Practical course

454 PHG

Introduction :

Non- Medical use of drugs , drug dependence …

" All the naturally accruing sedation , narcotics , euphoriants , hallucinogens , and excitants were discovered thousands of years ago, before the down of civilization.
By the late stone age man was systematically poisoning himself . The presence of poppy heads in the kitchen middew of the Swiss Lake Dewellers shows how early in his history man discovered the techniques of self-transcendence through drugs. There were dope addicts long before there were farmers "

The dividing-line between legitimate use of drugs for social purposes and their abuse as indistinct for it is not only a matter of which drug, but of amount of drug and of whether the effect is directed antisocially or not. " Normal " people seem to be able to use alcohol for their occasional purpose without harm but, given the appropriate degree of mental abnormality or environmental adversity, many may become dependent on it , both emotionally and physically.

Abuse potential of a drug is related to it's capacity to produce immediate satisfactions (e.g. amphetamine & heroine give immediate effect, antidepressant do not) and to it's route of administration in descending order, inhalation, IV ,IM, oral .

Some terms used :

- Drug abuse : What constituents abuse is determined by the opinions generally held in a particular society. Thus in Britain, temperate use of tobacco and alcohol to insulate from environmental stress and anxiety and to ease social intercourse, a form of self-medication, is generally accepted, a substantial minority do not consider occasional taking of cannabis to be abuse. Thus non-medical (non-prescription) (or social) during use may be a term preferable to abuse . drugs used for non-medical purposes are after divided into two groups :
  - Hard-use drugs : Where the drug is central in the user's life . These drugs disable the individual as a functioning member of society by including severe emotional and , in the case of cerebral depressants, physical dependence. The group includes heroin , morphine and cocaine
  - Soft-use drugs : Where the drug is merely incidental . These drugs are less dependence-producing. There may be emotional dependence, but there is little or no physical dependence except with heavy doses of depressant (alcohols, barbiturates) . This group includes : sedative ,trsnquillisers , amphetamines, cannabis , hallucinogens, alcohol and tobacco .

Abuse drug can be classified according to it's effect on CNS into :

1. CNS depressants
    a. Narcotic analgesics (Opioid) : e.g. Morphine
    b. Sedative and hypnotics : e.g. Barbiturates , Carbamates, Meprobanate .
    c. Alcoholic drinks .
    d. General depressants : general anesthetic : e.g. NO_2 . ether

2. CNS stimulants
    a. Caffeine and it's derivatives
    b. Cocaine
    c. Amphetamine and it's derivatives : e.g. Methamphetamine or methedrine .
    d. Khat – canthinone

3. Hallucinogens :
    a. Mescale (Mescaline alkaloids)
    b. LSD
    c. Cannabis
    d. Solanaceus drug e.g. Atropine
    e. Nutmeg
    f. Poisonous mushrooms

4. Others

PRACTICAL COURSE OF

PHG 454

lab No. 2 & 3

Detection of drugs of abuse
(using T.L.C. in urine sample)

Sample used :

Different urine samples containing one or more of the following drugs:

- ☒ Morphine
- ☒ Cocaine
- ☒ Amphetamine
- ☒ Phenoparbitone

🗗🗗 These sample can be prepared as 0.1% in dilute acids for lab use .

Principle of assay :

Different drug in a urine sample can be extracted by organic solvent(s) at certain PH . The drug(s) in the concentrated solvent extract can be identified by thin layer chromatography (TLC) .

Procedure :

1) Transfer, about 10ml of the urine sample into a 100 ml separatory funnel.
2) Add few drops of bromocresol purple reagent , until the sample acquires the same reagent color (purple) .
3) Adjust the PH to about 8 , using few drops of ammonia . The sample turns violet.
4) Extract the drug(s), twice each 20 ml of chloroform/propan-2-ol (3:1), receive the combined extract in another separatory funnel. containing about 5 ml of ammonia chloride (PH 9.4) , mix well.
5) Receive the lower organic solvent in a 100ml conical flask , through anhydrous sodium sulphate (Dehydration) , distill of the solvent .
6) Dissolve the residue in 1-2 ml of $CHCl_3$/MeOH and apply for TLC .

TLC of the separate compounds :

CONDITION :

- ❖ Stationary phase : Silica gel 60 F_{254} (5X10)
- ❖ Mobile phase : Ethylacetate:MeOH:Ammonia (17:2:1)
- ❖ Detection : by UV at 254 nm then spray with Dragendorf reagent
- ❖ References : Cocaine , morphine ,Phenobarbital and Amphetamine dissolved in $CHCl_3$: MeOH .

Notes on the Procedure :

In case that the ultraviolet lamp is not available , the following changes in the conditions can be done for TLC :

1. For morphine and cocaine solvent system will be :
   Ethyacetate : MeOH : NH_4OH (17:2:2) .
   And spray with iodoplatinate .
2. For amphetamine :
   Using plate pre-treated with 0.5 N NaOH:MeOH and the solvent system is MeOH:NH_4OH (100:1.5) and spray with iodine/chloroform (1%) or using iodine chamber .
3. For Phenobarbitone :
   Same with amphetamine but the detector is $HgSO_4$.

Reagent Used :

- Bromocresol/purple

It gives yellow with weak acid solution and bluish-violet in alkaline and neutral media , and extremely weak acids (5.6 – 6.8 PH) .

PREPARATION :

Warm 0.1 g bromocresol purple with 5 ml 95%EtOH until dissolved , add 100 ml of 20 % EtOH ,3.7 ml of 0.05 N aqueous NaOH solution and complete to 250 ml with 20 % EtOH .

- Ammonium chloride (PH 9.4) :
  Prepare 10% aqueous solution then adjust the PH to 9.4 using the PH meter, using conc. ammonia .

- Mercuric sulphate solution ($HgSO_4$) :
  Weigh 5 gm yellow mercuric oxide (HgO) , add 40 ml H_2O then (slowly and cautiously) add 20 ml conc. H_2SO_4 , stir until dissolved , leave to cool , then complete to 100 ml with H_2O .

- Iodoplatinate reagent :
  Mix 3 ml of 10% hexachloroplatinic acid (IV) solution with 97 ml H_2O , add 100 ml 6% aq. Potassium iodate solution , keep in dark .

PRACTICAL COURSE OF

PHG 454

lab No. 4

Detection of cannabis

in samples of different sources

Detection of cannabis
in samples of different sources

Cannabis OR Hashish :

Cannabis or hemp is an annual plant . Known by the name : *cannabis sativa* (F. Cannabinaceae) .

Generally two varieties are grown : the fiber type which is cultivated mainly for fiber production and the drug type which is cultivated to provide the narcotic drug known by the name : *Marijuana (Marihuana) or Hashish* .

CHEMICAL COMPOSITION :

It contains tetrahydrocannabinonol (THC$\triangle^9$) and cannabinol (CBN) . Cannabidiol also isolated (CBD) .

CBN is not present in the fresh plant and it is a measure of aging : as the sample ages , THC content declines and decompose to CBN , which means that fresh plant contains mainly $\triangle^9$ THC .

LAB WORK :

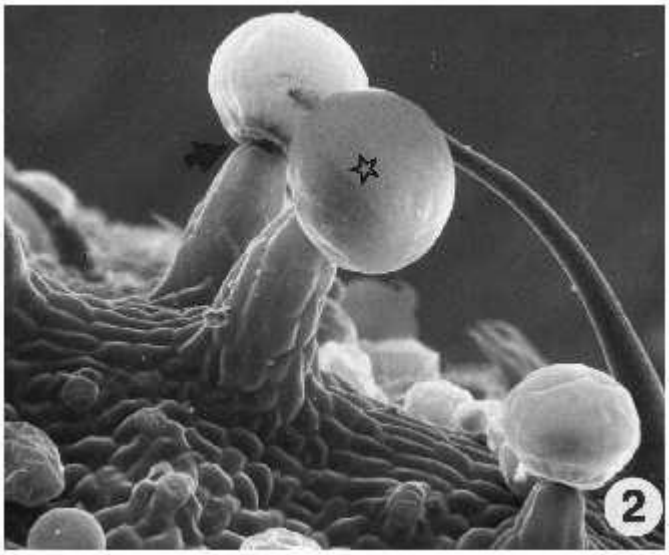
Screening of the plant including : Microscopically , chemical and chromatographic identification .

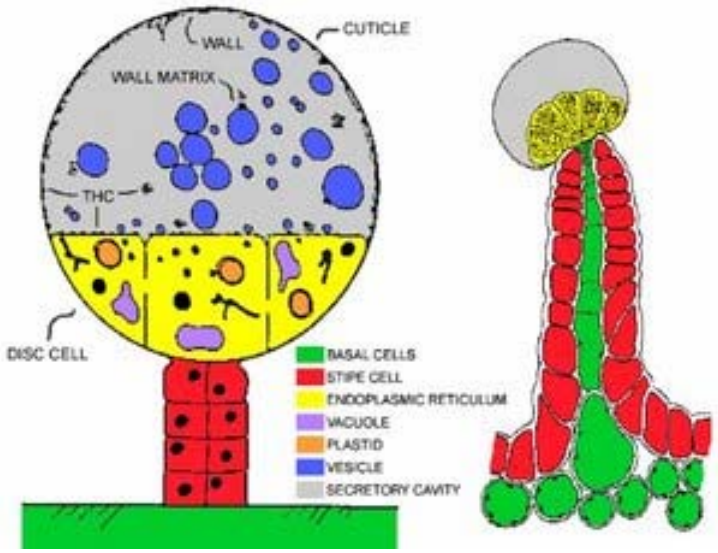
❖ Microscopy :

The resin is secreted by numerous glandular hairs . The head is 8-celled and the pedicel is multi seriate , multi cellular , Abundant conical , curved , unicellular hair are also found , having systolith of calcium carbonate in their enlarged bases.

Cluster crystals of calcium oxalate are abundant.

A slide is prepared by heating with chloralhydrate then the characteristic elements are identified microscopically .





❖ Chemical Identification :

The resin which contains the active constituent(s) is soluble in organic solvents which can be used for identification of the compounds as follow :

◎ Place few mgs of suspect material (preferably powdered) in a beaker and shake with 10 ml of petroleum ether. for the following tests:

a. Furfural test :

Evaporate 3 ml pet. ether extract (using BWB) to dryness and leave to cool. To the residue add 3-5 drops of ethanol, 2-3 drops of furfural reagent, and 1-2 drops of hydrochloric acid. Evaporate to dryness on a steam bath and to the residue add 1-2 drops of acid reagent. Observe the appearance of an intense purplish red residue which dissolves in the acid reagent upon swirling of the dish.

[Reagents : Furfural reagent: A 1 per cent solution (w/v) of furfural in ethanol; Acid reagent: A mixture of 55 ml of sulfuric acid and 45 ml of absolute ethanol; Alkaline Beam reagent: A 5 per cent solution of potassium hydroxide in ethanol.]

b. *Gamraway's* test :
Evaporate 3 ml pet. ether extract (using BWB) **to** dryness and leave to cool . Dissolve the residue in 2-3 ml acetone , then add few crystals of vanillin and mix well . Add 2 ml conc. HCl and notice the green color which develop slowly with 1-2 min .

c. *Najm's* test :
Evaporate 3 ml of pet.ether extract (using BWB) to dryness and leave to cool . Dissolve the residue in 2-3 ml of p-dimethylaminobenzaldehyde reagent. Add conc. H_2SO_4 in drop wise technique without mixing .
Notice a crimson color in the bottom of the solution .

◎ *Duquenois – Levine* test:

- Dissolve the material in ethanol in test tube
- Add five drops of (2.5 ml of acetaldehyde + 2 gm vanillin dissolved in 100 ml of 95 ethanol) , shake
- Add five drops of HCl , shake
- 10 drops of $CHCl_3$, shake
- Two layers are formed

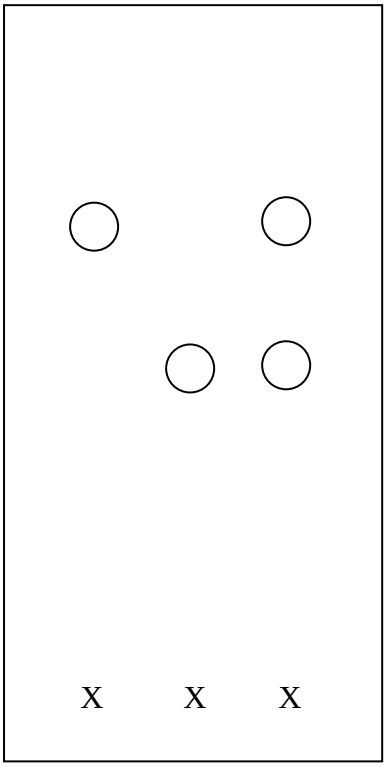
Cannabinoids rapidly form a blue/purple complex into the chloroform layer

❖ Chromatographic method (TLC)

Thin layer Chromatography provides a rapid , easy and cost-effective means for screening for cannabis materials.
This is a good technique by which the type and number of components can be identified .
The petroleum ether extract of the sample can be used in this technique after being concentrated to about 1 ml .

Condition :

- Stationary phase : Silica gel 60 F_{254} (5X10)
- Mobile phase :Hexane : ether (4:1)
- Detection : by UV at 254 nm then spray with Fast blue B salt
- References : THC$\triangle^9$, CBN dissolved in pet. ether .



Tetrahydrocannabinonol
[THC (orange)]

Cannabinol
[CBN (Violet)]

What is an additional value of TLC in cannabis analysis ?

*There are a number of reasons for carrying out TLC analysis of cannabis. This method can be used to identify the **pattern** of compound typical of cannabis. It can also be used to determine **whether or not cannabinol is present in the sample**. Cannabinol is the breakdown product of THC$\triangle^9$. If this present, the sample is understood to have **started to decompose** and it should not be used for comparative purposes. It is for this reason that analysis for comparative purpose is not generally carried out more than three month after sample seizure*

Cannabinoids

Trade Names	Marijuana, Hashish, Hashish Oil
Classification	Hallucinogen
Physical/ Psychological Dependence :	Unknown/moderate
Methods of Administration:	Swallowed or smoked
Physical Appearance :	Dry crushed leaves (marijuana), hand-rolled cigarettes (joints), hard chunks of resin of various colors (hashish), dark viscous liquid (hashish oil)
Approximate Detection Time in Urine :	Acute dosages of 1 or 2 joints – 2 to 3 days; chronic use of more than 5 joints/day – 14 to 18 days; oral ingestion (20ng) – 1 to 5 days
Clinical Effects/ Symptoms:	Hallucinations, euphoria, relaxed inhibitions

The Basics

Marijuana consists of the dried leaves and flowering tops of the Cannabis sativa plant and is a source of psychoactive agents, a major one being delta 9-tetrahydrocannabinol (D9-THC). The gastrointestinal tract and the respiratory system rapidly absorb this drug after oral or inhalation routes of ingestion. The drug is extensively and rapidly metabolized and can be detected in the urine within a couple of hours and for as long as several days after use. Regular users report feelings of euphoria, hallucinations, and relaxed inhibitions.

Sample

Random urine specimen in a properly sealed and labeled urine container.

Method and Instrument

Preliminary screening is performed by immunoassay, Bayer ADVIA 2400. Confirmation by Gas Chromatography/Mass Spectroscopy.

Stability

After proper collection, the concentration of cannabinoid in urine does not change significantly for several days at room temperature, for several weeks at refrigerated temperature or indefinitely when frozen.

Purpose

To detect recent usage.

Normal Results

Negative.

Abnormal Results

The original immunoassay has been confirmed by GC/MS. This concentration is consistent with ingestion of marijuana sometime within the 72-hour period preceding the urine collection. The screening cutoff for marijuana is 50 ng/mL. The confirmation cutoff for D9-THCA in those samples is 15 ng/mL.

Interferences

No compounds have been tested which cannot be separated from D9-THCA by GC/MS analysis.

454 PHG practical course

lab No. 5 & 6

qualitative and quantitative analysis of

Heroine

and other adulterants

INTRODUCTION

Heroin (diacetylmorphine) is a semisynthetic drug first made from morphine at St, May's hospital (London) in 1874 . it was introduce in 1898 as remedy for cough and for morphine addiction . Some years passed , however before it was appreciated that it cured morphine addiction by substituting itself as the addicting agent . It is converted to morphine in the body .

It is commonly stated that heroin is the most potent of all dependence-producing drugs .

The great advantage of heroin, from the point of view of illicit traffickers, is that it seems capable of almost unlimited dilution with other substances, while still the addict obtains from it some gratification for his desire. Nearly every small peddler who resells heroin first mixes his supply with at least an approximately equal amount of lactose or powdered sugar. As his heroin is usually highly adulterated and diluted even before he gets it, it frequently reaches the final user containing no more than 5% of the actual alkaloid, or even sometimes as little as1% . This illicit "heroin" is actually a salt of the base, namely the hydrochloride.

The impure heroine, containing small amounts of acetylcodeine and papaverine derived from the original opium, and of poorly acetylated heroine, containing more or less monoacetylmorphine and residual morphine.

CLASSIFICATION OF OTHER SUBSTANCES

The various substances that may be found in a heroin sample, besides diacetylmorphine, the substances may be classified as follows:

A. DILUENTS:

Generally lactose or powdered sugar, but sometimes mannitol or some other substance may be used.

B. ADULTERANTS:

While the diluents may also be called adulterants in the usual meaning of the term, it is convenient to distinguish the substances which are merely diluents from the drugs of more or less activity which are added either to conceal the comparative lack of real heroine, or to enhance its effect. **Quinine** is the most common adulterant and probably occurs in more than half the heroin samples in the United States and Canada. It is probably added, for one reason, *to enhance the bitter taste of the mixture, in case the addict tries to assay the strength by tasting*. It is most often added as the hydrochloride; sometimes as the sulfate. Some years ago, **procaine** was very commonly used. The amount of quinine usually equals or exceeds that of heroine, but the procaine was only about one-sixth to one-third the amount of the

heroine, and possibly it was used chiefly f*or its effect as a local anaesthetic*. Other adulterants that have been found are **barbiturates**, **caffeine**, **acetphenetidin**, **methadone**, and **amphetamine**.

C. IMPURITIES OF MANUFACTUR:

These include monoacetylmorphine and morphine, remaining from imperfect acetylation. The absence of such impurities may be significant of diversion from legal manufacture.

D. IMPURITIES OF ORIGIN:

These include codeine (present as acetylcodeine after the acetylation), papaverine, meconic acid, and brown coloring matter. The absence of these substances may not mean much, but their presence proves that a rather crude process was used, not only in the manufacture and purification of the heroine, but before that, in the extraction of the morphine.

LAB WORK

Lab work includes the qualitative and of heroin by color tests, Microscopically tests and TLC and quantitative analysis by HPLC.

◈ Colour Tests :

1. Marquis' reagent (Formaldehyde in concentrated or (6 + 1) H_2SO_4) -purple red changing to purple.
2. Frohde's reagent (Molybdate in concentrated H_2SO_4) strikes violet , quickly changing to strong purplish red, fading out to weaker brown or brownish, then developing green.
3. Mecke's (or Lafon's) reagent (Selenious acid in concentrated H_2SO_4) green,quickly greenish blue, changing to blue, slowly to bluish green with yellow-brown edge, then olivaceous green.

   Comment on tests 1 to 3: These sulfuric acid reagents provide highly characteristic colour tests for the spot-plate, and most adulterants and diluents do not seriously interfere. Rarely, a sample may contain so little heroine that charring of the diluent sugar with the concentrated acid will obscure the colours. Of course in that case, a separation is necessary. Imperfect acetylation does not matter as morphine and monoacetylmorphine give the same colours. The initial colour with the hydrochloride is slightly different from that with the free alkaloid or sulfate, bluer with Frohde's reagent and more yellow-brown with Mecke's; and this effect is increased if additional chloride is present.

4. Nitric acid-light yellow solution, gradually bright green. Concentrated HNO_3 is usually used, but a (4 + 1) acid is somewhat better (4 parts concentrated HNO_3 mixed with 1 part H_2O).

   Comment: This highly characteristic test is not very sensitive and often enough it may not be obtained on adulterated, diluted samples. Morphine gives an orange red color fading to yellow; sometimes with a little morphine present a red-orange is obtained at first and later the green of diacetylmorphine develops. Monoacetylmorphine is similar to morphine.

5. Copper test. To a little of the powder on the spot plate add several drops of water, 2 or 3 drops of 3% H_2O_2, a drop or two of NH 4OH and stir with a piece of copper. A pink to red color is produced. This test is given by various phenols and their acetyl derivatives including morphine and diacetylmorphine.

◈ Microscopic Tests :

1. Platinum chloride, H_2PtCl_6 . A little of the powder being examined is dissolved in a drop of water, or better in diluted acetic acid, on the microscpoe slide, and a drop of the 5% reagent solution is added, then crystals are looked for under the microscope. They form gradually and are needles in rosettes.The crystals grow larger and form blades in rosettes in the presence of acetic acid, which also diminishes the interface of quinine, and may be used up to (1+1) strength. (Lews, New York International Revenue Laboratory.)
2. Mercuric iodide in HCl. (The solvent solution contains about 27% by volume of concentrated HCl, or 10% by weight of HCl, and is saturated with Hgl_2) This reagent is applied to a little of the dry substance on the microscope slide. The crystals are branching threads and splinter-plates.

   Comment: The test is extremely sensitive and usually succeeds even with highly adulterated samples. The reagent can also be applied to the aqueous solution.

◈ Thin Layer Chromatography (TLC) :

Sample preparation :

- If the sample provided ,suspected to contain heroin, is in powdered from : It is boiled with dichloromethane (CH_2Cl_3) , Filtered then the residue (after evaporation of solvent) is dissolved in 1 ml $CHCl_3$:MeOH .
- If the sample is provided in solution , it should be alkalinizing (using NH_4OH) then extracted with CH_2Cl_2 as mentioned before.

Condition :

- Stationary phase : Silica gel 60 F_{254} (5X10)
- Mobile phase :MeOH (10ml) :NH_4OH (5 drops) OR 5% MeOH/$CHCl_3$ (using 4-5 drops)
- Detection : by UV at 254 nm then spray with Dragendorff's reagent
- References : Heroin 0.05% in $CHCl_3$/MeOH .

N.B. Heroin, distributed in the market is usually mixed with adulterants and diluents. The average purity of wholesale samples (45%) was only slightly higher than the purity of retail samples (30%). It may contain: Paracetamol, phenobarbitone, caffeine, which a can be detected easily on TLC plate by using references of these compounds.

Tests for adulterants :

1. Quinine or other fluorescent compounds. A little of the powder is dissolved in dilute sulfuric acid in a test tube or on the microscope slide and observed under ultraviolet light. Nupercaine also fluoresces strongly.
2. Sanchez test for procaine and other primary aromatic amines. A solution of furfural in acetic acid, applied to the dry powder on the spot plate, yields a bright red colour with any speck of procaine or other primary aromatic amine.
3. Chromium sulfate-chloride reagent for methadone. This reagent can be applied either to a drop of solution or directly to a little of the dry powder on the microscope slide, and will readily yield the methadone crystals even in the presence of much more heroine or quinine or both.

High Performance Liquid Chromatogarphy (HPLC)

Conditions:

Column : μ-porasil [Normal phase] (150 X 3.9 mm)
Mobile phase : 3% MeOH in $CHCl_3$.
Detector : UV at 254 nm
Flow rate : 2 ml/ min
CS : 1 cm / min
Volume injection : 20 μl

Procedure:

1. For standard curve:

Heroin is prepared in concentration (0.05%) in CH_2Cl_2 (0.5 mg/1 ml). Then dilute different volumes : 4, 3, 2, and 1 ml to each to 5 ml (in a volumetric flask) with CH_2Cl_2 , representing: 0.4, 0.3, 0.2, and 0.1 mg/ml.

Inject 20 ml, in duplicate, into the HPLC system. The standard curve is plotted using different concentrations of heroin (mg/1mg) versus area under the peak.

A linear relationship confirm the possible use of this curve for quantization.

2. For the Sample:

For determination of heroin in the sample provided : prepare 0.1 – 0.2 % in CH_2Cl_2 and inject 20μl, in duplicate, into the HPLC system. Obtain from the read-out sheat the area under the peak corresponding to the injected samples refer to the standard curve to calculate the percentage of heroin in the sample.

Opiates

Trade Names	Morphine, Heroin, Codeine, Hydromorphone, Hydrocodone
Classification	Narcotic analgesic
Physical/ Psychological Dependence :	Morphine-high; Heroin-high; Codeine-moderate; hydromorphone-high
Methods of Administration:	Swallowed or injected
Physical Appearance :	White, brown, or black powder, liquids (injectable), tablets, capsules of various sizes and colors
Approximate Detection Time in Urine :	3 days
Clinical Effects/ Symptoms:	Euphoria, analgesia, drowsiness, respiratory depression

The Basics

Opiates are drugs such as heroin, codeine and morphine, which can produce a very high physical and/or psychological dependence by their users. Samples may be tested for codeine and morphine (a metabolite of both codeine and heroin), and follow-up testing for 6 acetylmorphine, also a metabolite of heroin, is allowed on morphine-positive samples.

Testing for the opiates, hydrocodone (Vicodin, Lortabs), hydromorphone (Dilaudid), is performed on nonregulated samples. Feelings of euphoria, analgesia, drowsiness, and respiratory depression are reported by users.

Sample

Random urine specimen in a properly sealed and labeled urine container.

Method and Instrument

Preliminary screening for drugs of abuse in urine is performed by immunoassay, Bayer ADVIA 2400. Confirmation by Gas Chromatography/Mass Spectroscopy.

Stability

After proper collection, concentration of codeine, morphine or other opiates in urine will not change significantly for several days at room temperature, for several weeks at refrigerated temperature or indefinitely when frozen.

Purpose:

Urine positive indicates recent usage.

Normal Results

Negative.

Abnormal Results

The original immunoassay has been confirmed by GC/MS. The screening cutoff for opiates in regulated samples is 2000 ng/mL. The confirmation cutoff for either codeine or morphine in these samples is 2000 ng/mL. This concentration is consistent with ingestion of the analyte or the analyte-producing medication sometime within the 72-hour period preceding the urine collection.

Interferences

Though no compounds have been tested that cannot be separated from codeine and morphine by GC/MS analysis, there are numerous prescription medications which contain codeine. In addition, poppy seeds contain morphine and codeine in varying amounts, so careful evaluation of confirmed opiate-positive samples by an experienced professional can be essential in avoiding a false accusation of drug abuse.

454 PHG practical course

lab No. 7

Detection of Active Constituents of Khat

(*Catha edulis*)

and its metabolites

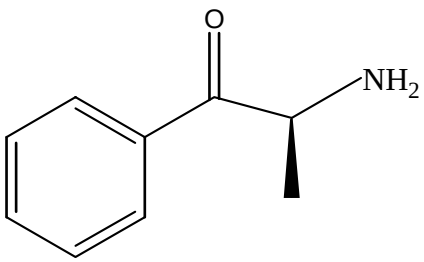
INTRODUCTION

A number of plants are known to contain drugs which are subject to control. Of the less common seizures that a forensic scientist might encounter are included products of *Catha edulis* Forsk. (Celastraceae), which have the street names 'Khat', 'Cat', 'Qat', 'Jeff and 'Mulka'.

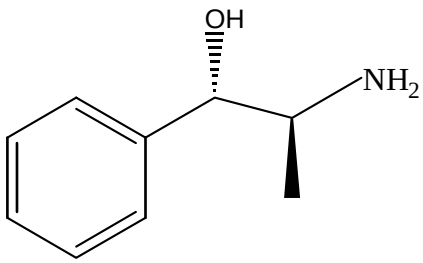
Khat is an evergreen shrub, which is cultivated as a bush or small tree. The leaves have an aromatic odour. The taste is astringent and slightly sweet. The plant is seedless and hardy, growing in a variety of climates and soils. Khat can be grown in droughts where other crops have failed and also at high altitudes.

The plant and/or its constituent phytochemicals have come under national control in a number of countries, as well as under international control as a result of the United Nations Convention on Psychotropic Substances, 1971.
Cathinone [(S)-1-aminopropiophenone] and **cathine** [(+)-norpseudoephedrine, (S,S)-norpseudoephedrine] are the major constituents of khat. However, the parent plant material is not controlled in the United Kingdom, *although it is controlled in some European countries (Norway and Sweden)*, the USA and Canada. The plant material contains these drugs at levels of between 0.3—2.0% of the dry weight of the plant.

Cathinone



Cathine



Khat is purchased as bundles of dried and fresh leaves and buds. For transport purposes, it is usually wrapped in plastic bags or banana skins to preserve its moisture content-loss of 'activity' is observed after 48 h if the plant material dries out.

The major part of the pharmacological action is due to **cathinone**, which acts as a central nervous system (CNS) stimulant, promoting excitation, reducing the need to sleep and enhancing communication.

Identification and Quantification of Khat

There are few reports in the literature of analyses of material from *C. edulis* and the only known prosecution for the production of cathine and cathinone from Khat was unsuccessful . Nevertheless, it is still necessary and useful to understand how to identify, quantify and compare such materials.

Basic forensic science principles should be adhered to when carrying out the analysis of a Khat seizure. Prior to any chemical investigation, a full physical description of the material should be made and appropriate samples removed for subsequent analytical work.

- *What details should be reported during a full physical description of a Khat sample?*

The form of the drug, and its size, weight, dimensions and smell should all be recorded. If the sample includes plant material, a botanical description might also be useful. For the analysis of such material, leaf and bark (twig) samples should be analysed separately.

Having obtained and described a suitable sample, the next stage is to analyse the material for the presence of cathinone and cathine. In addition to these drugs, other structurally similar compounds found in the *C. edulis* plant include norephedrine . It is well accepted that cathinone is converted into this compound by the process of enzymatic reduction. Because cathinone is a ketoamine base which is extremely unstable. Withering , drying and clean up of the plant material results in various degradation products.
Enzymatic reduction transforms (-)- cathinone into less active cathine, norephedrine and (+)- norpseudoephedrine.

In terms of identification of the material as Khat, from C. *edulis*. it is necessary to demonstrate the presence of Cathinone, since this is not found in other members of the genus.

Typical drug identifications based on chemical analysis depend upon presumptive (color) test techniques, followed by chromatographic separation. However. a slightly different approach is required for the analysis of Khat because the spot tests traditionally used for some drugs of abuse (e.g. the Marquis, Simons and Mandelin

tests) do not work for Cathinone. In general, the material is extracted and the drugs present are determined by using TLC, followed by either HPLC or GC-MS.

In order for chromatographic investigations to be carried out, a representative extract must be obtained which is suitable for the analysis of the drugs. A number of different methods have been employed for extraction of the drug components. All of these are based on the conversion of the drugs to their water-soluble salt forms (for example, as the hydrochlorides) by triturating of the ***freeze-dried*** [(*)] plant material in dilute acid (for example, 1 M HCl), basification of the extracts, back-extraction of the drug free bases into an organic solvent which is immiscible with water, drying of the solvent, concentration of the extracts, and then finally analysis of the drug components

*(*) **Freeze drying** (also known as **lyophilization**) is a dehydration process typically used to preserve a perishable material or make the material more convenient for transport. Freeze drying works by freezing the material and then reducing the surrounding pressure to allow the frozen water in the material to sublime directly from the solid phase to gas. The greatly reduced water content that results inhibits the action of microorganisms and enzymes that would normally spoil or degrade the substance.*

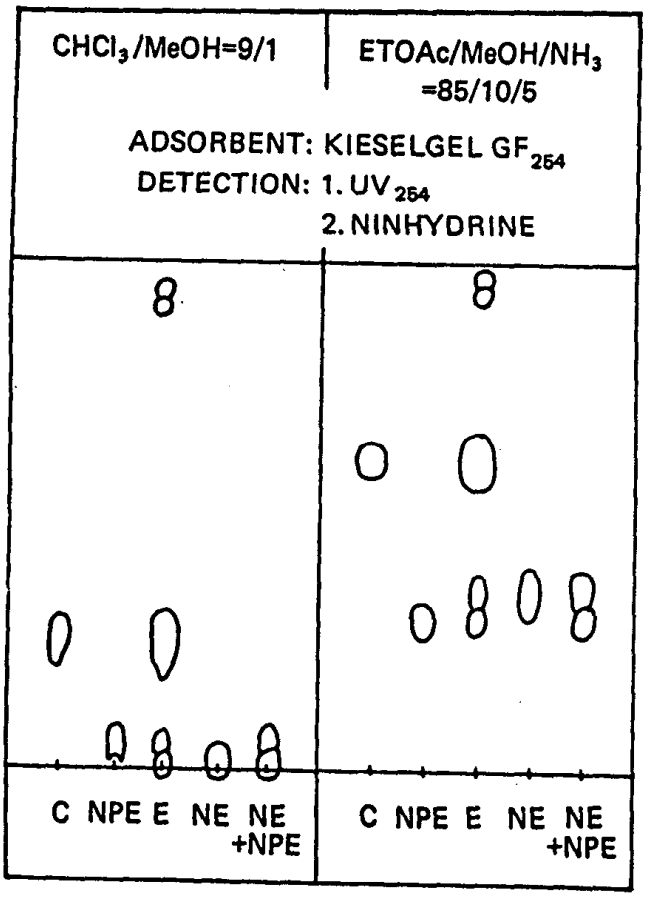
LAB WORK

◈ Thin Layer Chromatography (TLC) :

Procedure :

1. Cut the few fresh leaves of khat into small pieces, add suitable volume of methanol or $CHCl_3$ (using a mortar)
2. Triturate the cut leaves with methanol and filter, receiving the methanol in porcelain dish.
3. Repeat step 2.
4. Concentrate the filtrate, preferably under vacuum at low temperature, to about 0.5 ml.
5. Apply for TLC according to the following condition.
   Condition :
   - Stationary phase : Silica gel 60 F_{254} (5X10)
   - Mobile phase : ethylacetate : MeOH : ammonia (17 : 2 : 1)
     or $CHCl_3$: MeOH (9 : 1)
   - Detection :
     1. by UV at 254 nm
     2. spray with Ninhydrine (0.1%) reagent then heat at 110^oC
   - References : Cathinone and cathin in MeOH .
   - Result : Cathinone has high Rf value with brownish color while cathine gives violet color at low Rf value.

Thin-layer chromatogram of the chloroform fraction of khat



C = CATHINONE **NE = NOREPHEDRINE** **NPE = NORPSEUDOEPHEDRINE** **E = EXTRACT**

Pharmacokinetics and Detection

The euphoric effect appears shortly after the chewing begins, suggesting absorption from the oral mucosa. The effect of cathinone is maximum after 15–30 minutes. ***Metabolism of cathinone is rapid, occurring mainly during first passage through the liver. Only a small fraction (about 2%) appears unchanged in the urine. Most cathinone is metabolised to norephedrine and is excreted in this form***. The rate of inactivation is about the same as the rate of absorption, which limits the cathinone blood levels attainable by chewing.

Cathine has a slower onset of action, ***with a serum half-life in humans of about 3 hours. It is excreted unchanged in the urine within about 24 hours.*** When taking khat, large amounts of non-alcoholic drinks are consumed. There is pharmacological synergism with drinks containing methylxanthines (e.g. tea and cola), which therefore enhances the effects of khat.

In the UK, a commercially available biochemical test to detect khat constituents in the urine can now be used for confirmation of a suspected khat-induced state. Initially, a rapid screen by immunoassay detects amphetamine-related compounds. Then gas chromatography mass spectrometry is performed. ***This cannot detect cathinone directly, but a positive result indicates the presence of norephedrine, a cathinone metabolite. The test will give a positive result for up to about 48 hours after consumption of khat,*** although this is dependent on many factors, for example chronic consumption as opposed to a single episode of use, the quantity taken, the user's metabolism, and dilution of urine following consumption of fluids. The test is highly sensitive, but not highly specific, and there are some cross-reactions with other metabolites (Lehmann et al, 1990). However, in the context of the clinical presentation together with the history of khat consumption, the urinalysis is a useful additional test. In many areas urine testing is not available, and an accurate history of (increased) khat consumption prior to the onset of clinical symptoms is equally important, if not more so.

HPLC of Alkaloids from Khat

HPLC can be used to identify the drugs present in this controlled substance, with resolution of cathinone, norpseudoephedrine and norephedrine being achieved on silica gel (as the stationary phase), with a mobile phase consisting of 1,2-dichloroethane/methanol/acetic acid/diethvlamine/water (800:200:10:5:5, by volume), employing UV detection at 257 nm. This method has also been used to quantify the drug components present in these samples. However, retention time data were not provided in reference paper.

GC-MS of Alkaloids from Khat

Another approach to identifying the drug is to use GC-MS. Two such methods have been described recently. One of these involves components analysis of the underivatized alkaloids on a 30 m HP-5 column (i.d. 0.25 mm; d_f 0.25 µm) under the following conditions: split ratio of 50:1; carrier gas flow rate of 0.87 $mlmin^{-1}$; temperature programme. starting at 40^oC, rising to 95^oC at a rate of 25^oCmin^{-1} , holding for 18 mm, then rising to 270^oC at a rate of $20^oC\ min^{-1}$, and holding for 10 min. The retention times of cathinone and cathine were 18 and 19.5 mm, respectively. However, it was found in this case that the mass spectra of the two compounds were very similar.

454 PHG practical course

lab No. 8 & 9

analysis of Cocaine, its metabolites and other adulterants

INTRODUCTION

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 insuffiacated (‘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.

Qualitative Identification of Cocaine

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 colour being formed. Interestingly, diamorphine and temazepam also result in a blue reaction, but none of the other opiates or benzodiazepines result in this colour reaction. In addition, a number of local anaesthetics also produce a positive colour 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*

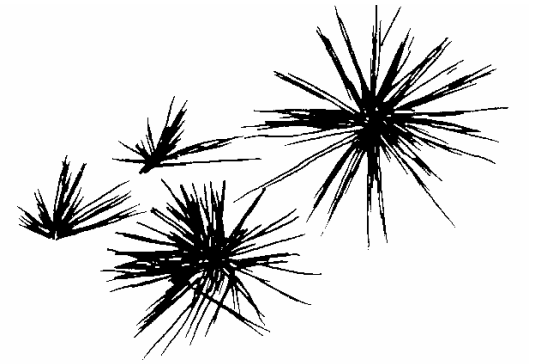
This test relies on the hydrolysis of the benzoate ester of cocaine and benzoyl ecgonine by potassium or sodium hydroxide . A 5% (wt/vol) solution of the hydroxide is prepared in methanol. The sample under test is mixed with a small volume of this reagent in a suitable container and warmed gently, for 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

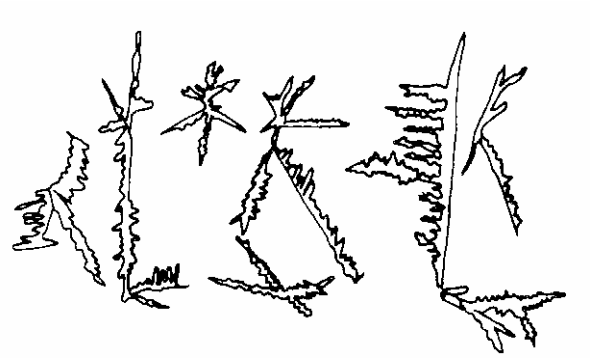
A. Place a drop of the alkaloidal solution on a clean glass slides, add a drop of reagents (platonic chloride, lead iodide and gold chloride) to the slides, and without stirring or covering, examine under the microscope. Please note that you should examine the crystals within 15 minutes and they should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

B. $KMnO_4$ test: Dissolve 10 mg of cocaine in 1 ml of O.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 KMnO4 solution. A violet crystalline precipitate is formed which shows characteristic violet red crystalline aggregates, when examined microscopically.

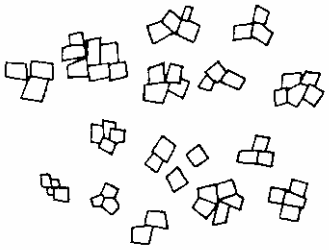
Cocaine + Platinic chloride



Cocaine + Lead iodide



Cocaine + Gold chloride



Cocaine + $KMnO_4$

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 rnL4 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 colours 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.

LAB WORK

A. Preparation of Benzylecogonin (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 mental.
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/$CHCl_3$.

B. Thin layer Chromatography :

Condition :

- **Stationary phase :** Silica gel 60 F_{254} (5X10)
- **Mobile phase :** MeOH (10ml) : NH_4OH (5 drops) or
  Cyclohexane : toluene : Et_2NH
- **Detection :** by UV at 254 nm, long-wavelength UV light (360 nm) then spray with Dragendorff's reagent
- **References :** benzylecogonin , cocaine, procaine and lidocaine in $CHCl_3$/MeOH .

C. Chrystalography :

By using platonic chloride solution and potassium permanganate test.

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

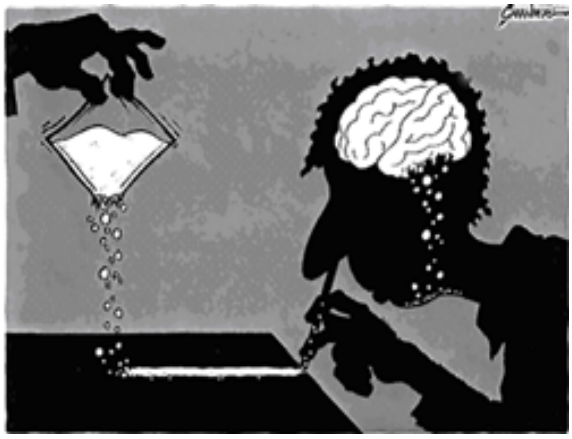
Typical GC operating conditions and parameters suitable for the analysis of cocaine by using GC-FID or GC-MS

System/parameter	Description/conditions
Column	OV-1: 25 m x 0.222 mm i.d.; d_f, *0.5* µm
Injection temperature	300^0C
Column oven temperature programme	170^0 C for 2 mins; increased to 280^0 C at 16^0C min^{-1} held for 2 min.
Carrier gas	He [a] or N_2 [b], at a flow rate of 1 m/min.
Split ratio	50:1
Detector	Flame-ionization or mass spectrometric detection
Detector temperature (FID)	300^0 C
Derivatization reagent	*N, 0* -(bistrimethylsilyl)acetamide

a: with mass spectrometric detection
b: with Flam Ionization Detection

Cocaine

Trade Names	Cocaine
Classification	Stimulant/local anesthetic
Physical/ Psychological Dependence :	Possible/high
Methods of Administration:	Sniffed, swallowed or injected
Physical Appearance :	Odorless, white crystalline powder with bitter numbing taste.
Approximate Detection Time in Urine :	2 to 4 days
Clinical Effects/ Symptoms:	Euphoria, motor and verbal hyperactivity, mood elevation, inflated self-esteem, grandiose delusions



The Basics

Cocaine is a central nervous system stimulant. It usually appears in the form of a fine crystal-like powder, although it can come in larger pieces called rocks. It may be injected, snorted or smoked as the free base. The effects of the drug begin within minutes and peak within 15 to 20 minutes. The effects include dilated pupils, increase in blood pressure, heart rate, breathing rate, and body temperature. The dangers associated with cocaine use vary depending on how the drug is taken, the dose, and the individual. Feelings of restlessness, irritability, anxiety, and sleeplessness are reported by some regular users. Even low doses of cocaine may create psychological problems. Use of high doses of cocaine over a prolonged period of time may lead to paranoia, commonly called cocaine psychosis, which includes hallucinations of touch, sight, taste, and smell.

Sample

Random urine specimen in properly sealed and labeled urine container.

Method and Instrument

Enzyme immunoassay measured spectrophotometri cally, Bayer ADVIA 2400. Confirmation by Gas Chromatography/Mass Spectroscopy.

Stability

All sample types are stable ten days at room temperature, two weeks when refrigerated, indefinitely when frozen.

Purpose

Urine and saliva metabolite positive indicate recent usage.

Normal Results

Negative.

Abnormal Results

The original enzyme immunoassay has been confirmed by GC/MS. The screening cutoff is different than the confirmation cutoff because the screening test detects all forms of the drug whereas the confirmation test measures only one form (metabolite) of the drug in question. The screening cutoff for cocaine metabolites is 300 ng/mL. The confirmation cutoff for the metabolite benzoylecgonine is 150 ng/mL. This concentration is consistent with ingestion of cocaine within a 48-hour period preceding the urine collection.

Interferences

No compounds have been tested which cannot be separated from benzoylecgonine by GC/MS analysis.