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Recommended methods for the **Identification and Analysis of Cocaine in Seized Materials**

MANUAL FOR USE BY NATIONAL DRUG ANALYSIS LABORATORIES

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Laboratory and Scientific Section
UNITED NATIONS OFFICE ON DRUGS AND CRIME
Vienna

Recommended Methods for the Identification and Analysis of Cocaine in Seized Materials

(Revised and updated)

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UNITED NATIONS
New York, 2012

Note

Operating and experimental conditions are reproduced from the original reference materials, including unpublished methods, validated and used in selected national laboratories as per the list of references. A number of alternative conditions and substitution of named commercial products may provide comparable results in many cases, but any modification has to be validated before it is integrated into laboratory routines.

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ST/NAR/7/REV.1

Original language: English

This publication has not been formally edited.

Publishing production: English, Publishing and Library Section, United Nations Office at Vienna.

Contents

Acknowledgements

UNODC's Laboratory and Scientific Section (LSS, headed by Dr. Justice Tettey) wishes to express its appreciation and thanks to Dr. Michael Collins, Dr. Helen Salouros and Mr. Hilton Swan for the preparation of the first draft of the present revised and updated manual.

LSS would also like to thank Prof. Niamh Nic Daeid, Dr. Kathleen Savage, Dr. Udo Zerell and Dr. Pierre Esseiva for their expert reviews and valuable contributions.

The preparation of the present manual was coordinated by Ms. Yen Ling Wong, staff of LSS. The contribution of other UNODC staff is gratefully acknowledged.

1. Introduction

1.1 Background

Cocaine is a highly addictive stimulant that occurs naturally as an alkaloid of the coca plant (*Erythroxylon coca* or *Erythroxylon novogranatense*). Traditionally, the coca leaves are chewed or brewed as tea. In the early 1900s, purified cocaine was used in most tonics and elixirs to treat a wide variety of illnesses.

Due to its high potential for abuse and dependence, coca leaf and cocaine were put under international control in the Schedule I of the Single Convention on Narcotics Drugs, 1961. However, in some countries, cocaine still has legitimate medical uses, such as local anaesthesia for eye, ear and throat surgeries.

The two main chemical forms of cocaine are the water-soluble salt form and the water-insoluble cocaine base form. Generally, the salt form can be injected or snorted while the base form ("crack") is usually smoked.

With excessive or prolonged abuse, cocaine can cause development of tolerance, strong psychological dependence, malnutrition, disorientation, hallucination and paranoid psychosis.

1.2 Purpose and use of the manual

The present manual is one in a series of similar publications dealing with the identification and analysis of various types of drugs under international control. These manuals are the outcome of a programme pursued by UNODC since the early 1980s, aimed at the harmonization and establishment of recommended methods of analysis for national drug analysis laboratories.

The present manual is a revision of the manual on *Recommended methods for testing cocaine* (ST/NAR/7), which was published in 1986. It has been prepared taking into account developments in analytical technology with a view to providing the basis for reliable forensic evidence on cocaine-containing seized materials.

In line with the overall objective of the series, the present manual 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, leaving room also for adaptation to the level of sophistication of different laboratories and the various legal needs. The majority of methods included in the present manual are validated methods, which have been used for a number of years in reputable laboratories and as part of inter-laboratory studies, collaborative exercises and proficiency tests. The reader should be aware, however, that there are a number of other methods, including those published in the forensic science literature, which may also produce acceptable results. *Any new method that is about to be used in the reader's laboratory must be validated and/or verified prior to routine use.*

In addition, there are a number of more sophisticated approaches, but they 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 not normally change the validity of the results. The choice of the methodology and approach to analysis as well as the decision whether or not additional methods are required remain with the analyst and may also be dependent on the availability of appropriate instrumentation and the level of legally acceptable proof in the jurisdiction within which the analyst works.

Attention is also drawn to the vital importance of the availability to drug analysts of reference materials and books on drugs of abuse and analytical techniques. Moreover, the analyst must of necessity keep abreast of current trends in drug analysis, consistently following current analytical and forensic science literature.

UNODC's 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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All manuals, as well as guidelines and other scientific-technical publications may be requested by contacting the address above.

2. Physical appearance and chemical characteristics of coca leaf and illicit materials containing cocaine

Coca leaf

Coca leaves are somewhat similar in appearance to *Laurus nobilis* leaves. Different *Erythroxylon* species produce leaves varying in size and appearance. In all species the upper side of the leaf is darker than the underside which may be grey-green in colour. On the underside of the leaves are found two lines parallel to the midrib which are considered to be characteristic of coca leaf.

The leaves of *Erythroxylon coca Lam*. are characteristically large and thick, broadly elliptic in shape, more or less pointed at the apex and dark green in colour. The leaves of *Erythroxylon novogranatense (Morris) Hieron* are smaller, narrower, thinner and rounded at the apex. They are bright yellow-green coloured. The leaves of *Erythroxylon novograntense var. truxillense (Rusby) Plowman* are even smaller and narrower. However, they are thicker then the other types and have a rich green colour.

Coca paste

This is an off-white, creamy or beige-coloured powder; it is rarely fine, often contains aggregates and is generally damp. Unless the aggregates are crystalline (which is rare) they usually break down under slight pressure. It has a characteristic odour.

Cocaine

Although produced from a somewhat variable natural product, by a batch process capable of wide variation, cocaine varies comparatively little when compared for example to heroin products. Nevertheless no two illicit samples of cocaine are exactly identical. For the most part it is a white or off-white crystalline powder which is often fine, and rarely damp.

Adulteration is comparatively rare (but not unknown) for material being internationally trafficked with a purity often of 80-90% (as cocaine hydrochloride). Subsequent adulteration and transformation for trafficking purposes usually involves the addition of uncontrolled substances such as levamisole (and/or tetramisole), phenacetin, lidocaine, caffeine, diltiazam, hydroxyzine, procaine, benzocaine or sugars (e.g. mannitol, lactose or glucose). In either case the physical appearance is changed only slightly, since all the known adulterants are themselves fine dry white powders.

For trafficking within countries, the cocaine purity is typically about 30%; the internationally trafficked material is subjected to adulteration with about three times its own weight of the diluent.

"Crack" cocaine

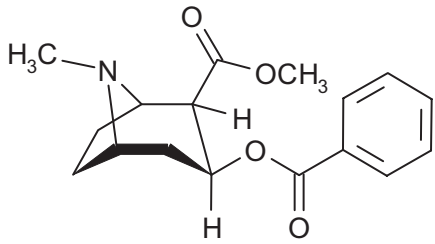
A flaky, hard material obtained by adding ammonia or sodium bicarbonate (baking soda) and water to cocaine hydrochloride and heating the resulting precipitated powder. The term "crack" which is the street name given to freebase cocaine, refers to the crackling sound produced when the mixture is heated.

Deviations/variations of materials submitted for forensic examination to the physical characteristics described here, should not be interpreted to mean the absence of cocaine or a cocaine containing product.

3. Description of the pure compounds

The compounds listed below include cocaine, major components (> 1% by weight) and minor components (usually < 1% by weight). Trace components (usually < 0.1% by weight and typically requiring an extraction step) are not described here.

Cocaine



Synonyms: [1R-(*exo,exo*)]-3-(Benzoyloxy)-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylic acid methyl ester

3β-Hydroxy-1αH,5αH-tropane-2β-carboxylic acid methyl ester benzoate

Ecgonine methyl ester benzoate

l-Cocaine

β-Cocaine

Benzoylmethylecgonine

$C_{17}H_{21}NO_4$
Molecular Weight = 303.4 (base), 339.8 (hydrochloride)
Melting point: 98° C (base), 195° C (hydrochloride)

Solubilities (1g/ml):	*Base*	*Hydrochlorid*e
Water	slightly soluble (1 in 600)	soluble (1 in 0.4)
Ethanol	soluble (1 in 6.5)	soluble (1 in 3.2)
Diethyl ether	soluble (1 in 3.5)	practically insoluble
Chloroform	soluble (1 in 0.7)	soluble (1 in 12.5)

GC-MS data (percentage abundance):

303 (M^+, 17), 182 (71), 105 (29), 96 (24), 94 (36), 82 (100), 77 (35) m/z

NMR data (hydrochloride):

^{1}H NMR 600 MHz; (D_2O): δ 2.90 (3H, s), 3.63 (3H, s), 3.65 (1H, dd,), 4.10 (1H, bm), 4.24 (1H, bm), 5.59 (1H, ddd), 7.54, (1H, t), 7.70 (1H, t), 7.96 (1H, d) ppm

^{13}C NMR (151 MHz; D_2O): δ 22.8, 23.9, 32.8, 39.1, 46.3, 53.6, 63.4, 64.1, 64.7, 128.7, 129.2, 129.8, 134.7, 167.5, 173.6 ppm

Infrared data:

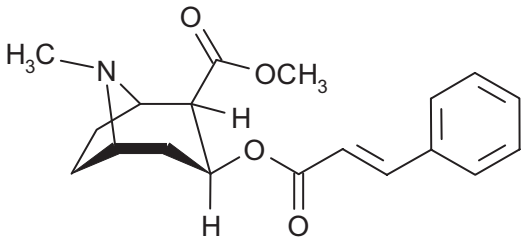
Principal peaks at wavenumbers 1710, 1738, 1275, 1110, 712, 1037 cm^{-1} (KBr disk).

UV Data:

Aqueous acid—233 nm (A¦ = 430), 275 nm

Major and minor components

Cinnamoylcocaine



Synonyms: [1R-(*exo,exo*)]-8-Methyl-3-[(1-oxo-3-phenyl-2-propenyl)oxy]-8-azabicyclo[3.2.1]octane-2-carboxylic acid methyl ester

Ecgonine cinnamate methyl ester

Cinnamoylecgoninmethyl ester

Cinnamoylmethylecgonine

Cinnamylcocaine

$C_{19}H_{23}NO_4$

Molecular Weight: 329.4

Melting point: 121° C (base)

Solubilities (1g/ml):	*Base*	*Hydrochloride*
Water	almost insoluble	soluble
Ethanol	soluble	soluble
Diethyl ether	soluble	soluble
Chloroform	soluble	slightly

GC-MS data (percentage abundance):

329 (M^+, 15), 238 (14), 182 (72), 131 (33), 103 (24), 96 (59), 94 (35), 82 (100), 42 (27) m/z

NMR data (hydrochloride):

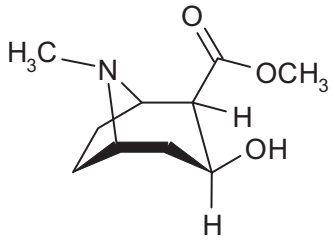
^{1}H NMR (300 MHz; $CDCl_3$): (Key spectral data): δ 2.21 (3H, s), 2.40 (1H, ddd), 3.71 (3H, s), 5.11 (1H, ddd), 6.44 (1H, d), 7.36 (3H, m), 7.51 (2H, m), 7.65 (1H, d) ppm

^{13}C NMR (75.5 MHz; $CDCl_3$): δ 25.2, 25.4, 35.5, 41.2, 50.1, 51.4, 61.6, 64.8, 66.6, 118.3, 128.1 (x 2), 128.8 (x 2), 130.2, 134.4, 144.9, 166.7, 170.8 ppm

Infrared data:

Principal peaks at wavenumbers 2959, 2856, 2804, 1749, 1699, 1630, 1319, 1179, 1037, 1008, 767, 683 cm^{-1} (KBr disk)

Methylecgonine



Synonyms: [1R-(*exo,exo*)]-3-Hydroxy-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylic acid methyl ester

Ecgonine methyl ester

3β-Hydroxy-1αH,5αH-tropane-2β-carboxylic acid methyl ester

$C_{10}H_{17}NO_3$

Molecular Weight: 199.3 (base), 235.7 (hydrochloride)

Melting point: oil (base), 215° C (hydrochloride)

GC-MS data (percentage abundance):

199 (M^+, 30), 168 (18), 112 (12), 96 (78), 94 (38), 82 (100), 68 (8), 42 (32) m/z

NMR data (hydrochloride):

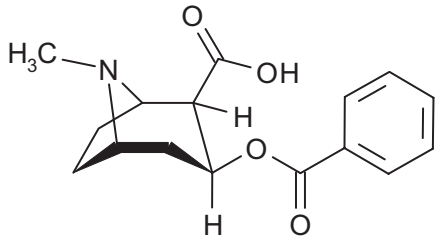
^{1}H NMR (500 MHz; D_2O): δ 2.03-2.14 (3H, m), 2.20-2.24 (1H, m), 2.30-2.48 (2H, m), 2.83 (3H, s), 3.31 (1H, dd, *J* = 2.2, 7.2 Hz), 3.80 (3H, s) 3.99 (1H, m), 4.15 (1H, bd, *J* = 7.0 Hz), 4.43-4.48 (1H, m) ppm

^{13}C NMR (125 MHz; D_2O): δ 22.5, 23.5, 34.8, 38.4, 48.8, 52.8, 60.3, 63.1, 63.8, 174.2 ppm

Infrared data:

Principal peaks at wavenumbers 3269, 2963, 2132, 1704, 1481, 1428, 1350, 1215, 1140, 1049, 1013, 968, 777, 616 cm^{-1} (KBr disk).

Benzoylecgonine



Synonyms: 3-(Benzoyloxy)-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylic acid
3β-Hydroxy-1αH,5αH-tropane-2β-carboxylic acid benzoate

Ecgonine benzoate

$C_{16}H_{19}NO_4$

Molecular Weight: 289.3

Melting point: 195° C (anhydrous) (decomposes), 86-92° C (tetrahydrate), 200° C hydrochloride

Solubilities (1g/ml):	*Base*	*Hydrochloride*
Water, boiling	soluble	soluble
Ethanol	soluble	soluble

GC-MS data (percentage abundance):

289 (M^+, 5), 168 (26), 124 (100), 105 (31), 96 (19), 94 (26), 82 (61), 77 (40), 67 (11) m/z

NMR data (hydrochloride):

^{1}H NMR (300 MHz; D_2O): (Key spectral data): δ 2.61-2.17 (6H, m) 2.88 (3H, s), 3.22 (1H, dd), 4.07 (2H, bd), 5.54 (1H, m), 7.59 (2H, dd), 7.76 (1H, dd), 8.06 (2H, d) ppm

^{13}C NMR (75.5 MHz; D_2O): δ 23.2, 32.6, 37.6, 48.8, 62.3, 64.8, 128.7, 128.9, 129.5, 133.9, 167.2, 176.9 ppm

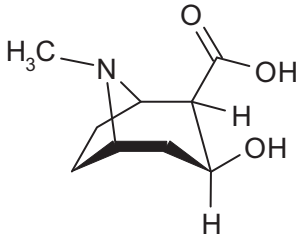
Infrared data:

Principal peaks at wavenumbers 1275, 1720, 1618, 717, 1116, 1316 cm^{-1}

UV Data:

Aqueous acid—234 nm (A¦ = 376), 274 nm

Ecgonine



Synonyms: [1R-(*exo,exo*)]-3-Hydroxy-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylic acid

3β-Hydroxy-1α*H*,5α*H*-tropane-2β-carboxylic acid

$C_9H_{15}NO_3$

Molecular Weight: 185.2 (base), 221.7 (hydrochloride)

Melting point: 198° C (base), 246° C (hydrochloride)

Solubilities (1g/ml):	*Base*	*Hydrochloride*
Water	soluble	soluble
Ethanol	slightly soluble	slightly soluble
Diethyl ether	sparingly soluble	
Chloroform	sparingly soluble	

GC-MS data (percentage abundance):

185 (M^+, 9), 124 (33), 96 (82), 82 (100), 57 (54), 42 (89) m/z

NMR data (hydrochloride):

^{1}H NMR (600 MHz; D_2O): δ 1.98-2.19 (4H, m), 2.25-2.41 (2H, m), 2.78 (3H, s), 3.18 (1H, dd, *J* = 2.3, 7.1 Hz), 3.92 (1H, m), 4.10 (1H, d, *J* = 7.3 Hz), 4.41 (1H, m) ppm

^{13}C NMR (150 MHz; D_2O): δ 23.2, 24.0, 35.5, 38.9, 49.5, 60.8, 63.6, 64.6, 176.4 ppm

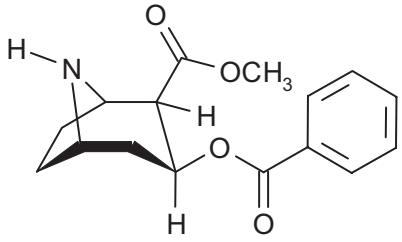
Infrared data:

Principle peaks at wavenumbers 1688, 1210, 1200, 1223, 1134, 1179 cm^{-1} (ecgonine hydrochloride, KBr disk)

UV Data:

Ethanol—275 nm

Norcocaine



Synonyms: 1R-(*exo,exo*)-3-(Benzoyloxy)-8-azabicyclo[3.2.1]octane-2-carboxylic acid methyl ester

$C_{16}H_{19}NO_4$

Molecular Weight: 289.3 (base), 325.8 (hydrochloride)

Melting point: 115-116° C (hydrochloride)

Solubilities (1g/ml):	*Hydrochloride*
Water	soluble
Ethanol	slightly soluble

GC-MS data (percentage abundance):

289 (M^+, 11), 168 (100), 136 (37), 108 (25), 105 (23), 82 (13), 80 (23), 77 (33), 68 (41) m/z

NMR data (hydrochloride):

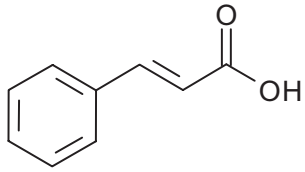
^{1}H NMR (500 MHz; D_2O): (Key spectral data): δ 3.59 (1H, dd), 3.64 (3H, s), 4.38 (1H, bd), 5.56 (1H, ddd), 7.54 (2H, t), 7.71 (1H, t), 7.95 (1H, d) ppm

^{13}C NMR (75.5 MHz; $CDCl_3$): δ 24.4, 25.1, 31.1, 44.9, 53.0, 54.3, 55.4, 65.0, 128.4, 128.8, 129.4, 134.3, 167.0, 173.0 ppm

Infrared data:

Principle peaks at wavenumbers 3597, 3408, 3152, 2951, 2772, 2744, 2527, 1721, 1440, 1350, 1275, 717 cm^{-1}

*Cinnamic acid (*trans-*)*



Synonyms: 3-phenyl-2-propenoic acid

β-phenylacrylic acid

$C_9H_8O_2$

Molecular Weight: 148.2

Melting point: 133° C (base)

Solubilities (1g/ml):	*Base*
Water	slightly soluble
Ethanol	soluble
Diethyl ether	soluble
Chloroform	soluble

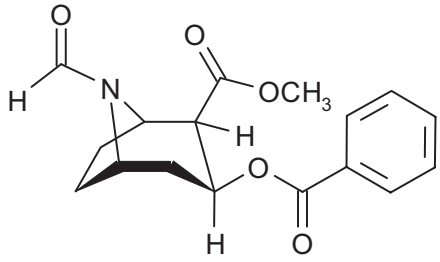
GC-MS data (percentage abundance):

148 (M^+, 74), 148 (100), 147 (100), 131 (22), 103 (61), 77 (47), 51 (40) m/z

UV Data:

Ethanol—273 nm

N-*Formylnorcocaine*



Synonyms: [1R-(*exo,exo*)]-3-(Benzoyloxy)-8-formyl-8-azabicyclo[3.2.1]octane-2-carboxylic acid methyl ester

N-Formylcocaine

$C_{17}H_{19}NO_5$

Molecular Weight: 317.3

GC-MS data (percentage abundance):

289 (38), 195 (39), 168 (100), 136 (42), 105 (94), 77 (58), 68 (48) m/z.

NMR data:

^{1}H NMR (600 MHz; $CDCl_3$): Double resonances observed due to restricted rotation about the amide bond. Rotamers present in ca. 1:1 ratio at room temperature. Key spectral data:

Rotamer A δ 2.37 (1H, ddd), 3.25 (1H, bdd,), 3.65 (3H, s), 4.30 (1H, bd), 4.81 (1H, m), 5.53 (1H, ddd,), 8.02 (0.5H, s) ppm

Rotamer B δ 2.53 (1H, ddd), 3.17 (1H, bdd), 3.68 (3H, s), 4.27 (1H, m), 4.95 (1H, bd), 5.49 (1H, ddd), 8.16 (1H, s) ppm

^{13}C NMR ($CDCl_3$): δ 26.9, 27.4, 27.9, 28.4, 33.4, 35.4, 48.5, 48.7, 49.3, 51.1, 51.9, 52.0, 53.6, 55.5, 66.2, 66.3, 128.4, 129.6, 129.7, 133.3, 157.8, 158.0, 165.6, 165.7, 169.6, 170.0 ppm

For additional details related to the substances, the reader is referred to the *Multilingual Dictionary of Narcotic Drugs and Psychotropic Substances Under International Control* (http://www.unodc.org/unodc/en/scientists/multilingual-dictionary-of-narcotic-drugs-and-psychotropic-substances-under-international-control.html.html), the widely used Merck Index [1] and *Clarke's Analysis of Drugs and Poisons* [2].

4. Illicit production of cocaine

4.1 Production from coca leaves

The production of illicit cocaine can be achieved in a number of ways. The details outlined here represent one of the approaches to the illicit production of cocaine [3]. In illicit production, variation of technique, reagents and quantities are to be expected.

The first step involves the extraction of the crude coca paste from the coca leaf. The coca leaves are stripped from the plant. The fresh or dried leaves are mixed with water and lime. The alkaline mixture is crushed and kerosene, or some other hydrocarbon, is added so as to extract the cocaine from the leaves. The kerosene contains the coca alkaloids and may additionally contain waxy material from the leaves. This waxy material can be removed by heating then cooling the kerosene mixture which results in solidification of the unwanted wax. The kerosene is separated from the coca leaves and wax.

The kerosene is then back-extracted with dilute acidified water (such as sulphuric acid). The acid converts the cocaine free base to cocaine sulphate and this process extracts the alkaloids into the aqueous layer. At this point the kerosene is discarded and the aqueous layer is made alkaline with lime or ammonia. This converts the cocaine sulphate back to the free-base, which results in precipitation of crude cocaine, the more basic alkaloids as well as inorganic salts. The product is then filtered and dried to give coca paste.

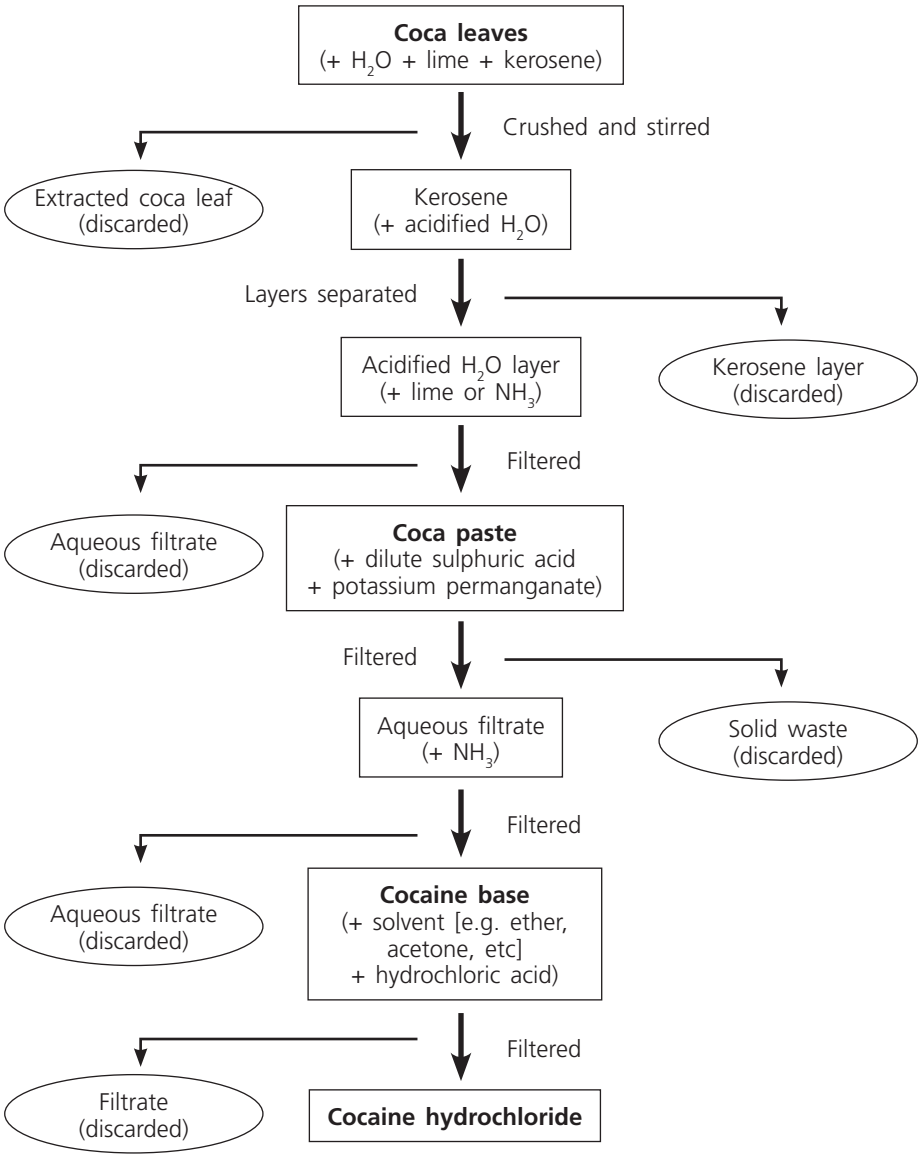
Another technique employed in the extraction of the crude coca paste from the coca leaf is the acid extraction method. The coca leaves are directly extracted with dilute sulphuric acid which converts the cocaine free base to cocaine sulphate. The mixture is filtered and to the aqueous layer is then added excess lime or carbonate resulting in precipitation of crude coca paste. The coca paste is then back extracted with kerosene and the kerosene layer is treated as mentioned above in the earlier method.

The second step in the production of illicit cocaine is the purification of the coca paste to cocaine base. The coca paste is dissolved in dilute sulphuric acid. The solution has a yellow-brown colour and is treated with potassium permanganate. The potassium permanganate is slowly added until the solution turns from a yellow-brown colour to a colourless liquid. The purpose of the addition of potassium permanganate is to oxidise the cinnamoylcocaine isomers present in the cocaine. The oxidation process also makes the cocaine whiter in appearance. The solution is filtered, and

the filtrate is made basic with ammonia, resulting in precipitation of cocaine base and other alkaloids. The cocaine base is filtered, washed with water and dried.

The final stage of the production involves the conversion of the crude cocaine base to cocaine hydrochloride. The cocaine base is dissolved in diethyl ether. The solution is filtered and concentrated hydrochloric acid and acetone are added, resulting is precipitation of cocaine hydrochloride. The cocaine hydrochloride is then filtered and dried.

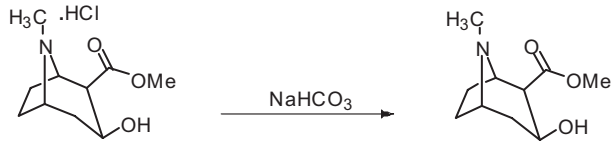
Figure 1. Flow chart showing the illicit production of cocaine from coca leaves



4.2 Chemical synthesis of cocaine

One of the routes for synthesizing cocaine from the starting material, methyl ecgonine hydrochloride is described as follows [4]:

Step 1. Conversion of methyl ecgonine hydrochloride to methyl ecgonine base

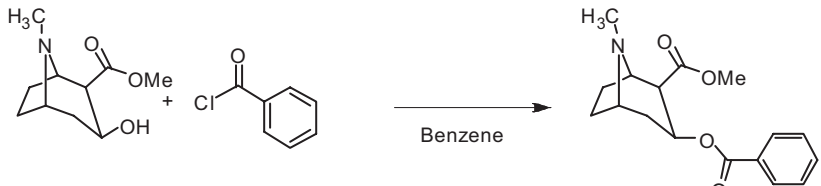


$C_{10}H_{18}ClNO_3^+$
Exact Mass: 235.10

$C_{10}H_{17}NO_3$
Exact Mass: 199.12

Methyl ecgonine hydrochloride (5.0 g) is dissolved in aqueous $NaHCO_3$ solution (10%, 100 ml) and extracted with chloroform (5 x 50 ml). The chloroform layer is washed with brine (2 x 50 ml), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to yield an oil. The oil is then dried under high vacuum for 2 hours to yield methyl ecgonine as an oil (3.62 g, 85%).

Step 2. Benzoylation of methyl ecgonine to cocaine base



$C_{10}H_{17}NO_3$
Exact Mass: 199.12

C_7H_5ClO
Exact Mass: 140.00

$C_{17}H_{21}NO_4$
Exact Mass: 303.15

Methyl ecgonine base (3.62 g) is dissolved in dry benzene (200 ml) and cooled in an ice bath. Benzoyl chloride (3.05 g) in dry benzene (50 ml) is added slowly to the methyl ecgonine solution and the mixture is refluxed for 16 hours. The reaction mixture becomes more viscous and white crystals of cocaine HCl are observed to precipitate. The mixture is cooled and extracted with 1M aqueous HCl solution (3 x 50 ml). The combined aqueous HCl extract is then basified with aqueous ammonia solution and the resulting white precipitate of cocaine base is filtered, and washed with water. The cocaine base is dissolved in diethyl ether, washed with water (1 x 50 ml), brine (1 x 50 ml), dried (Na_2SO_4), filtered and concentrated under reduced pressure to a white solid. The solid is purified by flash column chromatography (eluting with $CHCl_3$/EtOAc 80/20) to yield cocaine base (2.23 g, 41% yield). The cocaine base is recrystallized from n-hexane to give white needle like crystals. The crystals are then ground and dried under high vacuum at 40° C to give cocaine base (1.57 g, 29% yield).

5. Qualitative and quantitative analysis of materials containing cocaine

Generally, in attempting to establish the identity of a controlled drug in suspect material, the analytical approach must entail the determination of at least two uncorrelated parameters, one of which should provide information on the chemical structure of the analyte (for example, IR, MS; or tandem methods such as GC-MS).

It is recognized that the selection of these parameters in any particular case would take into account the drug involved and the laboratory resources available to the analyst. It is also accepted that unique requirements in different jurisdictions may dictate the actual practices followed by a particular laboratory.

5.1 Sampling

The principal reason for a sampling procedure is to permit an accurate and meaningful chemical analysis. Because most methods—qualitative and quantitative—used in forensic drug analysis laboratories require very small aliquots of material, it is vital that these small aliquots be representative of the bulk from which they have been drawn. Sampling should conform to the principles of analytical chemistry, as laid down, for example, in national pharmacopoeias or by regional or international organizations. For general aspects of qualitative sampling of multi-unit samples, refer to the *Guidelines on Representative Drug Sampling* (http://www.unodc.org/unodc/en/scientists/publications_manuals.html). For seized material with obvious external characteristics, a sampling method based on the Bayes' model may be preferred over the hypergeometric approach.

The use of an approved sampling system also helps to preserve valuable resources and time by reducing the number of determinations needed. It is recognized that there may be situations where, for legal reasons, the normal rules of sampling and homogenization cannot be followed.

5.2 Analysis of coca leaf

Coca leaf, being a vegetable product, requires a different analytical approach to that to be applied to the extracted material, whether it be impure coca paste or the purer cocaine. The sampling methods may be used on seizures of coca leaf, provided the analyst varies the sampling procedure to allow for the different physical makeup of leaf material as opposed to powder.

Trafficking in coca leaf is rare (but not unknown) outside those countries where coca is grown. This section has therefore been included in the manual to assist the analyst on those rare occasions when he or she may be required to deal with this material.

The identification of both coca leaf and powdered coca leaf material should be by a two part process—botanical and chemical. Ideally the analyst should be trained in both botany and chemistry and should have appropriate reference materials for both techniques.

5.2.1 Physical identification

(i) Whole coca leaf

This is described in section 2. Confirmation should include microscopy.

(ii) Powdered coca leaf

This may be identified by microscopy. The standard textbooks dealing with powdered vegetable drugs usually contain a section devoted to coca leaf (e.g. *Trease and Evans Pharmacognosy*, 15th edition, W.C. Evans, W.B. Saunders, 2002, 349 and 529).

5.2.2 Chemical analysis of coca leaf (whole or powdered)

Short immersion in boiling ethanol produces effective extraction of ecgonine-type alkaloids and minimizes the breakdown of cocaine. Extraction with hot methanol has also been found to be effective.

If quantitative extraction of the alkaloids is not required, a short extraction at room temperature may be sufficient. The leaves (preferably chopped or powdered) may be pounded with ethanol or methanol in a mortar.

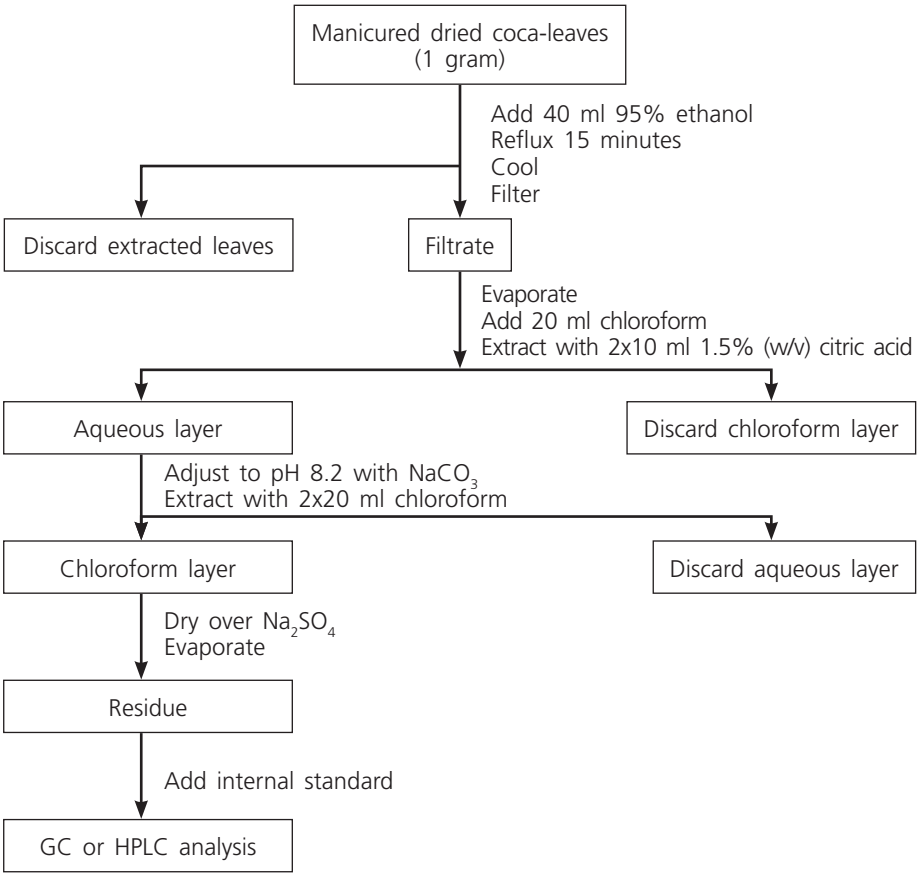
The alcoholic extract is subjected to TLC (with visualization by spray reagent) or GC-MS for qualitative analysis of the coca leaf (see sections 5.3.2 and 5.3.3 for detailed procedures).

For quantitative analysis, a systematic extraction procedure follows the scheme illustrated below (figure 2) [5]. The resulting extract may be subjected to GC or HPLC analysis to estimate the cocaine/alkaloidal content of the coca leaf (see sections 5.3.4 and 5.3.5 for detailed procedures). Typical amounts of cocaine present in dried coca leaf could be found in table 1.

Table 1. Typical amounts of cocaine present in three *Erythroxylon* varieties

Erythroxylon variety	*Typical cocaine content in dry leaf*
coca var. ipadu	0.11-0.41%
novo var. novogranatense	0.17-0.76%
novo var. truxillense	0.42-1.02%

Figure 2. Flow chart showing the extraction procedures for the chemical analysis of coca leaves



5.3 Analysis of coca paste and cocaine

Cocaine exhibits generally come in powder form and commonly contain adulterants or cutting agents such as levamisole (and/or tetramisole) phenacetin, lidocaine, caffeine, diltiazem, hydroxyzine, procaine, benzocaine or sugars (e.g. mannitol, lactose or glucose).

For cocaine containing exhibits, combinations of testing methods such as Scott's colour test, TLC, FTIR, GC coupled with FID and/or MS and HPLC are considered to be appropriate tests for positive identification of cocaine. Recommended minimum guidelines for method selection have been formulated by the Scientific Working Group on Drugs (SWGDRUG) [8].

In cases where the cocaine is present in other matrices such as clothing, synthetic resins or liquids it must be first extracted before these tests are applied.

5.3.1 Presumptive tests for cocaine

Presumptive tests are fast screening procedures that are designed to provide an indication of the presence or absence of drug classes in the test sample and quickly eliminate negative samples. Good presumptive test techniques, as all analytical techniques, maximize the probability of a "true" result, and minimize the probability of a false positive. However, presumptive tests are not considered sufficient for drug identification and results must be confirmed by additional laboratory tests.

5.3.1.1 Colour test [9,10]

Colour reactions are produced by compounds with a particular chemical structure. The colour obtained in any particular test may vary depending on the conditions of the test, amount of substance present and extraneous material in the test sample. Colour test reagents must be checked with known substances when prepared. A blank should be run to preclude false positive results.

It must be stressed that positive results to colour tests are only a presumptive indication of the possible presence of cocaine. The colour tests for cocaine are especially prone to produce false positives. Many other materials, often harmless and uncontrolled by national legislation or international treaties, may give similar colours with the test reagents. A number of these are either other controlled drugs, often encountered as white powders (e.g. methaqualone), or the synthetic local anaesthetics which are often substituted for cocaine in the illicit traffic. It is mandatory for analysts to confirm such results by the use of alternative techniques.

The colour test described below is known as the Scott's Test (modified Cobalt Thiocyanate Test).

Reagent 1: Dissolve 1.0 g cobalt thiocyanate in 50 ml of 10% (vol/vol) acetic acid, then add 50 ml of glycerine

Reagent 2: Hydrochloric acid (concentrated)

Reagent 3: Chloroform

Method:

Step 1: Place a small amount (no more than 1 mg) of the suspected material in a test tube. Add 5 drops of Reagent 1 and shake the test tube for 10 seconds. Cocaine and related substances produce a blue precipitate and a blue solution.

Step 2: Add a drop of Reagent 2 and shake the mixture for a few seconds. The blue solution should turn pink. If the blue colour still does not change, add one further drop. If still no change, repeat the test with a smaller portion of suspected material.

Step 3: Add 5 drops of Reagent 3 and shake. If cocaine is present, the lower chloroform layer will develop an intense blue colour, while the upper layer will be pink.

Results:

A positive result at each stage is required in order to be considered as a positive test for cocaine. Only a few non-controlled or controlled drugs will give a similar colour sequence.

Analytical notes

- It is important to use less than 1 mg of sample.
- The amounts used of Reagents 1 and 3 are not critical. However, the ratio of Reagent 1 to 2 is critical. If excess hydrochloric acid is added to Reagent 1 after the blue colour has developed with cocaine, a blue rather than pink solution will result; this blue will not be extracted into the chloroform layer. If excess cocaine is used in Step 1, then it is sometimes necessary to add 1-2 drops of hydrochloric acid; no more should be used.

5.3.1.2 Odour test [11]

As is typical of many drugs, cocaine's biological activity is not matched by a comparable degree of chemical reactivity. However, it is unique among commonly abused drugs in being a benzoate ester. While colour tests for this functional group are not available, the lower alkyl esters of benzoic acid have quite distinctive odours detectable at very low concentrations relative to the average colour test. The transfer of the benzoate function of cocaine from methyl ecgonine to methanol is readily accomplished in the presence of dry methanolic potassium or sodium hydroxide.

Evaporation of the excess methanol leaves a residue containing methyl benzoate readily apparent by its odour.

Reagent:

Dissolve 1 g of potassium or sodium hydroxide in 20 ml of methanol to give methanolic sodium or potassium hydroxide.

Method:

Thoroughly moisten dried test material with the reagent. After allowing excess alcohol to evaporate, compare the odour characteristic of the sample with that of standard cocaine material.

Analytical notes

- Over one hundred drugs were tested for positive interference and only piperocaine (also a benzoate ester) gave a positive result. Certain amines such as amphetamines will produce a "weak, fishy odour".
- The sensitivity is greater than that of existing field tests, e.g. the Scott's Test.
- The sample and reagent must be kept free of water which interferes with the reaction.
- Ideally the test should be performed concomitantly with a test on standard cocaine material and the odours compared.

5.3.1.3 Microcrystal tests [12]

Microcrystal tests are quick, simple and extremely sensitive test for the identification of substances. They involve the formation of crystals from the reaction of the target compound with a chemical reagent, followed by the analysis of the resulting crystals by means of a polarizing microscope and comparison with reference material.

Platinic chloride test

Reagent: Dissolve 1 g of platinic chloride in 20 ml of distilled water.

Method:

Place 2 drops of sample solution (approximately 2-3 mg of the sample/5 drops of 10% hydrochloric acid) on a clean microscope slide. Then place 2 drops of the reagent near the drops of the sample and use a glass rod to create a tiny channel connecting the solutions. Observe the reaction and resulting crystals without a cover slip at 100-200 magnification using a polarized microscope.

Results:

Cocaine forms V-shaped long, thin needles with branching.

Figure 3. Cocaine and platinic chloride, 100x

Source: Reprinted, with permission, from *Journal of Forensic Sciences*, vol. 48, Issue 3, copyright ASTM International, 100 Barr Harbor Drive, West Conshohocken, PA 19428.

Gold chloride test

Reagent: Dissolve 1 g of gold chloride in 20 ml of distilled water.

Method:

Place 2 drops of sample solution (approximately 2-3 mg of the sample/5 drops of 10% hydrochloric acid) on a clean microscope slide. Then place 2 drops of the reagent near the drops of the sample and use a glass rod to create a tiny channel connecting the solutions. Observe the reaction and resulting crystals without a cover slip at 100-200 magnification using a polarized microscope.

Results:

Cocaine forms radiating clusters of fine needles with perpendicular branches.

Figure 4. Cocaine and gold chloride, 200x

Source: Reprinted, with permission, from *Journal of Forensic Sciences*, vol. 48, Issue 3, copyright ASTM International, 100 Barr Harbor Drive, West Conshohocken, PA 19428.

Analytical notes

- Standard cocaine should be analysed concomitantly.
- The dilution of the test material or the hydrochloric acid may be varied to give the optimum results.

5.3.1.4 Solubility tests

If it is suspected that the material has cutting agents added to it, then a solubility test is typically carried out. The solubility of a small amount of the material in water and in ethanol may give an indication of the form in which the drug is present. Cocaine hydrochloride is soluble in water and ethanol. Whereas, cocaine base is soluble in ethanol and almost insoluble in water as are many adulterants. The presence and amount of insoluble material may give some indication of the likely purity, as sugar diluents are largely insoluble in ethanol. The insoluble material may be filtered, dried and subjected to further testing by, for example IR spectroscopy.

Method:

Step 1: Dissolve a portion (approximately 1 g) of the powder or material in approximately 5 ml of distilled or deionized water. For small seizures of 0.1 g, 0.5 ml of water should be used.

Step 2: Dissolve a portion (approximately 1 g) of the powder or material in approximately 5 ml of ethanol. For small seizures 0.1 g, 0.5 ml of ethanol should be used. This will show the presence of any ethanol insolubles such as carbohydrates. The carbohydrates have low solubility in ethanol.

Analytical notes

- This test is most useful when the sample size is large and a considerable quantity of the powder can be used without seriously reducing the total amount of exhibit that can be produced in court.
- It may be used on small seizures by reducing both the amount of test material and solvent.

5.3.1.5 Anion tests

Anion testing for forensic purposes typically makes use of solubilities combined with selected reactions where results are determined by the presence or absence, and solubility, of a precipitate.

The hydrochloride salt of cocaine is by far the most commonly encountered while sulphates salts are rarely encountered in cocaine products with the exception of coca

paste. This test should be confirmed, if possible, by IR spectroscopy and/or X-ray diffraction methods.

Chlorides—Silver nitrate test

Reagent: Dissolve 1.0 g of silver nitrate in 20 ml of distilled water (to result in a 5% aqueous silver nitrate solution).

Method:

Dissolve a small amount of solid material in distilled water. Test pH with indicator paper, and acidify if necessary with a few drops of nitric acid. Add 1-2 drops of reagent and observe for precipitate. If a white or yellow precipitate develops, add ammonia until alkaline.

Results:

Solutions of chlorides, when treated with silver nitrate solution, yield a white curdy precipitate which is insoluble in nitric acid. After washing with water, the precipitate is soluble in ammonia solution, from which it can be re-precipitated by the addition of nitric acid.

Sulphates—Barium chloride test

Reagent: Dissolve 1.0 g of barium chloride in 10 ml of distilled water. (to result in a 10% aqueous barium chloride solution).

Method:

Dissolve a small amount of material in distilled water, acidify with a few drops of dilute hydrochloric acid and then add 1-2 drops of reagent.

Results:

Solutions of sulphates, when treated with barium chloride solution, yield a white precipitate which is insoluble in hydrochloric acid.

Analytical notes

- Washing the precipitate with water before performing the test for dissolution of the precipitate is critical to remove any soluble (non-precipitated) anion.
- A blank should be carried out concurrently to preclude false positive results.

5.3.2 Thin Layer Chromatography (TLC)

TLC is a commonly used technique for the separation and identification of illicitly manufactured drugs. It is inexpensive, rapid, sensitive (sub-milligram quantities of analyte required), flexible in the selection of both the stationary and mobile phase and amenable to a wide variety of substances, in base and salt form, ranging from the most polar to non-polar materials.

TLC plates (stationary phases)

Coating: Silica gel G with layer thickness of 0.25 mm and containing an inert indicator, which fluoresces under UV light wavelength 254 nm (silica gel GF254).

Typical plate sizes: 20 x 20 cm; 20 x 10 cm; 10 x 5 cm (the latter should be used with the 10 cm side vertical with the TLC tank).

Plates prepared by the analyst must be activated before use by placing them into an oven at 120° C for at least 10 to 30 minutes. Plates are then stored in a grease-free desiccator over blue silica gel. Heat activation is not required for commercially available coated plates.

Methods:

Solvent systems

Prepare developing solvent system (System A, B or C as shown in table 2) as accurately as possible by use of pipettes, dispensers and measuring cylinders. Leave the solvent system in the TLC tank for a time sufficient to allow vapour phase saturation to be achieved prior to the analysis (with adsorbent paper-lined tanks, this takes approximately 5 minutes).

Table 2. Developing solvent systems suitable for the analysis of cocaine

System	*Solvents*	*Solvent proportions (by volume)*
System A	Chloroform	25
	Dioxane	60
	Ethyl acetate	10
	Ammonia (29%)	5
System B	Methanol	100
	Ammonia (29%)	1.5
System C	Cyclohexane	75
	Toluene	15
	Diethylamine	10

Preparation of standard and sample solutions

Standard and sample solutions are prepared at a concentration of 1 mg per ml in methanol. The form of cocaine standard used, i.e., salt or base, is unimportant as on the TLC plates, compounds move as the free base.

Spotting and developing

Apply as separate spots 1 µl and 5 µl aliquots of sample solution, 2 µl of the standard solutions and 2 µl of solvent (as a negative control) on the TLC plate. Spotting must be done carefully to avoid damaging the surface of the plate.

Analytical notes

- The starting point of the run i.e. the "spotting line" should be 2 cm from the bottom of the plate.
- The spacing between applications of sample (spotting points) should be at least 1 cm and spots should not be placed closer than 1.5 cm to the side edge of the plate.
- To avoid diffuse spots during development, the size of the sample spot should be as small as possible (2 mm) by applying solutions in aliquots rather than a single discharge.
- Allow spots to dry and place plate into solvent-saturated tank (saturation of the vapour phase is achieved by using solvent-saturated pads or filter paper as lining of the tank).
- Remove plate from the development tank as soon as possible as the solvent reaches the development line (10 cm from starting line) marked beforehand; otherwise, diffused spots will occur.

Visualization/detection

The plates must be dried prior to visualization. The solvent can be allowed to evaporate at room temperature or with a hot air blower. In the later case, care must be exercised that no component of interest is thermally labile. It is important for proper colour development that all traces of ammonia or other bases are removed from the plate.

Visualization methods

A. UV light at 254 nm

   Dark spots against a green background are observed.

B. Acidified potassium iodoplatinate reagent

   Dissolve 0.25 g of platonic chloride and 5 g of potassium iodide in distilled water and make up to 100 ml; add 2 ml of concentrated hydrochloric acid to

the resulting solution. Cocaine appears as a blue spot when the plate is sprayed with the reagent.

C. Dragendorff's reagent (Munier)

Solution 1: Dissolve 2 g of bismuth subnitrate in 25 ml of concentrated (glacial) acetic acid and add 100 ml of distilled water.

Solution 2: Dissolve 40 g of potassium iodide in 100 ml of distilled water.

Mix 10 ml of solution 1, 10 ml of solution 2, 20 ml of concentrated (glacial) acetic acid and 100 ml of distilled water to produce Dragendorff's reagent. Cocaine appears as an orange spot when the plate is sprayed with the reagent.

Interpretation

After visualization, mark spots (e.g. by pencil) and calculate retardation factor (R_f) values.

$$R_f = \frac{\text{Migration distance: from origin to centre of spot}}{\text{Development distance: from origin to solvent front}}$$

Results

	Developing System (R_f x 100)		
Compound	*A*	*B*	*C*
Cocaine	81	59	56
Ecgonine	0	84	0
Methylecgonine	61	65	44
Benzoylecgonine	0	25	0
Cinnamoylcocaine	83	59	51
Tetracaine	63	56	25
Benzocaine	77	80	11
Lidocaine	77	69	40-55 (s)*
Pethidine	61	49	69
Methaqualone	81	78	38
Methadone	75	31-45 (s)*	74
Procaine	61	55	8-16 (s)*

*(s): streak, not spot produced on the TLC plate.

Analytical notes

- R_f values are not always reproducible due to small changes in plate composition and activation, in solvent systems, tank saturation or development distance. Therefore, the R_f values provided are indications of the chromatographic behaviour of the substances listed.
- It is essential that reference standards be run simultaneously on the same plate.
- For identification purposes, both the R_f value and the colour of the spots after spraying with the appropriate visualization reagents should always be considered.

5.3.3 Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS is one of the most commonly used techniques for the identification of forensic drug samples. As a hyphenated technique, it unifies the separation power and sensitivity of a GC with the analyte specificity of a spectroscopic technique. It can provide high specific spectral data on individual compounds in a complex mixture without prior isolation.

Sample preparation and extraction procedure

Solid samples are pulverized and homogenized with a mortar and pestle. A suitable quantity of sample material is extracted with an appropriate solvent (e.g., methanol, chloroform or 1:1 methanol:chloroform) to approximately 1 mg/ml sample solution. Some sample presentations and analytes may necessitate the use of other solvents or solvent mixtures.

Preparation of standard solutions

Prepare a standard solution of cocaine at a concentration of 1 mg/ml with an appropriate solvent (e.g., methanol, chloroform or 1:1 methanol:chloroform).

Preparation of Internal Standard (IS) e.g. benzopinacolone (for Retention Locking if required)

Dissolve 50 mg of 2,2,2-triphenylacetophenone (benzopinacolone) in 1l of an appropriate solvent (e.g. chloroform or 1:1 chloroform:methanol). Add an aliquot of the internal standard to the sample/standard solution if retention time locking of the analysis is required.

GC-MS operating conditions

GC oven conditions:	Column temperature initially set at 60° C and held isothermal for 3 minutes immediately after injection and ramped to 300° C at a rate of 40° C/min with a final isotherm of 6 minutes.
Column:	DB-5MS, HP-5MS, 30 m x 250 µm; d_f 0.25 µm.
Inlet:	Mode: splitless (purge flow 60 ml/min at 0.5 min); Temp: 240° C; Carrier gas: Helium, 1 ml/min, constant flow.
Detector:	Ionization mode: EI mode, 70 eV; Transfer line temp: 300° C; Ion source temp: 230° C.
MS parameters:	Solvent delay 3.00 minutes; Scan parameters: TIC, Scan range: 40–450 amu at 1 scan/sec.

Note: The above conditions may be altered as long as appropriate validation is carried out.

Results:

Identification is accomplished by comparing the retention time and mass spectrum of the analyte with that of a reference standard. All compounds identified by GC-MS and reported by the analyst must be compared to a current mass spectrum of the appropriate reference standard, preferably obtained from the same instrument, operated under the same conditions. Commercial mass spectral libraries or user generated spectra should be used for reference purposes only.

GC retention times for cocaine and known adulterants using the above operating conditions are as follows:

Compound	*GC RT (mins)*	*Base, P1, P2, M^+ ions (m/z)*
Benzocaine	7.59	120, 165M^+, 92, 65
Phenacetin	7.97	108, 109, 179M^+
Caffeine	8.40	194M^+, 109, 55, 67
Lignocaine	8.53	86, 58, 30, 234M^+
Levamisole	8.83	204M^+, 148, 73, 101
Procaine	8.91	86, 99, 120, 236M^+
Cocaine	*9.36*	*82, 182, 94, 303*M^+
Benzopinacolone (IS)	11.11	243, 165, 105, 348M^+
Hydroxyzine	11.83	201, 165, 299, 374M^+
Diltiazem	12.36	58, 71, 73, 414M^+

5.3.4 Gas Chromatography (GC) with flame ionization detection (GC-FID)

The GC instrument of choice for routine analytical work is the narrow bore capillary gas chromatograph, using columns with internal diameter between 0.2 and 0.32 mm. The packed column technique is no longer included in this manual as GC systems are now typically equipped with capillary columns. Information on packed column techniques for the analysis of cocaine can be found in an earlier edition of this manual.

The method provided below for the quantitative GC-FID analysis of cocaine does not require derivatization and is described here for general use.

Single standard method without derivatization [13]

This method involves the preparation of only one cocaine standard solution in a concentration similar to the anticipated concentration of the analyte.

Preparation of internal standard solution (IS)

Dissolve isopropylcocaine hydrochloride (IPC) in chloroform to give a concentration of 1.0 mg/ml. The solution is stored at 4° C when not in use. Solutions can be stored for one year at 4° C without detectable degradation. The solution should be warmed to room temperature before use. Each analysis requires 5 ml.

Preparation of standard solution

Accurately weigh 20 mg of cocaine (hydrochloride or base) standard and transfer quantitatively into a 50 ml Erlenmeyer flask. Accurately transfer 5 ml of internal standard solution to the flask followed by 20 ml of chloroform containing 50 µl of diethylamine. Allow the solution to sit for 5 minutes and then inject 1-2 µl of the solution into the gas chromatograph.

Preparation of sample solution

Obtain a representative sample from the seized material. Grind and homogenize to a fine powder. Accurately weigh a quantity of seized material containing approximately 20 mg of cocaine hydrochloride and transfer quantitatively to a 50 ml Erlenmeyer flask. Accurately transfer 5 ml of internal standard solution to the flask followed by 20 ml of chloroform containing 50 µl of diethylamine. Allow the solution to sit for 5 minutes and then inject 1-2 µl of the solution into the gas chromatograph.

GC operating conditions

Detector:	FID
Column:	HP-1 or equivalent, 30 m length, 0.25 mm ID, 0.25 µm film thickness
Carrier gas:	Hydrogen 1.1 ml/min
Injector temp:	280° C
Detector temp:	280° C
Oven temp:	250° C
Injection volume:	2 µl
Split ratio:	25:1
Run time:	7 min

Calculations

The percentage of cocaine (as base) in the sample can be calculated using the general formula below:

$$\text{Content (\%)} = \frac{Wsamp_{calc}}{Wsamp_{nom}} \text{ x } 100$$

where

$Wsamp_{calc} = \frac{PAR_{sam}}{PAR_{std}}$ x W_{std} = calculated weight of analyte in the sample

$Wsamp_{nom}$ = nominal amount of sample used in preparation of the sample solution

$$PAR_{sam} = \frac{\text{Area of cocaine peak in sample solution}}{\text{Area of internal standard peak in sample solution}}$$

$$PAR_{std} = \frac{\text{Area of cocaine peak in sample solution}}{\text{Area of internal standard peak in sample solution}}$$

W_{std} = amount of standard (as base) used in preparation of the standard solution

Results

Elution order and the corresponding relative retention time with respect to cocaine are as follows:

Compound	*Relative retention time (min)*
Ecgonine methyl ester	0.48
Benzocaine	0.49
Acetaminophen	0.52
Caffeine	0.61
Lidocaine	0.65
Procaine	0.76
Cocaine	1.00
Isopropylcocaine (IS)	1.14
cis-Cinnamoylcocaine	1.36
trans-Cinnamoylcocaine	1.78
Benzoylecgonine	1.89

Analytical notes

- This method can be modified from a single to a multiple standard method by sequentially diluting aliquots of a concentrated cocaine standard stock solution with the internal standard solution using volumetric flasks.
- The use of a structurally related internal standard such as IPC maximizes precision and accuracy. If they are not available, alternative internal standards such as n-eicosane, n-tetracosane or tetraphenylethylene may be used. Selection of a suitable solvent will be required to ensure that both the internal standard and the cocaine sample are fully dissolved.

5.3.5 High Performance Liquid Chromatography (HPLC)

In addition to GC, HPLC is another major separation technique commonly used in forensic drug analysis. For ease of sample preparation, best reproducibility and detectability, reversed phase chromatography is recommended for the analysis of cocaine. The most universal and versatile column is a bonded octadecyl silica column (C18). Column length, diameter, particle size, pore size and carbon load should be considered before final selection of the column.

Since there is a large variety of stationary and mobile phases available to the analyst, one method for quantitative HPLC method is described below where it can be modified for best performance. All methods must be properly validated and/or verified prior to routine use.

Method

Preparation of cocaine standard and sample solutions

Dissolve an appropriate amount of standard or sample in the mobile phase, targeting a concentration of the cocaine between 0.05-0.50 mg/ml.

Stock and standard solutions must be prepared from reference standards. Working standards should be within the linear range of the detector and approximately 80-120% of the target concentration. Multiple point calibration is desirable but a single standard method is also acceptable.

Chromatographic conditions

Column:	C18 Hypersil (or equivalent), 160 mm x 5.0 mm ID
Mobile phase:	methanol:water:1% phosphoric acid:n-hexylamine (300:700:1000:14 by volume; pH = 2.5)
Flow rate:	2.0 ml/min (slower flow values should be considered with shorter columns and smaller particle sizes)
Detection:	UV at 230 nm
Injection volume:	5-20 µl

Quantitation

External or internal standard calibration may be used. The use of peak area for HPLC quantitation is recommended, because peak broadening (decreases in peak height) may occur as a result of deterioration of the stationary phase, rendering peak height unsuitable for quantitation.

Results

Elution order and the corresponding capacity factor (k) are as follows:

Compound	*Capacity factor (k)*
Procaine	0
Lignocaine	0.79
Cocaine	2.68
Benzoylecgonine	5.68
cis-Cinnamolycocaine	6.30
Amylocaine	7.19
Butacaine	8.97
trans-Cinnamoylcocaine	10.65
Benzocaine	20.06

5.3.6 Fourier Transform Infrared (FTIR) Spectroscopy

The confirmation of the identity of a substance can be achieved by FTIR. Unequivocal identification of cocaine is thus possible from each unique spectrum. For powders, considered from prior chromatographic analysis to be reasonably pure, the infrared spectrum of the powder may be run directly in a KBr disc for comparison with those of cocaine free bases or HCl salts.

Analytical notes

- The KBr disc method consists of grinding a dry sample to a very fine powder, then mixing about 2 mg of homogenized sample powder with 200 mg of carefully dried and ground KBr. After grinding, the mixture is pressed into a thin transparent disk.
- KBr should be "IR Grade" and dried at 105° C for a minimum of one hour. It can be stored in a desiccator containing a strong desiccant (silica gel) or left in the oven and removed when required.

Results

Major peaks occur at the following wavenumbers (cm^{-1}) which are listed in order of magnitude of absorbance. The sequence may, however, vary from sample to sample.

Compound	*Principal peaks at wavenumbers*
Cocaine base	1275, 1700, 1106, 1728, 710, 1040, 1280 cm^{-1}
Cocaine HCl	1712, 1730, 1276, 1230 (side peak), 732, 1106, 1075, 1025 cm^{-1}

Cis- and *trans*-cinnamoylcocaine could be differentiated by:

- The large absorbance at 1320 cm^{-1} in the spectrum of *trans*-cinnamoylcocaine is absent in the spectrum of *cis*-cinnamoylcocaine.
- In the spectrum of *trans*-cinnamoylcocaine the absorbance at 1625 cm^{-1} is of about the same magnitude as the absorbance at 1745 and 1695 cm^{-1}, whereas in the spectrum of *cis*-cinnamoylcocaine the absorbance at 1625 cm^{-1} is smaller than the absorbances at 1745 and 1715 cm^{-1}.

5.3.7 Ultraviolet (UV) Spectrophotometry

Cocaine show the following absorption peaks in aqueous acid media: 233 nm {A (1% 1 cm) = 430}, 275 nm.

5.4 The analysis of cocaine enantiomers

Four pairs of enantiomers can be predicted from the structural formula of cocaine. Each member of a given pair of enantiomers has a diastereoisomeric relationship to members of all the other pairs. All diastereoisomers have been synthesized and their configurations and conformations determined by various methods. The only natural occurring cocaine enantiomer is the l-cocaine.

Many methods have been developed to differentiate these cocaine enantiomers as certain countries control only the l-cocaine enantiomer. The microcrystal test method is described in detail below:

Microcrystal test

Reagents

1. Dissolve 10 mg of di-p-toluoyl-d-tartaric acid (TDTA) in 1 ml of ethanol in a 10 ml volumetric flask. Add 1 ml of glycerine and top up to mark with distilled water.
2. Dissolve 10 mg of di-l-toluoyl-d-tartaric acid (TLTA) in 1 ml of ethanol in a 10 ml volumetric flask. Add 1 ml of glycerine and top up to mark with distilled water.

Analytical notes

- Crystals form in reagents after about three months. Fresh solutions should be made if old ones fail to give results with authentic cocaine.
- If cocaine is not present as the hydrochloride salt, it must be converted to this form.

Method

Place 1 drop of reagent onto the microscope slide, then add a small quantity of sample to the reagent and stir. View the resulting crystals with a microscopic magnification of about 100.

Results

With TDTA, l-cocaine HCl, after about 1 minute gives almost perfectly symmetrical rosettes. The crystals, when first formed, will be greyish-white to white under polarized light. After growing for a few minutes, some rosettes will exhibit different colours, (red, blue, green, yellow) on the arms of the rosettes, depending on orientation.

With TLTA, l-cocaine HCl immediately forms greyish-white crystals. The formation of these crystals varies from a multitude of single needles, to tufts, to fan-shaped, to sheaves.

D-cocaine HCl gives the completely opposite crystal formation as l-cocaine HCl, i.e. after about one minute, it gives almost perfectly symmetrical rosettes with TLTA and crystals varying from single needles, to tufts, to fan-shaped, to sheaves with TDTA.

Alternative methods to differentiate cocaine enantiomers

There are a variety of other methods and instrumentation (e.g. HPLC, GC, GC-MS, TLC, IR and NMR) that could be used to differentiate cocaine enantiomers which would not be mentioned here. Please refer to references [15], [16] and [17] for more information.

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United Nations publication
Printed in Austria
ST/NAR/7/REV.1

V.11-87637—March 2012—300