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METHODS FOR

IMPURITY PROFILING OF HEROIN AND COCAINE

MANUAL FOR USE BY NATIONAL DRUG TESTING LABORATORIES

Laboratory and Scientific Section
United Nations Office on Drugs and Crime
Vienna

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Note

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Contents

I. INTRODUCTION

A. Background

In order to successfully counter the ever-growing drug problem, there is an increasing need, inter alia, to identify conspiracy links and trafficking routes and to gather background intelligence concerning both the number of sources of drugs and whether those sources are within a country or are "internationally" based and also the points of distribution and distribution networks.

A scientific tool to complement routine law enforcement investigative work in this field is the characterization and impurity profiling of seized drugs. Drug characterization studies have shown that it is possible to link samples, to classify material from different seizures into groups of related samples and to identify the origin of samples. Such information can be used for evidential (judicial, court) purposes or it can be used as a source of intelligence to identify samples that may have a common origin or history. Drug characterization and impurity profiling may also assist in the identification of output from new illicit laboratories and in the monitoring of common methods used for drug manufacture, which, in turn, may provide information helpful to the maintenance of other intelligence-gathering tools, for example, precursor-monitoring programmes. Finally, drug characterization and impurity profiling may also provide supporting evidence in cases where illicitly manufactured drugs need to be differentiated from those diverted from licit sources.

Beyond areas for application in the law enforcement and regulatory field, more in-depth analysis of drug samples, such as in drug characterization and impurity profiling studies, can also generate information essential for health authorities. The identification of unusual new drugs or drug combinations, for example, is a key element of early warning systems concerning unexpected adverse health consequences. Finally, systematic analysis of the composition of seized drugs also contributes to improved understanding of drug abuse trends.

The laboratory of the United Nations Office on Drugs and Crime (UNODC) has a long history of involvement in drug characterization and impurity profiling. Opium characterization studies were initiated in 1948 and such study was one of the first and most comprehensive research efforts in international drug control under the aegis of the United Nations. In the late 1960s, the increasing abuse of heroin resulted in a shift in focus from opium to heroin. Pursuant to a request by the Commission on Narcotic Drugs, an expert group to determine the feasibility of using chemical characteristics for identifying sources and distribution patterns of heroin was convened in 1977 [1]. A follow-up meeting was held in Vienna in

1982 to coordinate research on the physical and chemical characteristics of heroin for the purpose of tracing origin and movement in the illicit traffic [2]. In 1992, a consultative meeting on chemical characterization and impurity profiling of drug seizures was held in Vienna; that meeting expanded coverage beyond heroin to include cocaine and synthetic drugs and, in particular, amphetamine [3].

At its thirty-ninth session, in 1996, the Commission on Narcotic Drugs recognized the need for a cohesive international strategy in the field of drug characterization and impurity profiling. In so doing the Commission requested the Executive Director of the United Nations International Drug Control Programme (now called UNODC) to develop standard protocols and methods for the profiling/ signature analysis of key narcotic drugs and psychotropic substances [4]. The objectives of the Office in this area are to develop guidelines for characterization and impurity profiling of key narcotic drugs and psychotropic substances and to make the guidelines available to national forensic laboratories with the aim of providing them with a scientific tool to support law enforcement work for both evidential and strategic intelligence purposes. At the same time, the UNODC reference services, which include the provision of training, reference samples, material and scientific literature, were expanded to include specific aspects of drug characterization and impurity profiling as well.

In this context and in view of the focus of the General Assembly at its twentieth special session on, inter alia, the need for enhanced measures to counter illicit manufacture of and trafficking in drugs, it was considered timely to review profiling methods for heroin and cocaine, along with the broader concepts of drug characterization and impurity profiling of both plant-based and synthetic drugs.

To this end, the Laboratory and Scientific Section of UNODC convened a Consultative Meeting on Recommended Methods for the Impurity Profiling of Heroin and Cocaine, in cooperation with the Government of Australia, in Sydney, Australia, in November 1999.

B. Purpose of the manual

The present manual, prepared by the Laboratory and Scientific Section of UNODC, reflects the discussions and conclusions of the Consultative Meeting held in Sydney. It is aimed at providing practical guidance to national drug testing laboratories that want to embark on heroin and/or cocaine profiling activities. The broader aspects of how drug characterization and impurity profiling can be used effectively as a scientific tool to support law enforcement operational investigative work can be found in a separate manual entitled *Drug Characterization/ Impurity Profiling: Background and Concepts* [5], which was endorsed by the participants at the Consultative Meeting. Both manuals form part of a series of publications dealing with the identification and analysis of various groups of drugs under international control. Specifically, the present manual complements United Nations manuals on recommended methods for testing opium, morphine and heroin [6] and on cocaine [7]. These and other manuals in the series, including those

on the detection and assay of various groups of drugs in biological specimens, can be requested from the Laboratory and Scientific Section (see address below). Copies of articles from the scientific literature on drug characterization and impurity profiling can also be requested from the same address.

It is important to note that these manuals are provided to laboratories as educational documents within the training remit of UNODC and as a means of encouraging laboratories to collaborate and participate with the United Nations in the global effort to combat illicit drugs. In addition, these publications are also an attempt to help promote national efforts by providing internationally accepted guidelines.

C. Use of the manual

The present manual, as was the case with previous manuals, suggests approaches that may assist drug analysts in the selection of methods appropriate to the sample under examination and provide data suitable for the purpose at hand. The majority of methods described here are published in the scientific literature. They represent a summary of the experience of scientists from several reputable laboratories around the world and have been routinely used for a number of years in those laboratories. The reader should be aware, however, that there are a number of other published methods not mentioned in this manual that may also produce acceptable results. There are a number of more sophisticated additional approaches that may provide insight into specific aspects of sample history, but may not be necessary for routine operational applications. Therefore, the methods described here should be understood as guidance, that is, minor modifications to suit local circumstances should normally not change the validity of the results.

Methods provided here are chosen on the basis of proven suitability and reliability, important prerequisites, especially if the results are to be used for evidential purposes. However, the reader should be aware that the conclusions drawn using different analytical methods will each have a different level of certainty. Since similarity based on one method alone is usually not sufficient for evidential purposes and in order to make the overall process more rigorous, the expert group recommends the use of at least a second method where each method used addresses a different set of target analytes. However, the decision whether or not additional methods are required remains with the chemist on a case-by-case basis and may also be dependent on national requirements. As a guiding principle, the rigorousness of the method employed determines the strength of the evidence produced.

In this context, it should also be understood that drug profiling is not a routine analytical technique. Dedicated equipment and experienced chemists are required and close cooperation between forensic laboratories and law enforcement personnel is critical for effective use of results.

For further details on the background and operational value, as well as limitations, of drug characterization and impurity profiling, the reader is referred to

the United Nations manual entitled *Drug Characterization/Impurity Profiling: Background and Concepts* [5].

The Laboratory and Scientific Section would welcome observations on the contents and usefulness of the present manual. Comments and suggestions may be addressed to:

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II. ASPECTS OF DRUG CHARACTERIZATION AND IMPURITY PROFILING

A. Background, concepts, purpose and strategies

Drug characterization and impurity profiling studies can be valuable scientific tools to support law enforcement operations. As a general concept, when impurity profiling is employed for sample comparisons (linkage determinations), all possible means for the generation of information and data should be considered, including the use of physical characteristics, packaging, adulterants and diluents.

In organic chemistry the term "characterization" is often utilized to describe the process of determining the exact molecular structure of an organic compound. However, here the term "drug characterization" is used to mean the process of determining the major features of a drug sample, both its physical and chemical characteristics, and also including the presence and nature of cutting agents, the quantification of significant sample components and so on. In contrast, impurity profiling typically involves several analyses that are designed to produce a detailed picture (profile) of a drug sample, usually in the form of chromatographic data. The resulting chemical profiles, also known as "signatures" or "impurity profiles", result in the identification and, usually, the quantification of major components in the sample just as may also be accomplished in drug characterization. However, unlike drug characterization work, impurity profiling will also include one or more additional analyses designed to target the minor (trace) components in the sample.* When performing impurity profiling work, the analyst's ultimate goal is to obtain profiles of the major and minor components in formats that allow him/her to use the data as a comparative tool for the purpose of locating other samples having similar profiles.

An introduction to drug characterization and impurity profiling, its potential and limitations is given in the United Nations manual *Drug Characterization/ Impurity Profiling: Background and Concepts* [5], which addresses both laboratory and law enforcement personnel who may wish to start an impurity profiling programme. While the reader is referred to this United Nations manual, some of the parts most relevant to the forensic chemist are the uses and the limitations of the technique and these are discussed briefly below.

*For the purposes of this manual, major components are components present above 1% relative to the target compound (heroin or cocaine), while minor components are generally below 1% relative to the target compound and, in general, can be found only after an extraction or pre-concentration step.

Impurity profiling applications fall principally into one of two overlapping categories. They are tactical (evidential/judicial) and strategic (intelligence). An example of a tactical application of impurity profiling is the establishment of distribution and/or trafficking links between multiple seized samples that have been obtained at different locations or in the possession of different individuals. Strategic uses can include the identification of synthesis methods utilized in clandestine laboratories, the identification of chemicals, reagents and/or solvents employed by those laboratories and, for drugs derived from botanical sources, a scientifically defensible determination of the geographical origin of the sample.

In order to successfully apply impurity profiling results, all parties involved need to understand both the goals and the limitations of the technique. First and foremost, it must be understood that drug impurity profiling is not a stand-alone technique, but rather a scientific approach that complements law enforcement investigative information. A necessary requirement for the successful implementation of this work, therefore, is an information exchange mechanism that allows continuous feedback between laboratory and law enforcement personnel. Not only must the forensic chemist be very familiar with the chemistry of all target analytes, he or she must also fully understand the purpose of the investigation in order to select the most appropriate analytical approach and interpret the results correctly.

The value of the analytical data obtained using impurity profiling procedures is limited by several considerations, principal among them the limits imposed by:

(a) The quality of the samples under investigation;
(b) The quality of the information provided by law enforcement personnel;
(c) The extent of the analyst's knowledge of the relevant analytical and synthetic (drug processing) chemistry;
(d) Uncertainties arising because clandestine processing techniques and trafficking practices are often not well known;
(e) The availability and quality of a database of analytical results.

Nevertheless, and despite these limitations, drug impurity profiling studies can be a valuable tool in support of operational investigations by law enforcement agencies and are used successfully for that purpose.

B. Analytical implications of heroin and cocaine manufacture and supply

1. Determination of origin

The first and absolutely necessary prerequisite for the successful implementation of any drug origin determination programme is the acquisition of relevant data sets derived from drug samples obtained from all known sources. Such a compilation

is generally referred to as an "authentic database". Obviously the acquisition of these authentic database samples directly from the growing fields and directly from the illicit laboratories is a difficult, dangerous and costly task that in general constitutes a primary impediment to creating a successful origin determination programme for any illicit drug.

For drugs derived from plant sources it is most often possible to assign geographical origin, if at all, in only the most tentative sense when the analytical technique employed exclusively targets the major alkaloids. However, there are notable exceptions, perhaps the most notable being those heroin samples originating in South-East Asia. The relative ratios of the alkaloids present in the majority of South-East Asian heroin samples are distinctly different from those found in heroin samples originating in other source regions. As a result, many South-East Asian heroin samples can, with reasonable certainty, be distinguished from those samples originating elsewhere simply by comparison of major alkaloid analyses. On the other hand, many samples of highly refined heroin from South-West Asia are impossible to distinguish even tentatively from heroin from South America using only major alkaloid data.

Fortunately, analyses of minor alkaloids, processing by-products and occluded solvents provide powerful and useful tools for both the determination of sample origin and trafficking links. Those laboratories fortunate enough to have the capability of trace element and isotopic abundance analyses have two additional powerful tools that are also applicable to both of these tasks. The abundances of carbon^{13}, oxygen^{18} and nitrogen^{15} (relative to the most abundant isotope) are a complex interplay between latitude, altitude, the underlying sources for the target elements and the overall biological fractionation events of the different isotopes [8-13]. On the other hand, trace elements present in clandestine samples arise almost entirely from inadvertent contamination of the sample from a myriad of possible sources to even include the remote possibility that some of the contamination is related to the soil in which the drug crop was cultivated. For both isotopic abundance and trace elemental analyses, proper interpretation of the analytical data requires that the analyst have an in-depth understanding of the relevant physical, chemical and biochemical principles.

The analysis of trace elements using inductively coupled plasma-mass spectrometry (ICP-MS) has also been employed for sample comparison studies. Additional criteria for the classification and the comparison of heroin samples have been developed using ICP-MS to screen 96 samples of known geographical origin for 35 elements [14]. The limitations of this approach arise from non-homogeneity of illicit heroin samples, from the lack of an appropriate database and from those contaminations which are typically introduced through the addition of cutting agents. At present there is a very limited understanding of the relationships that may exist between the various observed elemental compositions versus geographical origin, processing in the source areas and the process of cutting.

Relative alkaloid ratios within samples are most closely associated with plant varietal differences and, to a lesser extent, agronomic differences. An even more important influence on the relative ratios of all alkaloidal constituents, to include

all minor by-products, are the processing methods employed in the conversion of the raw plant product to a street-ready product. Isotopic abundances are also strongly affected by processing; however, isotopic abundance measurements are the only validated method that can actually relate a specific sample to a specific geographical origin. Processing issues apply equally to the illicit products of heroin and cocaine; however, heroin profiling is further complicated by the additional synthetic step required to convert the morphine into heroin.

Isotopic abundance and trace element measurements are not widely available to the forensic analyst owing in part to the cost of the hardware and in part to the significant training requirement mandated by the techniques. More frequently the forensic analyst will use chromatographic techniques for impurity profiling that target major alkaloids, minor alkaloids, processing by-products, adulterants and diluents, and occluded solvents. As noted previously, it is important to understand that these techniques only provide information that is indirectly related to a specific geographical origin. When using chromatographic techniques, too, it is important to understand that, in general, there is a direct and inverse relationship between the quality (purity) of the sample and one's ability to associate that sample with a specific geographical origin or to establish definitive linkages between two or more samples (i.e. the more highly refined or the more highly diluted a sample, the more difficult it becomes to detect components other than the major alkaloids; hence, the number of comparison points are reduced).

The fact that the relative ratios of the major alkaloids in opium and coca leaf are closely associated with plant varietal, cultivar and agronomic differences can be exploited for the assignment of geographical origin. It is the case that a significant portion of the illicit opium poppy grown shows relatively little varietal or cultivar variation within each major growing region, while varietal or cultivar variation between the major growing regions can be significant. For instance, opium poppy cultivated in the Americas is quite distinct both morphologically and chemically from opium poppy grown in Asia. However, most of the illicit coca bush grown is *Erythroxylum coca v. coca*, with the illicit cultivation of other varieties being limited mostly to Colombia. Hence for cocaine, it is possible that the environmental differences existing between the various coca bush growing regions of South America may be the most significant influence as regards both trace level alkaloid content and ^{13}C and ^{15}N abundance. For both cocaine and heroin, the processing methods employed are most often region-specific and, in many instances, differ quite substantially from one source region to the next. Therefore, in the absence of post-processing hydrolysis, the quantities of alkaloidal constituents as compared with the primary alkaloid (i.e. cocaine and heroin) and the processing by-products found in the "street" product are primarily a function of the processing methods employed by the clandestine laboratory, the source plant species, variant or cultivar grown and, perhaps, agronomic differences.

It has been successfully demonstrated that, when suitable authentic databases are at hand, samples of seized cocaine can be related to relatively specific growing regions. For instance, Ehleringer and others [15] have successfully related cocaine samples to the growing regions of the Guaviare and Putumayo-Caqueta

areas of Colombia, the Apurimac valley and the Huallaga-Ucayali valleys of Peru and the Chapare valley of Bolivia. This work was accomplished using isotopic abundance measurements in conjunction with chromatographic methods targeting both major and minor alkaloid impurities, and occluded solvents. For cocaine, the use of occluded solvent data does not provide information about the growing region; however, it does allow for a distinction to be drawn about the region where the cocaine was processed into the hydrochloride salt.

Unfortunately, such precise results are not at present available for determination of the origin of heroin samples. This is because of two limitations inherent in work on heroin origin determination. One of those limitations is the additional synthetic step that is required for converting morphine to heroin, as this process obscures much of the minor alkaloid information. The second and perhaps most important limitation is the very large number of authentic samples required from frequently inaccessible growing and processing sites. For heroin, the acquisition of such a large number of authentic samples is as yet beyond any practical scope of work. Nevertheless, practical experience has shown that a large percentage of heroin samples can be assigned to general regions of origin. Those regions of origin are reasonably well known and are four not well-defined areas best designated as South-West Asia, South-East Asia, Mexico and South America, principally Colombia.

Origin information for heroin and cocaine may also be derived from other law enforcement information. However, since traditional law enforcement information sources are frequently anecdotal in nature and/or inferred from other related information, origin determination by scientific studies can be invaluable in negating or confirming such information.

2. Establishing trafficking and distribution networks

Although the same analytical tools can be employed for both work on origin determination and the elucidation of trafficking and distribution linkages, there exists a quite different set of data handling requirements. Obviously impurity profiling used for the purpose of establishing trafficking and distribution linkages does not require the existence of an origin-related authentic database. Additionally, impurity profiling for origin determination requires that the analytical data from a single sample be correlated to one of several large groups of samples that exhibit similar features where those features can be related to geographical origin, while the successful determination of a trafficking linkage requires that the analytical data generated from no less than two samples must share a significant number of essentially identical features. The ideal case for a positive linkage determination, of course, is when all measurable features of the comparison samples are identical. Although rare, it is possible for the data utilized in a successful linkage determination to be derived from relatively few sample-specific features regardless of when or where the features were introduced into the sample, as long as the features are sufficiently unique. However, in order to maximize the probability of successful linkage determinations, it is strongly recommended that comparison

data should be gathered from all available sources, that is, to include data on cutting agents (adulterants and diluents), packaging methods and materials, logos and fingerprints and, in short, all comparable information.

As noted above, there are significant limitations associated with origin determination work and, not surprisingly, there are also other equally important limitations associated with linkage determination work. These limitations are due primarily to the variable nature of the clandestine production processes, subsequent sample modifications during distribution and sample changes related to widely varying transportation histories. Variations in processing impurities across consecutive batches from a given laboratory have not been well studied, but it is well known that such variations can be relatively large. Moreover, large illicit consignments of cocaine and heroin rarely constitute the product of a single laboratory operation. As a result, the analyst may be able to form an opinion as to the likelihood that two samples are consistent with their being from two different batches made in the same laboratory, but most often such a link cannot be established with certainty. When the samples are from the same processing batch and their locations in the distribution chain are close, then the probability that the analyst will be able to link them with reasonable certainty is markedly improved, but still not guaranteed. However, when two samples are not chemically related, it is often possible for an analyst to state that fact with certainty.

Considering the previously noted limitations, it would be unrealistic to think a sufficiently large information base of regional and/or international impurity profiling data could be developed that would enable the identification of related sample seizures in large networks and/or major trafficking organizations. However, with regard to smaller scale or local drug supply networks, impurity profiling databases can be useful in a number of ways beyond the obvious establishment of possible network linkages. One common such usage is to estimate the size of a particular drug operation, which is information that can be used to estimate the monetary value of the operation and, in turn, aid in the confiscation of financial assets.

C. Types and investigational value of sample components

In general, the impurity profile of a drug sample reflects its history. For a drug derived from a natural product, the history the analyst will attempt to measure starts when the farmer selects the seed for planting and ends only upon the completion of all analytical work. As a consequence, impurities detected by an analyst can arise from many sources, but usually those impurities most easily detected belong to one of the following four categories:

(a) Compounds co-extracted from the raw plant materials;

(b) Impurities and by-products to include occluded solvents and/or reagents:

(i) Due principally to laboratory processing;

(ii) Also contributed to by transportation and distribution practices;

(c) Cutting agents added at any point in the distribution chain;
(d) Analytical procedure-generated artefacts.

A number of laboratories throughout the world are at present involved in the work of impurity profiling. Those laboratories do not all have the same purpose for this work nor do all of the laboratories pursue the same "easily detected impurities" (analytical targets). Following this paragraph is a listing of the most common analytical targets that these laboratories do pursue, two of which require the use of relatively expensive analytical hardware not typically found in the majority of forensic drug laboratories.

*1. Major components**

The resulting data are useful for:

(a) Characterizing the target analyte heroin or cocaine;
(b) Screening samples for possible linkages (similar samples);
(c) Indicating region of sample origin;
(d) Characterizing cutting agents (adulterants and diluents).

*2. Trace components***

The resulting data is useful for:

(a) Determining possible sample links;
(b) Indicating region of sample origin;
(c) Indication of processing or synthetic methods utilized.

3. Residual and/or occluded solvents

The resulting data are useful for:

(a) Determining possible sample links;
(b) Indicating region of sample origin (heroin only);***
(c) Identification of "last-step" processing solvents.

*Major components are generally the most abundant alkaloids, diluents and adulterants.

**Analysis of trace components generally requires an extraction step and is most frequently followed by a concentration step.

***For cocaine, the analysis of residual and/or occluded solvents may only indicate the region in which the hydrochloride was produced, but not where the coca bush was grown.

4. Trace elements (ICP-MS)

The resulting data are useful for:

(a) Determining possible sample links;
(b) Possible indication of origin for "uncut" samples.

5. Isotopic abundance (isotope ratio mass spectrometry (IRMS))

The resulting data are useful for:

(a) Determining possible sample links;
(b) Indicating region of sample origin.

To aid the reader in assigning the significance of individual peaks in the chromatogram, table 1 of annex II summarizes many of the impurities that have been identified in illicit heroin samples and gives, to the extent possible, an indication as to their "source", that is, whether they are related to the plant material, the manufacturing process, subsequent distribution or the analytical procedure (artefacts). Table 2 of annex II summarizes the same information for samples of illicit cocaine.

III. METHODS FOR IMPURITY PROFILING

A. General comments

The methods discussed in this manual are provided as a general guide for the interested reader. For those who wish to utilize any of these methods as part of the establishment of profiling programmes for heroin and/or cocaine, it is advisable to study the original publications.* Not only will such study ensure that the method selected will meet requirements, but most certainly the reader will gain much additional practical and theoretical information. Quantification allows a more rigorous basis for comparison, but frequently only qualitative data are used. The recommended qualitative approach is to normalize the data by determining area count ratios of the various detected impurities relative to the appropriate target alkaloid (heroin or cocaine). It is the case that this method is actually more reliable for purposes of comparing samples, as both quantification errors and biases from the introduction of adulterants and/or diluents are greatly reduced.

Whatever the approach, it is essential that all methods, whether published in this manual or in the scientific literature, be evaluated in the analyst's own laboratory prior to use.

After the Consultative Meeting held in Sydney, Australia, in 1999, a review [16] and several other methods for heroin profiling, using mostly advanced statistical procedures for data evaluation, were published [17-28]. However, in terms of analytical technique, publications since 1999, for the most part, still indicate gas chromatography as the analytical tool of choice.

For those who do decide to create an impurity profiling programme, it cannot be overemphasized that the successful implementation of any programme requires meticulous control of all analytical method and sample storage variables. Additionally, it is the opinion of several experts in the field that instrumentation utilized in an ongoing impurity profiling programme should not be used for any other purpose. Finally, all procedures utilized for receiving, processing and storage of samples for impurity profiling should adhere to good laboratory practice and custodial evidentiary procedures.

Impurity profiling results are typically used for court purposes (evidence, tactical operations) and/or for intelligence purposes (strategic intelligence operations). Since one rarely knows beforehand what samples will be used in an evidentiary manner, the integrity of both the sample handling procedures and the analytical

*A few of the methods listed here have not been published previously and in those instances the names of the source laboratory and a primary contact person are provided under the method heading; contact details are available from UNODC.

procedures must be maintained at the highest level. From an analytical standpoint, it is clear that for all chromatographic methods the frequent incorporation of standard and "check" samples can be invaluable in helping to maintain the integrity of the analytical product. As has already been noted, it is necessary for the analyst to understand exactly how the impurity profiling results will be utilized and to know the precise nature of all applicable requirements before attempting to set up an impurity profiling programme and/or choosing the appropriate analytical methods.

Needless to say, if impurity profiling results may ever be utilized as evidence, then it is likely that some aspects of the impurity profiling procedure will depend on court and/or legislative requirements. Irrespective of the approach utilized, the importance of taking a comprehensive approach to this work cannot be overemphasized. While it is true that the approach and the methods utilized by the forensic chemist can be constrained to some degree by legal requirements, typically the analyst still has a good deal of flexibility in the design of an impurity profiling procedure. Finally, and most importantly, when linkage determinations are performed for evidentiary purposes, it is quite clear that a positive correlation cannot be based only on the results of a single method. In those cases, the expert group recommends the additional use of other, preferably independent methods, with at least one of those methods designed to access a trace level alkaloidal fraction. An analysis of occluded solvents is always an excellent additional method and is very useful in the earlier stages of a comparative analysis; however, solvent analyses are of secondary importance relative to analyses that address the presence of plant-related constituents. Supplementary information is provided in annex I.

B. Heroin methods

Table 1 of annex II summarizes many of the major and trace level alkaloidal impurities found in heroin, together with some common adulterants and diluents.

1. Hydrolysis of heroin

Post-processing hydrolysis can occur readily for those samples containing non-bound water or excess acid. In those cases where both the alkaloidal content and the extent of hydrolysis are significant, the sample may become dark brown (almost black) and will finally become tar-like. In less severe cases, hydrolysis may not be obvious until the impurity profile data have been examined. An O^6-monoacetylmorphine (O6MAM) content greater than 10% relative to the heroin is an indication that post-processing hydrolysis may have occurred in the sample. Other indicators are very low levels of O^3-monoacetylmorphine (O3MAM) and relatively high morphine content (> 1% relative to the heroin). In some cases both the morphine and the O6MAM can be present at a higher level than the heroin

and, infrequently, even a significant amount of codeine. However, the analyst should be careful when making this assessment as a dark brown (black) tar sample containing high O6MAM can arise from causes other than post-processing hydrolysis. For instance, if a dark brown, tar-like sample contains high levels of O6MAM along with significant quantities of O3MAM and little or no codeine, then the sample could well have originated from a "homebake" process. Also the tar-like dark brown heroin samples that originate in Mexico typically have a processing-related O6MAM content higher than 6%, with 12% or greater (relative to heroin) not being at all uncommon. It is thus not always a simple matter to distinguish between heroin samples that have undergone significant post-processing hydrolysis and those samples where there was significant hydrolysis during processing. Not surprisingly, the task of comparing two samples where one has undergone significant post-process hydrolysis versus one that has not undergone any significant hydrolysis, although not impossible, is very much more difficult. There are several publications included in the list of references [29-33] that will enhance the reader's understanding of the conditions necessary to produce O3MAM and O6MAM.

Over time a properly prepared and stored heroin hydrochloride (or for that matter cocaine hydrochloride) will not degrade in any significant manner. In this context "properly prepared and stored" means fully hydrated +99.5% pure hydrochloride salt containing no unbound water or acid and stored in the dark at ambient temperature in a tightly sealed container. Obviously there are few properly prepared and stored drug samples in the illicit marketplace. As a result, some degradation over time is common for illicitly produced heroin, in particular when it is the free base, as the base is less stable than the hydrochloride salt. However, for high-purity illicit heroin samples the rate of degradation (hydrolysis) is so slow that it can be difficult to measure year-to-year.

The analyst does need to exercise care in order to avoid hydrolysis of the heroin when performing impurity profiling analyses. For instance, the hydrolysis rate for heroin is markedly increased at extremes of pH (e.g. pH < 3 and > 10) and as a result such routine tasks as liquid-liquid extractions need to be performed carefully. Gas chromatography (GC) is an analytical tool frequently used in impurity profiling and it can also result in hydrolysis of heroin and/or the trans-esterification of co-injection compounds. These problems are not limited to heroin, since most esters are more or less subject to these reactions, as is evidenced by the well known formation of O6MAM when morphine and aspirin are dissolved in methanol and co-injected into a gas chromatograph. It is for these reasons that GC methods utilizing direct dissolution into an injection solvent may not provide as rigorous a result as do impurity profiling techniques that incorporate a derivatization step. The previous two paragraphs apply equally to illicit cocaine.

GC analysis of heroin without the use of a derivatization step will result in the formation of three injection port artefacts. One of these compounds has not been identified (MW = 381), while the others are 15,16-didehydroheroin [34] and O^6-monoacetylmorphine. An injection of a heroin sample, in the absence of a derivatization step, will nearly always result in the production of some quantity

of O^6-monoacetylmorphine in the injection port, where the amount so produced is a function of injection port temperature and the quantity of activation sites within the injection port. The unknown compound and 15,16-didehydroheroin* are usually observed at trace levels (< 0.2% relative to heroin) and chromatograph immediately after heroin on a 100% methylsilicone column. The presence of either of these two compounds at a level greater than 0.2% relative to heroin may suggest the need for injection port maintenance.

2. Methods for the determination of major components

Methods for the determination of major impurities frequently incorporate quantification of the primary analyte, that is, heroin. The remaining components in the sample may or may not be quantified and, if not, the response of these remaining components is typically set up as a ratio relative to the primary analyte. These methods are often referred to as screening methods, or ratio methods. In actual fact nearly all impurity profiling methods are ratio methods. A ratio method is one where the various components of the sample are separated, generally using a chromatographic technique, concomitant with the tabulation of response measurements followed by determining the ratios of the response measurements against either an added internal standard or a common sample component. Major impurity analyses are typically used to eliminate samples from comparison that are clearly different from other samples under examination or to obtain an indication of the heroin sample origin, that is, South-East Asia, South-West Asia, Mexico or South America.**

All of the following methods are equally applicable to heroin base and heroin hydrochloride, although sample preparation may be different for base and hydrochloride samples. All methods should be regarded as guidance. In general, minor modification to suit local circumstances will not normally change the validity of the results. However, any modification must be carefully validated to ensure that the results have not been compromised. The analytical chemist should also be aware that not all methods described below are suitable for all types of heroin sample and that the probability that the method used provides a correct conclusion can vary significantly depending on both the method employed and the exact nature of the sample.

*Produced in the injection port from the precursor $\Delta^{16,17}$-dehydroheroinium hydrochloride.

**For a description of chemical characteristics of heroin samples from different source regions, see the United Nations manual *Recommended Methods for Testing Opium, Morphine and Heroin* ([6], pp. 8-11).

Method A1: High-performance liquid chromatography (HPLC) method*

Source: I. S. Lurie and S. M. Carr, "The quantitation of heroin and selected basic impurities via reversed phase HPLC: I. The analysis of unadulterated heroin samples", *Journal of Liquid Chromatography and Related Technologies*, vol. 9, No. 11 (1986), pp. 2485-2509.

Sample type: Major components: cut and uncut samples.

Operating conditions:

Detector:	Ultraviolet diode array Monitor 3 wavelengths: 210 nm, 228 nm and 240 nm			
Column:	Partisil 5, ODS 3, 125 mm x 3.2 mm ID			
Mobile phases:	*(a)* Phosphate buffer (pH 2.2) *(b)* Methanol			
Injection solvent:	HPLC-grade water, acetonitrile and glacial acetic acid (89:10:1), adjusted to pH 3.7 with 2M sodium hydroxide			
Flow rate:	0.76 ml/min			
Injection size:	20 µl			
Elution gradient:	*Equilibration*	*Time*	*MeOH (%)*	*Buffer (%)*
		15	5	95
	1	20	30	70
	2	6	30	70
	3	10	80	20
	4	4	80	20
	5	5	5	95

Internal standard: propiophenone at 0.5 mg/ml in injection solvent

Phosphate buffer: 870 ml of HPLC-grade water, 30 ml of 2N sodium hydroxide and 10 ml of phosphoric acid. Filter and degas, then add 3.0 ml hexylamine. The final pH is adjusted to pH 2.2 with 2M sodium hydroxide or phosphoric acid. Add additional hexylamine as necessary for baseline separation of papaverine and noscapine.

Sample preparation: Accurately weigh the sample into a 100-ml volumetric flask to give an approximate heroin concentration of 0.9 mg/ml. Add 10 ml of propiophenone internal standard solution and dilute to volume with injection solvent. Sonicate to complete solvation and filter.

Rationale for use: A robust method providing accurate quantification and excellent precision for heroin and all typical opium alkaloid impurities down to 1% relative to heroin content. In many cases minor alkaloids and adulterants can be quantified at

*This HPLC method is a slightly modified version of the "classic" heroin signature 1 method used by the Drug Enforcement Administration (DEA) of the United States of America in its heroin origin determination programme, until it was replaced in 2003 by a capillary electrophoresis (CE) method (method A6).

levels as low as 0.1% relative to heroin content along with significant but generally acceptable losses in precision. The minor alkaloids quantified are morphine, codeine, O3MAM, O6MAM, papaverine and noscapine. As is the case with every analytical procedure for heroin, hydrolysis can be an issue, but if the method is followed properly, hydrolysis will be kept to an absolute minimum. The original reference states: "Quantitative values of the various basic impurities relative to heroin in the samples analysed were found to vary over a large range and formed a basis for comparing illicit heroin samples." The on-column heroin content specified in this method typically produces an UV-detector response that is in the upper region of detector linearity. Hence, it is necessary for the analyst to obtain a "rough" quantification of the heroin prior to analysis by this method. Any of the following GC methods designed for major impurity analyses can be utilized for this purpose. The use of a photo-diode array (PDA) detector is recommended as it allows the facile detection of co-eluting compounds, that is, peak purity assessments. Precision and accuracy for morphine are somewhat limited, as morphine elutes very soon after column void volume. Common co-elution issues occur for acetaminophen (paracetamol) with codeine, cocaine with acetylcodeine and diphenhydramine with noscapine. Sugars are not detected.

Outcome: Indication of general source region (South-East Asia, South-West Asia, Mexico and South America). Sample comparisons for discrimination and evaluation of samples for case-to-case evidential purposes (linkage determinations). Additional information is required to confirm links between samples or to assign source regions, that is, the method should be used as one part within a broader analysis scheme.

Method A2: GC method, without derivatization

Source: Modified from C. Barnfield and others, "The routine profiling of forensic heroin samples", *Forensic Science International*, vol. 39, No. 2 (1988), pp. 107-117.

Sample type: Major components: cut and uncut samples.

Operating conditions:

Detector:	Flame ionization detector (FID)	
Column:	DB-1, PB1 or equivalent, 25 m x 0.32 mm x 0.5 µm	
Carrier gas:	Helium	
Make-up gas:	Not specified	
Injection:	1 µl; split 30:1	
Temperatures:	Injector:	280° C
	Detector:	320° C
	Oven:	200° C to 260° C at 10° C/min, to 310° C at 30° C/min, hold for 1 min

Internal standard: None: normalize all data relative to heroin response.

Sample preparation: Weigh out the powder and dissolve initially in one part of *N, N*-dimethylformamide,* then dilute with nine parts of ethanol to give a final concentration of 3-5 mg/ml of sample, depending on the heroin concentration.

*The use of *N,N*-dimethylformamide facilitates the dissolution of samples containing large proportions of caffeine, phenacetin and/or paracetamol.

Rationale for use: The outstanding virtues of the method are simple sample preparation (i.e. no extraction or derivatization), very quick instrumental analysis (< 10 minutes) and therefore fast sample throughput. Although the method is not the most sensitive, it has relatively good resolution and discrimination power and a broad range of compounds can be detected. The method detects major opiate or opiate-related compounds as well as many cutting agents. Sugars are not detected and a co-elution issue is known to exist between diazepam and morphine. The separation of O6MAM, acetylthebaol and acetylcodeine can, in some instances, be problematic.

Outcome: Sample comparisons for discrimination and evaluation of samples for case-to-case evidential purposes (linkage determinations). Additional information is required to confirm links between samples, that is, the method should be used as one part within a broader analysis scheme.

Method A3: GC method, without derivatization

Source: Adrian V. Kemmenoe, Forensic Science Service, Birmingham Laboratory, Birmingham, United Kingdom.

Sample type: Major components: cut and uncut samples.

Operating conditions:

Detector:	FID at 35 ml/min hydrogen, air at 350 ml/min	
Column:	HP1, 25 m x 0.2 mm x 0.33 µm	
Carrier gas:	Helium (0.9 ml/min to 1.5 ml/min at 0.1 ml/min, final hold for 5 min)	
Injection:	1 µl; split 100:1	
Make-up gas:	Nitrogen at 30 ml/min	
Temperatures:	Injector:	280° C
	Detector:	300° C
	Oven:	220° C to 300° C at 10° C/min, final hold for 3 min

Internal standard: n-Tetracosane at 0.5 mg/ml (±0.0001 mg/ml) in chloroform*:* ethanol:isopropyl alcohol (8:1:1)

Calibration standard preparation: Prepare two calibration standards for each target analyte, with one standard solution prepared at the highest expected concentration and the other at a lower concentration. For the high concentration heroin HCl, dissolve 20 mg standard into 5 ml internal standard solution, and for the lower concentration standard, dissolve 10 mg standard in 5 ml of internal standard solution.

Sample preparation: Dissolve sufficient sample in 5 ml of internal standard solution to give a final total heroin content (calculated as the hydrochloride salt) of 10-20 mg (2-4 mg/ml).

Rationale for use: As in the previous method, the outstanding virtues of the method are simple sample preparation (i.e. no extraction or derivatization), quick instrumental analysis (ca. 11 minutes) and therefore fast sample throughput. The method is reasonably sensitive; given the use of the non-polar methyl silicone column phase, it has relatively good resolution and discrimination power; and a broad range of compounds can be detected. The method detects major opiate or opiate-related compounds as well as many cutting agents. Sugars are not detected and a co-elution issue is known to exist between diazepam and morphine. The separation of O6MAM, acetylthebaol and acetylcodeine can, in some instances, be problematic.

Outcome: Sample comparisons for discrimination and evaluation of samples for case-to-case evidential purposes (linkage determinations). Additional information is required to confirm links between samples, that is, the method should be used as one part within a broader analysis scheme.

Method A4: GC method, with derivatization

Sources: Modified from M. Gloger and H. Neumann, "Analysis of heroin samples by capillary gas chromatography: comparison of glass capillary column and packed column", *Forensic Science International*, vol. 22, No. 1 (1983), pp. 63-74; H. Neumann, "Vergleichende Heroinanalyse mit der Kapillar-GC: Bestimmung charakteristischer Größen", *Toxichem+Krimtech*, vol. 59, Nos. 3-4 (1992), pp. 121-124.

Sample type: Major components: cut and uncut samples.

Operating conditions:

Detector:	FID at 30 ml/min hydrogen and 400 ml/min air
Column:	HP-1, DB-1 or equivalent, 30 m x 0.32 mm x 0.25 µm
Carrier gas:	Helium at 61 cm/sec, measured at 150° C oven temperature
Injection:	1 µl; split, 15:1
Make-up gas:	Argon or nitrogen at 25 ml/min
Temperatures:	Injector: 250° C
	Detector: 310° C
	Oven: 150° C to 300° C at 9° C/min, hold for 2.4 min

Internal standard: n-Tetracosane (see sample preparation)

Sample preparation: Weigh accurately approximately 5 mg of sample together with 1 mg of *n*-tetracosane as internal standard. Dissolve the mixture in 1 ml of chloroform and 200 µl of pyridine. Silylation is performed with 150 µl of *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA). Heat for 10 minutes at 70° C and then let stand one hour at room temperature.

Reference chromatograms: See annex III, figure I.

Rationale for use: This is a robust method* that can be modified in many ways. As a result of the derivatization step the method provides a nearly complete picture of the major organic components to include most adulterants and many cutting agents. The combination of a derivatization step coupled with the use of a fused silica non-polar capillary column results in superior resolution and excellent discrimination power and also avoids problems associated with transacetylation. Mono- and disaccharides are detected with high sensitivity and high chromatographic resolution. Known co-elution issues are present with diazepam, quinine and phenylbutazone with *n*-tetracosane and heroin, respectively. A modification using a 30 m x 0.25 mm x 0.25 µm DB-1 with H_2 as the carrier gas (60-70 cm/sec constant velocity) allows complete separation of those compounds; however, chloroquine and heroin were found to co-elute in this system.

Outcome: Indication of general source region (South-East Asia, South-West Asia, Mexico, South America). Sample comparisons for discrimination and evaluation of samples for case-to-case evidential purposes (linkage determinations). Additional information is required to confirm links between samples or to assign source regions, that is, the method should be used as one part within a broader analysis scheme.

*Note that this method is the same as the GC method recommended in the United Nations manual *Recommended Methods for Testing Opium, Morphine and Heroin* [6].

Method A5: GC method, with derivatization

Source: James Wong, Bureau of Drug Analysis Services, Health Canada, Western Region Health Protection Branch, Burnaby, British Columbia, Canada.

Sample type: Major components: cut and uncut samples.

Operating conditions:

Detector:	FID
Column:	DB-5 or equivalent, 25 m x 0.32 mm x 0.52 µm
Carrier gas:	Helium
Injection:	2 µl; split 25:1
Temperatures:	Injector: 250° C Detector: 310° C Oven: 200° C for 0.5 min, 20° C/min to 280° C, hold for 17 min

Internal standard: None; normalize by ratios of the area counts for O^3-benzoyl-O6MAM to heroin and acetylcodeine to heroin.

Derivatization solution: 10 µl benzoyl chloride/ml chloroform

Sample preparation: Dissolve 15-20 mg of the sample in 10 ml 0.1N HCl. Accurately pipette 1 ml of this solution to a 15-ml round-bottomed centrifuge or test tube, add

1 ml 0.1N HCl, add solid sodium carbonate until basic, then add exactly 1 ml derivatizing solution, vortex for about 30 seconds, centrifuge to separate phases. Aspirate and discard top (aqueous) phase, bottom chloroform layer is subjected to GC-FID analysis, where quantification and determination of the two pairs of ratios are examined.

Rationale for use: Good resolution and discrimination power with a simple derivatization scheme. The benzoyl derivative of O6MAM is completely resolved from acetylcodeine and derivatization also ensures that the O6MAM detected is not from the degradation of heroin.

Outcome: Sample comparisons for discrimination and evaluation of samples for case-to-case evidential purposes. Additional information is required to confirm links between samples or to assign source regions, that is, the method should be used as one part within a broader analysis scheme.

Method A6: Capillary electrophoresis (CE) methods

Source: I. S. Lurie and others, "Use of dynamically coated capillaries for the determination of heroin, basic impurities and adulterants with capillary electrophoresis", *Journal of Chromatography A*, vol. 1034, Nos. 1-2 (2004), pp. 227-235. (current DEA Signature I)

Method A6.1: Capillary zone electrophoresis (CZE)

Sample type: Major basic components: cut and uncut samples.

Operating conditions:	Agilent model HP^{3D}CE
CZE:	Column maintained at 25° C with an applied potential of 30 kV
Detector:	UV diode array Monitored wavelengths: 195 nm, 205 nm and 260 nm
Column:	64 cm x 50 µm fused silica (55.5 cm to detector window)*
Run buffers:	*(a)* 100-mM dimethyl-ß-cyclodextrin in CElixir reagent B (pH 2.5)** *(b)* 100-mM hydroxypropyl-*ß*-cyclodextrin in CElixir reagent B (pH 2.5)
Injection solvent:	2:8 mixture of methanol and 3.75-mM monobasic sodium phosphate buffer adjusted to pH 2.6 with phosphoric acid
Injection:	500 mbar*s

*In the original reference, the fused silica tubing was obtained from Polymicro Technologies, Phoenix, Arizona, United States. Columns can also be obtained from Agilent that are already cut to length with the appropriately located detector window.

**CElirix reagents are proprietary products from MicroSolv Technology, Eatontown, New Jersey, United States.

Initial column conditioning: Flush the capillary with 0.1M NaOH, then with water, then with CElixir reagent A (each for two minutes) and finish with run buffer for four minutes.

Pre-injection column conditioning: Same procedure as initial.

External standards: Accurately weigh approximately 40 mg heroin HCl, 0.5 mg morphine HCl, 0.5 mg O3MAM sulfamate, 0.5 mg codeine HCl, 0.5 mg papaverine HCl, 0.5 mg noscapine and 1.0 mg acetylcodeine HCl into a 100-ml volumetric flask and dilute to volume with injection solvent. As a separate standard solution, accurately weigh approximately 1.5 mg O6MAM into a 100-ml volumetric flask and dilute to volume with injection solvent.

Sample preparation–heroin HCl: Accurately weigh an amount of sample approximately equivalent to 20 mg of heroin HCl into a 50-ml volumetric flask and dilute to volume with injection solvent. Assure dissolution before diluting to final volume. Sonication for 15 minutes is recommended.

Sample preparation–heroin base: Accurately weigh an amount of sample approximately equivalent to 10 mg of heroin HCl into a 50-ml volumetric flask and dilute to volume with injection solvent. Samples containing both heroin base and heroin HCl can be treated as hydrochloride samples if the base content is less than the hydrochloride content. Assure dissolution before diluting to final volume. Sonication for 30 minutes is recommended.

Reference chromatograms: see annex III, figure II and table 1.

Rationale for use: A rugged method providing good quantitative accuracy and precision for heroin and all typical opium alkaloid impurities down to 0.2% relative to heroin content. The minor alkaloids quantified are morphine, codeine, O3MAM, O6MAM, papaverine and noscapine. Instrumentation cost is relatively modest and the cost of consumables is due primarily to the use of the proprietary run buffers. However, the use of the proprietary run buffers saves valuable operator time and significantly enhances reproducibility. If followed faithfully this method provides for an on-column heroin content that is in the middle of the quantification linearity range. Therefore, it is not absolutely necessary for the analyst to obtain a "rough" quantification of the heroin prior to analysis; however, this is highly recommended, as doing so assures that consistent results are obtained in conjunction with detection and quantification of the minor alkaloids at the 0.2% level relative to heroin content. The use of a photodiode array (PDA) detector is also recommended as it allows for the confirmation of peak identity and the facile detection of co-eluting compounds, that is, peak purity assessments. For run buffer *(a)*, co-elution issues occur for lidocaine and aminopyrene with O3MAM and for dipyrone with O6MAM. The use of CZE run buffer *(b)* in place of *(a)* will allow the determination of O3MAM and O6MAM in those situations. Most weakly basic, acidic and neutral compounds are not detected and as a result many co-elution issues are avoided. Effective mobilities are very reproducible, but increases in absolute migration times are observed over time. This occurs as a

result of changes in electro-osmotic flow, which in turn results in an improvement in resolution over time. A chromatogram taken from Lurie and others [35] showing a typical separation obtained with a selection of basic compounds frequently found in heroin samples and a table showing the corresponding relative migration times for these and additional compounds appear in annex III as figure II and table 1.

Outcome: Indication of general source region (South-East Asia, South-West Asia, Mexico, South America). Sample comparisons for discrimination and evaluation of samples for case-to-case evidential purposes (linkage determinations). Additional information is required to confirm links between samples or to assign source regions, that is, the method should be used as one part within a broader analysis scheme.

Method A6.2: Miscellar Electrokinetic Chromatography (MEKC)

Sample type: Major weakly basic, acidic and neutral components: cut and uncut samples.

Operating conditions: Agilent model HP[3D]CE

MEKC:	Column maintained at 15° C with an applied potential of 8.5 kV
Detector:	UV diode array Monitored wavelength: 195 nm
Column:	32 cm x 50 µm fused silica (23.5 cm to detector window)
Run buffer:	103.2 mM sodium dodecylsulfate in 50 mM dibasic phosphate-borate buffer (pH 6.5)*
Injection solvent:	2:8 mixture of methanol and 3.75 mM monobasic sodium phosphate buffer adjusted to pH 2.6 with phosphoric acid
Injection:	100 mbar*s

Initial column conditioning: Flush with 0.1M NaOH, then with water, then with CElixir reagent A and then with 50 mM phosphate-borate buffer (each flush for one minute). Finish with a six-minute run buffer flush.

Pre-injection column conditioning: Flush for two minutes with run buffer.

External standards: Accurately weigh approximately 10 mg of the appropriate standard material for each target analyte into a 100-ml volumetric flask. Dilute to volume with injection solvent. Assure dissolution before diluting to final volume. Sonication for 15 minutes is recommended.

Sample preparation: Use the sample prepared from the CZE run.

Reference chromatogram: see annex III, figure III and table 2.

*In the original reference, the run buffer was obtained from MicroSolv Technology, Eatontown, New Jersey, United States.

Rationale for use: A highly selective and rugged method that provides good quantitative accuracy and precision. As noted above the costs associated with the method are relatively low. Analyses times are short, resulting in a method capable of high sample throughput. Effective mobilities are very reproducible, but increases in absolute migration times are observed over time. The effect occurs owing to changes in electroosmotic flow, which in turn results in an improvement in resolution over time. Sugars are not detected. A chromatogram taken from Lurie and others [35] showing a typical separation obtained with a selection of weakly basic, acidic and neutral compounds found frequently in heroin samples and a table showing the corresponding relative migration times for these and additional compounds appear in annex III as figure III and table 2.

Outcome: Sample comparisons for discrimination and evaluation of samples for case-to-case evidential purposes (linkage determinations). Additional information is required to confirm links between samples, that is, the method should be used as one part within a broader analysis scheme.

3. Methods for the determination of trace components

Methods described in this subsection are used to substantiate the results of the methods for the analysis of major components described above.

All of the methods described below are designed for high-resolution capillary GC and employ a liquid-liquid extraction step to isolate the acidic and neutral components from the bulk basic fraction. The resulting extract produces an analytical product that can be quite complex. It is not uncommon for the acidic and neutral components extracted from a South-West Asian crudely refined heroin base sample to yield a 250+ component high-resolution GC chromatogram. Only a relative few of these compounds have been fully characterized [34, 36-39]. The most significant chemistry underlying the generation of a majority of these 250+ compounds is found in the works of Polonovski and Polonovski and of Mariella and the associated papers [40-46].

The routine application of a computer algorithm for the comparison of such complex data sets is not present in many laboratories. Rather, in those laboratories lacking the appropriate computerized comparison capabilities, comparison of trace impurity profiles is carried out by (visual) superimposition of chromatograms.

Virtually every manipulation of a sample carries with it some risk of sample degradation and, even in the hands of the most meticulous analyst, some sample degradation often occurs during an analytical process. For instance, two common sources of degradation are contact with acid during the extraction process and interactions with glass surfaces. When in the hands of a competent analyst, degradation of a neutrals extract is typically not noticeable when the extract contains at least 500 micrograms of total material. However, when the total amount of extracted material is decreased significantly, as would be the case with highly refined samples, then sample degradation during analysis becomes much more of a concern. An example would be an extract obtained from a 50-mg sample

(morphine equivalent) of a highly refined South-East Asian heroin HCl. The neutrals extract from such a sample may contain a total of only 30-40 µgm of alkaloidal-related neutral compounds with the triacetylnormorphine content accounting for 10+% of the total. At these levels, a significant portion of the triacetylnormorphine is most likely to undergo hydrolysis during the analytical process. Therefore, when comparing data obtained from neutrals extracts containing less than 100 µgm total content, it is recommended that the response for those neutral compounds having a clear degradation pathway available to them be summed with their corresponding degradation products (i.e. add the response obtained for triacetylnormorphine to the response obtained for *N*,6-diacetylnormorphine). Additionally, the well-known potential for sample degradation within the injection port of the GC is definitely worth extra consideration. The strong polar character of the amidic alkaloidal-derived neutrals enhances the probability of interactions with hot glass injection port liners. It is especially important for the analysis of highly refined samples to use deactivated fused silica injection port liners along with the addition of a relatively large excess of derivatization reagent (such as MSTFA) to the sample, as these measures greatly reduce unwanted interactions. Additionally, care should be exercised when carrying out the liquid-liquid extraction in order to avoid degradation of the more susceptible compounds. The dissolution of the sample in organic phase before the addition of the acid is one very useful precaution. Typically, crudely refined sample extracts can be stored as a dried residue in deactivated glassware for up to 24 hours without detectable degradation of the sample. However, extracts obtained from highly refined samples should be analysed immediately upon preparation.

For certain sample types, the analytical data obtained from a neutrals extract, when compared with the analytical data obtained from the major components, affords a much higher comparison specificity. This is due to three significant considerations:

(a) Frequently there are many more valid comparison data points available;

(b) Typically the bulk of the data points provide specific sample history (such as processing information);

(c) Typically larger arrays of opium alkaloids are represented in the neutrals fraction than are observed in the major component fraction. A few of the important compounds that are represented in the neutrals fraction, but absent in the major component fraction, are norlaudanosine, thebaine, oripavine and the papaverubines.

However, a neutrals extract of an uncut highly refined heroin sample may provide less than six useable data points and, as a result, comparison specificity for linkage purposes is often rather poor for those sample types.

For those interested in the underlying chemistry of the neutral fraction compounds, see Polonovski and Polonovski [41, 42] on the acetylation of *N*-oxides and the articles by Mariella and Brown and related articles [44-46] on secondary

products obtained from the action of acetic anhydride on ring-bound tertiary amines [37, 40-47]. Additionally, such compounds as *N*-acetylnorlaudanosine and the *N*-acetylated papaverubine isomers arise as simple acetylation addition products of alkaloids that are naturally present in opium as secondary amines.

Method B1: GC method, without derivatization

Source: L. Strömberg and others, "Heroin impurity profiling: a harmonization study for retrospective comparisons", *Forensic Science International*, vol. 114, No. 2 (2000), pp. 67-88.

Operating conditions:

Detector:	FID at 30 ml/min hydrogen, air at 400 ml/min
Column:	HP Ultra-2 or equivalent, 25 m x 0.20 mm x 0.11 µm
Carrier gas:	Helium at about 0.6 ml/min
Injection technique:	1 µl; split, 25:1
Make-up gas:	Helium at 30 ml/min.
Temperatures:	Injector: 300° C Detector: 330° C Oven: 160° C to 320° C at 6° C/min, hold for 6 min

Internal standard: Tetracontane at 10 mg/l in toluene

Sample preparation: Place an amount of sample equivalent to 15 mg of pure heroin (equivalent to base) into a glass tube. Add 5.0 ml of 0.5N sulphuric acid to dissolve the sample; then add 5.0 ml of internal standard solution. Mix thoroughly and then centrifuge to separate the phases. Remove 3.2 ml of the internal standard solution and evaporate to dryness. Add 200 µl of toluene and sonicate.

Reference chromatogram: See annex III, figure IV and table 3. The 16 selected reference peaks marked in the figure and presented in annex III, table 3, are used for retrospective comparisons with a computerized data retrieval system. The experience gained during a study of the national forensic science laboratories of Germany, the Netherlands and Sweden is reported in reference [48].

Rationale for use: No derivatization,* relatively simple sample preparation and good separations for nearly all components. Will not detect sugars and sensitivity probably not sufficient for highly refined heroin samples.

Outcome: Sample comparisons for discrimination and evaluation of samples for case-to-case evidential purposes (linkage determinations). Provides additional information required to confirm links between samples, that is, the method should be used in conjunction with a major component analysis.

*See the subsection entitled "Hydrolysis of heroin" for possible problems related to the use of methods that do not employ derivatization.

Method B2: GC method, without derivatization*

Source: James Wong, Bureau of Drug Analysis Services, Health Canada, Western Region Health Protection Branch, Burnaby, British Columbia, Canada.

Operating conditions:

Detector:	FID
Column:	DB-5 or equivalent (i.e., cross-linked 5% phenylmethylsilicone), 25 m x 0.32 mm x 0.52 µm
Carrier gas:	Helium
Injection size:	2 µl; split, 25:1
Temperatures:	Injector: 250° C Detector: 310° C Oven: 160° C, hold for 3 min, 3° C/min to 255° C, hold for 1 min, 20° C/min to 295° C, isothermal for 10 min

Internal standard: None

Sample preparation: Dissolve 200-250 mg of heroin sample in 5 ml 0.5N sulphuric acid, add 5 ml of glass-distilled $CHCl_3$ and thoroughly mix the two phases. Centrifuge to separate phases and discard the aqueous phase. Add to the $CHCl_3$ solution 5 ml 0.5N sulphuric acid, mix thoroughly, centrifuge and discard the aqueous layer. Transfer the $CHCl_3$ layer to a clean tube and evaporate to dryness at 45° C under a stream of dry air of nitrogen. Reconstitute dried residue in 50 µl acetonitrile:toluene (1:9).**

Rationale for use: No derivatization*** and relatively simple sample preparation. Will not detect sugars. Use of a moderately thick film column allows for high sample loading.

Outcome: Sample comparisons for discrimination and evaluation of samples for case-to-case evidential purposes (linkage determinations). Provides additional information required to confirm links between samples, that is, the method should be used in conjunction with a major component analysis.

*The Health Canada Laboratory uses this method, with identical parameters and sample preparation, also for cocaine (method D3). However, wherever possible, and especially for forensic comparative purposes, the use of dedicated instrumentation and optimized methodologies is always preferred.

**The dried residue of the heroin acid and neutral extract may be crusty. Therefore, it may be necessary to add 0.5 ml of 0.5N sulphuric acid and partition with the acetonitrile:toluene mixture.

***See the subsection entitled “Hydrolysis of heroin” for possible problems related to the use of methods that do not employ derivatization.

Method B3: GC method, with derivatization

Sources: A. C. Allen and others, "Illicit heroin manufacturing byproducts: capillary gas chromatographic determination and structural elucidation of narcotine- and norlaudanosine-related compounds", *Analytical Chemistry*, vol. 56, No. 14 (1984), pp. 2940-2947; H. Neumann and M. Gloger, "Profiling of illicit heroin samples by high-resolution capillary gas chromatography for forensic application", *Chromatographia*, vol. 16, 1982, pp. 261-264.

Method B3 is the analytical basis for the previously described method by Strömberg and others [48] (method B1).

Operating conditions:

Detector:	FID
Column:	DB-1 or SE-54, 25 m x 0.25 mm x 0.25 µm
Carrier gas:	Hydrogen at 50 cm/sec
Injection technique:	3 µl; split, 60:1
Make-up gas:	Nitrogen at 30 ml/min
Temperatures:	Injector: 300° C Detector: 300° C Oven: 200° C to 330° C at 4° C/min, hold for 4 min

Internal standard: n-Dotetracontane at 0.30 mg/ml in toluene or chloroform

Derivatization reagent: MSTFA (*N*-methyl-*N*-trimethylsilyltriflouroacetamide)

Sample preparation: Weigh out the sample in an amount equivalent to 15 mg of pure heroin base. Add 5.0 ml of 0.5N sulphuric acid and dissolve the sample, add 5.0 ml of toluene and mix thoroughly, then centrifuge to separate phases. Remove approximately 4 ml of the toluene layer and evaporate to dryness. Add to the residue 75 µl of internal standard solution and 25 µl of MSTFA. Heat the solution at 75° C for 3 min.

(Modification by Olivier Guéniat)

Operating conditions:

Detector:	FID at 45 ml/min hydrogen and 450 ml/min air
Column:	DB-1 or equivalent, 30 m x 0.25 mm x 0.25 µm
Carrier gas:	Helium at 1 ml/min
Injection size:	2 µl
Temperatures:	Injector: 290° C Detector: 320° C Oven: 200° C to 320 °C at 4° C/min, hold for 4 min

Internal standard: Heneicosane at 0.10 mg/ml in methylene chloride

Sample preparation: Weigh out about 20 mg of the sample. Add 2.0 ml of 1M sulphuric acid, then add 2.0 ml of toluene and mix thoroughly. Remove 1 ml of the toluene layer and evaporate to dryness under a nitrogen stream. Add to the residue 75 µl of methylene chloride and 25 µl of MSTFA. Heat the solution at 75° C for 30 min.

Rationale for use: Several modifications of the above methods are in use worldwide. Provides synthesis and processing information and alkaloidal information that can be linked back to the original opium poppy. Typically the sample amount can be increased two to three times in order to gain the sensitivity necessary for highly refined heroin samples.

Outcome: Indication of general source region (South-East Asia, South-West Asia, Mexico, South America). Sample comparisons for discrimination and evaluation of samples for case-to-case evidential purposes (linkage determinations). Provides additional information required to confirm links between samples, that is, the method should be used in conjunction with a major component analysis.

Method B4: GC/MS method, with derivatization

Sources: Jana Skopec, Australian Government Analytical Laboratories, Pymble, New South Wales, Australia; R. B. Myors and others, "Investigation of heroin profiling using trace organic impurities", *The Analyst*, vol. 126. No. 5 (2001), pp. 679-689.

Operating conditions:

Detector:	Mass spectrometer, EI, 40-600 amu, 1 sec scan cycle (or SIM mode)*
Column:	DB-5 or equivalent, 30 m x 0.25 mm x 0.25 µm
Carrier gas:	Helium at 41 cm/sec, constant flow
Injection technique:	1 µl splitless
Temperatures:	Injector: 260° C Detector: 280° C (MS transfer line); 230° C (MS source) Oven: 100° C for 1 min, to 230° C at 6° C/min, to 280° C at 3° C/min, to 320° C at 6° C/min, hold for 4 min

Internal standard: Benzopinacolone at 1.0 mg/ml in methylene chloride

Derivatization solution: Dilute 1 ml of BSTFA (N,O-bis-(trimethylsilyl)triflouroacetamide) containing 1% TMCS (trimethylchlorosilane) to 2 ml with dichloromethane.**

*Personal communication from Jana Skopec, 2005.

**Derivatization solutions can be stored for a maximum of 24 hours.

Sample preparation: All glassware is silylated. Add 200 mg of the heroin sample to 4 ml of 1M sulphuric acid and 4 ml of a 3:2 mixture of diethyl ether and dichloromethane, mix thoroughly. Centrifuge to separate phases and transfer 3 ml of the organic phase into a clean reaction vial. Dry under dry nitrogen at 60° C.* Add 300 µl of the derivatization solution and 60 µl of the internal standard. Seal the reaction vial tightly, mix thoroughly and heat at 70° C for 30 min.

If method retention times shift more than 0.2 min, the instrument is recalibrated and/or the retention time of the internal standard is adjusted by adjusting the carrier gas flow.

Rationale for use: The method allows a total impurity profile of all alkaloidal trace impurities. Several modifications of the above method are in use worldwide. This version should provide the sensitivity and the specificity necessary for the analysis of highly refined heroin samples, that is, highly refined hydrochloride samples from South-East Asia.

Outcome: Indication of general source region (South-East Asia, South-West Asia, Mexico, South America). Sample comparisons for discrimination and evaluation of samples for case-to-case evidential purposes (linkage determinations). Provides additional information required to confirm links between samples, that is, the method should be used in conjunction with a major component analysis.

*In this form, a tightly capped reaction vial can be stored for up to a week at -5° C prior to further analysis.

4. Residual solvent analysis

Method B5: Dynamic headspace (thermal desorption) gas chromatography (Conventional GC instrument)

Source: J. Cartier, O. Guéniat and M. D. Cole, "Headspace analysis of solvents in cocaine and heroin samples", *Science and Justice*, vol. 37, No. 3 (1997), pp. 175-181.

Operating conditions:

Detector:	FID, at 45 ml/min hydrogen and 450 ml/min air
Column:	DB-1 or equivalent , 60 m x 0.33 mm x 3.0 µm
Carrier gas:	Helium at 0.7 ml/min (column pressure 17 psig)
Injection technique:	1 µl; split, 30:1
Temperatures:	Injector: 280° C Detector: 280° C Oven: 35° C for 14 min, to 100° C at 5° C/min, to 245° C at 7° C/min, no final hold (if petrol/gasoline was detected, the temperature of the column was held at 245° C for 10 min)

Internal standard: None

*Sample preparation:** Weigh 250-300 mg of the powdered heroin sample into a 2-ml GC derivatization vial. Add a 0.25-ml insert, containing approximately 100 mg of activated carbon (charcoal strip). After sealing the vial, heat the contents for 60 min at 80° C and allow to cool. Extract the activated carbon with 50 µl of carbon disulphide and inject 1 µl of this solution** into the GC.

Rationale for use: The method provides for the detection of 12 solvents in heroin samples, at detection limits between 2-15 parts per million (ppm) for 250-300 mg powder samples. No special injector device is required; the method uses a conventional GC instrument. Solvent analyses may be carried out on samples up to two years old.

Outcome: Provides additional information and an independent means of confirming links from organic impurity and inorganic ion determinations.

*Preparation of headspace samples must be performed under clean laboratory conditions to avoid solvent cross-contamination.

**The remainder of this sample can be reused for ICP-MS analysis.

Method B6: Static headspace-GC/MS (Special injector device required)

Source: Modified from D. R. Morello and R. P. Meyers, "Qualitative and quantitative determination of residual solvents in illicit cocaine HCl and heroin HCl", *Journal of Forensic Sciences,* vol. 40, No. 6 (1995), pp. 957-963.

Operating conditions:

Detector:	Mass spectrometer, 70eV EI, 20-220 amu, 0.5 sec scan cycle
Column:	DB-1, 60 m x 25 mm x 1.0 µm
Carrier gas:	Helium, constant velocity approx. 30 cm/s
Injector technique:	Split 38:1, Tekmar 7000/7050 headspace autosampler 22 ml vials
Equilibration:	14 min at 85° C 2-ml loop; injection time: 0.3 min
Temperatures:	Loop: 175° C Injector: 190° C Injector transfer line: 175° C MS transfer line: 190° C Oven: 35° C to 150° C at 6° C/min to 180° C at 15° C/min, hold for 10 min

*Internal standard stock (ISS):** d_6-acetone at 6 mg/ml, d_9-2-chloro-2-methylpropane at 3.5 mg/ml, d_{14}-n-hexane at 1.5 mg/ml, d_8-isopropanol at 7.5 mg/ml and d_8-toluene at 1.5 mg/ml in dimethylsulfoxide (DMSO).** All standards should be +99.5% pure.

22% sodium sulfate solution: 220 gm of +99% anhydrous sodium sulfate in one litre of deionized water. Between use, store at room temperature.

Dilute internal standard solution (DISS): Add 100 µl of the ISS for each 100 ml of 22% Na_2SO_4 solution. Prepare daily.

Standard stock solutions (SSS): Methanol, ethanol, acetone, isopropanol, *n*-butanol and isobutanol at 0.4 mg/ml; methyl ethyl ketone, ethyl acetate, chloroform, methylene chloride, 1,1,2-dichloroethane, ethyl ether, methyl acetate, cyclopentane, isobutyl acetate, *n*-butylethyl acetate and 1,1,1-trichloroethane at 0.2 mg/ml; cyclohexane, benzene, toluene, *n*-hexane, *o*-xylene, *p*-xylene, mesitylene and mesityl oxide at 0.08 mg/ml. Two additional calibrations solutions are created by making two 1:5 serial dilutions.*** All dilutions are made using a solution of DMSO:water (5:1). Label the solutions SSSH, SSSM, SSSL respectively from the highest to the lowest concentration solution.

Calibration solutions: Add 5 ml of DISS to four 22-ml headspace vials, then add 50 µl of SSSH to the first vial, 50 µl of SSSM to the second vial and 50 µl of SSSL to the third vial. The fourth vial is used as a run blank (standard zero point) as it contains only the DISS. Immediately seal each vial tightly with a silicon/polytetrafluoroethylene (PTFE)-lined cap.

Sample preparation: Accurately weigh approximately 50 mg equivalent of heroin base into a 22-ml headspace vial, add 5 ml of DISS. Immediately seal each vial tightly with a silicon/PTFE-lined cap.

Rationale for use: The method provides facile detection to the 0.00001% level and reproducible quantification to the low 0.0001% level relative to the heroin content and, with minor modification, is utilized for both heroin and cocaine samples. The method requires a special injector device. Also requires the acquisition of appropriately deuterated standard materials; however, a few millilitres of the standard materials will last for a considerable period of time.

Outcome: Allows access to solvents trapped within the crystal matrices of samples without decomposition of solvents or other sample components. The method provides nearly comprehensive detection of all sample solvents and, very importantly, allows the accurate assessment of the relative importance of the detected solvents.

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*The internal standard stock is stable for up to two months if stored in the dark at -10° C between uses.

**Deuterated standards were from the Cambridge Isotope Laboratories, 50 Frontage Rd., Andover, Massachusetts, United States.

***The calibration standards solutions were found to be stable up to four weeks if maintained in the dark, at -10° C, between uses.

5. Additional methods

Method B7: Elemental analysis by ICP-MS

Source: R. J. Wells and others, "Trace element analysis of heroin by ICP-MS", *Chemistry in Australia*, vol. 62, No. 7 (1995), p. 14.

Rationale for use: The method measures an abundance of sample trace elements and is most useful for unadulterated samples. Elemental analysis has been shown to be a powerful sample-to-sample comparison tool.

Outcome: Aids in the evaluation of samples for case-to-case evidential purposes (linkage determinations). Provides additional information required to confirm links between samples, that is, the method should be used in conjunction with a major component analysis.

Method B8: Abundance of stable isotopes ^{15}N and ^{13}C by Isotope Ratio MS (IRMS)

Sources: J. R. Ehleringer and others, "Geo-location of heroin and cocaine by stable isotope ratios", *Forensic Science International*, vol. 106, No. 1 (1999), pp. 27-35; F. Besacier and others, "Isotopic analysis of ^{13}C as a tool for comparison and origin assignment of seized heroin samples", *Journal of Forensic Sciences*, vol. 42, No. 3 (1997), pp. 429-434; S. Dautraix and others, "^{13}C Isotopic analysis of an acetaminophen and diacetylmorphine mixture", *Journal of Chromatography A*, vol. 756, Nos. 1-2 (1996), pp. 203-210; M. Desage and others, "Gas chromatography with mass spectrometry or isotope-ratio mass spectrometry in studying the geographical origin of heroin", *Analytica Chimica Acta*, vol. 247, No. 2 (1991), pp. 249-254.

Rationale for use: An IRMS method for the measurement of the relative abundance of stable isotopes ^{15}N and ^{13}C. The method is designed for unadulterated samples. Work is ongoing for the application of GC-IRMS to adulterated samples.*

Outcome: Provides geo-specific information. Aids in the evaluation of samples for case-to-case evidential purposes (linkage determinations). Provides additional information required to confirm links between samples, that is, the method should be used in conjunction with a major component analysis.

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*Personal communication from David Morello, Special Testing and Research Laboratory, Drug Enforcement Administration, Dulles, Virginia, United States, 2005.

C. Cocaine methods

The reader is referred to chapter III, section A, above for general procedural comments as, for the most part, they apply equally to both heroin and cocaine impurity profiling work. Some useful comments, applicable to both heroin and cocaine, can also be found in the subsection entitled "Hydrolysis of heroin" in chapter III, section B, above.

Heroin profiling studies were first initiated during the 1960s and by the mid-1970s such studies were ongoing in several national laboratories. In contrast, one of the first works describing a cocaine profiling method was published by Casale and Waggoner in 1991 [49]. Hence, one might properly expect profiling methodologies for cocaine to be significantly less well developed than those for heroin. However, a relatively small cadre of researchers have taken the art of cocaine profiling to a level at least on a par with heroin profiling. While the strides made by those researchers are quite remarkable, they did enjoy some significant advantages. Certainly the 20 or more years of experience gained with heroin profiling gave them a significant knowledge base from which to start. Additionally, both the number of and the relative concentration of alkaloidal impurities are frequently higher in cocaine samples than they are in heroin samples and the cocaine alkaloidal impurities have not been modified by a synthetic step.

All of the following cocaine impurity profiling methods can be used for comparative analysis work. However, as in the case of heroin profiling, no single method provides a result specific enough to be used as a stand-alone method for evidentiary purposes.

As for heroin, an impurity profile database can be built and subsequently used for retrospective comparisons. Also similar to heroin, there are considerable difficulties attendant with the acquisition of a comprehensive compilation of current samples of known origin. As a result, it is likely that most laboratories will encounter significant difficulties in establishing regions and/or countries of origin.

All of the methods described are equally applicable to cocaine base, crack cocaine and cocaine hydrochloride. However, at the present time it is not possible to establish links between cocaine base and the cocaine hydrochloride from which it was prepared.

As was noted for heroin, those methods which do not include a derivatization step prior to GC chromatography all carry a risk of sample component decomposition during analysis. Scrupulous maintenance of the GC injection port does minimize that risk; however, benzoic acid measurements will remain very problematic. Typically, the detection of anhydroecgonine methylester by GC is also not useful. In nearly all instances this compound is a GC degradation product (artefact), owing to the decomposition of large tropine alkaloids, principally the 11 truxilline alkaloids. Since the truxilline isomers do not undergo derivatization, the inclusion of a derivatization step does not provide a viable remedy.

Annex II, table 4, summarizes many of the major and trace-level alkaloidal impurities found in cocaine samples.

1. Methods for the determination of major components

Method C1: GC method, with derivatization (Chromatographic Impurity Signature Profile Analysis (CISPA))

Source: J. F. Casale and R. W. Waggoner, Jr. "A chromatographic impurity signature profile analysis for cocaine using capillary gas chromatography", *Journal of Forensic Sciences*, vol. 36, No. 5 (1991), pp. 1312-1330.

Operating conditions:

Detector:	FID
Column:	DB-1701, or equivalent, 30 m x 0.25 mm x 0.25 µm
Carrier gas:	Helium at 30 cm/sec
Injection technique:	5 µl; split 50:1
Make-up gas:	Nitrogen at 30 ml/min
Temperatures:	Injector: 230° C Detector: 280° C Oven: 180° C for 1 min, to 200° C at 4° C/min, to 275° C at 6° C/min, final hold of 11.5 min

Internal standard: p-fluorococaine at a concentration of 175 µg/ml in chloroform

Sample preparation: Accurately weigh out a sample of about 4-5 mg of unadulterated cocaine base or hydrochloride into a small vial. Add 200 µl of internal standard and 200 µl of MSTFA. Cap the vial and heat it at 80° C for 15 min. Cool to room temperature before analysis.

Reference chromatograms: See annex III, figure V and table 4.

Rationale for use: Nearly complete picture of major components, including adulterants and cutting agents. The authors noted that the method was designed for the analysis of unadulterated samples. However, others have also found the method to be suitable for many cut samples.* Derivatization allows for the facile detection of sugars and avoids decomposition of the coca alkaloids in the GC injection port. This method requires the synthesis of the internal standard, *p*-fluorococaine; however, this internal standard is easily made from commercially available precursors. The use of a structurally related internal standard significantly enhances precision and reproducibility. The authors successfully apply a neural network pattern recognition programme for retrospective database searches.

Outcome: Sample comparisons for discrimination and evaluation of samples for case-to-case evidential purposes. Additional information is required to confirm links between samples or to assign source regions, that is, the method should be used as one part within a broader analysis scheme.

*The method can identify most of the common adulterants and diluents and some of the trace components such as tropacocaine, norcocaine and *N*-formylcocaine (personal communication from Olivier Guéniat, Police de sûreté, Neuchâtel, Switzerland, 2005).

Method C2: GC method, with derivatization

Source: Olivier Guéniat, Police de sûreté, Neuchâtel, Switzerland.

This method is a modification of the previously described method C1.

Operating conditions:

Detector:	FID at 45 ml/min hydrogen and 450 ml/min air
Column:	DB-1, or equivalent, 30 m x 0.25 mm x 0.25 µm*
Carrier gas:	Helium at 1 ml/min
Injection:	3 µl; split 50:1
Temperatures:	Injector: 230° C Detector: 320° C Oven: 180° C, hold for 1 min, then to 275° C at 4° C/min, hold for 5.25 min

Internal standard: Heneicosane at a concentration of 1 mg/ml in chloroform:pyridine (5:1)

Sample preparation: Weigh approximately 8 mg of cocaine sample. Add 500 µl of the internal standard and 100 µl of MSTFA. Cap the vial and heat it at 80° C for 30 min. After cooling the vial to room temperature for one hour, inject the solution.

Rationale for use: Nearly complete picture of major components, including adulterants and cutting agents. Derivatization allows for the facile detection of sugars and avoids decomposition of the coca alkaloids in the GC injection port. Uses a commercially available internal standard.

Outcome: Sample comparisons for discrimination and evaluation of samples for case-to-case evidential purposes. Additional information is required to confirm links between samples or to assign source regions, that is, the method should be used as one part within a broader analysis scheme.

*A DB-5 column has also been found to be suitable (personal communication from Jana Skopec, 2005).

Method C3: GC method, without derivatization

Source: James Wong, Bureau of Drug Analysis Services, Health Canada, Western Region Health Protection Branch, Burnaby, British Columbia, Canada.

Operating conditions:

Detector:	FID
Column:	DB-5 or equivalent, 25 m x 0.32 mm x 0.50 µm
Carrier gas:	Helium
Injection size:	1 µl; split 25:1
Temperatures:	Injector: 250° C Detector: 310° C Oven: 200° C, hold for 0.5 min, 20° C/min to 295° C, final hold 3 min

Internal standard: None; normalize by ratio of area counts for *cis*- and *trans*-cinnamoylcocaine relative to cocaine.

Sample preparation: Dissolve 15-20 mg of the sample in 10 ml methanol.

Rationale for use: Simple sample preparation and a less than nine-minute GC analysis provides for excellent sample throughput. Data reduction only relies on two alkaloidal ratios (i.e. the area counts of *cis*- and *trans*-cinnamoylcocaine to cocaine).

Outcome: Sample comparisons for discrimination and evaluation of samples for case-to-case evidential purposes. Additional information is required to confirm links between samples or to assign source regions, that is, the method should be used as one part within a broader analysis scheme.

Method C4: GC method, without derivatization, nitrogen phosphorous detector (NPD)

Sources: K. E. Janzen, L. Walter and A. R. Fernando, "Comparison analysis of illicit cocaine samples", *Journal of Forensic Sciences*, vol. 37, No. 2 (1992), pp. 436-445; see also K. E. Janzen, "Cross-matching of cocaine samples: a case study", *Canadian Society of Forensic Science Journal*, vol. 20, 1987, pp. 77-81; K. E. Janzen, A. R. Fernando and L. Walter, "A database for comparison analysis of illicit cocaine samples", *Forensic Science International*, vol. 69, No. 1 (1994), pp. 23-29.

Operating conditions:

Detector:	NPD, at 3 ml/min hydrogen and 80 ml/min air
Column:	HP-1, DB-1 or equivalent, 12.5 m x 0.2 mm x 0.5 µm
Carrier gas:	Helium at 1.5 ml/min
Make-up gas:	Helium at 30 ml/min
Injection technique:	1 µl; split 50:1

Temperatures:	Injector: 215° C Detector: 325° C Oven: 120° C, hold for 2 min, 6° C/min to 320° C, hold for 5 min

Internal standard: None; normalize by peak area ratios for tropacocaine, norcocaine and *cis*- and *trans*-cinnamoylcocaine relative to cocaine.

Sample preparation: Weigh the equivalent of 30 mg of cocaine base and dissolve in 2 ml of absolute ethanol.

Rationale for use: Not the most sensitive method, but a broad range of nitrogen-containing compounds can be detected (by the same token, the majority of nitrogen-free adulterants and diluents cannot be detected). Simple sample preparation (no extraction or derivatization) enhances sample throughput.

Outcome: Sample comparisons for discrimination and evaluation of samples for case-to-case evidential purposes. Additional information is required to confirm links between samples or to assign source regions, that is, the method should be used as one part within a broader analysis scheme.

2. Methods for the determination of trace components

The methods described below are used to substantiate the results obtained from methods C1-C4. A potential limitation for the methods D1 and D2, presented below, is that they are designed for the analysis of unadulterated samples. However, methods D3 and D4 are not limited to unadulterated samples, but they do not employ a derivatization step and, as a result, artefact production is a concern. In addition, many of the by-products and decomposition products generated during cocaine processing are not observed unless the sample is derivatized. Hence, it is likely that no one of these methods will be suitable for every laboratory and/or for every sample.

Some researchers have observed that the truxillines and the corresponding truxillic/truxinic acids may not be ideal parameters for profiling purposes owing to what is thought to be trans-isomerization and/or artefact formation.* However, Moore and others [50-52] examined this issue in detail and found no evidence to support this view. Rather, Moore and others describe a truxilline analysis method and state that the method results are very reproducible and very useful for indicating the country of origin. On the matter of benzoic acid, however, there seems to be general agreement that it may not provide a very useful parameter for either sample-to-sample comparison or origin determination.

*Personal communication from Olivier Guéniat, Police de sûreté, Neuchâtel, Switzerland, at the Consultative Meeting held in Sydney, Australia, in 1999; see also Rivier [53].

Method D1: GC method, with derivatization: "trimethoxy method"

Source: J. F. Casale and J. M. Moore, "3',4',5'-Trimethoxy-substituted analogs of cocaine, *cis-/trans*-cinnamoylcocaine and tropacocaine: characterization and quantitation of new alkaloids in coca leaf, coca paste and refined illicit cocaine", *Journal of Forensic Sciences*, vol. 39, No. 2 (1994), pp. 462-472.

Operating conditions:

Detector:	FID
Column:	DB-1 or equivalent, 30 m x 0.25 mm x 0.25 µm
Carrier gas:	Hydrogen at 35 cm/sec
Injection size:	2 µl, split 20:1
Temperatures:	Injector: 230° C Detector: 280° C Oven: 150° C for 1 min, 6° C/min to 275° C, hold for 8 min

Internal standard: 3', 4', 5'-Trimethoxyethylcocaine at 0.1 mg/ml in chloroform

Sample preparation: 100 mg equivalent of cocaine base is added to 0.5 ml of internal standard solution and 0.5 ml of saturated aqueous sodium bicarbonate. Mix thoroughly, allow the phases to separate and mix the chloroform layer into 0.5 gm of Celite 545 and pack the Celite sample mixture onto the top of a previously prepared ion-pairing column. Elute with 22 ml of water-saturated chloroform, discarding the first 10 ml and capturing the remainder. Reduce to dryness and reconstitute in 1.0 ml of chloroform for GC-FID analysis.

The ion-pairing column is prepared by packing a 0.5-ml saturated aqueous sodium bicarbonate Celite 545 bottom layer followed by 2 ml of 1N HCl:2N NaCl in a 4-gm Celite 545 top layer in a 260 x 22 mm glass column. The column is then primed by eluting with 10 ml of water-saturated chloroform.

Rationale for use: The ion-pairing column removes the vast majority of the cocaine and thereby allows for the quantification of 3',4',5'-trimethoxycocaine (TMC, MW 393), 3',4',5'-trimethoxytropacocaine (TMT, MW 335) and *cis*- and *trans*-3',4',5'-trimethoxycinnamoyl-cocaine (TMCC, MW 419) in unadulterated cocaine hydrochloride. When cocaine = 1.0, the approximate relative retention times are TMT = 1.3, TMC = 1.65, cTMCC = 1.82, tTMCC = 2.1. Tropacocaine and norcocaine are also easily detected by this method.

Outcome: As with method D2, this is one of the methods that are necessary to distinguish between coca grown in different countries. It allows sample comparisons for discrimination and evaluation of samples for case-to-case evidential purposes (linkage determinations). Provides additional information required to confirm links between samples, that is, the method should be used in conjunction with a major component analysis.

Method D2: GC method, with derivatization, electron capture detection (ECD)

Sources: J. M. Moore and others, "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", *Journal of Chromatography A*, vol. 410, 1987, pp. 297-318; J. M. Moore, J. F. Casale and D. A. Cooper, "Comparative determination of total isomeric truxillines in illicit, refined, South American cocaine hydrochloride using capillary gas chromatography-electron capture detection", *Journal of Chromatography A*, vol. 756, Nos. 1-2 (1996), pp. 193-201.

Operating conditions:

Detector:	ECD – ^{63}Ni (Agilent 6890 GC)
Make-up gas:	argon:methane (95:5) at 35 ml/min
Column:	DB-1701* or equivalent, 30 m x 0.25 mm x 0.25 µm
Carrier gas:	Hydrogen at 40-45 cm/sec as measured at 90° C
Injection size:	1 µl, splitless, Merlin microseal septa
Temperatures:	Injector: 275° C Detector: 300° C Oven: 90° C for 1.6 min, 25° C/min to 160° C for 1 min, 4° C /min to 275° C, hold for 5 min

Internal standard: µ-truxinic acid and aldrin at 200 pg/µl in isooctane

Sample preparation: To an amount of unadulterated sample equivalent to 50 mg of cocaine base is added 100 µg of µ-truxinic acid (internal standard) followed by 0.5 ml of boron trifluoride (BF_3-MeOH from Pierce, Rockford, Illinois, United States); the solution is heated for two hours at 95° C. Cool solution to -10° C and with mixing add 2 ml of ice water and 6 ml of 20% NaOH (also at 10° C). Immediately extract five times each with 5 ml of ethyl ether. Pass the extracts through anhydrous Na_2SO_4 into a 25-ml volumetric flask, dilute to volume with dry ether.** Evaporate 2 ml of the ether solution to dryness under dry N_2 and then add 200 µl $CHCl_3$ and heat at 75° C for 3 min. Add 4 ml of anhydrous ether and 200 µl of 1*M* $LiAlH_4$ (ethereal solution from Aldrich, Milwaukee, Wisconsin, United States). Reduce the volume to approximately 500 µl at 50° C then carefully add 5 ml of H_2SO_4, mix and extract three times with 5 ml of ethyl ether, passing each extract through anhydrous Na_2SO_4, and then reduce just to dryness under a stream of dry N_2. Add 1 ml of CH_3CN and 50 µl of heptafluorobutyric anhydride (HFBA) and heat at 75° C for 10 minutes. Add 10 µl of "clean" pyridine and heat for two more minutes. Cool and add 8 ml of aldrin internal standard solution and 5 ml of saturated solution of $NaHCO_3$, mix and separate phases by centrifugation. Separate isooctane and dry it over anhydrous Na_2SO_4.***

Reference chromatograms: see annex III, figures VI and VII.

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*The use of a moderately polar column is required in order to effect the separation of all 11 truxilline isomers.

**Ethyl ether maintained over 5 Å molecular sieve.

***Moore and Casale note in their article [54] that they are currently using an unpublished modification of this method that significantly reduces analysis time.

Rationale for use: Relatively complex method that employs a reduction step with lithium aluminium hydride. The use of the isomeric µ-truxinic acid* as an internal standard greatly enhances method reproducibility and precision. The aldrin internal standard functions only as a GC check standard. The method provides a summed quantity for each of the 10 isomeric diphenylcyclobutyldicarboxylic acids found in *E. coca* products by reducing the truxillines, and their hydrolyzed counterparts, to their corresponding diols. Derivatization of the diols with HFBA and the use of a moderately polar column allows for the separation of all 11 possible isomers and when coupled with electron capture detection (ECD) provides more than ample sensitivity.

Outcome: Indicates the country of origin and allows sample comparisons for discrimination and evaluation of samples for case-to-case evidential purposes (linkage determinations). Provides additional information required to confirm links between samples, that is, the method should be used in conjunction with a major component analysis.

*The µ-truxinic isomer is frequently not detected or, when detected, is at ultra-trace levels in coca extracts; hence, it is a near perfect internal standard for the other 10 isomeric structures.

Method D3: GC method, without derivatization**

Source: James Wong, Bureau of Drug Analysis Services, Health Canada, Burnaby, British Columbia, Canada.

Operating conditions:

Detector:	FID
Column:	DB-5 or equivalent, 25 m x 0.32 mm x 0.52 µm
Carrier gas:	Helium
Injection size:	1µl, split 25:1
Temperatures:	Injector: 250° C Detector: 310° C Oven: 160° C, hold for 3 min, 3° C/min to 255° C, hold for 1 min, 20° C/min to 295° C, hold for 10 min

Internal standard: None

Sample preparation: Dissolve 200-250 mg of cocaine sample in 5 ml of 0.5N sulphuric acid, add 5 ml of glass-distilled $CHCl_3$ and thoroughly mix the two phases. Centrifuge to separate phases and discard the aqueous phase. Add to the $CHCl_3$ solution 5 ml of 0.5N sulphuric acid, mix thoroughly, centrifuge and discard the aqueous layer. Transfer the $CHCl_3$ layer to a clean tube and evaporate to dryness at 45° C under a stream of dry air of nitrogen. Reconstitute the dried residue in 50 µl of acetonitrile:toluene (1:9).

**The Health Canada Laboratory uses this method, with identical parameters and sample preparation, also for heroin (method B2). However, as pointed out, wherever possible, and especially for comparative forensic purposes, the use of dedicated instrumentation and optimized methodologies is always preferred.

Rationale for use: No derivatization and relatively simple sample preparation. Good retrospective data searches via comparison of the ratios of *cis*-cinnamoylcocaine and *trans*-cinnamoylcocaine to cocaine.

Outcome: Sample comparisons for discrimination and evaluation of samples for case-to-case evidential purposes (linkage determinations). Should be used to substantiate results of the methods used for major components.

Method D4: GC or GC/MS method, without derivatization

Source: M. LeBelle and others, "Comparison of illicit cocaine by determination of minor components", *Journal of Forensic Sciences*, vol. 36, No. 4 (1991), pp. 1102-1120.

Method D4.1: GC/MS method, without derivatization

Operating conditions (GC/MS method):

Detector:	Finnigan Mat Model 800 Ion Trap Detector, electron impact mode 40 to 500 amu, 1 sec scan cycle
Column:	DB-5 or equivalent, 15 m x 0.25 mm x 0.25 µm
Carrier gas:	Helium at 55 cm/sec at 160° C oven temperature
Injection technique:	1 µl; split 25:1
Temperatures:	Injector: 275° C Oven: 160° C for 3 min, 3° C/min to 255° C for 1 min, 20° C/min to 295° C, final hold for 1 min

Sample preparation: To a 400 mg uncut sample add 5 ml of 0.5 M sulphuric acid and 5 ml of toluene and mix thoroughly. Centrifuge to separate phases, aspirate as much toluene as possible, add 5 ml of 0.5 M H_2SO_4, mix, centrifuge and aspirate the toluene layer. Combine the toluene extracts and evaporate under a stream of nitrogen at 40° C. Immediately dissolve the residue in 50 µl of ethyl acetate. Inject, in duplicate.*

Method D4.2: GC method, without derivatization

Modification of method D4.1

Operating conditions (GC method):

Detector:	FID, at 30 ml/min hydrogen and 400 ml/min air
Column:	DB-5 or equivalent, 15 m x 0.25 mm x 0.25 µm
Carrier gas:	Helium at 40 cm/sec at 160° C oven temperature; head pressure 85 kPa

*If a sample cannot be analysed immediately, evaporate it almost to dryness and store the resulting sample in a refrigerator (5° C) for future analysis.

Injection technique:	1 µl; split 25:1
Make-up gas:	Nitrogen at 30-40 ml/min
Temperatures:	Injector: 275° C Detector: 275° C Oven: 160° C for 3 min, 3° C/min to 255° C for 1 min, 20° C/min to 295° C, hold for 1 min

Internal standard: None

Sample preparation: See method D4.1 above

Rationale for use: Provides a peak rich impurity profile of minor and trace impurities many of which are unknown. No derivatization and relatively simple sample preparation. Good retrospective data searches via comparison of the ratios of *cis*-cinnamoylcocaine and *trans*-cinnamoylcocaine to cocaine.

Outcome: Sample comparisons for discrimination and evaluation of samples for case-to-case evidential purposes (linkage determinations). Should be used to substantiate results of the methods used for major components.

3. Residual solvent analysis

Method D5: Thermal desorption-GC

Source: J. Cartier, O. Guéniat and M. D. Cole, "Headspace analysis of solvents in cocaine and heroin samples", *Science and Justice*, vol. 37, No. 3 (1997), pp. 175-181.

Method is identical to that used for heroin (see method B5 in subsection 4, above). For cocaine, it provides for the detection of 16 solvents, at detection limits of 2-15 ppm for 250-300 mg powder samples.

Method D6: Static headspace, GC/MS

Source: D. R. Morello and R. P. Meyers, "Qualitative and quantitative determination of residual solvents in illicit cocaine HCl and heroin HCl", *Journal of Forensic Sciences,* vol. 40, No. 6 (1995), pp. 957-963.

The method is identical to that used for heroin (see method B6 in subsection 4, above), except for sample preparation, where a sample weight of 100 mg of cocaine equivalent is used.

4. Additional methods

Method D7: GC-ECD method for unadulterated samples

Source: J. M. Moore and D. A. Cooper, "The application of capillary gas chromatography-electron capture detection in the comparative analyses of illicit cocaine samples", *Journal of Forensic Sciences*, vol. 38, No. 6 (1993), pp. 1286-1304.

Rationale for use: This GC-ECD method provides for the detection and quantification of 6- and 7-*exo* and 6- and 7-*endo*hydroxycocaines along with manufacturing by-products of norcocaine and benzoylnorecgonine methyl ester. The method is designed for unadulterated samples.

Outcome: Aids in the evaluation of samples for case-to-case evidential purposes (linkage determinations). Provides additional information required to confirm links between samples, that is, the method should be used in conjunction with a major component analysis.

Method D8: GC/MS method for unadulterated samples

Sources: J. F. Casale and J. M. Moore, "Lesser alkaloids of cocaine-bearing plants: III. 2-Carbomethoxy-3-oxo substituted tropane esters; detection and gas-chromatographic-mass spectrometric characterization of new minor alkaloids found in South American *Erythroxylum coca var. coca*", *Journal of Chromatography A*, vol. 756, Nos. 1-2 (1996), pp. 185-192; J. F. Casale, J. M. Moore and N. G. Odeneal, "Comparative determination of 2-carbomethoxy-3-alkyloxy- and heteroaroyloxy-substituted tropanes in illicit South American cocaine using capillary gas chromatography-single ion monitoring", *Journal of Forensic Sciences*, vol. 43, No. 1 (1998), pp. 125-132.

Rationale for use: This GC-MS method provides for the detection and quantification of 3-oxo-heteroaryl and 3-oxo-hydrocarbon substituted 2-carbomethoxytropanes. Detection is accomplished in the selected ion monitoring mode. The method is designed for unadulterated samples.

Outcome: Aids in the evaluation of samples for case-to-case evidential purposes (linkage determinations). Provides additional information required to confirm links between samples, that is, the method should be used in conjunction with a major component analysis.

Method D9: Abundance of stable isotopes ^{15}N and ^{13}C by Isotope Ratio MS (IRMS)

Sources: J. R. Ehleringer and others, "Tracing the geographical origin of cocaine", *Nature*, vol. 408, 2000, pp. 311-312; see also E. Ihle and H. L. Schmidt, "Multielement isotope analysis on drugs of abuse: possibility for their origin assignment", *Isotopes in Environmental and Health Studies*, vol. 32, 1996, pp. 226-228.

Rationale for use: This IRMS method measures the relative abundance of the stable isotopes ^{15}N and ^{13}C. The method is designed for unadulterated samples.

Outcome: Provides geo-specific information. Aids in the evaluation of samples for case-to-case evidential purposes (linkage determinations [55]). Provides additional information required to confirm links between samples, that is, the method should be used in conjunction with a major component analysis.

IV. DATA HANDLING, INTERPRETATION OF RESULTS AND APPROACH TO SETTING UP PROFILING DATA COLLECTIONS

A critical factor for successful drug characterization and impurity profiling programmes is the availability of sufficiently comprehensive data collections for comparative purposes, within and between laboratories. While such data collections can only be built gradually, the process must be continuous and ongoing.

With regard to inter-laboratory comparison of data, the experience gained in heroin analyses, both in the 1980s in the United States by DEA and, more recently, by a group of European forensic laboratories in a harmonization study [48], has shown that retrospective inter-laboratory database searches are not likely to be an attainable goal in the immediate future. These experiences have shown that inter-laboratory comparison of data generated using only major component analyses can even be problematic.

The most significant issue is quantitative reproducibility (variance) for secondary targets (i.e. not heroin or cocaine but other secondary alkaloids and impurities). While this problem does not constitute an evidentiary issue, the variance in the data is too large to allow for successful inter-laboratory database searches, in other words, the average difference between samples of dissimilar origin history become ever smaller with increasing database size, because more groups of samples from different origins start to overlap. In principle the incorporation of trace impurity analysis data into a database search algorithm should greatly enhance sample-to-sample comparison. Unfortunately, trace component analyses generally have many more target analytes and typically the coefficients of variance in trace analysis are significantly greater than for major component analysis. Hence, it is expected that the inclusion of trace component analysis data into inter-laboratory database searches will significantly increase the complexity of the comparison parameters without a comparable increase in comparison specificity. It is because of these issues that nearly all retrospective database searches are performed as an intra-laboratory operation.

A. Data handling

It is clear that the use of ratios of quantities, rather than absolute quantities, provides significantly better comparison data as this approach greatly reduces the

magnitude of the analytical variance, in particular for the less abundant sample components. For heroin and cocaine, one laboratory* has successfully applied ratios for all major and minor components by expressing the ratios in terms of the appropriate major analyte, morphine base or cocaine base. It is thought that there is a significant advantage gained in employing this normalization calculation, as it allows the ready assessment of sample hydrolysis and, in some instances, the positive comparison of two samples that are identical except for the extent of hydrolysis. For cocaine, this entails summing the sample quantities of ecgonine, ecgonine methyl ester, benzoylecgonine and cocaine, while for morphine it is calculated from the sum of morphine, O3MAM, O6MAM and heroin. These calculations can be performed in several different manners where the exact method employed for this calculation is much less important than is consistency in the application. One example of such a calculation for morphine from a heroin sample is as follows:

Heroin hydrochloride sample
Quantitation results: Heroin hydrochloride•H2O = Hhcl
O6MAM hydrochloride•2-H2O = O6hcl
O3MAM hydrochloride•2-H2O = O3hcl
Morphine hydrochloride•3-H2O = Mhcl
(303.45/375.85 * Mhcl) + (303.45/399.87 * (O3hcl +O6hcl)) +
(303.45/423.89 * Hhcl) = total morphine base content expressed as the monohydrate.

While this calculation would be a tedious operation if performed by hand, it is a trivial calculation for the computer. Using the data derived from major component analyses, some laboratories have used in-house database search and classification algorithms, in some cases with commercial neural network programs. The success of those heroin and cocaine algorithms in determining sample origin is due largely to the fact that both algorithms rely on the normalization of all component ratios to morphine or cocaine content, respectively.

There are several other data handling approaches in current use by various laboratories that also rely on the use of ratios in order to normalize the data. Typically the ratios for *cis*- and *trans*-cinnamoylcocaine to cocaine and O6MAM and acetylcodeine to heroin are determined. Some will also include ratios for tropacocaine and/or norcocaine to cocaine, and papaverine and noscapine to heroin. Then when two samples are found with very similar ratios, they are considered to be possible "matches" and are selected for additional comparative analyses, usually for trace components.

The majority of those who perform these additional trace component analyses for heroin use the procedure of Neumann and Gloger [56] or a modification of that procedure. This procedure is a simple sulphuric acid extract whereby the

*Personal communication from Don Cooper, retired, Special Testing and Research Laboratory, Drug Enforcement Administration, Dulles, Virginia, United States, 2005.

bulk of the heroin and other alkaloidal bases are retained in the aqueous phase while extracting many of the alkaloidal-related amidic compounds into an organic phase. High-resolution capillary gas chromatograms of these extracts are typically quite complex and many analysts have found that visual comparison of the overlaid chromatograms is the most efficient direct comparison method (see annex II, tables 1 and 2, for a listing of many of the known alkaloidal compounds found in heroin and cocaine).

B. Interpretation of results

Irrespective of the equipment and software available in a laboratory carrying out, or planning to carry out, impurity profiling, the results have to be interpreted carefully, taking all relevant considerations into account, including analytical implications of illicit processing and distribution, as well as those arising, for example, from differences in storage conditions. Experience in the interpretation of profiling results can only be built gradually, but good analytical skills and knowledge of relevant chemistry are essential. In addition, for analytical results to be operationally useful, they have to be communicated in an adequate way to the requesting authority. The United Nations manual *Drug Characterization/Impurity Profiling: Background and Concepts* [5] provides an overview of relevant practical aspects related to the interpretation of results, addressing the following areas:

(a) The significance of chemical similarities and differences between drug samples;

(b) Establishing specific links between two or more samples;

(c) Establishing drug distribution patterns;

(d) Identifying the source of drug samples:

(i) Natural and semi-synthetic drugs;

(ii) Synthetic drugs;

(e) Identifying and characterizing the specific starting materials employed in clandestine drug manufacture.

C. Approach to setting up profiling data collections

As explained previously, the availability of appropriate databases is critical for both types of comparison, case-to-case comparisons for evidential purposes and retrospective comparisons for intelligence purposes. A recommended approach to the compilation of a profiling data collection is as follows:

(a) Generate a data bank of comparison data (i.e. analytical data plus any appropriate physical data). *(The samples used for generating compari-*

*son data will depend on the purpose of the profiling work: it will be day-to-day casework samples for a programme focusing on case-to-case comparisons or a collection of appropriate samples from known sources for a focus on determining sample origin);**

(b) Determine those data points collected within the data bank which have statistically useful comparison value;

(c) Develop a search algorithm to identify possible "matches". *(Typically either a relational database or spread sheet program is utilized. A simple search algorithm using peak area ratios of two to four of the statistically useful major components will keep the search times to a minimum and may be sufficient for this work);*

(d) Develop an enhanced search algorithm to extend comparison to other statistically useful data points to include trace alkaloid-related components. *(As a first step in developing a profiling programme, comparison of trace impurity profiles can be carried out by visual superimposition of chromatograms);*

(e) Develop and define a statistically defensible method for determining the probability that "matches" are made correctly. *(The use of a commercially available statistical software program can greatly simplify this process).*

Any database should be periodically re-evaluated to identify obvious outliers and to ensure its appropriateness for the intended purpose.

The process of building appropriate databases may be efficiently assisted by the exchange of information and data, in a standardized form, between laboratories.

In conclusion, the methods and approaches published in this manual provide guidance for the establishment of profiling programmes for heroin and/or cocaine. As has already been noted, it is necessary for the analyst to understand exactly how the impurity profiling results will be utilized and to know the precise nature of all applicable requirements before attempting to set up an impurity profiling programme and/or choosing the appropriate analytical methods. Irrespective of the purpose, the importance of taking a comprehensive approach to profiling work cannot be over emphasized.

*The accuracy of sample source assignments directly determines the limits of accuracy and quality of the profiling programme.

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ANNEX I
SUPPLEMENTARY INFORMATION (FOR BOTH HEROIN AND COCAINE)

Physical examination of seizures

The physical examination of seizures has considerable value in establishing a possible linkage between samples. In that regard, the detailed examination of packaging materials has proved particularly useful. The following brief outline provides the basis for one successful approach:

(a) Encode a description of known sample history;

(b) Encode a description of packaging to include:

(i) Appearance of packaging material (colour, design, etc.);

(ii) Number of wrapping layers;

(iii) Adhesives;

(iv) Chemical analysis of packaging materials.

X-ray diffraction

This is a well established method of acquiring information on cutting agents. The technique is especially useful for inorganic components. For some organic compounds, crystal polymorphism can be a problem.

ANNEX II
IMPURITIES IN HEROIN AND COCAINE AND THEIR "SOURCES"

A. Impurities in heroin and their sources

Detailed information on the primary alkaloidal constituents of heroin is available in the United Nations manual *Recommended Methods for Testing Opium, Morphine and Heroin: Manual for Use by National Drug Testing Laboratories* (ST/NAR/29/Rev.1).

In the tables below, major components are > 1% by weight (w/w); minor components are usually < 1% w/w; and trace components are usually < 0.1% w/w (typically requiring an extraction step).

Table 1. Alkaloidal impurities in heroin and their sources

(a) Major and minor components	*Typical source*
Acetylcodeine	Opium, codeine + Ac_2O[a]
3-O-Acetylmorphine	Opium, morphine + Ac_2O
6-O-Acetylmorphine	Heroin + hydrolysis (small quantities from morphine + Ac_2O)
Codeine	Opium
Heroin (diacetylmorphine, diamorphine)	Opium, morphine + Ac_2O
Morphine	Opium
Noscapine *{levo}* narcotine – *{racemic}*	Opium
Papaverine	Opium

(b) Heroin trace-level impurities	*Typical source*
(1R,9S)-1-Acetoxy-*N*-acetyl-1,9-dihydro-anhydronornarceine	Noscapine + Ac_2O
4-Acetoxy-3,6-dimethoxy-5-[2-(*N*-methyl-acetamido)]ethylphenanthrene	Thebaine + Ac_2O
4-Acetoxy-3,6-dimethoxy-8-[2-(*N*-methyl-acetamido)]ethylphenanthrene	Thebaine + Ac_2O
Delta$^{5,7,9(14)}$-*N*-Acetyldesthebaine =unstable=	Thebaine + Ac_2O
(E)-*N*-Acetylanhydronornarceine	Noscapine + Ac_2O
(Z)-*N*-Acetylanhydronornarceine	Noscapine + Ac_2O
N-Acetylnorlaudanosine	Norlaudanosine + Ac_2O
N-Acetylnormorphine	Morphine + O_2[b] + Ac_2O + hydrolysis
N-Acetylnornarcotine	Noscapine + O_2 + Ac_2O
4-O-Acetylthebaol[c]	Thebaine + Ac_2O

Table 1. Alkaloidal impurities in heroin and their sources *(continued)*

(b) Heroin trace-level impurities	*Typical source*
Desthebaine	Thebaine + Ac_2O
4,6-Diacetoxy-3-methoxy-5-[2-(*N*-methyl-acetamido)]ethylphenanthrene	Thebaine + Ac_2O
4,6-Diacetoxy-3-methoxy-8-[2-(*N*-methyl-acetamido)]ethylphenanthrene	Thebaine + Ac_2O
4,6-Diacetoxy-3-methoxyphenanthrene	Thebaine + Ac_2O
6-O-,*N*-Diacetylnorcodeine	Codeine + O_2 + Ac_2O
6-O-,*N*-Diacetylnormorphine	Morphine + O_2 + Ac_2O + hydrolysis
3,6-Dimethoxy-4,5-epoxyphenanthrene	Thebaine + Ac_2O
Hydrocotarnine	Noscapine + O_2
Meconine	Opium; also a GC degradation product
(E)-3-[2-(*N*-Methylacetamido)ethyl)-4,5-methylendioxy-6-methoxyphenyl]acrilic acid	Nocapine + Ac_2O
Alpha-Methylmorphimethine	Morphine + Ac_2O
Papaveraldine	Papaverine + O_2
Thebaol	Thebaine + Ac_2O + hydrolysis
Triacetylnormorphine	Morphine + O_2 + Ac_2O

[a]Ac_2O = acetic anhydride.
[b]O_2 = oxidation.
[c]Major decomposition product.

Table 2. Heroin: adulterants (non-opiate cutting agents with pharmacological effects)

Acetylsalicylic acid	Diphenhydramine	*N*-Phenyl-2-Naphthalene
Allobarbital	Gluthetimide	*N*-Phenyl-2-Naphthylamine
Aminophenazon	Griseofulvin	Procaine
Antipyrine	Lidocaine (lignocaine)	Quinine
Ascorbic acid	Methaqualone	Salicylamide
Barbital	Methylphenobarbitone	Salicylic acid
Benzocaine	Nicotinamide	Strychnine
Bisphenol-A	Paracetamol (acetaminophen) (+ acetyl-paracetamol)	Theophylline
Caffeine	Phenacetin	Thiamine
Chloroquine	Phenazon	Xylazine
Cocaine	Phenobarbitone (phenobarbital)	
Diazepam	Phenolphthalein	

Table 3. Heroin: diluents (cutting agents to dilute the sample) and processing by-products

Calcium carbonate[a]	Iditol hexa-acetate	Sodium chloride
Calcium chloride	Lactose/saccharose	Starch (usually corn)
Citric acid	Mannitol/mannit/sorbit	Sucrose
Fructose	Phthalic acid	Sucrose octa-acetate
Glucose	Potassium chloride	Tartaric acid
Glycine	Sodium carbonate	

[a]Calcium carbonate is almost always present as a processing by-product, almost never as a cutting agent (diluent).

B. Impurities in cocaine and their sources

Detailed information on the primary alkaloidal constituents of cocaine is available in the United Nations manual *Recommended Methods for Testing Cocaine: Manual for Use by National Narcotics Laboratories* (ST/NAR/7).

In the tables below, major components are > 1% by weight (w/w); minor components are usually < 1% w/w; and trace components are usually < 0.1% w/w (typically requiring an extraction step).

Table 4. Alkaloidal impurities in cocaine and their sources

(a) Major and minor components	*Typical source*
Anhydroecgonine	Gas chromatography (GC) artefact
Anhydroecgonine methyl ester	GC artefact
Benzoic acid	Hydrolysis of cocaine
Benzoylecgonine	Hydrolysis of cocaine, also found in coca leaf but thought not to be extracted in the clandestine process
Trans-cinnamic acid	Hydrolysis of *cis*- and *trans*-cinnamoylcocaine
Cis-cinnamoylecgonine (often at trace level)	Hydrolysis of *cis*-cinnamoylcocaine
Trans-cinnamoylecgonine (often at trace level)	Hydrolysis of *trans*-cinnamoylcocaine
Cis-cinnamoylcocaine	Coca leaf
Trans-cinnamoylcocaine	Coca leaf
Ecgonine	Hydrolysis of cocaine, also found in coca leaf, but thought not to be extracted in the clandestine process
Ecgonine methyl ester	Found in coca leaf and occurs due to cocaine hydrolysis; most probably is not extracted in the clandestine process
N-formyl-cocaine	O_2,[a] not present in coca leaf
Norcocaine	O_2, not known to be present in coca leaf
Pseudoecgonine methyl ester	GC artefact: direct injection in MeOH of cocaine base (crack) containing traces of $NaHCO_3$
Truxillic acid(s)	Hydrolysis of truxillines
Truxillines	Coca leaf, the total content of all isomers can be more than 10% of an unadulterated cocaine sample. 11 theoretical diastereomers–11 observed.
Truxinic acid(s)	Hydrolysis of truxillines

(b) Cocaine trace-level impurities	*Typical source*
Acetoxyecgonine methyl ester	Coca leaf
N-Benzoylnorcocaine	Possibly a GC artefact
6-*exo*-Benzoyloxytropine	Coca leaf
Benzoyltropine	Coca leaf
Butyroylecgonine methyl ester	Coca leaf
Cis-cinnamoyltropacocaine	Coca leaf
Trans-cinnamoyltropacocaine	Coca leaf
2'-Furanoylecgonine methyl ester	Coca leaf
3'-Furanoylecgonine methyl ester	Coca leaf

Table 4. Alkaloidal impurities in cocaine and their sources *(continued)*

(b) Cocaine trace-level impurities	*Typical source*
Heptadienoylecgonine methyl ester	Coca leaf
Cis-3-heptenoylecgonine methyl ester	Coca leaf
Trans-3-heptenoylecgonine methyl ester	Coca leaf
Trans-, *trans*-2,4-hexadienoylecgonine methyl ester	Coca leaf
Hexanoylecgonine methyl ester	Coca leaf
Trans-2-hexenoylecgonine methyl ester	Coca leaf
Trans-3-hexenoylecgonine methyl ester	Coca leaf
Trans-4-hexenoylecgonine methyl ester	Coca leaf
2'-Hydroxybenzoylpseudotropine	Coca leaf
1-Hydroxycocaine	Coca leaf
3'-Hydroxycocaine	Coca leaf; no 2' or 4' isomers detected
4α-Hydroxycocaine	Coca leaf
4β-Hydroxycocaine	Coca leaf
5-Hydroxycocaine	Coca leaf
6-*endo*-Hydroxycocaine	Coca leaf
6-*exo*-Hydroxycocaine	Coca leaf
7-*endo*-Hydroxycocaine	Coca leaf
7-*exo*-Hydroxycocaine	Coca leaf
1-Hydroxytropacocaine	Coca leaf
6-*exo*-Hydroxytropacocaine	Coca leaf
Isobutyroylecgonine methyl ester	Coca leaf
Isovaleroylecgonine methyl ester	Coca leaf
Nicotinoylecgonine methyl ester	Coca leaf
3α-Phenylacetoxytropane	Coca leaf
Pseudococaine	Coca leaf
Pseudoecgonine	Hydrolysis of pseudococaine
Pseudoecgonine methyl ester	GC: solvent MeOH + trace bicarbonate (crack) Hydrolysis and epimerization of cocaine (the very low levels of pseudococaine in sample make it an unlikely co-source)
Propionoylecgonine methyl ester	Coca leaf
2'-Pyrroloylecgonine methyl ester	Coca leaf
Senecioylecgonine methyl ester	Coca leaf
Tigloylecgonine methyl ester	Coca leaf
3',4',5'-Trimethoxybenzoyltropine	Coca leaf
Cis-3',4',5'-trimethoxycinnamoylcocaine	Coca leaf
Trans-3',4',5'-trimethoxycinnamoylcocaine	Coca leaf
Trans-3',4',5'-trimethoxycinnamoylpseudotropine	Coca leaf
Trans-3',4',5'-trimethoxycinnamoyltropine	Coca leaf
3',4',5'-Trimethoxycocaine	Coca leaf
3',4',5'-Trimethoxytropacocaine	Coca leaf
Tropacocaine	Coca leaf (levels higher than 1% relative to cocaine may indicate addition of the commercially available product)
Tropacocalline	Coca leaf: presence in leaf not confirmed. 16 theoretical diastereomers–10 observed.

[a]O_2 = oxidation.

Table 5. Cocaine: adulterants (non-coca-related cutting agents with pharmacological effects)

Allobarbital	Ephedrine	Nicotinamide
Amphetamine	Fentanyl	Nitrazepam
Antipyrine	Flunitrazepam	Paracetamol (acetaminophen)
Aspirin	Flurazepam	Phenacetin
Atropine	Lidocaine (lignocaine)	Phenobarbital
Benzocaine	MDEA[a]	Piracetam
Benzoic acid	MDMA[b]	Procaine
Caffeine	Methadone	Quinine
Diazepam	Methamphetamine	Tetracaine
Dipyrone	Methaqualone	Theophylline

[a]3,4-Methylenedioxyethylamphetamine.
[b]3,4-Methylenedioxymethamphetamine.

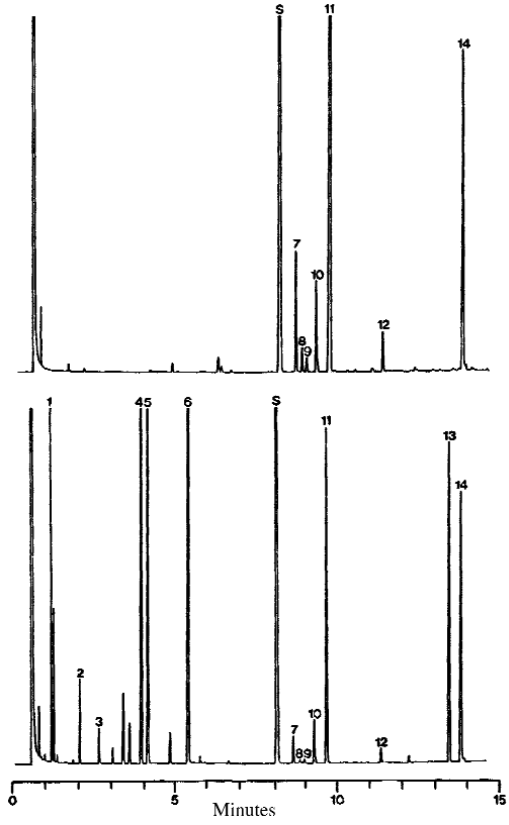
Table 6. Cocaine: diluents (cutting agents to dilute the sample)

Ascorbic acid	Inositol	Mannitol
Citric acid	Lactose	Mannose
Fructose	Lysine	Sorbitol
Glucose	Maltose	Sucrose

ANNEX III
REFERENCE CHROMATOGRAMS AND PEAK IDENTIFICATION TABLES

Figure I. Capillary gas chromatograms of illicit heroin samples

(Top = uncut heroin sample; bottom = cut heroin sample)



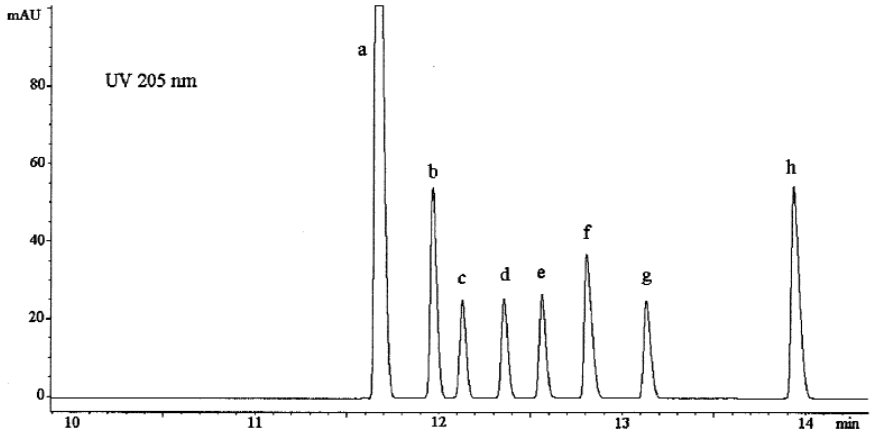
Peaks:
1 = nicotinamide-TMS
2 = meconin
3 = caffeine
4 = glucose-TMS
5 = phenobarbital-TMS
6 = methaqualone
7 = acetylcodeine
8 = acetylthebaol
9 = morphine-TMS
10 = 6-O-acetylmorphine-TMS
11 = diacetylmorphine
12 = papaverine
13 = phenolphthalein-TMS
14 = narcotine
S = *n*-tetracosane (internal standard)

Note: TMS = trimethylsilyl derivative.

Conditions: Fused silica capillary column 25 m x 0.32 mm i.d. coated with OV-1 cross-linked; carrier gas 0.7 bar hydrogen, split 20 ml/min; make-up gas 18 ml argon/min; oven temperature programme 150° C to 280° C at 9° C/min, then 0.5 min isothermal; and injector/detector (FID) temperatures 250° C/280° C.

Source: Reprinted from *Forensic Science International*, vol. 44, No. 1, H. Neumann, "Comments on the routine profiling of illicit heroin samples", pp. 85-87. Copyright 1990, with permission from Elsevier.

Figure II. lectropherogram of a standard mixture of heroin, O^6-monoacetylmorphine, O^3-monoacetylmorphine, morphine, acetylcodeine, papaverine, codeine and noscapine



Peaks:

a = heroin (0.40 mg/ml)
b = O^6-monoacetylmorphine (0.09 mg/ml)
c = O^3-monoacetylmorphine (0.04 mg/ml)
d = morphine (0.04 mg/ml)
e = acetylcodeine (0.04 mg/ml)
f = papaverine (0.04 mg/ml)
g = codeine (0.04 mg/ml)
h = noscapine (0.06 mg/ml)

Conditions: A 64-cm (55.5 cm to detector window) x 50-µm i.d. fused silica capillary operating at 25° C and 30 kV with UV detection at 205 nm was used. Pressure injections of 500 mbar*s were used with a run buffer consisting of Celixir reagent B (pH 2.5) + 100 mM DM-ß-CD. For migration times, see table 1.

Source: Reprinted from *Journal of Chromatography A*, vol. 1034, Nos. 1-2, I. S. Lurie and others, "Use of dynamically coated capillaries for the determination of heroin, basic impurities and adulterants with capillary electrophoresis", pp. 227-235, Copyright 2004, with permission from Elsevier.

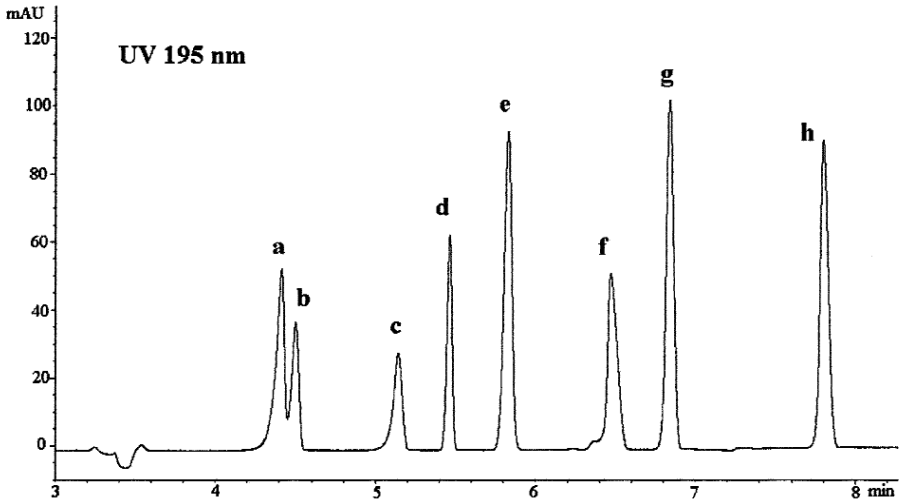
Table 1. Relative migration times of heroin, basic impurities and basic adulterants for the electropherogram in figure II

Solute	*Relative[a] migration time (min)*
Thiamine	0.562
Nicotinamide	0.666
D- or L- Chloroquine	0.713
D- or L- Chloroquine	0.717
Quinine	0.815
Quinine impurity	0.856
Heroin	0.927
O^6-monoacetylmorphine	0.953
Dipyrone	0.957
O^3-monoacetylmorphine	0.965
Aminopyrene	0.968
Lidocaine	0.968
Morphine	0.983
Acetylcodeine	1.00 (12.4)
Papaverine	1.02
Strychnine	1.03
Codeine	1.05
L-Ephedrine	1.07
L-Pseudoephedrine	1.07
D-Ephedrine	1.08
Xylazine	1.10
D-Pseudoephedrine	1.10
Noscapine	1.11
Thebaine	1.12
Procaine	1.14
Chlorpheniramine	1.15
Brompheniramine	1.16
Cocaine	1.22
trans-Doxepin	1.23
Diphenhydramine	1.23
Tetracaine	1.23
cis-Doxepin	1.24
t_0	1.85

Source: Reprinted from *Journal of Chromatography A,* vol. 1034, Nos. 1-2, I. S. Lurie and others, "Use of dynamically coated capillaries for the determination of heroin, basic impurities and adulterants with capillary electrophoresis", pp. 227-235, Copyright 2004, with permission from Elsevier.

[a]Relative to acetylcodeine.

Figure III. Electropherogram of a standard mixture of acetaminophen, theophylline, caffeine, aspirin, salicylic acid, antipyrene, phenobarbitol and phenacetin



Peaks:

a = acetaminophen (0.08 mg/ml)
b = theophylline (0.09 mg/ml
c = caffeine (0.09 mg/ml)
d = aspirin (0.09 mg/ml)
e = salicylic acid (0.09 mg/ml)
f = antipyrene (0.10 mg/ml)
g = phenobarbital (0.10 mg/ml)
h = phenacetin (0.10 mg/ml)

Conditions: A 32-cm (23.5 cm to detector window) x 50-µm i.d. fused silica capillary operating at 15° C and 8.5 kV with UV detection at 195 nm was used. Pressure injections of 100 mbar*s were used with a run buffer consisting of 50 mM phosphate-borate (pH 6.5) + 103.2 mM (3%, w/w) SDS: For migration times, see table 2.

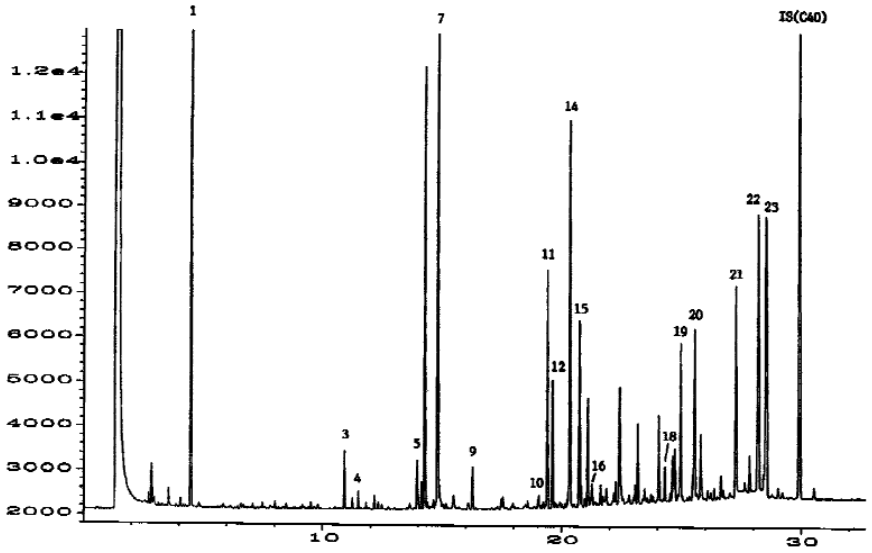
Source: Reprinted from *Journal of Chromatography A*, vol. 1034, Nos. 1-2, I. S. Lurie and others, "Use of dynamically coated capillaries for the determination of heroin, basic impurities and adulterants with capillary electrophoresis", pp. 227-235, Copyright 2004, with permission from Elsevier.

Table 2. Relative migration times of acidic and neutral adulterants, basic impurities and basic adulterants for the electropherogram in figure III

Solute	*Relative migration time (min)*
t_0	0.504
Nicotinamide	0.591
Acetominophen	0.660
Theophylline	0.673
Dipyrone (1)	0.738
Caffeine	0.764
Aspirin	0.809
Salicylic acid	0.860
Anitipyrene	0.948
Phenobarbital	1.00 (6.8)
Ibuprofen	1.11
Aminopyrene	1.11
Phenacetin	1.13
Dipyrone (2)	1.13
Benzocaine	1.25
Thiamine	1.32
Morphine	1.35
Codeine	1.44
O^3-monoacetylmorphine	1.44
Procaine	1.45
Pseudoephedrine	1.45
Ephedrine	1.45
Lidocaine	1.46
Heroin	1.47
O^6-monoacetylmorphine	1.47
Acetylcodeine	1.47
Noscapine	1.47
Quinine	1.48
Chloroquine	1.48
Yohimbine	1.48
Strychnine	1.48
Thebaine	1.48
Xylazine	1.49
Cocaine	1.49
Tetracaine	1.49
cis- and *trans*-Doxepin	1.50
Brompheniramine	1.50
Methorphan	1.50
Papaverine	1.50
Chlorpheniramine	1.51
Diphenhydramine	1.51

Source: Reprinted from *Journal of Chromatography A*, vol. 1034, Nos. 1-2, I. S. Lurie and others, "Use of dynamically coated capillaries for the determination of heroin, basic impurities and adulterants with capillary electrophoresis", pp. 227-235, Copyright 2004, with permission from Elsevier.

Figure IV. Typical capillary gas chromatogram of (trace) impurity compounds detected after extraction from a sample of heroin of Southwest Asian origin using methodology similar to that of method B1[a]



Peaks:

1 = Meconine
3 = Unknown
4 = 3,6-Dimethoxy-4,5-epoxyphenanthrene
5 = 4-O-Thebaol
7 = 4-O-Acetylthebaol
9 = 4,6-Diacetoxy-3-methoxyphenanthrene
10 = Unknown
11 = 6-O,*N*-Diacetylnorcodeine
12 = Unknown
14 = 4-Acetoxy-3,6-dimethoxy-5-[2-(*N*-methyl-acetamido)]ethylphenanthrene
15 = 3-O,6-O,N-Triacetylmorphine
16 = *N*-Acetylnorlaudanosine
18 = Unknown
19 = 4-Acetoxy-3,6-dimethoxy-8-[2-(*N*-methyl-acetamido)]ethylphenanthrene
20 = *N*-Acetylnornarcotine
21 = *(E)*-*N*-Acetylanhydronornarceine
22 = (1*R*,9*S*)-1-Acetoxy-*N*-acetyl-1,9-dihydroanhydronornarceine
23 = *(Z)*-*N*-Acetylanhydronornarceine
IS (C40) = *n*-Tetracontane (internal standard)

Source: Reprinted from *Forensic Science International*, vol. 114, No. 2, L. Strömberg and others, "Heroin impurity profiling: a harmonization study for retrospective comparisons", pp. 67-88, Copyright 2000, with permission from Elsevier.

[a]For details on peak identification, see table 3.

Table 3. Identification of peaks in the capillary gas chromatogram in figure IV

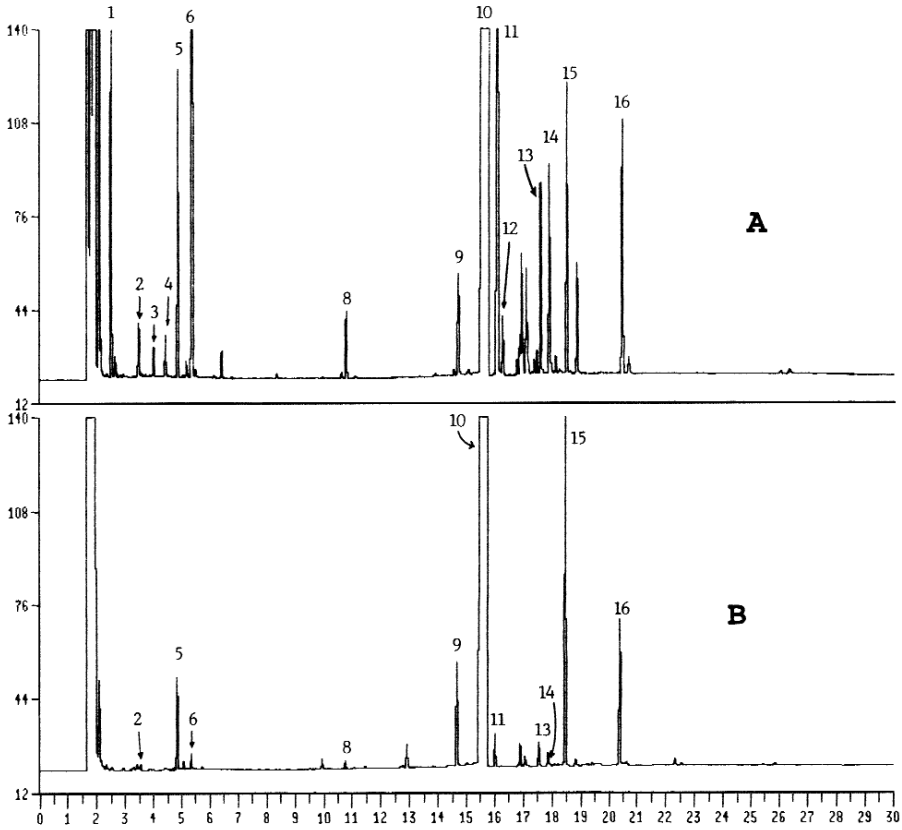
Peak	*Identification*	*Molecular weight*	*Base peak*	*Relative retention time*[a]	*Retention index*
1	Meconine	194	165	0.152	1765
3	Unknown		204	0.364	2290
4	3,6-Dimethoxy-4,5-epoxyphenanthrene	252	237	0.383	2333
5	4-O-Thebaol	254	254	0.465	2522
7	4-O-Acetylthebaol	296	254	0.494	2591
9	4,6-Diacetoxy-3-methoxyphenanthrene	324	240	0.542	2708
10	Unknown		252	0.634	2944
11	6-O,*N*-Diacetylnorcodeine	369	87	0.646	2977
12	Unknown		254	0.653	2995
14	4-Acetoxy-3,6-dimethoxy-5-[2-(*N*-methylacetamido)]ethylphenanthrene	395	265	0.677	3064
15	3-O,6-O,N-Triacetylmorphine	397	87	0.690	3100
16	*N*-Acetylnorlaudanosine	385	234	0.707	3147
18	Unknown		151	0.808	3446
19	4-Acetoxy-3,6-dimethoxy-8-[2-(*N*-methylacetamido)]ethylphenanthrene	395	280	0.830	3515
20	*N*-Acetylnornarcotine	441	248	0.857	3601
21	*(E)*-*N*-Acetylanhydronornarceine	455	193	0.907	3757
22	(1R,9*S*)-1-Acetoxy-*N*-acetyl-1,9-dihydro-anhydronornarceine	515	280	0.939	3848
23	*(Z)*-*N*-Acetylanhydronornarceine	455	193	0.950	3876
IS (C40)	*n*-Tetracontane (internal standard)	562		1.000	4000

Source: Reprinted from *Forensic Science International*, vol. 114, No. 2, L. Strömberg and others, "Heroin impurity profiling: a harmonization study for retrospective comparisons", pp. 67-88, Copyright 2000, with permission from Elsevier.

[a]Based on *n*-tetracontane = 1.000.

Figure V. Capillary gas chromatograms of a cocaine hydrochloride sample and a cocaine base sample[a]

(A = cocaine hydrochloride sample; B = cocaine base sample)



Source: J. F. Casale and R. W. Waggoner, Jr., "A chromatographic impurity signature profile analysis for cocaine using capillary gas chromatography", *Journal of Forensic Sciences*, vol. 36, No. 5 (1991), pp. 1312-1330. Copyright ASTM International. Reprinted with permission.

[a]For peak identification, see table 4.

Table 4: Retention times for cocaine impurities in the capillary gas chromatograms in figure V

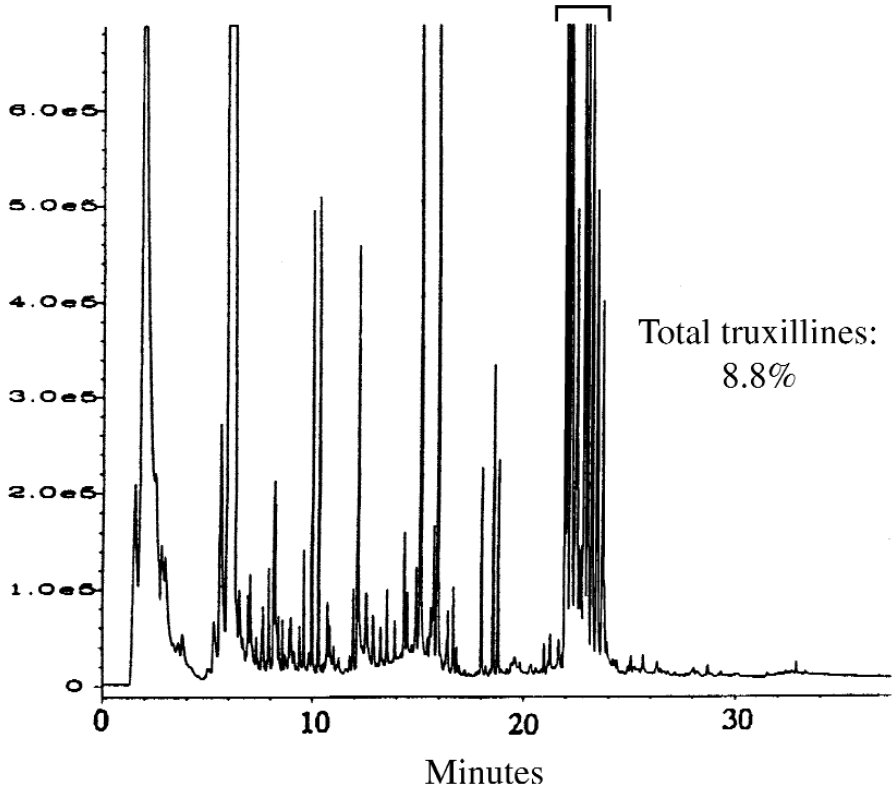
Peak	*Compound*	*Retention time (min)*
1	Benzoic acid[a]	2.60
2	Anhydroecgonine methyl ester	3.52
3	Anhydroecgonine[a]	4.10
4	*trans*-Cinnamic acid[a]	4.36
5	Ecgonine methyl ester[a]	4.94
6	Ecgonine[b]	5.45
8	Tropacocaine	10.81
9	*para*-Fluorococaine[c]	14.68
10	Cocaine	15.45
11	Benzoylecgonine[a]	16.04
12	Norcocaine[d]	16.26
13	*beta*-Truxinic acid[b]	17.61
14	*alpha*-Truxillic acid[b]	17.89
15	*cis*-Cinnamoylecgonine methyl ester	18.47
16	*trans*-Cinnamoylecgonine methyl ester	20.55
17	*N*-Formyl cocaine	22.93

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

Source: J. F. Casale and R. W. Waggoner, Jr., "A chromatographic impurity signature profile analysis for cocaine using capillary gas chromatography", *Journal of Forensic Sciences*, vol. 36, No. 5 (1991), pp. 1312-1330.

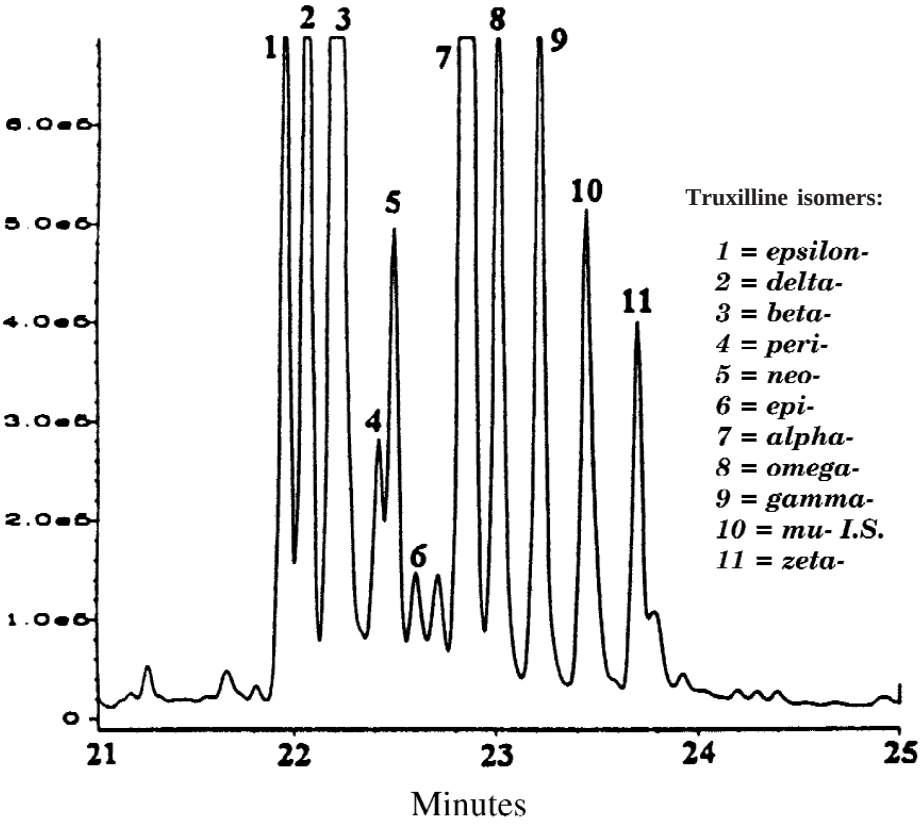
Figure VI. GC-ECD chromatogram of the determination of truxillines in a cocaine comparison case

(Truxillines indicated by square bracket)



Source: J. M. Moore and J. F. Casale, "Cocaine profiling methodology: recent advances", *Forensic Science Review*, vol. 10, 1998, pp. 13-46. Copyright Forensic Science Review. Reprinted with permission.

Figure VII. Expanded "window" of the chromatogram in figure VI, showing chromatography and identification of the individual truxillines



Source: J. M. Moore and J. F. Casale, "Cocaine profiling methodology: recent advances", *Forensic Science Review*, vol. 10, 1998, pp. 13-46. Copyright Forensic Science Review. Reprinted with permission.

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