

**Qualitative and Quantitative  
Analysis of**

# **Cocaine,**

**its metabolites and other  
adulterants**

**PHG 454 Practical course**  
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## INTRODUCTION

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There is anecdotal evidence that cultivation of 'Coca leaf' from *Erythroxylum spp.* has occurred for the last 20000 years, although the first archaeological evidence appears to occur at around 3000 BC . The species *Erythroxylum spp.* are of significance because they provide the raw material for the production of cocaine . Of the approximately 200 species of *Erythroxylon*, 17 members of the genus are reported to produce cocaine, but of greatest significance are *E. coca* Lam. The dried plant materials are either chewed or ingested directly, or a tea can be prepared from them.

Coca leaf itself can be chewed as a drug, or 'coca paste' or cocaine can be produced. Coca paste is an off-white or beige coloured powder comprising damp friable aggregates. Cocaine, as the hydrochloride salt (sometimes known as 'snow'), is itself a white, or off-white, powder, which typically smells of HCl.

In the United Kingdom, cocaine is listed as a Class A drug in Schedule 2 to the Misuse of Drugs Act, 1971, as are ecgonine , benzoyl ecgonine , ecgonine methyl ester and any derivative of ecgonine which is convertible to ecgonine or cocaine. In addition, coca leaf is also a Class A drug. Although material' containing 0.1 % or less of cocaine is still, by definition, a Class A drug, through Schedule 5 of .the regulations it is lawful for anyone to possess such material. This is because the concentration of the cocaine in this case is so low that it is deemed to be harmless. In the United States, cocaine and crack cocaine are both classified as Schedule II drugs.

Cocaine can be administered via a variety of different routes. Popularly, the hydrochloride salt is insufflated ('snorted') from a line of white powder and the drug absorbed across the mucous membranes of the nose. Alternatively, it may be administered by injection. 'Crack' cocaine, or 'Rocks' may be administered by smoking.

## **❖ Extraction and Preparation of Coca Paste**

Following cultivation of the plant material, the leaves from which the cocaine will be prepared are harvested and dried in the sun. From these, coca paste and, subsequently, cocaine is produced. In general, coca paste is prepared by one of two methods. The first involves wetting the leaves and macerating them with dilute sulfuric acid, thus forming the water-soluble sulfate salts of the alkaloids. The mixture is then extracted with kerosene. After phase separation, the aqueous layer is basified with ammonia, lime or sodium carbonate, and the alkaloids precipitated. They are then recovered by filtration.

The second method involves basifying the leaves with sodium or potassium carbonate, macerating the leaves, and adding kerosene, into which the alkaloids are extracted. Dilute aqueous sulfuric acid is then used to collect the alkaloids as the sulfate salts. The aqueous layer is then basified and the alkaloids filtered and recovered.

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## **Qualitative Identification of Cocaine**

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### **1. *Presumptive Tests for Cocaine***

There are a number of presumptive tests for cocaine available from the literature. These are described below.

#### **❖ The Cobalt Isothiocyanate Test**

Although there are several presumptive tests available for cocaine and related compounds, none alone is specific to cocaine itself. A very simple test for cocaine is the addition of the material under investigation to a 2% (wt/vol) solution of cobalt isothiocyanate in water. The presence of cocaine and related alkaloids results in a blue color being formed. Interestingly, diamorphine and temazepam also result in a blue reaction, but none of the other opiates or benzodiazepines result in this color reaction. In addition, a number of local anaesthetics also produce a positive color reaction. It should be remembered that positive and negative controls should always be undertaken at the same time as the sample is being examined.

### **❖ The Scott Test**

This is a modification of the cobalt isothiocyanate test, and involves a 2% (wt/vol) solution of cobalt isothiocyanate in water, diluted with an equal volume of glycerine (Reagent 1), concentrated hydrochloric acid (Reagent 2) and chloroform (Reagent 3). In order to test the sample for the presence of cocaine, a small amount of material is placed in a test-tube and 5 drops of the first reagent are added. A blue colour develops if cocaine is present. One drop of concentrated hydrochloric acid is then added and the blue colour, if it results from the presence of cocaine, should disappear, leaving a pink solution. Several drops of chloroform should then be added. An intense blue colour forms in the chloroform (lower) layer if cocaine is present. Again, positive and negative controls should be performed concurrently with analysis of the test sample.

### **❖ The Methyl Benzoate Odour Test**

This test relies on the hydrolysis of the benzoate ester of cocaine and benzoyl ecgonine by potassium or sodium hydroxide . A 5% (wt/vol) solution of the hydroxide is prepared in methanol. The sample under test is mixed with a small volume of this reagent in a suitable container and warmed gently, for example, on the palm of the hand. The excess alcohol is allowed to evaporate and the smell arising from the mixture noted (very great care should be exercised when assessing this). A positive and a negative control should also be undertaken. The resulting smell is that of methyl benzoate (familiar in the smell of oil of wintergreen). Piperocaine and benzoyiecgonine (both benzoate esters) also result in a positive test.

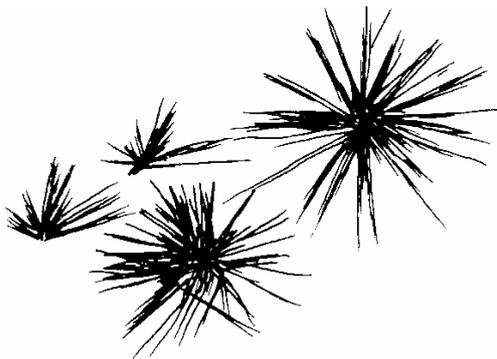
## ***2. Crystallography***

A. Place a drop of the alkaloidal solution on a clean glass slides, add a drop of reagents (platonite chloride, lead iodide and gold chloride) to the slides, and without stirring or covering, examine under the microscope. Please note that you should examine the crystals within 15 minutes and they should not be allowed to dry up.

**B.  $\text{KMnO}_4$  test:** Dissolve 10 mg of cocaine in 1 ml of 0.02 N HCl in an evaporating dish, and evaporate the solution on a water-bath to dryness. Dissolve the residue in few drops of water and add 1 ml of 0.1 N  $\text{KMnO}_4$  solution. A violet crystalline precipitate is formed which shows characteristic violet red crystalline aggregates when examined microscopically.



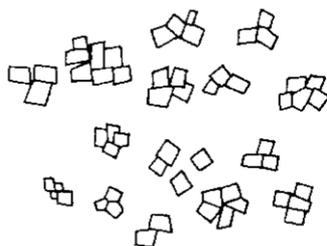
Cocaine + Platinic chloride



Cocaine + Lead iodide



Cocaine + Gold chloride



Cocaine +  $\text{KMnO}_4$

*If positive tests are obtained in the presumptive tests and crystallography , the next stage in the analysis is to undertake TLC or to proceed directly to a spectroscopic evaluation. The use of TLC will allow a rapid, inexpensive means of discriminating samples containing cocaine from the false positives and a determination of what should be used in the standard mixtures for GC—MS analyses.*

### **3. Thin Layer Chromatography**

Prior to the use of any further method for the analysis of the material and identification of cocaine, the latter must be extracted. For powdered samples, which have been thoroughly homogenized, a 1 mg/mL solution in methanol will suffice.

The presence or absence of cocaine in a sample may qualitatively be determined by using TLC, carried out on silica gel. A number of different solvent systems are available for this. The compounds can be examined visually under

1. white light,
2. short-wavelength UV light (254 nm), and
3. long-wavelength UV light (360 nm),
4. and after using visualization reagent (Dragendorff's reagent).

*As any analysis of controlled drugs, it should be remembered that positive and negative controls should be included in every chromatographic investigation.*

**What are the difficulties associated with interpreting data from  
TLC?**

*There are two principle problems. The first is lack of resolution, for example, in the methanol/ammonia system, cocaine/procaine and ecgonine methyl ester/lidocaine are not well resolved and yet may occur together in the mixture. A further difficulty is added by the lack of specificity of the visualization reagents. None of the reagents used here are specific to this group of compounds.*

By comparison of the Rf data and the colors observed under different light conditions and with different visualization reagents, the compounds thought to be present in the mixture can be determined and standard mixes prepared for GC--MS analysis. In addition, it may be possible to make comparisons between samples.

## Manufacturing Crack Cocaine

" Crack " is the street name given to cocaine that has been processed from cocaine hydrochloride to a free base for smoking. Rather than requiring the more volatile method of processing cocaine using ether, crack cocaine is processed with ammonia or sodium bicarbonate (baking soda) and water and heated to remove the hydrochloride, thus producing a form of cocaine that can be smoked. The term "crack" refers to the crackling sound heard when the mixture is smoked (heated), presumably from the sodium bicarbonate. The solid is then dried and cut up into small nuggets, or "rocks."

### Procedure :

Step 1 : Dissolving powder cocaine in hot water.

Step 2 : Adding sodium bicarbonate to the mixture.

Step 3 : Boiling the solution to separate out the solids.

Step 4 : Cooling the separated mixture.

Step 5: Filtering the cooled mixture to isolate the solids.

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## LAB WORK

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### A. Preparation of Benzylecgonin (Major metabolite of cocaine)

*The main metabolites are benzoylecgonine and ecgonine methyl ester (methylecgonine). They are formed by enzymatic (pseudocholinesterase) or spontaneous hydrolysis. Anhydroecgonine methyl ester is a specific marker for "crack" consumption, while cocaethylene is detectable after the simultaneous consumption of alcohol.*

### **Procedure :**

1. Place, in round bottom flask, of 250 ml capacity, 200 mg of cocaine hydrochloride.
2. Add 40 ml of distilled water.
3. Boil under reflux for 2 hrs, using heating mantle.
4. Cool under tap, then adjust the PH of the hydrolyzed solution to about 7 PH, using PH meter (use ammonium hydroxide solution)
5. Transfer to a separator funnel , then extract twice with chloroform (each 25 ml).
6. Dehydrate the extract, using anhydrous sodium sulphate.
7. Concentrate to dryness, then dissolve in 1-2 ml of MeOH/CHCl<sub>3</sub>.

### **B. Thin layer Chromatography :**

#### **Condition :**

- **Stationary phase** : Silica gel 60 F<sub>254</sub> (5X10)
- **Mobile phase** : MeOH (10ml) : NH<sub>4</sub>OH ( 5 drops) or  
Cyclohexane : toluene : Et<sub>2</sub>NH
- **Detection** : by UV at 254 nm, long-wavelength UV light (360 nm) then spray with Dragendorff's reagent
- **References** : benzylecgonin , cocaine, procaine and lidocaine in CHCl<sub>3</sub>/MeOH .

### **C. Crystallography :**

By using platonic chloride solution and potassium permanganate test.

***D. Gas -Flam Ionization Detection [GS-FID] :***

Typical GC operating conditions and parameters suitable for the analysis of cocaine by using GC-FID or GC-MS

System/parameter	Description/conditions
<b><i>Column</i></b>	OV-1: 25 m x 0.222 mm i.d.; $d_f$ , 0.5 $\mu$ m
<b><i>Injection temperature</i></b>	300 <sup>0</sup> C
<b><i>Column oven temperature programme</i></b>	170 <sup>0</sup> C for 2 mins; increased to 280 <sup>0</sup> C at 16 <sup>0</sup> C min <sup>-1</sup> held for 2 min.
<b><i>Carrier gas</i></b>	He <sup>a</sup> or N <sub>2</sub> <sup>b</sup> , at a flow rate of 1 m/min.
<b><i>Split ratio</i></b>	50:1
<b><i>Detector</i></b>	Flame-ionization or mass spectrometric detection
<b><i>Detector temperature (FID)</i></b>	300 <sup>0</sup> C
<b><i>Derivatization reagent</i></b>	<i>N, O</i> -(bistrimethylsilyl)acetamide

*a: with mass spectrometric detection*

*b: with Flam Ionization Detection*

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