min. Injector and detector temperatures were 250 °C and 270 °C, respectively. Helium was the carrier gas at a linear velocity of 33.7 cm/sec; split flow was 20 mL/min and split ratio was 24:1.)

Figure 2. Alumina column chromatographic scheme of cocaine profiling methodology.

B. Alumina Column FRAC C (Ions Monitored: m/z 94, 124, 182, 185, 196, 212, 270, 391)

FRAC C residue is quantitatively transferred to a smaller tube with chloroform and then evaporated to residue. To the tube is added 100 µL of chloroform and 100 µL MSTFA, vortexed and then heated at 75 °C for 20 min. This solution is set aside for cGC-MSD-SIM analysis.

Of the four alumina fractions, FRAC C consistently exhibited the most peak-rich chromatograms. As mentioned above, virtually all of the alkaloids in this fraction were chromatographed as O-trimethylsilyl (O-TMS) derivatives. Figure 4 illustrates a typical FRAC C chromatogram, which represents the summation of ions at m/z 94, 124, 182, 185, 196, 212, 270, 391.

The alkaloidal composition of this fraction is described below.
1. Hydroxy-Substituted Conjugated Diene and Triene Tropane Alkaloidal Impurities

The largest group of compounds in FRAC C were the hydroxy-substituted carbonyl-conjugated diene and triene tropane alkaloids. Figure 5 illustrates the SIM 182 reconstructed cGC. As seen for this cocaine comparison sample, the most intense peak in the partial chromatogram was identified as 8'-hydroxy-2',4',6'-decatrienylecgonine methyl ester (chromatographed as the O-TMS derivative). Figure 6d shows the electron impact (EI) mass spectrum of this alkaloid (as O-TMS derivative). In coca leaf extracts, this is usually the most abundant alkaloid of this type. In refined illicit cocaine, however, this alkaloidal impurity can be significantly diminished by rigorous treatment of the sample with potassium permanganate during the manufacturing process. This treatment appears to be less effective on the carbonyl-conjugated diene alkaloids. It is also seen in Figure 5 that there are a significantly greater number of carbonyl-conjugated diene alkaloids (molecule ion: m/z 437) than carbonyl-conjugated trienes (molecule ion: m/z 435). This was the case for virtually all of the 51 cocaine exhibits examined. All refined cocaine samples examined in this study contained the hydroxy-substituted carbonyl-conjugated dienes as alkaloidal impurities. Conversely, the alkaloid, whose structure is seen in Figure 5, was sometimes not detected in cocaine exhibits because of permanganate oxidation. In Peruvian and Bolivian coca leaf extracts, as many as 50-75 of these alkaloids were detected [13]. Five of the most abundant ones are illustrated in Figure 7 [17]. In refined cocaine samples a lesser number was found, due to losses during processing. In Figure 6a and 6b are EI mass spectra (as O-TMS derivatives) for two of the carbonyl-conjugated diene tropane alkaloidal impurities. Figure 6c illustrates the FS-EI mass spectrum of what is believed to be one of five significant isomeric manufacturing byproducts, and all of which yielded virtually identical mass spectra, and all with a molecule ion at m/z 495 (as O-TMS derivatives). The chromatograms for these compounds are seen in Figure 8 and are identified as peaks 3, 4, 5, 7 and 8. Peaks 1 and 2 are tropane alkaloidal impurities containing the carbonyl-conjugated, hydroxy-substituted diene moieties; peak 6 is the tropane alkaloidal impurity with the carbonyl-conjugated, hydroxy-substituted triene moiety, as seen in Figure 5.

Bar graphs in Figures 9 are illustrations (at two different attenuations) of the comparative determination of 8'-hydroxy-2',4',6'-decatrienylecgonine methyl ester (Figure 5) for the 51 cocaine samples. In the bar graphs, the concentration of this impurity is reported as ng of the alkaloid in 500 mg of UA cocaine HCl. The internal standard used for these determinations was the lesser of a diastereomeric pair of tropoylecgonine ethyl ester (Figure...
3). (In the preparation of this internal standard, a diastereomeric pair were formed in a ratio of about 4:1.) The base peak ion of this internal standard at m/z 196 was used in all calculations. As seen in Figure 9, most of the samples had detectable levels of this alkaloidal impurity. Furthermore, the levels of this alkaloid exhibited a wide dynamic range (0–42,805 ng/500 mg cocaine).

Figure 6. Electron-impact mass spectra of alumina column FRAC C alkaloidal and manufacturing impurities, isolated from refined illicit cocaine: carbonyl-conjugated dienoylecgonine methyl ester alkaloidal impurities (a and b); suspected cocaine manufacturing byproduct (c, molecule ion = m/z 495); and alkaloidal impurity 8'-hydroxy-2',4',6'-decatrienoylglucine methyl ester (d).

Figure 7. Structures of some alkaloidal impurities, isolated from refined illicit cocaine, and present in alumina column FRAC C.

Figure 8. Partial reconstructed cGC-MS (SIM) chromatogram (62–66 min) of alumina column FRAC C alkaloidal impurities and manufacturing byproducts, isolated from refined illicit cocaine; peaks 1 and 2 = hydroxy-substituted conjugated diene alkaloidal impurities as O-TMS derivatives (e.g., see Figure 7); peaks 3, 4, 5, 7, 8 = suspected isomeric manufacturing byproducts, each having a molecular weight of 495 Daltons (as TMS derivative); and, peak 6 = alkaloidal impurity 8'-hydroxy-2',4',6'-decatrienoylglucine methyl ester (as O-TMS derivative).
2. Hydroxycocaine Alkaloidal Impurities

Another group of alkaloidal impurities detected in all cocaine samples were those with hydroxy substitution on the tropane ring. Figure 10 illustrates the structures of those hydroxycocaines known or suspected to be present in illicit cocaine. The presumptive presence of 6- and 7-exo- and 6- and 7-endo-hydroxycocaines in refined illicit cocaine samples has been reported recently [18]. Since that report, their identities have been confirmed via syntheses of their standards along with comparison of cGC retention times and mass spectra [1]. These four alkaloids were detected in all 51 cocaine samples. Figure 11 illustrates the cGC-MSD-SIM chromatogram of these alkaloidal impurities in one of the exhibits from the cocaine comparison case. In all samples examined to date, the levels of the exo-isomers were markedly similar and greater than the endo-isomers, which were also similar to one another in concentration (Figure 11). As seen from the chromatogram, the fragment ion at m/z 94 was monitored to provide sensitive detection of these alkaloids. (The m/z 94 fragment ion represents about 50% of the total ion current from an FS-EI spectrum.) Furthermore, a base peak at m/z 94 strongly suggests OH-substitution at the C-6 or C-7 position.

Bar graphs shown in Figures 12 are for the comparative determination of 7-exo-hydroxycocaine (at two different attenuations). Again, there is observed marked discrimination between samples. The concentration range for 7-exo-hydroxycocaine varied from 355-46,525 ng/500 mg cocaine. The internal standard used for these determinations was the greater of the two tropylecgonine ethyl ester diastereomers. The internal standard fragment ion used for calculations was at m/z 94. Since the m/z 94 ion in 7-exo-hydroxycocaine was significantly greater than that for the same ion (assuming equal concentrations) in the internal standard, a factor was introduced into the calculations for normalization purposes.

As an aside, it has been recently proposed [6] that the 6- and 7-hydroxycocaines in refined illicit cocaine may come from two different sources. It has been amply demonstrated that the hydroxycocaines are bona fide alkaloids in South American coca leaf [13] and are prob-

![Figure 9](image-url)  
**Figure 9.** Bar graphs, at different attenuations, for the comparative determinations of the FRAC C alkaloidal impurity 8'-hydroxy-2,4,6-decatrienylecgonine methyl ester in 51 refined illicit cocaine samples. (Sample 18 is a cocaine base exhibit; Sample 19 is an anomalous result.)

![Figure 10](image-url)  
**Figure 10.** Structures of some suspected and known hydroxycocaines (with hydroxy substituent on tropane ring) detected as alkaloidal impurities in refined illicit cocaine.
ably "carried-through" the manufacturing process, to a certain extent. The other source, however, may be responsible for most of the 6- and 7-hydroxycocaines in illicit cocaine. It has been shown that there may be an inverse relationship between the levels of hydroxycocaines and the cinnamoyl cocaine in illicit cocaine [6]. When the cinnamoyl cocaine levels were minuscule, due to rigorous treatment with potassium permanganate during processing, the 6- and 7-hydroxycocaine concentrations were much higher [6]. These observations suggested that in the coca leaf, there are precursor alkaloids to the 6- and 7-hydroxycocaines that have easily oxidizable functional groups at the C-6 or C-7 site; upon potassium permanganate treatment, these groups (perhaps conjugated esters?) underwent oxidation, thereby liberating the 6- and 7-hydroxycocaines. These unknown alkaloids are believed present in FRAC A of the new cocaine signature methodology [6]. This subject will be investigated further.

In addition to the 6- and 7-exo and endo-hydroxycocaines, other alkaloidal impurities have been detected in FRAC C that are also believed to be hydroxy-substituted on the tropane ring. These possibilities are illustrated in Figure 10. Seen in Figure 13, for the two comparison case samples, is the chromatography for four of these suspected alkaloidal impurities (as TMS derivatives), detected by monitoring ions at m/z 270 and 391. Three of these alkaloids, represented by peaks 2, 3, and 4 in Figure 13, were isolated from a concentrated, commercial extract of South American coca leaf. Their FS-EI mass spectra, as O-TMS derivatives, were all markedly similar, each yielding a suspected molecule ion at m/z 391, a base peak at m/z 270 and a diagnostic fragment ion at m/z 82 (the last ion indicative of the N-methyl five-membered ring in the tropane moiety). This data appears to support the presence, in refined cocaine, of the alkaloids 1- and 5-hydroxycocaine, and perhaps also 4-hydroxycocaine (Figures 10 and 13). It is interesting to note that the recently identified alkaloid 1-hydroxytropacocaine yields fragment ions at m/z 82 and 212 and a molecule ion at m/z 333 [20]. These ions are analogous to those for the suspected 1- and 5-hydroxycocaines, minus the carbomethoxy group at C-2.

3. 3'-Hydroxy-3'-phenylpropionylecgonine Methyl Ester and Unknown Alkaloidal Isomeric Impurities

Also found in FRAC C for all 51 cocaine samples was an alkaloidal impurity the precursor of which Leete [10] had proposed as an intermediate in the biosynthesis of cocaine. The chromatography and structure of 3'-hydroxy-3'-phenylpropionylecgonine methyl ester are shown in Figure 14 as peak 2 for both comparison case samples.
As seen, Sample 2 had more than a two-fold greater concentration of this trace alkaloid than Sample 1. Its EI mass spectrum is found in Figure 15 (upper).

Also present in the Figure 14 chromatograms are peaks 3 and 4, representing a pair of unidentified alkaloidal isomers. These two impurities were present in almost all 51 samples at varying levels. As seen from their virtually identical mass spectra in Figures 15 (middle and lower), fragment ions at m/z 82 and 182 were present (diagnostic for the presence of the 2-carboxymethoxytropane moiety), as well as molecular ions at m/z 431 as TMS derivatives. This alkaloid pair was also detected in a commercial extract of South American coca leaf and in South American coca analyzed by STRL [13]. Also present, as peaks 5a and 5b, in the Figure 14 chromatograms are the diastereomeric isomers of the tropoylecgonine ethyl ester internal standard. As seen, the area reproducibility of the internal standard in the two samples is excellent; this is also the case for the area ratios of one isomer to the other. The latter result demonstrates the value of using epimers as internal standards.

Figure 16 illustrates, in bar graph form, the levels of the 3'-hydroxy-3'-phenylpropionylecgonine methyl ester alkaloidal impurity in the 51 cocaine samples. This alkaloid ranged from 1–65 µg/500 mg cocaine. All calculations were done using SIM m/z 182 for the alkaloidal impurity and SIM m/z 196 for the lesser of the two internal standard isomers (Figure 14).

4. 6-exo-Benzoyloxytropine

Another alkaloidal impurity in FRAC C was 6-exo-benzoyloxytropine, whose structure and chromatography (as the O-TMS derivative), for the cocaine comparison case samples, are illustrated in Figure 17. Its FS-EI mass spectrum (as TMS derivative) is seen in Figure 18; the base peak ion at m/z 185 was used in the SIM chromatography. The confirmation of this impurity as a bona fide coca alkaloid was accomplished by its detection, isolation and structural characterization in a commercial coca leaf extract. Sample 2 had more than a two-fold greater concentration of this trace alkaloid than Sample 1. Its EI mass spectrum is found in Figure 15 (upper).
5. 3-Oxo-substituted Alkaloidal Impurities with Hydroxy-Substituent on the Tropane Ring

In addition to the presence of the 2-carbomethoxy-3-oxo-tropane alkaloids that bear a hydroxy substituent on the tropane ring (e.g., 7-exo-hydroxcocaine), FRAC C also contained what is suspected to be a pair of alkaloids that also have hydroxy attachment on the tropane ring, but without the 2-carbomethoxy substituent. Their chromatography, as peaks 1 and 2, is illustrated in Figure 19 for the comparison case samples. These alkaloidal impurities were partially isolated from the commercial South American coca leaf extract and their FS-EI mass spectra ob-

Figure 15. Electron-impact mass spectra of alumina column FRAC C alkaloidal impurities, isolated from refined illicit cocaine: 3'-hydroxy-3'-phenylpropionyllecanine methyl ester; isomeric alkaloidal impurities of unknown structure (all as O-TMS derivatives).

extract and in South American leaf analyzed at STRL [13]. This alkaloidal impurity was present in some cocaine samples and undetected in others. As seen in Figure 17, it was found at a much higher level in Sample 2 than in Sample 1 of the comparison case.

Figure 16. Bar graph illustrating the comparative determinations of the FRAC C alkaloidal impurity 3'-hydroxy-3'-phenylpropionyllecanine methyl ester in 51 refined illicit cocaine samples. (Sample 18 is a cocaine base exhibit; Sample 19 is an anomalous result.)

Figure 17. Partial reconstructed cGC-MS (SIM) chromatogram (40–50 min) of an alumina column FRAC C alkaloidal impurities, isolated from refined illicit cocaine; the two identified peaks are the alkaloidal impurity 6-exo-benzoyloxytropine (as O-TMS derivative; ion monitored: m/z 185) and cocaine (ion monitored: m/z 182).
Figure 18. Electron-impact mass spectrum of alumina column FRAC C alkaloidal impurity 6-exo-benzoyltropine (as O-TMS derivative).

Obtained. These spectra were markedly similar, both exhibiting base peaks at m/z 212 and molecule ions at m/z 333. No significant ion at m/z 182 was present, indicating the absence of the C-2 carbomethoxy substituent. Furthermore, no intense fragment ion at m/z 94 was found. Their spectra were very similar to that for 1-hydroxytropacocaine [20], which elutes in FRAC D and will be described below. It was clear from their mass spectra that these impurities had a hydroxy substituent on the tropane ring.

Figure 19. Partial reconstructed cGC-MS (SIM) chromatograms (42-45 min) of alumina column FRAC C alkaloidal impurities, isolated from two refined illicit cocaine comparison case samples; peaks 1 and 2 are suspected hydroxytropacocaine alkaloidal impurities with -OH substitution on tropane ring, probably at C-2 or C-4. 3 = 6-exo-Benzoyltropine (alkaloidal impurity) as TMS derivative.

The lack of an intense ion at m/z 94 forbid hydroxy substitution at C-6 (or C-7, which is a chemically equivalent site). Substitution at C-1 (or the equivalent C-5) was not a consideration, because 1-hydroxytropacocaine had already been characterized, revealing a different retention time compared with the unknown pair (Figure 19). The only remaining positions for a hydroxy substituent on the tropane ring are at C-2, C-4 (chemically equivalent sites), or C-3. Since there are two of these alkaloidal impurities, this requires that there be epimeric isomers at C-2/C-4 sites or at the C-3 position.

For all the FRAC C alkaloidal impurities discussed thus far, the selected ions used for monitoring their chromatography were all base peaks in their respective FS-EI mass spectra. These included fragment ions at m/z 94 (for 6- and 7-hydroxyccocaines) and m/z 182 (for those 2-carbomethoxy-3-oxo-tropane alkaloids with no additional substituents on the tropane ring; the largest group of alkaloidal impurities detected in refined illicit cocaine samples). Other ions monitored were at m/z 185 (benzoyloxy-substitution at chemically equivalent C-6 or C-7, with no carbomethoxy substituent at C-2), m/z 212 (for 3-oxo-substituted alkaloids, with hydroxyl attachment at C-2, C-3, or C-4) and m/z 270 (for 2-carbomethoxy-3-oxo-tropane alkaloids with hydroxy substitution on the tropane ring at sites other than C-6 or C-7).

Yet another group of alkaloidal cocaine impurities in FRAC C are those that have no carbomethoxy moiety at C-2 and no substituent on the tropane ring (except at C-3). All of these compounds have an oxo-substituent at C-3 and yield FS-EI mass spectra with a base peak at m/z 124. Finally, none of these alkaloids formed TMS derivatives. These FRAC C impurities are now discussed below.

6. 3-oxo-Substituted Alkaloidal Impurities with No Derivatizable Substituents

Figure 20 illustrates the chromatography for the alkaloidal impurities benzoyltropine, tropacocaine (the bulk of which is found in FRAC B), and 3-alpha-phenylacetoxytropane for the cocaine comparison case samples. p-Fluorobenzoyltropine (peak 1) is the internal standard. It is interesting to note the major differences in the benzoyltropine and 3-alpha-phenylacetoxytropane content between the two samples. We have also observed that cocaine samples produced from Colombian coca leaf yield much higher levels of benzoyltropine, compared with samples processed from Peruvian or Bolivian leaf.

The comparative determinations for the benzoyltropine and phenylacetoxytropane alkaloidal impurities in the 51 refined illicit cocaine samples are presented in Figures 21 and 22. The internal standard for these determinations was p-fluorobenzoyltropine (Figures 3 and 20). The base peak ion at m/z 124 was used in all calculations. The levels of benzoyltropine and 3-alpha-phenylacetoxytropane ranged from 24-130,012 ng and 0-8,026 ng/500 mg cocaine, respectively. Figure 21 suggests that as many as 30-40%
of the 51 samples were derived from Colombian leaf. It is not known at this time whether the Colombian leaf was E. coca v. coca or E. novogranatense v. novogranatense, or both.

Another non-derivatizable alkaloid found as an impurity in FRAC C is 3',4',5'-trimethoxybenzoyltropine. Its C-3 epimer is 3',4',5'-trimethoxytropacocaine and is found in FRAC B. (In an analogous relationship, benzoyltropine and tropacocaine are found in FRAC C and FRAC B, respectively). Figure 23 illustrates the chromatography for 3',4',5'-trimethoxybenzoyltropine, with monitoring at m/z 124. Of the 51 cocaine samples examined, the one shown in Figure 23 was one of the more abundant in this alkaloid.

In Figure 24 are bar graphs illustrating the comparative determinations of 3',4',5'-trimethoxybenzoyltropine in the 51 cocaine samples; p-fluorobenzoyltropine was used as internal standard. As seen, there were only several samples in which this alkaloid was not detected. The levels of this alkaloidal impurity ranged from 0–24,602 ng/500 mg cocaine.

7. The Tropacocalline-A's
One of the most intriguing groups of new cocaine impurities in illicit cocaine are what we refer to as the tropacocalline-A's. They are also only one of two groups of cocaine alkaloidal impurities found in FRAC C that did not possess a hydroxy group and, consequently, did not form O-TMS derivatives. The structural possibilities for the tropacocalline-A's are shown in Figure 25. Individually, the structures for alpha-tropacocalline-A and beta-tropacocalline-A are illustrated in Figure 26. The tropacocalline-A's are similar in part to the truxillines.
In that a substituted cyclobutane ring and tropane moiety are present. The major differences are that, for the tropacocalline-A's, the cyclobutane ring is substituted with two phenyls and a carbomethoxy as well as a carboxypseudotropine moiety; in the truxillines the substituents on the cyclobutane ring are two phenyl and two ecgonine methyl ester groups (Figure 34). These structural differences account for the asymmetry of the tropacocalline-A's and, conversely, the symmetry of the truxillines, allowing more positional isomers for the former (Figure 25); eleven of the latter have been detected in illicit cocaine samples [19]. The tropacocalline-A's were presumptively identified in a refined illicit cocaine sample by comparison of their eGC retention times and EI mass spectra with standards synthesized at STRL [1].

Figure 27 is a chromatogram illustrating some of the tropacocalline-A's present at relatively high levels (compared to other samples) in a refined illicit cocaine HCl sample; this sample was from the survey set of 51 samples. As seen, peak 5 is alpha-tropacocalline-A; peak 6 represents the possible presence of epsilon- and/or gamma-tropacocalline-A. Because the tropacocalline-A's all contain a pseudotropine moiety, an intense fragment ion at m/z 124 was present in all of their EI mass spectra; therefore, it was used in the selected ion detection of these compounds.

It is interesting to note that in virtually all cocaine samples analyzed to date for the truxillines, the alpha- and beta-isomers were the most abundant [15]. Furthermore, the total truxilline content varied from less than 0.5% to as high as 19% (w/w relative to cocaine) [1]. The ratios, however, of the individual truxilline content to one another did not vary greatly from sample to sample. Conversely, alpha- and beta-tropacocalline-A's were not the most abundant in the 51 cocaine samples examined, and the overall levels of the tropacocalline-A's were at least 2-3 orders of magnitude below that for the truxillines. Finally, there appeared to be a larger variation in the ratios of individual tropacocalline-A isomeric content to one another, compared to the truxillines.

As seen in Figure 27, the tropacocalline-A's have rather long retention times (70-78 min), due in part to the size of the molecule (molecular weight = 433). It has been observed that their response and chromatography are
more sensitive to overall chromatographic conditions than are other cocaine processing impurities. For example, the condition of the injection liner and capillary column, (e.g., new column versus old column), as well as the presence of active sites, could have deleterious effects on the chromatography of the tropacocalline-A's. For these reasons, quantitative and reproducibility data for these compounds have not been reported, and awaits further study, including additional refinement of chromatographic conditions. It should be noted that the truxillines (molecular weight = 658) do not chromatograph intact under the conditions of this methodology.

There has been conflicting data supporting the presence of the carbomethoxy group in the coca leaf samples, using modified methodology [1]. Because of the variation in the relative ratios of the individual tropacocalline-A's and, due to their dynamic range, vis-a-vis concentration, it is unlikely that these compounds are produced as artifacts by either the method herein or during cocaine manufacture. Additional coca leaf samples, using the modified methodology, will be reanalyzed to determine if they are, indeed, bona fide coca-leaf alkaloids.

Finally, attempts will be made to isolate and characterize what we refer to as the tropacocalline-B's in coca leaf extracts and in refined illicit cocaine. The tropacocalline-B's differ from the tropacocalline-A's in that instead of the presence of the carbomethoxy group (Figure 25), an additional carboxypseudotropine substituent is attached to the cyclobutane ring. In other words, the tropacocaine-B's, if present, would be structurally identical to the truxillines, minus two C-2 carbomethoxy groups.

Figure 24. Bar graphs, at different attenuations, for the comparative determination of the FRAC C alkaloidal impurity 3',4',5'-trimethoxybenzoyltropine in 51 refined illicit cocaine samples. (Sample 18 is a cocaine base exhibit; Sample 19 is an anomalous result.)

Figure 25. Structural possibilities for the alumina column FRAC C cocaine impurities, the tropacocalline-A's: (1) alpha-A: R1 = pseudotropine ester, R2 = R3 = R6 = R7 = H, R4 = R5 = phenyl, R8 = carbomethoxy ester; (2) beta-A: R1 = carbomethoxy ester, R2 = pseudotropine ester, R3 = R4 = R5 = H, R7 = R8 = phenyl; (3) gamma-A: R1 = R2 = R6 = R7 = H, R3 = pseudotropine ester, R4 = R5 = phenyl, R8 = carbomethoxy ester; (4) delta-A: R1 = R4 = R6 = R7 = H, R2 = pseudotropine ester, R3 = carbomethoxy ester, R5 = R8 = phenyl; (5) neo-A1: R1 = R4 = R5 = R6 = H, R2 = pseudotropine ester, R3 = carbomethoxy ester, R7 = R8 = phenyl; (6) neo-A2: R1 = R4 = R5 = R6 = H, R2 = carbomethoxy ester, R3 = pseudotropine ester, R7 = R8 = phenyl; (7) epsilon-A: R1 = R4 = R5 = R6 = H, R2 = R3 = phenyl, R7 = R8 = pseudotropine ester; (8) epi-A1: R1 = R4 = R5 = R6 = H, R2 = R3 = carbomethoxy ester, R7 = R8 = pseudotropine ester; (9) epi-A2: R1 = carbomethoxy ester, R2 = R3 = phenyl, R4 = R5 = R6 = H, R7 = R8 = pseudotropine ester; (10) mu-A: R1 = carbomethoxy ester, R2 = R3 = R6 = R7 = H, R4 = pseudotropine ester, R5 = R8 = phenyl; (11) omega-A: R1 = carbomethoxy ester, R2 = pseudotropine ester, R3 = R4 = R5 = R6 = H, R7 = R8 = phenyl; (12) peri-A: R1 = pseudotropine ester, R2 = R3 = phenyl, R4 = R5 = R6 = H, R7 = R8 = carbomethoxy ester; (13) zeta-A1: R1 = carbomethoxy ester, R2 = pseudotropine ester, R3 = R4 = R5 = H, R6 = R7 = R8 = phenyl; (14) zeta-A2: R1 = pseudotropine ester, R2 = carbomethoxy ester, R3 = R4 = R5 = H, R6 = R7 = R8 = phenyl.
Figure 26. Structures for alpha-tropacocalline-A (upper) and beta-tropacocalline-A (lower), suspected impurities in FRAC C of refined illicit cocaine.

Figure 27. Partial reconstructed cGC-MS (SIM) chromatogram (68–78 min) of alumina column FRAC C alkaloidal or manufacturing impurities, isolated refined cocaine: presumptive identification: peak 1 = unknown tropacocalline-A, peak 2 = beta-tropacocalline-A, peak 3 = neo-tropacocalline-A (one of two possible positional isomers) and/or mu-tropacocalline-A, peak 4 = delta-tropacocalline-A, peak 5 = alpha-tropacocalline-A, peak 6 = epsilon- and/or gamma-tropacocalline-A, peak 7 = zeta-tropacocalline-A (one of two possible positional isomers), peak 8 = neo-tropacocalline-A (one of two possible positional isomers) and peaks 9 and 10 = unknown tropacocalline-A’s (ion monitored: m/z 124) illicit cocaine.

C. Alumina Column FRAC D (Ions Monitored: m/z 94, 124, 182, 185, 196, 212, 270, 391)

FRAC D residue is treated as above for FRAC C.

In alumina column FRAC D (acetone/methanol, 1:1) were additional alkaloids possessing derivatizable (with MSTFA) hydroxy groups. Atropine was chosen as an internal standard for this fraction, because:

1. The alkaloid atropine is not present in coca and, therefore, not found in refined cocaine,
2. It elutes entirely within FRAC D, and
3. It possesses a derivatizable hydroxy group.

Though the sample chromatograms for this fraction were not as peak-rich as FRAC C, there were, nonetheless, some interesting alkaloids present. Figure 28 illustrates, for FRAC D impurities, the reconstructed cGC-MSD chromatogram representing the sum of the ions at m/z 94, 124, 182, 185, 196, 212, 270, and 391. The sum total of these ions was used for determination of the following alkaloidal impurities.

1. 1-Hydroxytropacocaine

This alkaloid has been identified at relatively high levels in greenhouse-cultivated *Erythroxylum novogranatense var. novogranatense* (ENVN) [20]. Its presence is also believed to be about 1–2 orders of magnitude greater in Colombian-cultivated ENVN coca compared to *Erythroxylum coca var. coca* (ECVC) grown in Peruvian (Huallaga Valley) and Bolivian (Chapare region) coca. The content of 1-hydroxytropacocaine in the Huallaga and Chapare coca is at only ultratrace amounts. Although its levels in ECVC-cultivated leaf in Colombia are not known at this time, 1-hydroxytropacocaine is probably higher than in Peruvian or Bolivian leaf. This alkaloid is believed to be important in differentiating refined illicit cocaine derived from Colombian coca versus cocaine from Peruvian/Bolivian leaf. Presently, we are not certain whether refined illicit cocaine samples, high in 1-hydroxytropacocaine, are derived from Colombian ECVC or ENVN cultivars or both.

Figure 29 illustrates reconstructed, partial chromatograms from the sample comparison case, using the summation of the ions at m/z 94, 124, 182, 185, 196, 212, 270, and 391; also seen is the structure for 1-hydroxytropacocaine. Although the FS-EI mass spectrum of 1-hydroxytropacocaine (as the O-TMS derivative) yields a base peak at m/z 212, summation of all ions produced a more peak-rich and, therefore, informative chromatogram; furthermore, there were no apparent interferences with the 1-hydroxytropacocaine peak (as TMS derivative) when using summation of the multiple ions.

From Figure 29 it is seen that the 1-hydroxytropacocaine content of Sample 2 was markedly higher than that for Sample 1. This strongly suggested that the cocaine in Samples 1 and 2 were derived from taxonomically differ-
ent coca cultivars. As mentioned above, atropine was used as the internal standard. It is believed that the N-norcocaine (Figure 29) present in these chromatograms was formed, in part, as a manufacturing byproduct as well as created by the method herein, i.e., by the action of peroxides (present in solvents) upon cocaine.

The comparative determinations for the 1-hydroxytropacocaine alkaloidal content in the 51 cocaine samples are seen in Figures 30. It appears reasonable that because of their relatively high 1-hydroxytropacocaine content, at least 20-30% of these samples were derived from Colombian coca leaf or similar cultivars in neighboring regions, e.g., northern Ecuador or northern Peru. (The Huallaga valley is in central Peru.) From Figure 30, it is seen that there is a significant number of samples with no or minimal levels of 1-hydroxytropacocaine. The levels of this alkaloid for the 51 samples ranged from 0-95,500 ng/500 mg cocaine. For the determination of 1-hydroxytropacocaine in illicit cocaine, the sum of the multiple ions for atropine were used in all calculations.

2. m-Hydroxycocaine

Also present in FRAC D is the alkaloidal impurity m-hydroxycocaine; this has also been identified as an alkaloid in South American coca [13]. Figure 31 illustrates its chromatography (as the O-TMS derivative) for the two sample comparison exhibits. Although the meta-hydroxycocaine content for these two samples are similar, major differences can be seen for peak 7, an unidentified compound. Peak 7 is intriguing, because its levels in cocaine samples vary widely. The chromatography illustrated in Figure 31 was acquired at a relatively high attenuation; upon a decrease in this attenuation, the resultant chromatogram revealed the presence of other numerous unidentified peaks/compounds. Finally, the in-depth analyses of coca leaf and its commercial extracts failed to reveal the presence of the o- or p-hydroxycocaine isomers.

In Figure 32 are the comparative determinations for m-hydroxycocaine in the refined cocaine samples. The levels of this alkaloidal impurity varied from 323-11,994 ng/500 mg cocaine.

3. 6-exo-Hydroxytropacocaine

This alkaloid was not found at significant levels in most of the 51 cocaine samples analyzed. This was surprising, given the levels of 6- and 7-hydroxycocaines in those exhibits. As was the case for the hydroxycocaines, the FS-EI mass spectrum for standard 6-exo-hydroxytropacaine (the 7-exo isomer is chemically equivalent) yielded a base peak at m/z 94.

In a quite atypical cocaine sample (from the 51 sample database), 6-exo-hydroxytropacocaine (chromatographed as the O-TMS derivative) was found at a relatively high level compared with virtually all other samples. This is illustrated by the chromatogram in Figure 33. Also identified are 1-hydroxytropacocaine and atropine internal standard (as O-TMS derivatives), as well as cocaine. The peaks labeled "a" all gave a base peak at m/z 94, suggesting the presence of additional tropacocaines; this could include the endo-isomer at C-6, or OH-substitution at C-6 with different epimeric configurations at C-2 and C-3.
D. Alumina Column FRAC B (Ions Monitored: \( m/z \) 94, 124, 182, 185, 196, 212, 270, 391)

FRAC B residue is dissolved in 1.0 mL of chloroform; 250 \( \mu \)L of that solution and 250 \( \mu \)L of MSTFA are added to another tube, vortexed and then heated at 75 °C for 20 min. This solution is set aside for cGC-MSD-SIM analysis.

Unlike FRAC C and FRAC D, the bulk of the alkaloids in FRAC B (chloroform/acetone, 8:2) possessed no MSTFA-derivatizable hydroxy substituents. The largest group of alkaloids found in FRAC B were the eleven isomeric truxillines. Two such alkaloids, alpha- and beta-truxilline, are illustrated in Figure 34. The determination of the truxillines in FRAC B by the new cocaine signature method using cGC-MSD is still under investigation, so this data will appear in a future report. In some preliminary work that appears promising, an aliquot of FRAC B was subjected to hydrolysis with 6N hydrochloric acid, followed by evaporation to residue and MSTFA derivatization to form di-TMS esters, which were then determined by cGC-MSD [14]. If this method proves successful, it means that the detection and determination of the majority of known alkaloidal impurities in refined illicit cocaine can be accomplished using a single detector (MSD) and a single sample weight. In the new signature methodology described herein, the presence of the truxillines, and other similarly large alkaloidal impurities, is indicated by the presence of a significant methyl ecgonidine degradation peak (retention time = ca 18 min as shown in Figure 4).

In the original truxilline methodology developed by STRL, cGC-ECD was used in the determinative step [19]. Because the truxillines were difficult to determine directly by GC (because of their large mass), the sample was first converted to the free base, followed by reduction to their respective diols with lithium aluminum hydride (LiAlH\(_4\)), and then, extraction of the diols and derivatization with heptafluorobutyric anhydride (HFBA) [19]. This methodology was subsequently modified by first subjecting the

![Figure 30](image)

**Figure 30.** Bar graphs, at different attenuations, for the comparative determination of the alkaloidal impurity l-hydroxytropacocaine in 51 refined illicit cocaine samples. (Sample 18 is a cocaine base exhibit.)

![Figure 31](image)

**Figure 31.** Partial reconstructed cGC-MS chromatograms (46–59 min) of alumina column FRAC D alkaloidal impurities, isolated from two refined illicit cocaine comparison case samples; alkaloidal impurity meta-hydroxycocaine (peak 5) chromatographed as an O-TMS hydroxycocaine; peak 3 is a suspected alkaloidal impurity of unknown structure; chromatograms are summation of ions as in Figure 28.
Figure 32. Bar graph for the comparative determination of the alkaloidal impurity meta-hydroxyecgonine in refined illicit cocaine. (Sample 18 is a cocaine base exhibit.)

Figure 33. Partial reconstructed cGC-MS chromatogram (42–48 min) of alumina column FRAC D alkaloidal impurities, isolated from a refined illicit cocaine sample; alkaloidal impurities 1-hydroxytropacocaine (peak b), 6-exo-hydroxytropacocaine (peak c) and atropine internal standard (peak d) chromatographed as O-TMS derivatives; chromatograms are summation of ions as in Figure 28.

Sample to methyl esterification using boron trifluoride/methanol, followed by extraction, reduction with LiAlH₄ and derivatization with HFBA [11]. Using that methodology, it was possible to determine the total truxilline content for over 100 refined illicit cocaine samples [15].

Currently, the STRL determines the truxillines using recently modified methodology that reduces analysis time significantly [1]. In this method the cocaine hydrochloride sample is reduced directly with LiAlH₄, thereby eliminating the conversion-to-base step; this was followed by extraction of the diols and derivatization with HFBA. Determination of the di-heptafluorobutyryl derivatives was also accomplished by cGC-ECD. Seen in Figure 35 is the entire cGC-ECD chromatogram for the truxillines determination in Sample 2 of the comparison case. The total truxilline content of 8.8% is significantly higher than for most samples [15]. Figure 36 illustrates the chromatographic window and identified peaks for the truxillines. Note that the mu-isomer was selected as internal standard. This was possible because of its minuscule quantities in illicit cocaine.

In addition to the truxillines there are numerous other alkaloidal impurities in alumina column FRAC B, present at much lower levels. Figure 37 is a cGC-MSD-multiple ion chromatogram for the alkaloidal impurities in FRAC B. The truxilline degradation product, methyl ecgonidine, is represented by the intense peak at about 18 min. In addition to the truxillines, other alkaloids detected and/or determined in FRAC B include those listed below.

1. Tropacocaine
One of the more significant alkaloidal impurities determined in FRAC B is tropacocaine. This is because levels of tropacocaine derived from some coca cultivated...
Figure 35. GC-ECD chromatogram of the truxillines determination for Sample 2 of the cocaine comparison case. The truxillines are in brackets.

Figure 36. Expanded “window” from Figure 35 chromatogram showing chromatography and identification of the individual truxillines.

Figure 38 shows the comparison case chromatography for tropacocaine, alkaloidal impurity cis-cinnamoyltropacocaine and an unknown alkaloid; the internal standards are p-fluorotropacocaine and hydrocinnamoyltropacocaine (Figure 3). As seen from the peak areas, Sample 2 was somewhat higher in tropacocaine content than Sample 1. Conversely, the level of cis-cinnamoyltropacocaine is about 25 times more abundant in Sample 1 than in Sample 2, again demonstrating the selectivity of this methodology.

Figure 39 represents the comparative determinations for tropacocaine in the 51 samples. As seen, tropacocaine was detected in all samples, and from the y-axis, it is apparent that the overall levels of tropacocaine are significantly higher than the alkaloidal impurities discussed thus far (excepting the truxillines and the cinnamoylcocaines). The range for tropacocaine as an alkaloidal impurity was from 16–2,993 µg/500 mg cocaine; all calculations were done using the internal standard p-fluorotropacocaine.
Figure 37. Reconstructed cGC-MS chromatogram of alumina column FRAC B alkaloidal impurities, isolated from refined illicit cocaine; chromatogram is the summation of ions \(m/z\) 94, 124, 182, 185, 196, 212, 270, and 391.

Figure 38. Partial reconstructed cGC-MS chromatograms (34-46 min) of alumina column FRAC B alkaloidal impurities, isolated from two refined illicit cocaine comparison case samples; peaks 2 and 4 = \(p\)-fluorotropacocaine and hydrocinnamoyltropacocaine internal standards, respectively; peaks 3 and 5 = alkaloidal impurities tropacocaine and cis-cinnamoyltropacocaine, respectively.

Figure 39. Bar graphs, at different attenuations, for the comparative determination of the FRAC B alkaloidal impurity tropacocaine in 51 refined illicit cocaine samples. (Sample 18 is a cocaine base exhibit.)

2. cis- and trans-Cinnamoyltropacocaine and 3',4',5'-Trimethoxytropacocaine

The chromatography for these impurities is illustrated in Figure 40 (peaks 2, 5, and 8) for the comparison case samples. As seen, Sample 1 contained markedly higher levels of cis- and trans-cinnamoyltropacocaine than Sample 2. Other alkaloids in this chromatogram included cis- and trans-cinnamoyltropacocaine (also present at greater levels in FRAC A) and 3',4',5-trimethoxytropacocaine. (As discussed previously, the C-3 epimer of this impurity, namely, 3',4',5'-trimethoxybenzoyltropine, is present in FRAC C.) From Figure 40 it is also apparent for comparison case Sample 1 that the level of 3',4',5'-trimethoxytropacocaine is exceedingly small, yet much greater than for Sample 2. Hydrocinnamoyltropacocaine (Figure 3) internal standard is peak 1 in the Figure 40 chromatogram.

The bar graphs representing the comparative determinations for cis- and trans-cinnamoyltropacocaine content for the 51 cocaine samples are shown in Figure 41. The concentration ranges for cis- and trans-cinnamoyltropacocaine alkaloidal impurities were 0-130,891 ng and 0-56,427 ng/500 mg cocaine, respectively. The internal standard hydrocinnamoyltropacocaine was used in all...
calculations. About 20% of the 51 cocaine exhibits had barely or no detectable levels of these isomers. This is undoubtedly due to rigorous treatment with potassium permanganate during the manufacturing process. This oxidation has long been known to affect the reduction of the levels of the cinnamoylcocaines in illicit cocaine samples.

Figure 40. Partial reconstructed cGC-MS chromatograms (42-60 min) of alumina column FRAC B alkaloidal impurities, isolated from two refined illicit cocaine comparison case samples; peak 1 = hydrocinnamoyltropacocaine (internal standard) used for comparative determinations for alkaloidal impurities cis- and trans-cinnamoyltropacocaine (peaks 2 and 5) and 3',4',5'-trimethoxytropacocaine (peak 8); ion monitored: m/z 124.

Figure 41. Bar graphs for the comparative determination of the alumina column FRAC B alkaloidal impurities cis- and trans-cinnamoyltropacocaine in 51 refined illicit cocaine samples. (Sample 18 is a cocaine base exhibit.)

Bar graphs in Figures 42 show the comparative determinations of 3',4',5'-trimethoxytropacocaine in the 51 cocaine samples. This alkaloidal impurity was detected in nearly all samples. The levels of this impurity varied from 0-47,748 ng/500 mg cocaine. The internal standard hydrocinnamoyltropacocaine (Figure 3) was also used for these calculations.

3. 2'-Furanoylecgonine- and Nicotinoylecgonine Methyl Esters

As a result of the analyses of authentic Bolivian and Peruvian coca leaf, there was evidence suggesting that the alkaloid 2'-furanoylecgonine methyl ester, whose structure is illustrated in Figure 43, was at markedly higher levels in the latter leaf, compared with the former [13,14]. These findings still await further substantiation by analyzing additional coca leaf samples. Nonetheless, it was of special interest to determine the levels of this alkaloid as an impurity in cocaine samples.

Figure 43 illustrates the chromatography of 2'-furanoylecgonine for the two comparison case samples, after extraction of the ion at 182 Daltons. As seen from peak 1, there is little difference in this alkaloid's content.
between the two samples. (The level of 2'-furanoylcgonine methyl ester for these samples was much higher than for most of the 51-sample data base.) Other alkaloidal impurities of interest in this chromatogram are 3-alpha-phenylacetylecgonine and nicotinoyllecgonine methyl esters (peaks 4 and 5) and 3',4',5'-trimethoxycocaine (peak 8). It is observed that the trimethoxycocaine level for Sample 1 is 1-2 orders of magnitude greater than for Sample 2. The internal standards for 2'-furanoylcgonine methyl ester and 3',4',5'-trimethoxycocaine are their ethyl homologs (peaks 2 and 9, respectively; see Figures 3 and 43). Nicotinoylcgonine methyl ester was detected in virtually all of the samples examined; its levels were considerably below that for many of the other alkaloids in FRAC B.

Bar graphs in Figure 44 show the comparative determinations for 2'-furanoylcgonine methyl ester, using the fragment ion at m/z 182. The ion at m/z 196 was used for the internal standard 2'-furanoylcgonine ethyl ester. The levels of 2'-furanoylcgonine methyl ester for the 51 cocaine samples varied from 16-1,063 µg/500 mg cocaine.

Figure 42. Bar graphs, at different attenuations, for the comparative determination of the FRAC B alkaloidal impurity 3',4',5'-trimethoxypopacocaine in 51 refined illicit cocaine samples. (Sample 18 is a cocaine base exhibit.)

Figure 43. Partial reconstructed cGC-MS chromatograms (40-70 min) of alumina column FRAC B alkaloidal impurities isolated from two refined illicit cocaine comparison case samples; peaks 1 and 2 = 2'-furanoylcgonine methyl ester and its internal standard, 2'-furanoylcgonine ethyl ester, respectively; peaks 4 and 5 = alkaloidal impurities 3-alpha-phenylacetylecgonine methyl ester and nicotinoyllecgonine methyl ester; peaks 8 and 9 = alkaloidal impurity 3',4',5'-trimethoxycocaine and its internal standard 3',4',5'-trimethoxyoecocethylene (3',4',5'-trimethoxybenzoylecgonine ethyl ester), respectively; ions monitored: m/z 182 and 196.

Figure 44. Bar graphs in Figure 44 show the comparative determinations for 2'-furanoylcgonine methyl ester, using the fragment ion at m/z 182. The ion at m/z 196 was used for the internal standard 2'-furanoylcgonine ethyl ester. The levels of 2'-furanoylcgonine methyl ester for the 51 cocaine samples varied from 16-1,063 µg/500 mg cocaine.
Figure 44. Bar graphs, at different attenuations, for the comparative determination of the FRAC B alkaloidal impurity 2'-furancylecgonine methyl ester in 51 refined illicit cocaine samples. (Sample 18 is a cocaine base exhibit.)

4. 3',4',5'-Trimethoxycoacaine

The chromatography for this alkaloid is seen in Figure 43. The comparative determinations for this alkaloidal impurity are seen in Figures 45. As seen from the bar graph (Figure 45), the levels of 3',4',5'-trimethoxycoacaine are much higher than for most other trace-level alkaloidal impurities, excepting tropacocaine. The dynamic range for 3',4',5'-trimethoxycoacaine concentrations varied from 7-3,984 µg/500 mg cocaine.

Unlike 3',4',5'-trimethoxycoacaine, the related alkaloids cis- and trans-trimethoxyacinomoylcocaine and cis- and trans-trimethoxyacinomoyltropacocaine could not be determined accurately using the method herein. This is believed due to degradation on the alumina column, or less likely, from poor response on the DB-1701 capillary column.

Figure 45. Bar graph for the comparative determination of the FRAC B alkaloidal impurity 3',4',5'-trimethoxycoacaine (3',4',5'-trimethoxybenzoylecgonine methyl ester) in 51 refined illicit cocaine samples. (Sample 18 is a cocaine base exhibit.)

E. Alumina Column FRAC A (Ions Monitored: m/z 182, 196, 317, 321, 331, 343, 347, 349, 351, 365)

FRAC A residue is dissolved in 4.0 mL of hot hexane, allowed to cool to room temperature and then placed in a freezer overnight (not to exceed 16 hr); after centrifugation, the mother liquor is transferred to a smaller tube, reduced to residue and then redissolved in 1.0 mL of hot hexane; after allowing it to cool to room temperature, the tube is placed in a freezer for 3 hr; after centrifugation, the mother liquor is transferred to another tube and evaporated to residue. To the residue is added 500 µL of chloroform and 500 µL of N-methyl-N-trimethylsilyltrifluoroacetimide (MSTFA); the solution is heated at 75 °C for 20 min in a heating block and then set aside for cGC-MSD-SIM analysis.

Most of the alkaloids in FRAC A (chloroform/petroleum ether, 8:2) can be placed into two groups. One contains alkaloidal impurities all possessing a C-2 carbomethoxy moiety and 3-oxo- substituents that are hydrocarbon in character; furthermore, none of these impurities were derivatizable. Most of these Group I alkaloids were first detected, along with heteroaryl-substituted tropane alkaloids, as described in a recent study [13]. These alkaloids were also structurally characterized and determined in both South American coca and refined illicit cocaine [4, 7]. Group II alkaloids, now reported here for the first time in illicit cocaine samples, are believed to be, in part, similar to the hydroxy-substituted, carbonyl-conjugated diene and triene alkaloids described in FRAC C, except the former do not possess hydroxy substitutents. These alkaloids, which do not form O-TMS derivatives, have yet to be structurally characterized.
Figure 46 illustrates the total multiple ion reconstructed chromatogram for Sample 1 of the cocaine comparison case; nearly 50 detectable alkaloidal impurities are present in the FRAC A chromatogram. As seen in Figure 47, from 28–47 min, the fragment ions at m/z 182 and 196 were monitored for alkaloidal impurities in the sample and the internal standard, acetylcocaine ethyl ester (Figure 3), respectively. Although all of the alkaloidal impurities that eluted after 47 min exhibited the intense, diagnostic ion at m/z 182 (indicative of a 2-carbomethoxytropane moiety), that ion could not be monitored because of the overwhelming presence of cocaine and cis- and trans-cinnamoylcocaine, all of which have an intense 182 ion. However, the alkaloidal impurities eluting between 47 and 57 min did exhibit molecule ions of sufficient intensity to render them suitable for selected ion monitoring. When monitoring these ions, the cocaine and cinnamoylcocaine responses were effectively suppressed.

Figure 47 shows the x-axis-expanded chromatogram for FRAC A alkaloidal impurities present in the cocaine comparison case Sample 1. As described by the method herein, these alkaloids are present in the mother liquor after a double recrystallization of the cocaine matrix. It has been observed that if the recrystallization time is significantly prolonged, then major losses of the target analytes occur. (Reference [7] describes shorter, yet effective, recrystallization times, compared with the method herein, for FRAC A alkaloids.) The internal standards for FRAC A are seen in the Figure 47 chromatogram as acetylcocaine ethyl ester (peak 1, Figure 3), hydrocinnamoylcocaine (peak 37, Figure 3) and apotropoylecgonine ethyl ester (peak 38, Figure 3). Figure 47 also lists the identities or molecule ions for most of the FRAC A alkaloidal impurities. It has been reported recently that, after review of a very limited data base, some of the alkaloidal impurities found between 28 and 47 min in the Figure 47 chromatogram appeared diagnostic for some geographic discrimination [4,7]. It is also interesting to note that if the illicit cocaine sample had been subjected to rigorous potassium permanganate treatment during processing, most of the alkaloids that eluted between 50 and 57 min were significantly diminished.

Bar graphs for the comparative determinations for the two most consistently abundant alkaloidal impurities in FRAC A, hexanoyl- and phenylacetylcocaine methyl esters (peaks 8 and 26, respectively, in Figure 47), for the 51 samples are seen in Figures 48 and 49. The structures for these alkaloids are illustrated in Figure 50. Figure 51 shows structures, along with the chromatography, for Group I alkaloids in FRAC A. As seen in Figures 48 and 49, it appears that the bars for Samples 23, 24, and 28–31 for both impurities indicate the absence of these alkaloids. This was not the case, however, because these samples were mishandled during their analyses. For the hexanoylcocaine and phenylacetylcocaine methyl ester impurities, the concentrations ranged from 39–1,148 µg and 0–291 µg/500 mg cocaine, respectively (based upon 45 cocaine samples). The internal standards used in the calculations were acetylecgonine ethyl ester and hydrocinnamoylcocaine (Figure 2), for hexanoylcocaine and phenylacetylcocaine methyl ester impurities, respectively.

Figure 46. Reconstructed cGC-MS chromatogram of alumina column FRAC A alkaloidal impurities, isolated from refined illicit cocaine; chromatogram is the summation of ions at m/z 182 and 196 (28-47 min); 317, 321, 347, 349, 351, 365, 331, and 343 (47–53.2 min); and 317, 321, 347, 349, 351, 365, and 343 (53.2–58 min).
Refined Illicit Cocaine

Ions monitored:
- 28–47 min: m/z 182 and 196
- 47–53.2 min: m/z 317, 321, 347, 349, 351, 365, 331, and 343
- 53.2–58 min: m/z 317, 321, 343, 347, 349, 351, and 365

Figure 47. X-Axis-expanded cGC-MS chromatogram from Figure 46. Peak identities: peak 1 = acetylecgonine ethyl ester internal standard, peak 2 = butyryl-EME, peak 3 = 2-methylbutyroyl-EME, peak 4 = isovaleroyl-EME, peak 5 = valeroyl-EME, peak 6 = suspected isomer of hexanoyl-EME, peak 7 = tigloyl-EME, peak 8 = hexanoyl-EME, peak 9 = trans-3-hexenoyl-EME, peaks 10, 17, 22 = unknown alkaloidal impurities, peaks 11, 13 = suspected isomers of heptanoyl-EME, peak 12 = suspected isomer of hexenoyl-EME, peaks 14, 15 = suspected isomers of heptenoyl-EME, peaks 16, 20, 21 = suspected isomers of octenoyl-EME, peak 18 = suspected octadecenoyl-EME, peak 19 = suspected isomer of octanoyl-EME, and peak 23 = suspected isomer of undecanoyl-EME; peaks 24, 25 = suspected isomers of decadienoyl-EME, peak 26 = phenylacetyl-EME, peak 27 = unknown alkaloidal impurity (molecule ion = m/z 331?), peak 28 = suspected isomers of decatrienoyl-EME + decanoyl-EME and unknown alkaloidal impurity (molecule ion = m/z 317), peak 30 = suspected isomers of decatrienoyl-EME + decadienoyl-EME, peak 31 = suspected isomer of decadienoyl-EME, peaks 32–36 = suspected isomers of decadienoyl-EME, peak 37 = hydrocinnamoyl-EME internal standard (see Figure 3), peak 38 = apotropoyllecgonine ethyl ester internal standard (see Figure 3) and peak 39 = suspected isomer of undecanoyl-EME?, peaks 40, 47 = suspected isomers of decadienoyl-EME + decatrienoyl-EME, peaks 41, 43, 45, 46, 48 = isomers of suspected decatrienoyl-EME, peak 42 = suspected isomer of decadienoyl-EME, and peak 44 = unknown alkaloidal impurity (molecule ion = m/z 343) (EME = ecgonine methyl ester).

Figure 48. Bar graph for the comparative determination of the FRAC A alkaloidal impurity hexanoylecgonine methyl ester in 51 refined illicit cocaine samples. (Sample 18 is a cocaine base exhibit.)

Figure 49. Bar graph for the comparative determination of the FRAC A alkaloidal impurity phenylacetylecgonine methyl ester in 51 refined illicit cocaine samples. (Sample 18 is a cocaine base exhibit.)
II. IMPORTANT ANALYTICAL CONSIDERATIONS

A. Selection of Internal Standards for New Cocaine Signature Method

All internal standards used in this study were prepared at the STRL. The importance of incorporating structurally related internal standards in methods used for the comparative determinations of illicit drug manufacturing impurities and byproducts cannot be overemphasized; this is especially so when these sample analyses are done over a time period of, perhaps, years. The inclusion of internal standards usually improves method reproducibility and quantitative accuracy, both important criteria in cocaine comparison analyses.

When using a mass spectrometer in the determinative step, the most efficacious internal standards are those that are isotopic analogs of the target analytes. However, their use is often not possible for many compounds because of

Refined Illicit Cocaine
(Comparison Case)

SAMPLE #1

![Image of structures for two FRAC A alkaloidal impurities: hexanoylecgonine (upper) and phenylacetylene gonic methyl esters (lower).]

Figure 50.

![Image of the chromatography and structures for some FRAC A (Group I) alkaloidal impurities.]

Figure 51.

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the difficulty in their synthesis and cost of materials. However, there are alternate types of internal standards suitable for use in comparative determinations. In the methodology herein the following criteria were considered in the preparation of internal standards:

1. They should have a close structural relationship to the target alkaloidal impurity; the closer the structural relationship, the more enhanced will be the reproducibility.
2. They must be chemically stable, especially when in contact with alumina.
3. They should have similar extraction characteristics as the target alkaloid. (This is usually accomplished by using an internal standard that has a close structural relationship to the target alkaloid.)
4. Their retention times should be as close as possible to those for the target analytes.
5. They should be found in the same alumina column fraction containing the target alkaloidal impurity.
6. In selected ion monitoring, the internal standard should yield an intense fragment or molecule ion that is analogous in structure to the ion selected for the alkaloidal impurity.
7. Preparation of the internal standard should be simple and inexpensive.

As seen in Figure 3, some of the internal standards used in this methodology had a carboethoxy group at the C-2 site of the tropane ring, in lieu of a carbomethoxy substituent at the same position for the target analyte; this difference of only a single methylene unit accounts for good method reproducibility. Thus, for example, the 2'-furanoylecgonine ethyl ester internal standard was an excellent choice for use in the comparative determination of the target alkaloidal impurity 2'-furanoylecgonine methyl ester. An additional example is the use of the internal standard trimethoxycocaine (Figure 3) in the determination of trimethoxy cocaine.

For other internal standards used and target alkaloids detected in the method herein, the only difference in their structures was the presence of a fluorine substituent on the aromatic ring of the internal standards. Thus, para-fluorotropacocaine (Figure 3) internal standard was present in the same alumina column fraction as tropacocaine (FRAC B) and was used effectively in its determination; likewise, the internal standard para-fluorobenzyloptropine and alkaloidal impurity benzyloptropine (the epimer of tropacocaine) both elute in FRAC C, with the former used in the determination of the latter.

The internal standards hydrocinnamoyltropacocaine and hydrocinnamoylcocaine are also markedly similar in structure to their target alkaloids cis-/trans-cinnamoyltropacocaine and phenylacetylecgonine methyl ester, respectively. The only structural differences involve the presence/absence of a carbon-carbon double bond (for hydrocinnamoyltropacocaine internal standard and cis-/trans-cinnamoylcocaine target alkaloids) and the presence of an additional methylene unit (for hydrocinnamoylcocaine internal standard and phenylacetylecgonine methyl ester target alkaloid).

B. Reproducibility of New Cocaine Signature Method

Good method reproducibility is a requirement for the optimum evaluation of drug impurity signature data. This is not only so for geographic origin determinations, but is more critical when used in sample comparison cases; for the latter can often result in expert testimony in criminal conspiracy cases.

For the methodology described herein, a selected cocaine exhibit (and not one of the 51 cocaine exhibits used as a data base) was subjected to 15 repetitive analyses over a 5-week period. Relative standard deviation values were calculated for selected alkaloidal impurities when (a) internal standards were incorporated in the calculations, and (b) internal standards were not incorporated in the calculations. This data is presented in Table 1.

A review of Table 1 demonstrates well the value of including structurally related internal standards in the methodology versus the exclusion of internal standards altogether. It can be seen that in each instance in which reproducibility data was calculated for an alkaloidal impurity, both using and excluding the internal standard, the relative standard deviation (RSD) was lower for the impurity when the internal standard was used.

Also seen in Table 1 is that, when using internal standards, only two alkaloidal impurities exceeded a relative standard deviation of ±10% or greater. One of these impurities was phenylacetoxytropine, which yielded a standard deviation of ±10%. This was believed due, in part, to its low level in the cocaine sample selected for repetitive analyses. The reproducibility of the other impurity, m-hydroxy cocaine, which had an RSD of ±14.3%, was calculated using the atropine internal standard; a better choice as an internal standard would probably have been either o- or p-hydroxy cocaine, both of which are not detected in illicit cocaine samples.

It should be emphasized that the reproducibility data in Table 1 was generated over a 5-week period. If the cocaine sample had been analyzed repetitively over the course of only several days, it is expected that there would be an overall decrease in the RSDs of the alkaloidal impurities; this is relevant, because in most cocaine comparison cases, the submitted cocaine exhibits are usually analyzed concomitantly, over only a few days, or less.

C. Comparative Versus Absolute Determinations in Cocaine Comparison Analyses

In the classical, absolute determination of drugs and/or their manufacturing impurities and byproducts, quantitative values for the target analyte were obtained by using a structurally identical external standard for analytical determinations.
Table 1. Reproducibility, with and without internal standard (IS), for new cocaine profiling method

<table>
<thead>
<tr>
<th>Alkaloidal impurity</th>
<th>Relative standard deviation</th>
<th>Ions used</th>
<th>Analyte</th>
<th>IS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With IS</td>
<td>Without IS</td>
<td>2'-FEEME</td>
<td>196</td>
</tr>
<tr>
<td>1 2'-FEME</td>
<td>3.0%</td>
<td>11.7%</td>
<td>TEEE</td>
<td>182</td>
</tr>
<tr>
<td>2 HDEEME</td>
<td>6.2%</td>
<td>8.3%</td>
<td>TEEE</td>
<td>182</td>
</tr>
<tr>
<td>3 HPPEME</td>
<td>2.5%</td>
<td>9.7%</td>
<td>TEEE</td>
<td>182</td>
</tr>
<tr>
<td>4 BZT</td>
<td>3.0%</td>
<td>8.9%</td>
<td>PFBZT</td>
<td>124</td>
</tr>
<tr>
<td>5 CCTC</td>
<td>3.0%</td>
<td>11.8%</td>
<td>HCTC</td>
<td>124</td>
</tr>
<tr>
<td>6 TCTC</td>
<td>3.6%</td>
<td>11.8%</td>
<td>HCTC</td>
<td>124</td>
</tr>
<tr>
<td>7 TMC</td>
<td>2.0%</td>
<td>12.2%</td>
<td>TMCE</td>
<td>182</td>
</tr>
<tr>
<td>8 TRPC</td>
<td>1.3%</td>
<td>12.3%</td>
<td>PFBZT</td>
<td>124</td>
</tr>
<tr>
<td>9 PAT</td>
<td>10.0%</td>
<td>12.1%</td>
<td>PFBZT</td>
<td>124</td>
</tr>
<tr>
<td>10 PAEME</td>
<td>3.2%</td>
<td>19.4%</td>
<td>HCC</td>
<td>317</td>
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<tr>
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<td>17.8%</td>
<td>AEEME</td>
<td>182</td>
</tr>
<tr>
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<td>HCC</td>
<td>124</td>
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<td>TMCE</td>
<td>124</td>
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<tr>
<td>14 MOHCOC</td>
<td>14.3%</td>
<td>18.0%</td>
<td>ATRP</td>
<td>182</td>
</tr>
<tr>
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<td>2.7%</td>
<td>9.4%</td>
<td>TEEE</td>
<td>94</td>
</tr>
<tr>
<td>16 7XOHCOC</td>
<td>2.4%</td>
<td>–</td>
<td>TEEE</td>
<td>94</td>
</tr>
<tr>
<td>17 6NOHCOC</td>
<td>4.1%</td>
<td>–</td>
<td>TEEE</td>
<td>94</td>
</tr>
<tr>
<td>18 7NOHCOC</td>
<td>6.6%</td>
<td>–</td>
<td>TEEE</td>
<td>94</td>
</tr>
</tbody>
</table>

*a Illicit refined cocaine sample analyzed 15 times over a period of 5 weeks.

*b Abbreviations for alkaloidal impurities: 2'-FEME = 2'-furanoy lecgonine methyl ester; HDEEME = 8'-hydroxy-2',4',6'-deca trienoy lecgonine methyl ester; HPPEME = 3'-hydroxy-3'-phenylpropionyl lecgoninemethyl ester; BZT = benzoyltropine; CCTC, TCTC = cis- and trans-cinnamoyltropacocaine; TMC = 3',4',5'-trimethoxybenzoyltropine; TRPC = tropacocaine; PAT = phenylacetoxytropane (3-alpha-phenylacetyltropine); PAEME = phenylacety lecgonine methyl ester; HEME = hexanoylecgonine methyl ester; TMCT = 3',4',5'-trimethoxytropacocaine; TMBZT = 3',4',5'-trimethoxybenzoyltropane; MOHCOC = m-hydroxycocaine; 6XOHCOC, 7XOHCOC, 6NOHCOC, 7NOHCOC = 6-exo-, 7-exo-, 6-endo, and 7-endo-hydroxycocaine, respectively.

*c Abbreviations for internal standards: 2'-FEEME = 2'-furanoylecgonine ethyl ester; TEEE = tropoyl ecgonine ethyl ester; PFBZT = p-fluorobenzoyltropine; HCC = hydrocinnamoyltropacocaine; TMCE = 3',4',5'-trimethoxycocaethylene; PFTRPC = p-fluorotropacocaine; HCC = hydrocinnamoylcocaine; AEEME = phenylacety lecgonine ethyl ester; ATRP = atropine.

measurements and calculations; this would often be supported with analyte(s) linearity studies. This may be practical for samples that are of simple composition, but for refined illicit cocaine HCl samples, however, the matrix is quite complex; some cocaine samples may contain several hundred alkaloidal impurities and manufacturing byproducts. Doing absolute determinations on such samples is impractical, and also not necessary; for these complex matrices, comparative analyses, using structurally related internal standards, is the most reasonable approach.

For cocaine impurity signature analyses, the only absolute determination that need be done, and with good accuracy, is that for cocaine, because quantitative values for impurities/byproducts are reported relative to the cocaine content. As described above, the quantities of values for alkaloidal impurities are acquired in conjunction with structurally related internal standards. In the method described herein, ten such internal standards were used (Figure 3).

D. Sample Weight in Cocaine Comparative Determinations and Method Sensitivity

The new cocaine signature method uses a sample weight of 500 mg cocaine equivalents. This amount was chosen because it is the minimum weight required for one of the six signature methods (BAR Method) currently being used at STRL, and reviewed above. (Current cocaine signature methods, excepting solvent residue determinations, require a total sample weight of about 700 mg). After a review of all chromatography and alkaloidal impurity responses for the 51 illicit cocaine exhibits done by the new signature method, it was concluded that the majority of samples could be successfully analyzed using weights as low as 50-100 mg. This lower amount would depend, of course, upon the overall purity of the sample and the type and the quantity of trace-level alkaloids and their mass fragmentation. For example, a sample weight of 50-100 mg should easily accommodate the determinations of the 6- and 7-hydroxycocaines; as described else-
where in this paper, about 50% of the total ion current for the hydroxycocaines resides in the base peak fragment ion at m/z 94, allowing for their sensitive detection.

Since the new methodology involves an adsorptive chromatographic column step, as well as an initial extraction, prior to cGC-MS (SIM) determination, the use of lower sample weights would require a concomitant reduction in column dimensions and the amount of alumina and solvents used. The reconstitution and derivatization volumes could also be reduced, where practical.

The overall sensitivity of the new signature method for most alkaloidal impurities is enhanced over current methodology, in part, because the 500 mg sample weight is subjected to column chromatographic resolution. Most of the cocaine and cinnamoylcocaines are found in FRAC A, with lesser amounts present in FRAC B, along with the truxillines. This allows for the use of smaller reconstitution volumes for the most peak-rich fraction, FRAC C, and also for FRAC D. Furthermore the use of selected ion monitoring during the MS determinative step allows for enhanced sensitivity and selectivity.

A disadvantage of this method is that it does not determine the hydrolysis products of cocaine and other major alkaloidal impurities, e.g., the benzoyl- and cinnamoylecgonines and truxillic/truxinic acids. The currently-used CISPA Method is superior for those analyses.

E. Additional Cocaine Manufacturing Impurities

Most of the alkaloidal impurity chromatographic profiles illustrated herein were generated at mostly nominal or high instrumental attenuations. When the attenuation was reduced, e.g., below 1,000–2,000 units, many additional cocaine impurities were detected, especially in FRAC C, and to a lesser extent in FRAC's D and B. These impurities have yet to be structurally characterized, although it is reasonable to postulate that some, if not most of these, are alkaloidal. Some of these unidentified impurities, present in FRAC B and FRAC C, can be seen as unenumerated peaks in the chromatograms illustrated in Figures 52 and 53 for Sample 1 of the cocaine comparison case.

Also suspected to be present in refined illicit cocaine samples are alkaloidal impurities of high molecular weight (in addition to the truxillines). These would include the so-called hydroxytruxillines, 10–15 of which have been presumptively identified at trace levels in a commercial coca-leaf extract [13]. Some of these compounds might well be in alumina column FRAC C and/or FRAC D. Figure 54 illustrates the partial chromatogram from FRAC D for Sample 2 of the comparison case. The off-scale ecgonidine methyl ester (methyl ecgonidine) response strongly suggests the presence of yet-to-be identified high molecular weight alkaloidal impurities.

Moore and Casale • Cocaine Profiling Methodology
F. Diluted and Adulterated Cocaine Samples

Because of the liquid-liquid extraction and column chromatographic steps, the new cocaine signature methodology can more readily accommodate diluted and/or adulterated cocaine exhibits, compared with current signature procedures. Interfering sugar and inorganic diluents are easily removed in the initial liquid-liquid extraction step. Since most cocaine adulterants are drugs that do not contain hydroxyl groups, they would not be expected in aluminia column FRAC C or FRAC D. This is important, in that it has been seen that FRAC C contains most of the cocaine alkaloidal impurities. Most common cocaine adulterants would be expected in FRAC A, with lesser amounts found in FRAC B. Since FRAC A is, in part, the result of a double-recrystallization step (with the target alkaloidal impurities being present in the mother liquor), the presence of significant levels of most adulterants might be expected to co-crystallize with the cocaine. Furthermore, the high selectivity of MSD-SIM should suppress the response of common adulterants, since most of them do not yield as base-peak fragment ions those that are used for monitoring alkaloidal impurities, e.g., m/z 94, 124, 182, 185, 212, and 270. One of the more significant effects adulterants could have upon alkaloidal impurities determinations would be “overloading” the capillary column, causing anomalous chromatographic behavior.

IV. IN-DEPTH ANALYSES OF COCA LEAF, ITS EXTRACT, AND REFINED COCAINE

The new cocaine signature method, described above, was derived from methodology developed for the in-depth analyses of a concentrated, commercial extract of South American coca leaf (referred to as E. Harz) and coca leaf samples cultivated in Bolivia, Peru and Colombia. This recently published method [13] was used in the analyses of kilogram quantities of E. Harz and coca leaf. For the analysis of the latter, a powdered sample was basified and extracted with toluene. The toluene extract was passed through a dilute sulfuric acid/Celite column, which retained the alkaloids as a whole and allowed for the removal of non-alkaloidal leaf components. The retained bulk cocaine matrix and trace coca alkaloids were removed from the acid column using chloroform containing diethyl amine [13]. After removal of solvent, the residue was partitioned between chloroform and a pH 4.0 phthalate buffer. The buffer, containing the bulk of the alkaloids, was made basic and extracted with chloroform. After removal of the chloroform, the alkaloid residue was chromatographed on a basic alumina (containing 2-4% water) column using an allotropic series of solvents, beginning with chloroform, followed by chloroform/acetone and then acetone and ending with acetone/methanol [13]. These fractions were then subjected to additional alumina chromatography, and if necessary, semi-preparative reversed-phase HPLC. Most of the HPLC work were done on a C-18 column using a mobile phase of varying phosphate buffer (pH 2.1)/methanol compositions.

The chloroform phase containing some of the other alkaloids (from the chloroform/buffer extraction above) was dried over anhydrous sodium sulfate and the chloroform removed, resulting in an oily residue. This residue was subjected to ion-pairing/Celite chromatography using dilute HCl/NaCl as the stationary phase and HzO-saturated chloroform as the eluant [3,5,13]. The chloroform eluate was dried and then evaporated to an oily residue.

The analysis of E. Harz was similar to that for the coca leaf, except the initial extraction of this matrix with toluene was not needed. Instead, the E. Harz was first partitioned between chloroform and pH 4.0 phthalate buffer and then treated as above for the leaf [13].

Compared with an equivalent quantity of dried South American coca leaf, E. Harz contained much higher amounts of trace-level alkaloids. Most of the new coca alkaloids determined by the new cocaine signature methodology herein were first characterized in E. Harz. After isolation of the alkaloids, they were identified by GC-MS in conjunction with synthesized standards; for some of the more abundant new alkaloids, characterization was also accomplished using 1H and 13C nuclear resonance spectroscopy and high-resolution mass spectrometry [17].

After characterization of the new alkaloids in E. Harz, most were also detected in coca leaf from Bolivia and Peru. Qualitatively, the differences in the alkaloidal chromatographic profiles for these two countries were, upon first inspection, not major. Studies are continuing in order to determine if Bolivian and Peruvian coca leaf can be qualitatively differentiated. There were, however, quantitative differences in the alkaloidal chromatographic profiles for Bolivian and Peruvian coca. Of interest, of course, is whether these differences in leaf also exist in the illicit cocaine processed from that leaf. This also is the subject of continuing investigations.

After detection, isolation and structural characterization of the new coca alkaloids in E. Harz and coca leaf, the in-depth analysis of a large seizure of refined illicit cocaine was undertaken to determine if these alkaloids were present in that matrix. The methodology used was virtually identical as that for E. Harz. The results confirmed that many of the new (and old) alkaloids characterized in E. Harz and South American coca leaf were also present in the refined illicit cocaine HCI. As mentioned previously, it was this methodology that was modified in order to accommodate the detection and determination of the new (and old) alkaloids in smaller (500 mg or less) quantities of refined illicit cocaine, reported herein as the new cocaine signature methodology.

There were also other trace-level alkaloids in E. Harz that have been partially characterized; there has been no attempt, as yet, to determine their presence in refined...
illicit cocaine. These alkaloids included the ubiquitous carbolines and related indole alkaloids, 15 or so isomeric hydroxytruxillines, other high molecular weight alkaloids and an abundance of N-methylpyrroidine-related compounds, i.e., those that are related to cuscohygrine and hygrine, both of which are well-established coca alkaloids.

**SUMMARY**

Analytical methodology has been described for the isolation and the eGC-MSD-SIM detection and determination of more alkaloidal impurities in refined illicit cocaine than previously reported. This methodology also has high sensitivity and specificity, rendering it suitable for use in cocaine impurity signature analyses. The new alkaloidal impurities determined by this methodology were first characterized in a commercial coca-leaf extract, South American coca leaf, and in a large seizure of refined illicit cocaine hydrochloride. Quantitative and reproducibility data are also reported for selected alkaloids when using the new cocaine profiling methodology.

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**REFERENCES**

15. Moore JM, Casale JF, Cooper DA: Comparative determination of total isomeric truxillines in illicit, refined, South American cocaine hydrochloride using capillary gas chromatography-electron capture detection; J Chromatogr 756:193; 1996.
17. Moore JM, Casale JF, Hays PA, Cooper DA, Castelli DM: Unpublished data.
19. Moore JM, Cooper DA, Lurie IS, Kram TC, Carr S, Harper C, Yeh J: Capillary gas chromatographic-electron capture detection of coca leaf-related impurities in illicit cocaine: 2,4-diphenylcyclobutane-1,3-dicarboxylic acids, 1,4-diphenylcyclobutane-2,3-dicarboxylic acids and their alkaloidal precursors, the truxillines; J Chromatogr 410:297; 1987.
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