RECOMMENDED METHODS FOR TESTING

COCAINE

MANUAL FOR USE BY NATIONAL NARCOTICS LABORATORIES

UNITED NATIONS
DIVISION OF NARCOTIC DRUGS

DIVISION OF NARCOTIC DRUGS
Vienna

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INTRODUCTION

Background

Over the past few years there has been a considerable increase in the number of scheduled substances newly included under international control. This increase reflects a rapid diversification of drugs of abuse, and the consequent increase of regulatory efforts results in turn in a larger number of controlled substances and in better but, at the same time, more stringent national legislation and sentencing provisions. At the same time, the seized quantities of drugs already under control, such as the opiates, cocaine and coca paste, cannabis products, amphetamine and related compounds have also shown an alarming and unprecedented increase in certain regions. This new situation, involving an increase both in the frequency and volume of seizures, presents a challenge not only to national law enforcement authorities, but also to the technical and scientific staff of forensic laboratories.

Owing to the ingenuity of illicit producers and promoters, unexpected new illicit drugs or combinations of drugs appear on the illicit market, requiring rapid and adequate action as well as ingenuity on the part of forensic chemists. Similarly, the increased number of controlled substances and of related legislative provisions place additional pressure on national forensic and narcotic laboratories and their staff. Analysts have to be able to deal with more substances and preparations and to use faster, more accurate and more specific methods of identification and analysis. In addition, the international character of drug trafficking requires the speedy exchange of analytical data between laboratories and law enforcement authorities both on the national and the international levels. Development of internationally acceptable methods of testing would contribute greatly to the achievement of these objectives, and this possibility has been under consideration for some time.

At its eighth special session in February 1984, the Commission on Narcotic Drugs requested the Secretary-General "to investigate the possibility of reaching agreement at the regional and interregional levels on recommended methods of analysis of drugs seized from the traffic". The Commission was of the opinion that closer scrutiny and harmonisation of the wide variety of analytical methods in use at the national level would not only ease the task of the staff of national institutions but would also facilitate the exchange of information at regional and interregional levels.

Purpose of the manual

In response to the Commission's request, a group of fifteen experts was convened in October 1985 by the Division of Narcotic Drugs in Wiesbaden at the invitation of the Federal Republic of Germany. The present manual prepared by the United Nations Division of Narcotic Drugs reflects the conclusions of the group of experts and has been designed to provide practical assistance to national authorities by describing recommended methods to be used in forensic laboratories for the identification and analysis of illicit coca products. The manual may also serve as a guide to national authorities in assessing existing methods used within their own government and university laboratories.

This manual is the second in a series of similar publications dealing with the identification and analysis of various groups of drugs under international control; it was preceeded by a manual on heroin analysis (ST/NAR/6) and will be followed by a similar publication covering the analysis of illicit cannabis products.

These manuals suggest approaches that may help the forensic analyst to select a technique appropriate to the sample currently being examined. The analyst may then choose to follow any of the methods described in the manual, as each method can be expected to produce reliable analytical information with respect to the samples to which they are applied. Each method has been used for a number of years in reputable forensic laboratories and has been published in the scientific literature. In identifying these methods, the expert group was aware that many other useful and acceptable methods produce worthwhile analysis and information for the forensic analyst, and that a number of other acceptable options are recorded in the forensic scientific literature.

Use of the manual

Few methods are perfect, least of all in forensic drug analysis where the materials under examination are very likely to show significant variation both in their physical form and chemical composition. The choice of methodology and approach to analysis remains within the control of the analyst working within his or her own country. The analyst alone has seen the suspect material and can best judge the correct approach to the problem at hand. Furthermore the choice of methods may necessarily depend on the availability of reference materials and of instrumentation.

Not all the methods listed need to be applied to all samples suspected to contain cocaine. Requirements may vary, for example, as a result of local trends in samples encountered, facilities available, and the standard of proof acceptable in the prosecution system within which the analyst works. The more complex methods are needed only for certain forensic requirements, such as comparison of samples or the development of typology.

In order to establish the identity of any controlled drug, it is suggested that the criteria should be at least two independent analytical parameters. The selection of these parameters in any particular case would take into account the drug involved and the laboratory resources available to the analyst. For example, two uncorrelated TLC systems would count as two parameters. Uncorrelated TLC systems in this context means that either the solvent systems or the coating on the plates are completely different. When possible, three entirely different analytical techniques should be used, for example: colour test, chromatography (TLC, GLC or HPLC) and spectroscopy (IR or UV). The actual choice of parameters is left to the discretion of the chemist.

Attention is also drawn to the vital importance of the availability of textbooks on drugs of abuse and analytical techniques. Furthermore, the analyst must continually keep abreast of current trends in analysis, consistently following current analytical and forensic science literature. For this purpose, attention is drawn to the Multilingual Dictionary of Narcotic Drugs and Psychotropic Substances under International Control (ST/NAR/1), a vital tool for forensic laboratories, and to the Manual on Staff Skill Requirements and Basic Equipment for

Narcotics Laboratories (ST/NAR/2), both published by the Division of Narcotic Drugs. The latter publication lists bibliographic references as well as a selection of well-known journals in the field.

Close liaison with national law enforcement and judicial authorities as well as between national narcotic laboratories and those at the regional level can lead to greater awareness of the latest trends in drug presentation, the illicit traffic, smuggling techniques and the preparation of evidence to courts of law. These in turn will produce a more meaningful choice of analytical techniques to be applied to the latest submissions.

It is equally important that the latest information on changes in drugs available in the illicit traffic be quickly disseminated. This may often need to be done prior to publication in specialised periodicals dealing with forensic and other chemical analyses, since these publications are available to the forensic community some two to three years after the changes become known. The value of frequently published national reports on the latest information on such changes in drugs and on work being undertaken and analytical results obtained within individual laboratories cannot be overemphasized.

The Division of Narcotic Drugs would welcome observations on the contents and usefulness of the present manual. Comments and suggestions may be addressed to:

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Vienna International Centre
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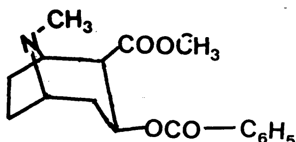
A-1400 Vienna, Austria

I. DESCRIPTION OF THE PURE COMPOUNDS

COCAINE
l-cocaine
Beta-cocaine
Methylbenzoylecgonine
Benzoylmethylecgonine

Melting points (oC)

Base	Hydrochloride
98	157 (200-202)



Solubilities (1g/ml)

	Base	Hydrochloride
Water	1300	0.5
Ethanol	7	4.5
Diethyl ether	4	almost insoluble
Chloroform	0.5	18

$C_{17}H_{21}NO_4$
MWt = 303.4

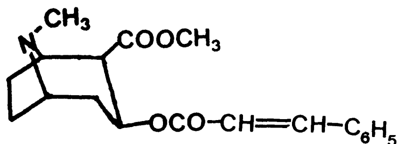
CINNAMOYLCOCAINE
Cinnamoylmethylecgonine
Cinnamylcocaine
Methylbenzoylecgonine
Benzoylmethylecgonine

Melting points (oC)

Base	Hydrochloride
121	

Solubilities (1g/ml)

	Base	Hydrochloride
Water	almost insoluble	soluble
Ethanol	soluble	soluble
Diethyl ether	soluble	soluble
Chloroform	soluble	slightly

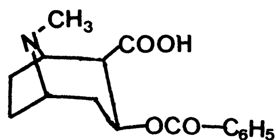


$C_{19}H_{23}NO_4$
MWt = 329.4

BENZOYLECGONINE
Ecgonine benzoylester

Melting points (oC)

Base	Hydrochloride
195 (anhydrous)(dec.) 86-92 (tetrahydrate)	200



Solubilities (lg/ml)

	Base	Hydrochloride
Water, boiling	soluble	soluble
Ethanol	soluble	soluble

$C_{16}H_{19}NO_4$
MWt = 289.34

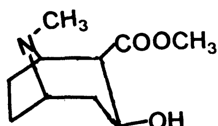
METHYLECGONINE
Ecgonine methylester

Melting points (oC)

Base	Hydrochloride
oil	215

Solubilities (lg/ml)

Base	Hydrochloride



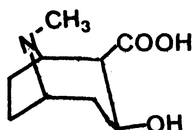
$C_{10}H_{17}NO_3$
MWt = 199.3

ECGONINE

Melting points (oC)

Base	Hydrochloride
198 (205)	241 (240-275)

Solubilities (1g/ml)



	Base	Hydrochloride
Water	5	soluble
Ethanol	67	slightly
Methanol	20	soluble
Ethylacetate	75	

$C_9H_{15}NO_3$
MWt = 185.22

For additional details related to the substances, the reader is referred to "The Multilingual Dictionary of Narcotic Drugs and Psychotropic Substances Under International Control (ST/NAR/1), and to the widely used Merck Index.

II. PRODUCTION OF ILLICIT COCAINE

The details here outlined represent one of the approaches to the production of illicit cocaine, which can be achieved in several ways. In the illicit production, variations of technique, reagents and quantities are to be expected. However, it would not be unreasonable to assume that the general approach will be similar to that given here.

1. Coca leaves are mixed with water and with a material such as lime, so as to produce an alkaline reaction in the resulting pulp. The mixture is crushed, kerosene (or an equivalent hydrocarbon) is added and the mixture stirred.

2. The kerosene is removed and the extracted coca leaf discarded. Acidified water is partitioned with the kerosene, extracting alkaloids into the aqueous layer. The kerosene is removed. If coca paste is required, the water is made alkaline with lime, ammonia or equivalent, which precipitates the more basic alkaloids. The precipitate, which often contains mixed inorganic salts as well as crude cocaine, is removed and dried. The result is coca paste.

3. To produce cocaine hydrochloride, the coca paste is dissolved in dilute sulphuric acid. Potassium permanaganate may be added at this stage until the solution remains pink. The potassium permanganate is added to destroy the cinnamoylcocaine isomers present as an impurity in the cocaine. The solution is allowed to stand, then filtered. The filtrate is made basic with ammonia, precipitating cocaine base and other alkaloids. The precipitate is filtered, washed with water and dried.

4. The crude cocaine base is dissolved in diethyl ether. The solution is filtered, and concentrated hydrochloric acid and acetone are added. The cocaine hydrochloride which precipitates is filtered and dried.

III. PHYSICAL APPEARANCE AND CHEMICAL CHARACTERISTICS OF COCA LEAF AND ILLICIT MATERIALS CONTAINING COCAINE

It must be stressed that no two samples of coca leaf, coca paste or cocaine hydrochloride will have exactly similar physical appearances.

A. 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, thiner 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 than the other types and have a rich green colour.

B. Coca paste

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

C. 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 with heroin products. Nevertheless no two illicit samples of cocaine are exactly identical. For the most part it is a white or off-white powder which is often fine, and rarely damp. It has a characteristic odour.

Adulteration is comparatively rare (but not unknown) within the economically developing countries, the material being internationally trafficked with a purity often of 80 to 90% (as cocaine hydrochloride). Subsequent adulteration and transformation for trafficking purposes within the economically developed countries usually involves the addition of either an uncontrolled synthetic local-anaesthetic (e.g., lidocaine, procaine or benzocaine) or a carbohydrate (e.g., mannitol, lactose or glucose). In either case the physical appearance is changed only slightly, for virtually all adulterants are themselves fine dry white powders.

The typical purity for trafficking in cocaine within the economically developed countries is about 30%; the internationally trafficked material is subjected to adulteration with about three times its own weight of the diluent.

Occasionally cocaine is encountered as material containing large, sometimes colourless crystals ("rock cocaine"). These crystals can be quite hard. Usually some, if not the major part, of such samples consist of material similar to ordinary "powder" cocaine.

If material submitted for forensic examination bears no physical relationship to the description given here, that does not mean, of course, that it is not cocaine or a cocaine containing product.

IV. THE ANALYSIS OF MATERIALS CONTAINING COCAINE

A. Sampling

The principal reason for a sampling procedure is to produce a correct and meaningful chemical analysis. Because most methods - qualitative and quantitative - used in forensic science laboratories for the examination of drugs require very small aliquots of material, it is vital that these small aliquots be entirely representative of the bulk from which they have been drawn. Sampling should be undertaken to conform to the principles of analytical chemistry, as laid down, for example, in national pharmacopoeias or by such organizations as the Association of Official Analytical Chemists.

There may be situations where, for legal reasons, the normal rules of sampling and homogenization cannot be followed if, for example, the analyst wishes to preserve some part of an exhibit as visual evidence. Alternatively, it may be necessary to perform separate assays on two powder items, rather than combining the powders prior to a single assay being performed on the mixture, because each has been separately exhibited by the seizing officer, and the legal system within which the analyst works requires individual results on every exhibit which is to be taken before the courts.

To preserve valuable resources and time, forensic analysts should seek, on all possible occasions, to use an approved sampling system and thereby reduce the number of quantitative determinations needed. To facilitate such an approach, the forensic analyst may need to discuss individual situations with both seizing officers and the legal personnel with whom he works.

Cocaine is most frequently encountered as a fine powder, although some presentations contain aggregates which may be hard or soft. The aggregates may be of any size. A seizure of cocaine may be of material within a single container or package, or the material may be inside a number of packages.

1. Sampling of single package items

The simplest sampling situation is where the submitted item consists of a single package of material - in the case of cocaine, the material will most often be a powder. The material should be removed from its container or wrappings, placed in a clean clear plastic bag and the net weight recorded. The material should then be thoroughly homogenised prior to the application of the sequence of chemical tests, although presumptive testing may be applied at this stage if it is thought that the sampling or homogenization process will be lengthy and there is still some doubt as to the identity of the material. The simplest way to homogenize a powder is to shake it thoroughly within the clear plastic bag to which it has been transferred. If the powder contains aggregates these may be broken down by passing through successively finer sieves, or by pounding with a mortar and pestle, or by use of an adapted commercial food-mixer or food-processor.

Alternatively, the technique of coning-and-quartering can be applied, as follows: the sample is mixed by shaking or stirring. Large fragments are reduced if necessary; the material is then poured on a flat surface to form a cone. The "cone" is flattened and the material is then divided at right angles, forming quarters. Opposite quarters are taken for a sample; the remainder of the material is returned to the receptacle from which it was removed. Should further coning-and-quartering be desired to reduce sample size, particle sizes are further reduced, the material mixed thoroughly, poured onto a flat surface, and divided as before.

2. Sampling of items consisting of more than one package

The analyst should examine the contents of all packages by eye, and possibly also by simple colour test or TLC to determine:

1. If all packages contain suspect cocaine or cocaine-containing material, and/or

2. If one or more packages contain material different to that of the majority of packages. The simplest indicator is the physical appearance of the powder. If one or more packages obviously differ in content, these should be segregated and subjected to separate analysis.

The compositing of multiple container items is as follows:

(a) If there are less than 10 packages - all packages should be sampled.

(b) If there are 10 - 100 packages - randomly select 10 packages.

(c) If there are more than 100 packages - randomly select a number of packages equal to the square root of the total number of packages rounded to the next highest integer.

If the powders are found to be the same then the contents of a number of packages may be combined; the combined bulk material may then be homogenized in, for example, an adapted commercial food-processor. Alternately, the bulk may be subjected to coning-and-quartering.

When different types of material have been identified in the various packages, then each sub-group should be composited in an identical fashion to that previously outlined.

Sampling errors for quantitative methods are reduced if large aliquots of material are subjected to sequential dilution with the dissolving solvent. If the total sample size is large, this approach may be adopted. However, when large amounts of material are used for the first dissolution, it may be necessary to add the solvent by pipette to avoid error due to insoluble materials. It will be rare to find large amounts of insolubles in cocaine samples seized within the economically developing countries or at importation points into economically developed countries. However, insoluble adulterants are a frequent occurrence in "street" samples seized within economically developed countries.

3. Sampling of materials containing large particles

If the particles can be easily reduced to powder, then this approach should be used and sampling procedure followed as outlined previously. Powdering may be achieved by mortar and pestle, commercial food-processor/mixer, or industrial grinder. If the material cannot be easily broken down, then random sized particles should be drawn from at least three different parts of the item. A minimum of 1 gramme should be collected, weighed accurately and subjected to assay.

B. The 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 more pure 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. The sampling methodology and the analytical methods described are equally applicable, with minor modification, to coca paste or cocaine.

Trafficking in coca leaf is rare (but not unknown) outside those countries of the developing world where coca is grown. This section has therefore been included in the manual to assist the analyst on those rare occasions 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 available standard reference materials for both techniques.

1. Physical identification

1. Whole coca leaf

This material was described on page 8. Confirmation should include microscopy.

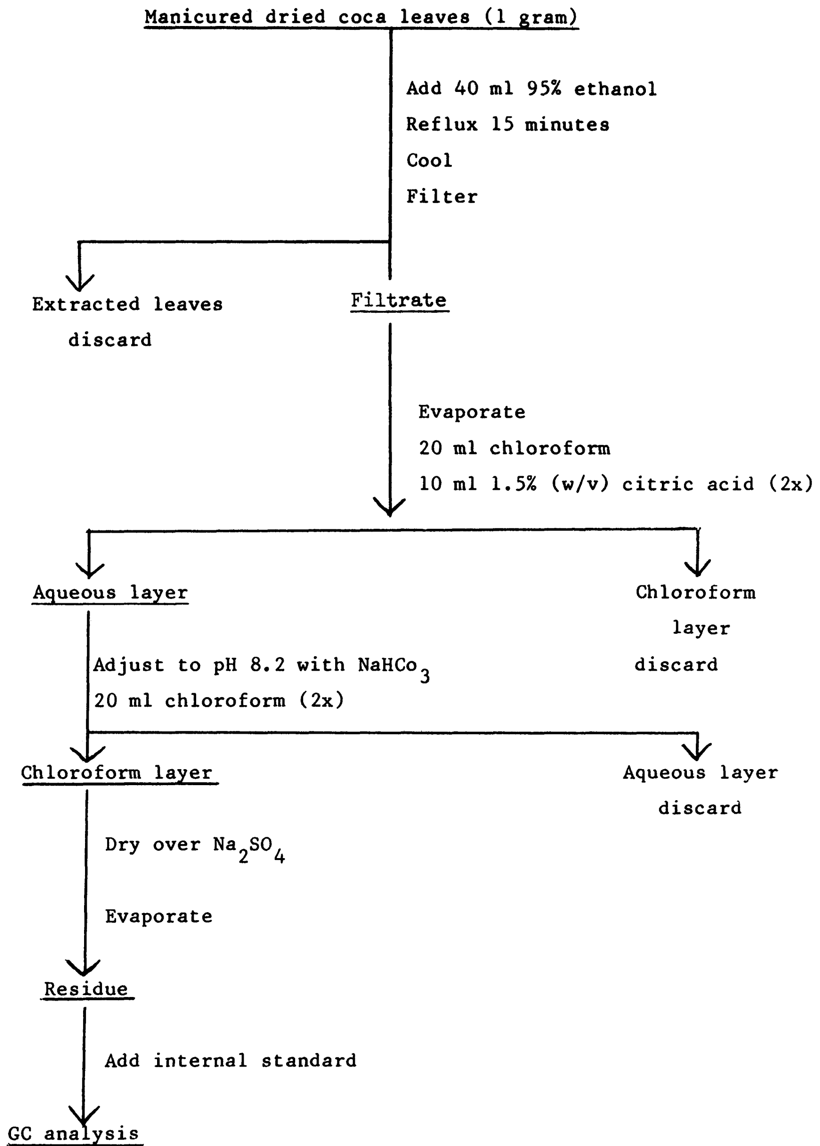
2. Powdered coca leaf

This may be identified by microscopy. The standard textbooks dealing with powdered vegetable drugs contain a section devoted to coca leaf (e.g. "Powdered Vegetable Drugs", Jackson, B.P. and Snowden, D.W., Churchill, London, 1968, p. 44)

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.

A systematic quantitative extraction procedure is to follow the scheme illustrated below (taken from Journal of Ethnopharmacology, 3 (1981). pp. 293-8).


Manicured dried coca leaves (1 gram)
Add 40 ml 95% ethanol
Reflux 15 minutes
Cool
Filter
Extracted leaves
discard
Filtrate
Evaporate
20 ml chloroform
10 ml 1.5% (w/v) citric acid (2x)
Aqueous layer
Chloroform
layer
discard
Adjust to pH 8.2 with NaHCo3
20 ml chloroform (2x)
Chloroform layer
Aqueous layer
discard
Dry over Na2SO4
Evaporate
Residue
Add internal standard
GC analysis

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 for qualitative analysis of the coca leaf, or (if quantitative extraction has been undertaken) may be subjected to GLC or HPLC to estimate the cocaine content of the leaf.

C. The analysis of coca paste and cocaine

1. Presumptive tests for cocaine

(a) Colour test

It must be stressed that positive results to colour tests are only presumptive indications 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 technique(s).

All colour-test reagents should be carefully scrutinized to ensure that they have not decomposed. Colour-test reagents that have themselves changed colour may lead to erroneous conclusions about the nature of the substance under test.

The colour test described below is known as the Scott Test, as follows:

REAGENTS

Solution 1. 2% cobalt thiocyanate - $Co(CNS)_2$ - dissolved in water and then diluted 1:1 with 96% glycerine (pharmaceutical grade).

Solution 2. Hydrochloric acid (concentrated).

Solution 3. Chloroform.

METHOD

The amount of test material should not exceed sufficient to cover the tip of a micro-spatula.

STEP 1 The test material is placed in a test tube, 5 drops of solution 1. are added and the mixture is shaken. If cocaine is present a blue colour develops at once. If a blue colour is not seen, add more test material. If a blue colour still does not develop, the sample does not contain cocaine.

STEP 2 Add one drop of solution 2. and shake. The blue colour will disappear and a clear pink solution should be seen. If all the blue colour does not disappear, add a second drop (no more) of solution 2.

STEP 3 Add several drops of solution 3. (chloroform) and shake. The chloroform layer will develop an intense blue colour if cocaine is present.

RESULTS

Phencyclidine, dibucaine, butacaine and methapyrilene, which give the same colour as cocaine in STEP 1, are all distinguished from cocaine by STEP 3, to which only cocaine gives a blue chloroform layer.

Notes

1. The amounts used of solutions 1. and 3. are not critical. However, the ratio of solution 1. to solution 2. is critical. If excess hydrochloric acid is added to solution 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 2 drops of hydrochloric acid; no more should be used.

2. The test works on samples containing as little as 1% cocaine.

3. The shelf-life of solution 1. has been shown to be at least 6 months.

(b) Odour test

The warning given about colour tests applies equally to the odour test for cocaine.

REAGENT

Methanolic sodium or potassium hydroxide (1 g of potassium or sodium hydroxide dissolved in 20 ml of methanol).

METHOD

The dried test material is thoroughly moistened with the reagent and, after allowing excess alcohol to evaporate, the odour characteristic of the sample is compared with that of standard cocaine material.

Notes

1. Over one hundred drugs were tested for possible interference and only piperocaine (also a benzoate ester) gave a positive result. Certain amines such as amphetamines will produce a "weak, fishy odour".

2. The sensitivity is greater than that of existing field tests, e.g. the Scott test.

3. The sample and reagent must be kept free of water which interfers with the reaction.

4. Ideally the test should be performed concomitantly with a test on standard cocaine material and the odours compared.

(c) Microcrystal test

The warnings given for colour and odour test apply equally to the microcrystal test.

PLATINUM CHLORIDE TEST

REAGENT

1 g of platinic chloride is dissolved in 20 ml of water.

METHOD

Approximately 2 mg of the sample is placed on a microscope slide and dissolved in 1 drop of 1N hydrochloric acid. One drop of the reagent is added. The resulting crystals should be viewed with a microscopic magnification of about 100. Standard cocaine should be analysed concomitantly. The dilution of the test material or the hydrochloric acid may be varied to give the optimum results.

2. Tests for anions associated with cocaine

(a) Solubility tests

METHOD

1. Dissolve a portion (approximately 1 gramme) of the powder or material in approximately 5 ml of distilled or de-ionised water. For small seizures 0.1 gramme should be used with 0.5 ml of water.

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

Note

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 of solvent.

(b) Precipitation tests

REAGENTS:

1. Nitric acid: concentrated (contains 70% w/w HNO_3)

2. Hydrochloric acid: concentrated (contains 35-38% w/w HCl)

3. Dilute ammonia solution: contains approximately 10% w/w of NH_3, and is made by dilution of concentrated ammonia solution (375 ml to 1 litre with water).

4. Silver nitrate solution: a 5.0% w/v solution of silver nitrate in water.

5. Barium chloride solution: a 10.0% w/v solution of barium chloride in water.

RESULTS

Chlorides

Solutions of chlorides, when treated with silver nitrate solution, yield a white curdy precipitate which is insoluble in nitric acid, but soluble, after being well washed with water, in dilute ammonia solution, from which it is reprecipitated by the addition of nitric acid.

SULPHATES

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

Water soluble illicit cocaine products are invariably found to be the chloride salt; the occurrence of any other anion is extremely rare, although sulphate has been encountered. This test should be confirmed, if possible by IR spectroscopy and/or by X-Ray diffraction methods.

Complete solubility of the powder or material in ethanol is to be expected for most suspected cocaine samples. Insoluble colourless crystals are a likely indication that the cocaine has been adulterated ("cut") by a carbohydrate such as lactose. The insoluble material may be filtered, dried and subjected to further testing by, for example, IR spectroscopy. The amount of insoluble material may give a rough guide to the extent to which the cocaine has been adulterated, but it must be noted that all the carbohydrates are soluble to various degrees in ethanol.

3. Thin layer chromatography of cocaine

(a) Standard technique

Coating: activated silica gel G on glass backed plates; the coating contains a fluorescing additive which fluoresces at 254 nm.

Layer thickness: 0.25 mm.

Plates should be stored in dry conditions, over blue silica gel inside a dessicator and should be protected from chemical vapours. Activation of plates before use should be at 110°C for a minimum of 30 minutes.

Size of plate: 20 x 20 cm; 20 x 10 cm; 10 x 5 cm; choice depends on number of samples to be developed simultaneously.

Starting point of run = "spotting line": 1 cm from bottom of plate.

Depth of developing solvent in TLC tank: not more than 0.5 cm, not less than 0.3 cm.

Distance between applications ("spotting points"): usually 1 cm, never less than 0.8 cm. Spots must be positioned at least 1.5 cm from edge of plate to overcome "side effect".

Length of run: optimum is 10 cm, for this figure allows easy calculation of R_f values (Method 1. below). However, if R_f values are not required, a simple approach is to allow the solvent to develop to the top of the TLC plate. In such circumstances the plates are arranged so that the maximum development does not exceed 10 cm (Method 2. below).

Method 1.

For 20 x 20 cm plates a line is drawn 11 cm from the "spotting end" which gives a 10 cm development for spots applied 1 cm from the bottom.

Method 2.

Plates of 20 x 10 cm and 10 x 5 cm dimension are placed in the TLC tank with the 10 cm sides vertical by allowing the solvent to flow to the top of the plate; a 9 cm development is produced.

It is important for the analyst to monitor the progress of solvent in both methods; plates must be removed from the TLC tank as soon as the solvent reaches the "development line" or the top of the TLC plate, otherwise diffuse spots will result.

Size of spot: the solution being applied to the plate spreads outwards from the "spotting point". This spreading of the solution should be restricted as much as possible, otherwise diffuse spots will be produced during development. The ideal size for the application spot is no more than 2 mm in diameter. To achieve, this it may be necessary to apply solutions in aliquots rather than by a single discharge of the spotting equipment. The aliquots may be dried by hot or cold air between discharges. If hot air is used, care must be taken to ensure that no component of the mixture under investigation is thermally labile.

The TLC tank and lid: preferably both should be of clear glass; the tank should be lined with adsorbent paper to assist saturation. The lid should be tight fitting to minimise solvent evaporation. The glass may be ground and/or a smear of petroleum jelly may be applied to the rim.

The developing solvent: if a mixture, it should be made as accurately as possible by careful use of measuring cylinders. If the same solvent systems are used daily, it may be convenient to obtain each component via an automatic dispenser. Mixing may be done within the TLC tank. The developing solvent, mixture or single component, should be placed within the TLC tank in sufficient time to allow saturation to be achieved. With paper-lined tanks this should take approximately 5 minutes.

It is important to note that, for certain developing systems, the solvent must be renewed after each development, or at least after 2 to 3 runs.

DEVELOPING SOLVENTS

SYSTEM A:	Chloroform	25% by volume
	Dioxane	60% by volume
	Ethyl acetate	10% by volume
	Ammonia (29%)	5% by volume
SYSTEM B:	Methanol	100 parts by volume
	Ammonia (29%)	1.5 parts by volume
SYSTEM C:	Cyclohexane	75% by volume
	Toluene	15% by volume
	Diethylamine	10% by volume

Preparation of solutions to be applied to the TLC plate

Illicit cocaine samples

At a concentration of 1 mg per ml in methanol.

Standards solutions*

All made at a concentration of 1 mg per ml in methanol.

*N.B. The form of standards used , salt or base, is unimportant. Either form will be satisfactory; on the TLC plates the compounds always move as the free base.

VISUALIZATION

The plates must be dried prior to visualization. This can be done at room temperature or, more quickly, by use of hot air. In the latter 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:

1. UV light at 254 nm.
2. Acidified potassium iodoplatinate reagent.
3. Dragendorff's reagent (Munier).

Preparation of spray reagents

ACIDIFIED POTASSIUM IODOPLATINATE REAGENT

Dissolve 0.25 gramme of platinic chloride and 5 grammes of potassium iodide in water to 100 ml and add 2 ml of concentrated hydrochloric acid is added.

DRAGENDORFF'S REAGENT

Mix together 2 g of bismuth subnitrate, 25 ml of concentrated (glacial) acetic acid and 100 ml of water to produce solution (1); dissolve 40 g of potassium iodide in 100 ml of water to produce solution (2).

Mix 10 ml of solution (1), 10 ml of solution (2), 20 ml of concentrated (glacial) acetic acid and 100 ml of water to produce Dragendorff's reagent.

RESULTS

R_f x 100 values:

COMPOUND	DEVELOPING SYSTEM		
	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.

4. Gas liquid chromatography of cocaine

(a) Packed column technique

Detector	FID (Hydrogen at 30 ml per minute, air at 450 ml per minute).
Column	6 ft (or 2 m), I.D. 2 to 4 mm.
Packing	SE-30; OV-1; OV-17.
Carrier gas	Nitrogen at 30 ml per minute.
Operating conditions	Injector temperature: 220°C. Oven temperature: 220°C. Detector temperature: 300°C.
Internal standard	n-tetracosane or tetraphenylethylene.
Derivatizing agent	N,O bis-trimethylsilylacetamide (BSA) or N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA).

Conditioning of packed columns

Prior to use, all packed columns must be conditioned. Usually the conditioning temperature should be at least 30°C above the temperature at which the analysis is to be performed, unless this would require exceeding the upper operating temperature of the column specified by the manufacturer. In this case, a smaller temperature differential must be used and the conditioning period substantially extended. Example: for a column to be used at 235°C, which has an upper working temperature of 300°C, an ideal conditioning temperature would be 270°C. A typical conditioning period is usually overnight, or a minimum of 15 hours. If, in the example, the upper recommended limit for the column was 280°C, then conditioning could be performed at 260°C for 30 hours.

For some columns, conditioning from Friday evening till Monday morning may be required. During conditioning, the carrier gas to be used experimentally is passed through the column at the same flow rate as in the analysis, e.g., nitrogen at 30 ml per minute measured at conditioning temperature. During conditioning, it is vital that the end of the GC column not be connected to the inlet line of the GC detector. This is because silica bleeds from the solid part of the stationary phase during conditioning and would soon build up within the detector. This would severely affect detector response and ultimately, in the case of an FID, prevent hydrogen combustion by blocking the orifice of the burner. In ordinary operation silica bleeds from the GC column, and the blocking of an FID burner is one of the most common causes of deterioration of detector response. In such circumstances a simple approach is to raise the operating temperature of the detector by as much as 50 to 100°C (if within the capability of the gas chromatograph). This is to volatilize ("burn-off") the deposited silica. If this is unsuccessful the only solution is removal of the burner from the FID, unit and mechanical removal of the silica deposit. Washing with water, detergent and abrasives, followed by drying with organic solvents has been found effective.

METHOD

Standard solution of cocaine hydrochloride is prepared at a concentration of 40 mg per 100 ml in ethanol or methanol.

Treat the illicit cocaine similarly, using at least 20 mg sample to give a cocaine concentration approximately equal to that of the standard solution. Derivatization procedure - see capillary column technique. Inject 2 to 5 microlitres as appropriate.

The content (%) of any component can be calculated using the general formula:

$$C_x\% = \frac{C_{r.\ std.}}{C_{sam.}} \times \frac{A_x \ / \ A_{int.std.in\ sam.\ chrom.}}{A_{r.std.} / A_{int.std.in\ std.\ chrom.}} \times 100$$

Where:

$C_x\%$ = content of component x in the sample (w/w %).

$C_{r.\ std.}$ = concentration of substance x in the standard reference solution (w/w%).

A_x = peak area for substance x during the sample chromatography.

$A_{int.\ std.\ in\ sam.\ chrom.}$ = peak area of the internal standard obtained during the sample chromatography

$A_{int.\ std.\ in\ std.\ chrom.}$ = peak area of the internal standard. obtained during the standard chromatography

$C_{sam.}$ = concentration of the sample (w/v %).

(b) Capillary column technique

Column	OV-1 - chemically bonded fused silica capillary.
Film thickness	0.15 um
Length	25 m. by 0.32 mm. i.d.
Carrier gas	Hydrogen.
Flow rate	2 ml per minute
Split ratio	1:50
Detector	FID.

Operating temperatures Injector.....250°C.
Detector.....280°C.
Programme....Start at 150°C., immediate increase at 9°C per minute to 280°C.

Sample preparation: 3-4 mg of the sample, 1-2 mg of internal standard ($n\text{-}C_{24}H_{50}$), 1 ml chloroform, 0.2 ml pyridine and 0.15 ml MSTFA (silylating reagent) are mixed and heated at 70°C for 10 minutes.

Injection volume: 1 ul

5. High performance liquid chromatography of cocaine

Operating conditions

Column	160 mm by 5.0 mm i.d.
Packing material	Octadecyl-silica HPLC grade 5 um
Mobile phases	
Eluent A	Methanol (300 ml), water (700 ml), 1% (v/v) phosphoric acid (1000 ml) and n-hexylamine (10.71g; 14 ml) (pH = 2.5).
Eluent B	Methanol (1000 ml), 1% (v/v) phosphoric acid (1000 ml) and n-hexylamine (10.71g; 14 ml) (pH = 2.8).

The 1% phosphoric acid is prepared by dissolving concentrated orthophosphoric acid (17 g) in distilled water (1000 ml).

Degassing of mobile phase

Dissolved atmospheric gas within the mobile phase must be removed prior to the start of the analysis. If this is not done, the gas comes out of solution and forms small bubbles either in the tubing between the solvent reservoir and the pump-head(s) or within the cylinder(s) of the pump-head(s). In either case, and especially the latter, pumping will cease and the chromatographic development will be ruined.

The simplest way of removing dissolved gas is to immerse the eluant mixture in an ultrasonic bath at high power for a minimum of 10 minutes. A problem which may arise with this approach is that the water of the ultrasonic bath may become warm during a long degassing period, and the heat may be transferred to the eluant. Addition of ice to the water in the ultrasonic bath will maintain the eluant at ambient temperature. Degassing by this method may be performed in situ by arranging for the dedicated use of an ultrasonic bath which is included as part of the entire HPLC system. The solvent reservoir is placed within the ultrasonic bath. Frequent and relatively short degassing periods should be used, possibly between each chromatographic development. The composition of the eluant must be taken into account when degassing. Components which are particularly volatile and/or which constitute only a small proportion of the eluant should not be exposed to frequent degassing. It is vital to keep the eluant reservoir closed to the atmosphere if the problem of dissolved gases is to be avoided.

It has been claimed that the ultrasonic technique is not completely effective, and that the only fully efficient way of degassing eluants is to pass helium or argon gas slowly through the solution. This approach may also be done in situ by having a gas cylinder of helium or argon connected via tubing into the solvent reservoir. Frequent degassing may be performed between chromatographic developments. It is important to maintain the solvent reservoir beneath an atmosphere of the inert gas chosen for degassing.

Flow rate	2.0 ml per minute for both eluents.
Detection	UV at 230 nm
Sample preparation	all materials are dissolved in the appropriate eluent.
Standard solutions	dissolve approximately 1 mg in 10 ml eluent of any of the following substances

Cocaine
Cis-cinnamoylcocaine
Trans-cinnamoylcocaine
Procaine
Lignocaine
Amylocaine
Butacaine
Benzocaine

Injection volume	20 µl by loop injector.
Quantitation	by peak areas, internal or external standard methods.

RESULTS

Elution order and the corresponding capacitance ratios (K')* for eluent A are as follows:

Procaine	0.0
Lignocaine	0.79
Cocaine	2.68
Cis-cinnamoylcocaine	6.3
Amylocaine	7.19
Butacaine	8.97
Trans-cinnamoylcocaine	10.65
Benzocaine	20.06

*Capacitance ratio $K' = \frac{tr - to}{t_o}$

where

t_r= time of elution of compound
t_o= time of elution of non-retained material (methanol injection)

6. Infra red spectroscopy of cocaine

Sample preparation

1. Halide disc method

The finely powdered dry material, about 2 mg, is mixed with an alkali halide, about 200 mg, ground either mechanically in an agate ball mill or by hand in an agate mortar, and pressed into a thin disc. The ideal is to produce a disc as nearly transparent as possible.

Originally, potassium bromide was used as the alkali halide, and the technique is often referred to as the "KBr disc method". Potassium chloride has also been widely used and is often considered to be superior to potassium bromide because it is less hygroscopic. Whatever halide is used, it should preferably be of "IR standard", dried at 105°C for a minimum of one hour and stored above a strong dessicant (for example phosphorus pentoxide) in a dessicator. Analytical grade halides, powdered to the same degree as "IR standard" material and dried to the same degree, are acceptable.

The major disadvantage of this method is the need for disc pressing equipment. However, at the present time, a number of commercially available disc pressing systems are very inexpensive relative to the cost of an IR spectrophotometer. Another disadvantage is the possible production of an erroneous spectrum if double decomposition occurs during the preparation of the disc:

$$\text{Base.HCl} \quad + \quad \text{KBr} \longrightarrow \text{Base.HBr} \quad + \quad \text{KCl}$$

Hydrochlorides should, therefore, always be examined in potassium chloride and not potassium bromide. The anion test using precipitating reagents should precede IR spectroscopy.

The major advantage of the halide disc method is that, provided the halide dispersant is free of water, it should contribute no interference to the resulting spectrum. Another minor advantage is that, with care, the discs may be stored indefinitely. This may be important in any subsequent legal proceeding. Also, the material under investigation can be recovered from the halide disc for further testing.

2. Micro halide disc method

There are commercially available dies which can produce a halide disc only 1 mm in diameter. In this case, the quantity of halide has to be drastically reduced (approximately 1 mg). The use of this technique finds most application in the IR examination of unknown components that have been eluted from TLC plates. It may also be used if the analyst disposes of less than 1 mg of sample.

3. Nujol mull method (Liquid paraffin method)

The finely powdered sample (2-3 mg) is mixed with one drop of the liquid paraffin and ground in an agate mortar. Sufficient liquid is then added so that the final mull is the consistency of a thin cream. The mull is spread on an alkali halide disc, usually NaCl or KBr, and a similar disc placed on top. The film between the halide discs should contain no air bubbles.

The obvious disadvantage of this method is the interference from the paraffin liquid in the IR spectrum. The advantage is that the only apparatus required are a mortar and pestle, and a pair of halide discs.

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 (Halide disc method):

Cocaine base....1275, 1700, 1106, 1728, 710, 1040, 1280.
Cocaine HCl.....1712, 1730, 1276, 1230 (side peak), 732, 1106, 1075, 1025.

Differentiation between _cis_- and _trans_-cinnamoylcocaine:

1. The large absorbance at 1320 cm^{-1} in the spectrum of _trans_-cinnamoylcocaine is absent in the spectrum of _cis_-cinnamoylcocaine;

2. In the spectrum of _trans_-cinnamoylcocaine the absorbance at 1625 cm^{-1} is of about the same magnitude as the absorbances 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}.

7. 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 enantiomer of cocaine occuring naturally is l-cocaine, and courts in some countries have ruled that l-cocaine alone is an illegal drug under the relevant legislation. Unless the analyst could satisfy the court that he had successfully determined that the cocaine was l-cocaine, the prosecution may fail in those countries.

Methods were developed to overcome this legal problem. One is described in detail and references are given for other approaches.

(a) Microcrystal test to differentiate cocaine enantiomers

REAGENTS

1. Di-p-toluoyl-d-tartaric acid (TDTA) in dilute alcohol with glycerin.

2. Di-p-toluoyl-l-tartaric acid (TLTA) in dilute alcohol with glycerin.

Concentration of solutions: 1 mg per ml.

10 mg of TDTA and TLTA are placed in separate 10 ml volumetric flasks, dissolved in 1 ml of ethyl alcohol, and then made to volume by adding 8 ml of water and 1 ml of glycerine.

N.B. Crystals form in these reagents after about three months. Fresh solutions should be made if old ones fail to give results with authentic cocaine.

METHOD

If the cocaine is not present as the hydrochloride salt, it must be converted to this form. The microcrystalline tests are performed on a microscope slide, viewed through a polarizing microscope at 100 to 125 times magnification, both with and without the analyser inserted. All tests are performed directly on the extracted cocaine. A drop of reagent is placed on the slide, then a small quantity of sample is added to the reagent and stirred.

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 complete 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.

The test has been applied to other synthetic local anaesthetics and none were found to interfere. It is important to extract the cocaine from the sample matrix and to convert it to the HCl salt for this determination to succeed.

(b) Alternative methods to differentiate the cocaine enantiomers

By HPLC:

Recueil des travaux chimiques des Pays-Bas, Vol 98, 1979, pp 501-2

By HPLC, GC, and GC/chemical ionisation mass spectrometry:

Journal of Chromatography, Vol 193, 1980, pp 371-380

By HPLC, GC, TLC, IR, NMR and electron impact mass spectrometry:

Journal of Forensic Sciences, Vol 26 (No. 1), 1981, pp 12-26

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