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NEW DELHI

WORKING PROCEDURE MANUAL: NARCOTICS

2021

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FOREWORD

Forensic Sciences have a critical role to play in criminal justice delivery system. Successful prosecution of offenders hinges on the quality of test reports. Exhibits in crime cases need to be examined timely, precisely and accurately. In order to standardise and benchmark forensic analysis and test reports in respect of crime cases in Forensic Science Laboratories across the country, the Directorate of Forensic Science Services (DFSS), Ministry of Home Affairs has taken the initiative to prepare Working Procedure Manuals (WPMs) for various forensic disciplines.

Due to advancement of technology and its global access, crime is continuously evolving and adapting. New crime trends are emerging with people committing crimes in cyberspace, trafficking occurring in new psychoactive substances and drugs etc. Therefore, incorporation of new technologies in the WPMs to meet the ever increasing challenges for solving crime is the need of the hour, which is systematically and comprehensively reflected in these WPMs.

A long-felt need for such uniform WPMs has been fulfilled and I hope that these will be of immense use to forensic professionals of India. I congratulate the DFSS team for developing these manuals and urge them to keep on updating these at regular intervals.

Punya Salila

(Punya Salia Srivastava)

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PREFACE

The analytical procedures for examination of forensic physical clue materials in forensic science laboratories involve high degree of skill & expertise and play a significant role in a wide range of legal proceedings. The occurrence of error(s) in any of the forensic analytical activities is a serious matter for both laboratories and end users. For a laboratory, it can lead to re-testing of samples, if available, and loss of its credibility. The analytical techniques adopted by the scientist(s) for the forensic analysis may be one of the causes for this serious error.

The risk of committing error can be eliminated if the scientists undertake two or more independent validated techniques while conducting forensic analysis of crime case exhibits in the laboratory. Essentially, the procedures adopted must conform to the quality, sensitivity, repeatability and reproducibility of the examination so that the chances of error are absolutely avoided. It is, therefore, one of the essential requirements of good laboratory practices to introduce a Working Procedure Manual, which contains validated laboratory methods/techniques for forensic analysis of the exhibits. It is also necessary for all the Central/State Forensic Science Laboratories to follow these manuals in the country to maintain uniformity in test reports.

Keeping in view the advancement in science & technology and use of various protocols & procedures in the international arena of forensic science, the Directorate of Forensic Science Services (DFSS), Ministry of Home Affairs(MHA), has taken the initiative for preparing a systematic and comprehensive Working Procedure Manual for the discipline of 'Forensic Narcotics' to bring uniformity and standardization in the examination methods. In this regard, DFSS/MHA formed Scientific Working Groups, comprising eminent forensic scientists from the CFSLs and FSLs for each forensic discipline to compile forensic analytical techniques in the form of Working Procedure Manuals. Several meetings were conducted with detailed deliberations among the scientists at National level and finally the manual has been prepared /updated in the present form.

I am sure that this Working Procedure Manual, which pertains to the discipline of 'Forensic Narcotics' will help the forensic science laboratories to continue to follow standard and latest updated procedures in the examination of clue materials as well as to adopt quality control/ quality assurance in the forensic practices and also for obtaining accreditation from National Accreditation Board for Testing and Calibration of Laboratories (NABL).

I understand that there is always a scope of improvement and perfection can be achieved with collective efforts, therefore, stakeholders are welcome to offer their feedback and suggestion, if any, in this regard.

19/3/2014

(Dr S K Jain)
Director-cum-Chief Forensic Scientist

TABLE OF CONTENTS

SECTION – 1

NARCOTIC DRUGS AND PSYCHOTROPIC SUBSTANCES

1.1 Introduction

Different chemical test methods are being adopted by different forensic analyst for the examination of the cases received in the laboratory to NDP Substances. The purpose of this manual/guidelines is to help the analyst in carrying out the analysis in the laboratory, to bring uniform system in the working procedures for the examination of crime cases and to prepare a written document for the working procedures of the chemistry division of this laboratory to meet the requirement of its accreditation. The test methods recommended in this manual are based on certain scientific principles/facts/experiments and selected after consulting the various books and publications etc. Forensic laboratories do the job of the chemical examination of the clue materials collected from the scene of crime. The purpose of the forensic chemical analysis is to examine the crime exhibits for its identification and determination to give unequivocal opinion to the Criminal Justice System. Sometimes the case has to be examined in a short period of time for which the analyst has to put his all efforts with the available resources in the laboratory. It is not possible to describe the entire chemical test methods for each and every type of NDPS exhibit in the manual. Though some of the test methods for the chemical examination of general routine cases have been recommended in the manual but depending upon the situation and need, other test methods/techniques/instructions available in the literature or validated can also be referred/followed for the examination of exhibits.

As the case, examining officer is the best judge for right approach to the case problem, the choice of the test methods recommended or other than recommended in the manual to be adopted for the examination of the exhibit lies with his discretion.

This manual suggests approaches that may help the forensic analyst to select a technique appropriate to the sample currently being examined. Each method described here 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 reputed Forensic Laboratories and has been published in the scientific literatures. In identifying these methods, the expert group was aware

that many of the useful and acceptable methods produce worthwhile analysis and information for the Forensic Analysts.

This manual has been primarily designed for use by the different Forensic Scientists working in the Laboratory. Not all the methods/techniques described in the manual need to be applied to all samples suspected to NDPS. The identity of the drug is to be established at least with two independent analytical parameters or till the satisfaction of analyst. Two uncorrelated TLC systems would count as two parameters which means either the solvent system or the coating on the plate or completely different. When possible three entirely different analytical techniques may be used, for example; colour test, chromatography (TLC, GLC, HPLC etc) and spectroscopy (IR/FTIR and UV-VIS). The actual choice of parameters is left to the discretion of the chemist.

1.2 Narcotic Drugs

The term "Narcotic" is derived from the Greek word "Narkotikos", which implies as state of lethargy or sluggishness. Narcotic drugs are those substances which gives relief from pain and induce sleep but socially not acceptable. Narcotic Drugs and Psychotropic Substances are of natural, semi synthetic and synthetic in nature. On ingestion into the body they act on the Central Nervous System and produce an altered state of mind of the subject. They produce physical and psychological dependencies, which depends on the drug.

The Opium Poppy is obtained from the plant 'Papaver somniferum'. The word "Papaver" is a Greek word meaning "Poppy", "Somniferum" is from Latin and means to dream or induce sleep. The opium poppy is indigenous to many climates. It also grows wild in many areas, but the largest quantities come from three main areas of the world, the areas known as Golden Triangle (Laos, Burma and Thailand) and the Golden Crescent (Afghanistan, Pakistan, Iran) and Mexico. The plant grows to 2 to 5 feet in height and displays a beautiful flower that ranges in colour from white to purple with the shades of red and orange being most common. After the flower petals have fallen off, raw opium is obtained by making a small incision either vertically or horizontally, on unripe poppy capsule which

allows the milky juice to come out from the plant. It is one of the major narcotic substances, by which various other powerful narcotic drugs.

Opium contains various organic and inorganic substances like alkaloids, acids, protein, sugar and inorganic elements etc., about 40 opium alkaloids have been known in the opium out of which 5 are major. They are morphine, codeine, thebaine, papavarine and narcotine. The first three alkaloids are covered under NDPS Act. Morphine is the alkaloid, which determines the identity and quality of opium. It should be more than 0.2% in the sample to be considered as opium. Since it is a obtained form the natural plant, the amount of these alkaloids may differ from crop to crop and area to area. These alkaloids are found in the following ranges **[1].**

Alkaloids % Ranges
Morphine 4 to 21%,
Codeine 0.7 to 3%
Thebaine 0.2 to 1%
Papavarine 0.5 to 1.3% and
Nicotine 2 to 8%

1.3 Common Terminologies of some of the drugs used in NDPS Acts [2]

"**Cannabis (hemp)**" means :-

a) Charas, that is, the separated resin, in whatever form, whether crude or purified, obtained from the cannabis plant and also includes concentrated preparation and resin known as hashish oil or liquid hashish.
b) Ganja, that is, the flowering or fruiting tops of the cannabis plant (excluding the seeds and leaves when not accompanied by the tops), by whatever name they may be known or designated; and
c) Any mixture, with or without any neutral material, of any of the above forms of cannabis or any drink prepared there from;

"**Cannabis plant**" means any plant of the genus cannabis.

"**Coca derivative**" means :-

a) Crude cocaine, that is, any extract of coca leaf which can be used, directly or indirectly, for the manufacture of cocaine,
b) Ecgonine and all the derivatives of ecgonine from which it can be recovered,
c) Cocaine, that is, methyl ester of benzoyl-ecgonine and its salts, and

d) All preparations containing more than 0.1 percent of cocaine.

"**Coca Leaf**" means :-

a) The leaf of the coca plant except a leaf from which all ecgonine, cocaine and any other ecgonine alkaloids have been removed.
b) Any mixture thereof with or without any neutral material, but does not include any preparation containing not more than 0.1 per cent of cocaine.

"**Coca plant**" means the plant of any species of the genus Erythroxylon.

"**Controlled substance**" means any substance, which the Central government may, having regard to the available information as to its possible use in the production or manufacturing of narcotic drugs or psychotropic substances or to the provisions of any International convention, by notification in the Official Gazette, declare to be a controlled substance.

"**Conveyance**" means a conveyance of any description whatsoever and includes any aircraft, vehicle or vessel.

"**Illicit traffic**" in relation to narcotic drugs and psychotropic substances means: -

a) Cultivating any coca plant or gathering any portion of coca plant
b) Cultivating the opium poppy or any cannabis plant
c) Engaging in the production, manufacture, possession, sale, purchase, transportation, warehousing, concealment, use or consumption, import inter-state, export inter-state, import into India, export from India or transshipment of narcotic drugs or psychotropic substances
d) Dealing in any activities in narcotic drugs or psychotropic substances other than those referred to in sub-clauses (a) to (c) or
e) Handing or letting out any premises for the carrying on of any of the activities referred to in sub-clauses (a) to (d); other than those permitted under this Act, or any rule or order made, or nay condition of any license, term or authorization issued, there under, and includes –
    1) Financing, directly or indirectly, any of the aforementioned activities

2) Abetting or conspiring in the furtherance of or in support of doing any of the aforementioned activities; and

3) Harbouring persons engaged in any of the aforementioned activities.

"**International Convention**" means :-

a) The Single Convention on Narcotic Drugs, 1961 adopted by the United Nations Conference at New York in March, 1961

b) The Protocol, amending the Convention mentioned in sub-clause (a), adopted by the United Nations Conference at Geneva in March, 1972

c) The Convention on Psychotropic Substances, 1971 adopted by the United Nations Conference at Vienna in February, 1971, and

d) Any other international convention, or protocol or other instrument amending an international convention, relating to narcotic drugs or psychotropic substances, which may be ratified or acceded to by India after the commencement of this Act.

"**Manufacture**" in relation to narcotic drugs or psychotropic substances, includes –

a) All processes other than production by which such drugs or substances may be obtained;

b) Refining of such drugs or substances;

c) Transformation of such drugs or substances, and

d) Making of preparation (otherwise than in a pharmacy on prescription) with oil containing such drugs or substances

"**Manufactured Drug**" means :-

a) All coca derivatives, medicinal cannabis, opium derivatives and poppy straw concentrate

b) Any other narcotic substance or preparation which the Central Government may, having regard to the available information as to its nature or to a decision, if any, under any International Convention, by notification in the Official Gazette, declare to be a manufactured drug. But does not include any narcotic substance or preparation which the Central Government may, having regard to the available information as to its nature or to a decision, if any, under International Convention, by notification in the Official Gazette, declare not to be a manufacture drug.

"**Medicinal Cannabis**" that is medicinal hemp, means any extract or tincture of cannabis (hemp)

"**Narcotics Commissioner**" means the Narcotics Commissioner appointed under Section 5.

"**Opium**" means :-

a) The coagulated juice of the opium poppy, and
b) Any mixture, with or without any neutral material, of the coagulated juice of the opium poppy, but does not include any preparation containing not more than 0.2 percent of morphine.

"**Opium derivatives**" means:-

a) Medicinal opium, that, is opium which has undergone the processes necessary to adapt it for medicinal use in accordance with the requirements of the Indian Pharmacopoeia or any other pharmacopoeia notified in this behalf by the Central Government, whether in powder form or granulated or otherwise or mixed with neutral materials
b) Prepared opium, that is, any product of opium obtained by any series of operations designed to transform opium into an extract suitable for smoking and the dross or other residue remaining after opium is smoked
c) Phenanthrene alkaloids, namely, morphine, codeine, thebaine and their salts
d) Diacetylmorphine, that is, the alkaloid also known as diamorphine or heroin and its salts, and
e) All preparations containing more than 0.2 per cent of morphine or containing any diacetylmorphine

"**Opium Poppy**" means:-

a) The plant of the species Papaver Somniferum L, and
b) The plant of any other species of Papaver from which opium or any phenanthrene alkaloid can be extracted and which the Central Government may, by notification in the Official Gazette, declare to be opium poppy for the purposes of this Act.

"**Poppy Straw**" means all parts (except the seeds) of the opium poppy after Harvesting whether in their original form or cut, crushed or powdered and whether or not juice has been extracted there from.

"**Poppy Straw Concentrate**" means the material arising when poppy straw has entered into a process for the concentration of its alkaloids.

"**Preparation**" in relation to a narcotic drug or psychotropic substance, means any one or more such drugs or substances in dosage form or any solution or mixture, in whatever physical state, containing one or more such drugs or substances.

References:

1. Recommended methods for testing opium/crude morphine, Manual for use by National Narcotics Laboratories, United Nations, New York, 1987.

2. The Narcotic Drugs and Psychotropic Substances Act, 1985, Government of India.

SECTION – 2

SAMPLING PROCEDURES

2.1 Title: Methods of Sampling

2.2 Scope: Sampling to profile various narcotic drugs of natural or synthetic origin under NDPS Act

2.3. Purpose: To obtain a representative sample of drugs of interest for their analysis.

2.4. Responsibility: Reporting officers and assisting scientific staff.

2.5. Methods: 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 draw. Sampling should be undertaken to conform to the principles of analytical chemistry, as laid down, for example, in national pharmacopoeias or the methods prescribed in the book by the Association of Official Analytical Chemists.

2.5.1 Single package [1]:

The simplest sampling situation is where the submitted item consists of a single package of material. The material should be removed from its container or wrappings and the net weight recorded.

The analyst should then visually examine the material to assess its homogeneity. Presumptive testing may be made at this stage if it is thought that the sampling or homogenization process will be lengthy or if there is still some doubt as to the identity of the material. For blocks, representative samples should be taken from alls surfaces including the center region or else the total brick is ground to a powder. For powders the material is placed in a clean clear plastic bag and shaken thoroughly.

If the powder contains aggregates, the same may be broken down by passing through successively finer sieves, or by pounding in a mortar pestle, or by use of an adapted commercial food mixer or food processor.

Alternately coning and quartering techniques 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 the sample size, particle sizes are further reduced, the material mixed thoroughly, poured on to a flat surface, and divided as before.

2.5.2 More than one package:

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

1. If all packages contain suspected drugs (opium or crude morphine-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 material. If one or more packages obviously differ in content, these should be segregated and subjected to separated analysis.

The composition 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; and
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 higher integer.

If the material in all the packages found to be similar by visual examination, one of the two following approaches may be followed:

1) The contents of a number of packages may be combined and the combined bulk material may then be homogenized.
2) Alternately, chemical testing may be applied to a number of each of the packages

When different types of materials have been identified in various packages, each sub-group should be composited in an identical fashion.

2.5.3 Items containing bulk aggregates [1]:

If the aggregates can be easily reduced to small particles then this should be the approach, and sampling procedure followed as outlined previously. If the material cannot be easily broken down, random samples should be drawn from at least two different parts of the item.

The above procedures may be followed for opium/crude morphine, cocaine, heroin, benzodiazepine, amphetamines, mescaline, psilocybe/ psilocybin and barbiturates.

2.5.4 Sampling procedure for LSD [2]:

Most LSD exhibits are either in paper, tablet or gelatin form. Powders are generally not encountered. The sampling procedure provided is applicable to these three forms. For the purposes of the sampling plan, one sheet of paper subdivided into smaller dosage units should be considered as one "Container".

In case of single container, determine the total number of dosage units and the average weight per dosage unit (du).

For sample sizes up to 10 du – screen all dosage units.

For sample sizes from 11 du to 27 du – randomly select and screen ¾ of all dosage units, rounding upward to the next higher integer.

For sample sizes from 28 dosage unit (du)– select randomly and screen ½ of all dosage units rounding upward to the next integer and selecting a minimum of 21 du and a maximum of 50 du.

In case of multiple containers having different contents, this should be segregated and subjected to separate analysis. Determine the square root of the total number of containers in each group. Randomly select a number of containers equivalent to the square root, rounded to the next highest integer.

Screen each unit using a presumptive test, based on the results of the screening test, proceed as follows: -

1. If all screened units appear the same, proceed as in case of single containers.
2. If all screened units do not appear the same, each container should be treated as a separate exhibit or entity. Thus for each container, proceed according to the direction above for a single container.

Reference:

1. Recommended methods for testing opium/crude morphine, Manual for use by National Narcotics Laboratories, United Nations, New York, 1987.
2. Recommended methods for testing LSD, Manual for use by National Narcotics Laboratories, United Nations, New York, 1987.

SECTION – 3

OPIUM/OPIUM ALKALOIDS/POPPY STRAW

3.1 Title: Testing of Opium /Opium Alkaloids/Poppy Straw/Husk.

3.2 Scope: To profile various opium alkaloids in various crime exhibits related to opium.

3.3 Purpose: To analyze various opium samples/crude morphine/Poppy straw/Husk.

3.4 Responsibility: Reporting Officers and supporting scientific staff.

3.5 Sampling Procedure:

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 draw. Sampling should be undertaken to conform to the principles of analytical chemistry, as laid down, for example, in national pharmacopoeias or in publications such as "Official Methods of Analysis" published by the Association of Official Analytical Chemists. This should include drying a representative sample to constant weight at 110^0C for moisture determination.

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 powers 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 an individual result 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.

Seizures of opium or crude morphine may consist of material in a single container or package, or in multiple packages.

3.5.1 Sampling of Single package items:

The simplest sampling situation is where the submitted item consists of a single package of material. The material should be removed from its container or wrappings and the net weight recorded.

The analyst should then visually examine the material to assess its homogeneity. Presumptive testing may be made at this stage if it is thought that the sampling or homogenization process will be lengthy or if there is still some doubt as to the identity of the material. For blocks, representative samples should be taken from all surfaces including the centre region or else the total brick is ground to a powder. For powders the material is placed in a clean clear plastic bag and shaken thoroughly.

If the powder contains aggregates, the same may be broken down by passing through successively finer sieves, or by pounding in a mortar pestle, or by use of an adapted commercial food mixer or food processor.

Alternately the technique of coning and quartering techniques 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 the sample size, particle sizes are further reduced, the material mixed thoroughly, poured on to a flat surface, and divided as before.

3.5.2 More than one package:

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If one or more packages obviously differ in content, these should be segregated and subjected to separated analysis.

The composition of multiple container items is as follows:

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e) If there are 10-100 packages – randomly select 10 packages; and

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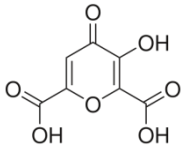
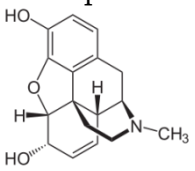
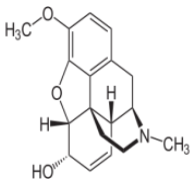
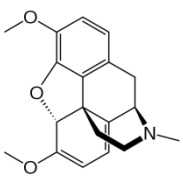
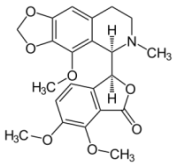
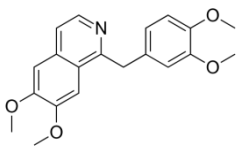
If the material in all the packages found to be similar by visual examination, one of the two following approaches may be followed:

1) The contents of a number of packages may be combined and the combined bulk material may then be homogenized.

2) Alternately, chemical testing may be applied to a number of each of the packages

When different types of materials have been identified in various packages, each sub-group should be composited in an identical fashion.

3.6 Major Chemical Constituents of the Opium

Poppy Capsule 	Poppy Straw 	Meconic Acid  $C_7H_4NO_7$ M.Wt-200.1	Morphine  $C_{17}H_{19}NO_3$ M.Wt-285.3
Codeine  $C_{18}H_{21}NO_3$ M.Wt-299.4	Thebaine  $C_{19}H_{21}NO_3$ M.Wt-311.4	Noscapine/Narcotine  $C_{22}H_{23}NO_7$ M.Wt-413.4	Papaverine  $C_{20}H_{21}NO_4$ M.Wt-339.4

3.7 Methods:

3.7.1 Colour Tests:

Positive results of these tests are only presumptive indication for the presence of opium alkaloids. It is mandatory for analyst to confirm such results by use of an alternate technique.

a) Marquis test [**1**]: Take a small amount of suspected sample in a test tube and add about 10 drops of water, crush the sample with a glass rod. Place a few drops of water solution through filter paper/supernatant liquid on a spotting plate and add few drops of Marquis reagent. The development of purple violet color indicates the presence of opium/crude morphine.

Preparation of Marquis Reagent: 8-10 drops of 40% formaldehyde solution is added to 10 ml of Con. Sulphuric acid.

b) Ferric Salt test [**1**]: Take small amount of suspected material on a spot plate and add about 2 drops of water, triturate the sample until the water becomes brown colour. Take a drop of brown liquid to another part of the spot plate, add one drop of reagent. Appearance of brown purple colour indicates the positive test for the presence of meconic acid. This meconic acid is present in raw and prepared opium, but it will not be detected in crude morphine.

Preparation of Ferric Salt Reagent: Dissolve 1 g of ferric sulphate in 20 ml of water.

Alternate Test for Meconic Acid [**2**]**:**

c) Ferric Chloride Test: Dissolve appropriate sample of opium in water and add a drop of dilute hydrochloric acid followed by few drops of 10% solution of ferric chloride. A red colour is appeared. Divide this solution into two parts. Take first part and add dilute hydrochloric acid to it in excess and warm. The red colour of the solution remains there. Take the second part and add a solution of mercuric chloride. The colour of the solution does not affect.

Preparation of Mercuric Chloride Reagent: Dissolve 5 gms. mercuric chloride in 100 ml of water.

Dilute Hydrochloric Acid [3]: About 10% W/W of HCl in water

d) Porphyroxine Test [**1**]: Take a small amount of suspected material on a spot plate and add two drops of water. Triturate it with glass rod. Take one drop of brown liquid from this mixture to another part of the plate, add one drop of 2 N hydrochloric acid and heat gently. Appearance of red colour indicates the presence of porphyroxine.

3.7.2 Thin Layer Chromatography

Stationary Phase: Activated silica gel (with or without fluorescing substance) TLC plates of 0.25/0.20 mm thickness.

Mobile Phase/Solvent systems:

System A [**1**]**:** Toluene, Acetone, Ethanol and Conc. Ammonia

(45 : 45 : 7 : 3)

System B [1]: Ethylacetate, Methanol and Concentrated Ammonia

(85 : 10 : 5)

System C [4]: Ethanol, Chloroform, Dioxane, Petroleum ether, benzene, ammonium hydroxide and ethyl acetate (5:10:50:15:10:15:5)

System D [4]: Ethanol, benzene, ammonium hydroxide and Dioxane

(5 : 50 : 5 : 40)

Visualization methods:

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

a) Preparation of Dragendorff's reagent [**1**]: Mix together 2 gms of bismuth subnitrate, 25 ml of 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 glacial acetic acid and 100 ml of water.

b) Preparation of Acidified potassium iodoplatinate reagent [**1**]: Dissolve 0.25 g of platinic chloride and 5 gms of potassium iodide in water to 100 ml. For the acidified version 2 ml of concentrated hydrochloric acid is added.

3. 7.3 Gas Liquid Chromatography

3. 7.3.1 Packed Column Technique [1]

Detector	: FID
Column	: 6 Ft (or 2 m), I.D. 2 to 4 mm
Packing	: 3 % SE-30 or OV-1
Carrier gas	: Nitrogen at 70 ml per minute
Injector temperature	: 275^{o}C
Oven temperature	: 230^{o}C
Detector temperature	: 275^{o}C
Internal standard	: Docosane or other n-alkanes (100 mg in 5.0 ml Methanol)
Derivatising reagent	: N,O bis trimethylsilylacetamide (BSA)

3. 7.3.2 Capillary Column Technique [1]

Detector	: FID
Column	: Fused silica, chemically bonded and cross-linked methyl silicone or methyl-phenyl silicone, such as OV-1, SE-54 or DB-1.
Film thickness	: 0.15 micrometer
Length	: 25 m by 0.27 mm i.d.
Carrier gas	: Hydrogen
Injection technique	: Split mode (ratio 1/60)
Makeup gas	: Argon at 18 ml per minute

Injector temperature : 250°C

Detector temperature : 280°C

Temperature program : Start at 150°C to 280°C @ 9°C per minute, then isothermal for 0.5 minute

Internal standard : Tetracosane or other n-alkanes

Silylating reagent :N-methyl-N-trimethylsilyltrifluoro acetamide (MSTFA)

3. 7.4 Gas Chromatography [3]

Detector : Flame ionization

Column : Glass (6ft x 2 mm ID)

Packing : 5% OV-1 on 80/100 chromosorb W-HP

Flow rate : 25 ml/min

Injector temperature : 275°C

Column temperature : 240°C

Detector temperature : 275°C

3. 7.4 High Performance Liquid Chromatography [1]

Method-1:

Column : 300 x 4 mm I.D.

Packing material : Cynoalkyl-silica HPLC 10 μm diameter (Nucleosil 10 CN or equivalent)

Mobile phase : Ammonium acetate (pH 5.8)- 80

Acetonitrile-10

Dioxane-10

Flow rate : 1.5 ml per minute

Detection : UV at 254 nm

Method - 2:

Column	: 300 x 3.9 mm I.D.	
Packing material	: Octadecyl-silica (μBondapak C_{18} or equivalent)	
Mobile phase	: Water	40
	Acetonitrile	60
	Triethylamine	0.1
Flow rate	: 2 ml per minute	
Detection	: UV at 280 nm	

3. 7.5 Spectroscopic methods:

3. 7.5.1 IR/FTIR Spectroscopy [3]:

Principal peak in KBr Disk are :

Morphine: 805, 1243, 1118, 945, 1146, 833.

Codeine: 1052, 1268, 1500, 1111, 793, 934.

Thebaine: 1234, 1605, 1144, 1270, 1030, 910.

3. 7.5.2 UV Spectrophotometry [3]:

Morphine:

Aqueous acid – 285 nm [A^{1}_{1} = 52 a]

Aqueous alkali – 298 nm [A^{1}_{1} = 92 a]

Codeine:

Aqueous acid – 285 nm [A^{1}_{1} = 55 a]

Aqueous alkali – no shift.

Thebaine:

Aqueous acid – 284 nm [A^{1}_{1} = 253 a]

Aqueous alkali – no shift.

Opium alkaloids may be studied by scanning the samples in appropriate organic solvents with the help of UV-Vis spectrophotometry. The spectrum and the value of λ_{max} of the spectrum can be compared with the standard value given in the literature or with using the standard sample of opium alkaloids.

3.7.5.3 Mass Spectrometry [3] :

Principal peaks at m/z :

Morphine: 285, 162, 42, 215, 286, 124, 44, 284.

Codeine: 299, 42, 162, 124, 229, 59, 300, 69.

Thebaine: 311, 255, 42, 44,296,310, 312, 174.

Gas Chromatography-Mass Spectrometry (GC-MS) [7]

Conditions:

The following conditions can be used for the analysis of morphine by GC-MS:

Column	: DB-5MS capillary column (fused silica, 30m x .25 mm)
Carrier gas	: Helium
Flow rate	: 1.8 ml/min
Temp. programme	: Start at 120^{0}C and increased to 320^{0}C at 15^{0}C/min
Injection volume	: 1 micro. litre
Mode	: Splitless
Ionisation	: EI (70 eV)
Mass spectra range	: m/z 40-650

3.7.5.4 Other Techniques/Methods

a) Capillary Electrophoresis

b) High Performance Thin Layer Chromatography (HPTLC)

References:

1. Recommended methods for Testing Opium, Morphine and heroin. Manual for use by National Drug Testing Laboratory by United Nations, New York, 1998.

2. Butler William P, Methods of Analysis for alkaloids, opiates, marihuana, barbiturates & miscellaneous drugs, Bureau of Narcotics & Dangerous drugs, Department of Justice, US.

3. Moffat AC, Clarke's Isolation & Identification of Drugs, 2nd edition, The Pharmaceutical Press, London, 1986.

4. Gunn John W., In Analytical Manual of Bureau of Narcotics & Dangerous Drugs, Department of Justice, US (By Stanely P Sobol & Richard A Moore).

5. Reddy M.M.; Suresh V.; Jayashanker G.; Sashidhar Rao B.; and Sarin R.K., Electrophoresis, 24, 1437-41, 2003.

6. Varshney K.M., Separation and Identification of opium alkaloids by High Performance Thin Layer Chromatography, Presented at 12th All India Forensic Science Conference, Port Blair, India, 2000.

7. Cingolani Mariano; Froldi Rino; Mencarelli Roberto; Mirtella Dora; and Rodriguez Daniel, J. of Analytical Toxicology, 25, 2001.

Other important references for the analysis of opium.

1. Indian Pharmacopoeia.

2. British Pharmacopoeia.

3. Quantitative analysis of drugs by DC Garratt, 3rd edition.

SECTION – 4

DIACETYL MORPHINE/HEROIN/SMACK/BROWN SUGAR

4.1 Title: Analysis of Heroin

4.2 Scope: To profile various types of heroin samples in crime exhibits.

4.3 Purpose: To analyze various types of exhibits of heroin.

4.4 Responsibility: Reporting officers and supporting scientific staff.

4.5 Sampling Procedure:

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 examinations 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 draw. Sampling should be undertaken to conform to the principles of analytical chemistry, as laid down, for example, in national pharmacopoeias or the methods prescribed in the book by the Association of Official Analytical Chemists.

4.5.1 Single package [1]:

The simplest sampling situation is where the submitted item consists of a single package of material. The material should be removed from its container or wrappings and the net weight recorded.

The analyst should then visually examine the material to assess its homogeneity. Presumptive testing may be made at this stage if it is thought that the sampling or homogenization process will be lengthy or if there is still some doubt as to the identity of the material. For powders the material is placed in a clean clear plastic bag and shaken thoroughly.

If the powder contains aggregates, the same may be broken down by passing through successively finer sieves, or by pounding in a mortar pestle, or by use of an adapted commercial food mixer or food processor.

4.5.2 More than one package:

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

1. If all packages contain suspect heroin or heroin-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 material. If one or more packages obviously differ in content, these should be segregated and subjected to separated analysis.

The composition of multiple container items is as follows:

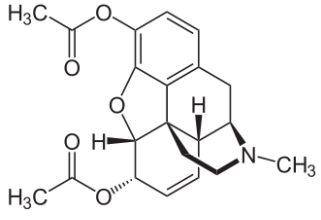
a) If there are less than 10 packages – all packages should be sampled;
b) If there are 10-100 packages – randomly select 10 packages; and
c) If there are more than 100 packages – randomly select a number of packages equal to the square root of the total number of packages rounded to the next higher integer.

If the material in all the packages found to be similar by visual examination, one of the two following approaches may be followed:

1) The contents of a number of packages may be combined and the combined bulk material may then be homogenized.
2) Alternately, chemical testing may be applied to a number of each of the packages

When different types of materials have been identified in various packages, each sub-group should be composited in an identical fashion.

4.6 Chemical Constituents of the Diacetyl Morphine:

Diacetyl Morphine	Heroin	Heroin
 $C_{21}H_{23}NO_5$ M.Wt-369.41		

4.7 Methods:

4.7.1 Colour Tests:

Positive results of these tests are only presumptive indication for the presence of heroin. It is mandatory for analyst to confirm such results by use of an alternate technique.

a) Marquis test [**1**]:- Take a small amount of suspected sample on a spotting plate and add few drops of Marquis Reagent. The appearance of purple violet colour indicates the presence of heroin.

Preparation of Marquis Reagent: 8-10 drops of 40% formaldehyde solution is added to 10 ml of Conc. Sulphuric acid.

b) Mecke's Test [1]: Take a small amount of suspected sample on a spotting plate and add few drops of reagent. The appearance of deep green colour indicates the presence of heroin.

Preparation of Mecke's Reagent: 0.25 gms of selenious acid is dissolved in 25 ml of concentrated sulphuric acid.

c) Frohde Test [1]: Take a small amount of suspected sample on a spotting plate and add few drops of reagent. The appearance of purple becoming grey/purple colour indicates the presence of heroin.

Preparation of Frohde's Reagent: 50 mgs of molybdic acid or sodium molybdate is dissolved in 10 ml of hot concentrated sulphuric acid. The resulting solution should be colourless.

d) Nitric Acid Test [2]: Take a small amount of suspected sample on a spotting plate and add few drops of conc. nitric acid. The appearance of yellow colour, which turns to green on standing, indicates the presence of heroin.

4. 7.2. Thin Layer Chromatography

Sample preparation: Take appropriate amount of suspected sample in methanol.

Stationary Phase: Activated silica gel (with or without fluorescing substance) TLC plates of 0.25/0.20 mm thickness.

Mobile Phase/Solvent systems:

System A [1]: Ethylacetate, Methanol and Concentrated Ammonia

(85 : 10 : 5)

System B [1]: Chloroform and Methanol (90:10)

System C [1]: Diethyl ether (water saturated), Acetone and Diethylamine (85 : 8 : 7)

Other Solvent Systems:

System 1 [3]: Ammonia, Benzene, Dioxane and Ethanol

(5 : 50 : 40 : 5)

System 2 [3]: Acetic acid, ethanol and water

(30 : 60 : 10)

System 3 [2]: Chloroform, Dioxane, Ethyl acetate and Ammonia

(25 : 60 : 10 : 5)

System 4 [2]: Ehtylalcohol, Chloroform, Dioxane, Petroleum ether (30-60^0), Benzene, Ammonia and Ethyl acetate (5:10:50:15:10:5:5)

System 6 [2]: Ethyl acetate, Benzene and Ammonia (60:35:5)

System 7 [2]: Ethyl acetate, n-butyl ether and Ammonia (60:35:5)

Visualization methods:

a. UV light at 254 nm.
b. Dragendorff's reagent spray.
c. Acidified potassium iodoplatinate reagent spray.

a) Preparation of Dragendorff's reagent [**1**]: Mix together 2 gms of bismuth subnitrate, 25 ml of 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 glacial acetic acid and 100 ml of water.

b) Preparation of Acidified potassium iodoplatinate reagent [1]: Dissolve 0.25 g of platinic chloride and 5 gms of potassium iodide in water to 100 ml. and add 2 ml of concentrated hydrochloric acid to make it acidic.

4.7.3 Gas Chromatography

4. 7.3.1 Packed column technique for heroin

Operating conditions:

Detector	: FID
Column	: 6 Ft (or 2 m), I.D. 2 to 4 mm
Packing	: SE-30 or OV-1; OV-17, i.e., methyl silicon or methyl phenyl silicon
Carrier gas	: Nitrogen
Flow rate	: 45 ml per minute
Injector temperature	: 275^{o}C
Oven temperature	: 250^{o}C
Detector temperature	: 275^{o}C
Internal standard	:Benzopinacolone(2,2,2-tripheyl acetophenone)or n-tetracosane or phenylbutazone

Derivatizing reagent: N,O bis trimethylsilylacetamide (BSA)

4. 7.3.2 Capillary column technique for heroin:

Detector	: FID
Column	: OV-1 cross linked, HP-17 (phenylmethylsilicone) or equivalent.
Film thickness	: 0.2 μm
Length	: 25 m by 0.32 mm ID
Carrier gas	: Hydrogen

Flow rate	: Circa 110 cm per second (about 30 ml per minute) measured at oven temperature of 150°C
Injector technique	: Split mode (ratio 1/60)
Makeup gas	: Argon at 18 ml per minute
Injector temperature	: 250°C
Detector temperature	: 280°C
Temperature program	: Start at 150°C to 280°C @ 9°C per minute, then isothermal for 0.5 minute
Detector Temp.	: 280°C

4. 7.3.2 Alternate/Additional methods/ of Gas Chromatography

Method 1 [3]:

Conditions :

Detector	: FID
Column	: Glass (6 ft x .25" i.d.)
Packing	: 3% OV-1
Carrier gas	: Nitrogen
Flow rate	: 60 ml/min.
Injector temperature	: 285°C
Column temperature	: 255°C
Detector temperature	: 285°C

Prepare a standard solution of heroin hydrochloride and n-triacontane (internal standard) in chloroform having a concentration of about 0.3 and 0.4 mg/ml respectively.

Prepare a working n-triacontane internal standard solution in chloroform having a concentration of 0.4 mg/ml.

Dilute an amount of sample with the working n-triacontane internal standard solution to give a final heroin hydrochloride concentration of between 0.08 and 0.5 mg/ml. Inject sample and standard solutions into gas chromatograph under the operating parameters given above. Calculate the amount of heroin hydrochloride in the sample from the formula:

Mg. Heroin HCl: A_s/A_{st} X I_s/I_{st} X C/W

Where:

A_s = Peak area of sample

A_{st} = Peak area of standard

L_s = Peak area of internal standard in sample solution

l_{st} = Peak area of internal standard in standard solution

C = Concentration of standard (mg./ml.)

W = Weight of sample (mg.)

Method 2 [4]

Gas Chromatography of Illicit heroin

The internal standard solutions of phenyl-butazone 0.2 mg/ml and chloramphemicol 2 mg/ml and standard solutions of caffeine 0.08 mg/ml, methaqualone 0.1 mg/ml and diazepam 0.1 mg/ml were also prepared in methanol. 2µl of standard mixture and internal standard solution (1:1) was injected. The brown sugar samples were well mixed, weighed and dissolved in methanol with internal standard and then filtered through Millipore filter paper of the type GV and pore size 0.22µm. 2µl of brown sugar (2-3 mg/ml) was injected. Different concentrations of heroin were prepared and mixed with internal standard phynyl-butazone (0.08 mg/ml) in 1:1 proportion; 2µl volume was injected to get response in the range of 25 to 100 ng. The attenuation of the integrator was increased to give good response for the low concentration of heroin. Run two or three time and plot the average.

Conditions:

GC : HP-5890 with HP-3396 integrator

Detector : FID

Column	: Fused silica wide bore
Column Temp.	: 250°C
Inj. Temp.	: 280°C
Detector Temp.	: 280°C
Flow carrier gas column	: 45 ml/min, Hydrogen
Aux. gas	: Nitrogen , 30 ml/min
Air	: 400 ml/min
Split ration	: 1.5:1

4. 7.4 High Performance Liquid Chromatography

4. 7.4.1 Isocratic Technique:-

Operating conditions:

Column	: 250mm by 4.6 mm i.d.
Packing material	: Silica HPLC grade 5 or 7 micron diameter.
Mobile phase (Degassed)	
Hexane	: 75% v/v
Dichloromethane	: 20% v/v
Methanol	: 5% v/v (The methanol contains 0.75% v/v diethlyamine)
Flow rate	: 2 ml per minute
Detection	: UV 227 nm Fluorescence – Excitation at 260 nm - Emission at 400 nm
Injection volume	: 10 µl
Quantitation	: By peak area, external standard method

4. 7.4.2 Gradient Technique [1]:

Operating conditions:

Column : 125 mm by 4.6 mm ID

Packing material : Octadecyl silane, 5µ, HPLC grade

Mobile phase :1) At start of chromatographic development:

5% MeOH, 95% phosphate buffer (0.023 M hexylamine, pH 2.2)

2) 20 minutes linear gradient

3) Final composition : 30% MeOH, 70% phosphate buffer

4) 8 minutes at final composition

5) End of chromatographic development

Flow rate : 1.5 ml per minute

Detection :(1) Two UV detectors in series at 218 & 228 nm.

(2) Photodiode array detector. Both signals on the photodiode array detector are time programmed so that the wavelength is switched on 12 minutes after the beginning of the run

Signal A: Detection first at 218 nm, then at 228 nm

Signal B: Detection first at 228 nm, then at 240 nm

Phospate buffer : 870 parts water
30 parts 2M sodium hydroxide

10 parts phosphoric acid.

The hexylamine is added to the buffer after degassing; otherwise it may be lost by evaporation. The final pH is obtained by further addition of sodium hydroxide of phosphoric acid as appropriate.

4. 7.5 Spectrophotometric methods [3]

4. 7.5.1 UV Spectrophotometry

Aqueous acid – 279nm (A^1_1 = 46 a)

Aqueous alkali – 299 nm (A^1_1=69 a)

Heroin may be studied by scanning the samples in appropriate organic solvents with the help of UV-Vis spectrophotometry. The spectrum and the value of λ_{max} of the spectrum can be compared with the standard value given in the literature or with using the standard sample of heroin.

4. 7.5.2 Infra-red/FTIR Spectroscopy

Sample preparation:

1. Halide disc method (KBr Pallet method)

2. Micro halide disc method

3. Nujol mull method (liquid paraffin method)

Major peaks are obtained in the IR spectra of heroin and monoacetyl-morphine at the following wave numbers (cm^{-1}). They are listed in the order of magnitude of absorbance. But the sequence may vary from sample to sample depending on the method of sample preparation.

Heroin Base:1243,1196,1727,1214,1444,1757,1054,1370

Heroin HCl : 1245,1736,1177,1194,1448,1765,1757,1368

0^6 – Monoacetylmorphine Base: 1239,1740,1018,1038,1374,1459,1505,915

0^6 – Monoacetylmorphine HCl : 1240,1723,1503,1039,1305,1368,1465,805

4. 7.5.3 Gas Chromatography-Mass Spectrometry (GC-MS) [6]

Conditions:

Column : Capillary column (30 m x .25 mm ID x .25 μm thickness

Packing : HP1 (100% dimethylpolysiloxane)

Detector	: MSD
Temp. program	: 180°C/min. increased to 240°C @ 10°C/min, hold for 4.5 min, continue the heating at 17°C/min upto 290°C & hold for 4 min.
Inj. Temp.	: 280°C
Detector Temp.	: 300°C
Carrier gas	: Helium
Flow rate	: 1.2ml./min
Inj. Volume	: 1µl - Split (1:20)
Mode	: EI (70eV)
Spectra range	: 40-450 amu for qualitative Selected ion monitoring (SIM) for quantitation.

Base (Heroin)	:	MAM: 327, 268, 42, 43, 215, 44, 328, 269.
	:	DAM: 327, 43, 369, 268, 310, 42, 215, 204.

4. 7.6 Other techniques/methods

a) High Performance Thin Layer Chromatography (HPTLC) **[5]**

b) Ion Mobility Spectrometry Technique

c) X-ray Diffraction Spectroscopy

d) GC-Ion Scan

[Details of the heroin and its examinations may also be referred from "**Recommended methods for testing of heroin"** described in the 'Manual for use by National Narcotics Laboratories, United Nations, New York, 1986]

References:

1. Recommended methods for testing Heroin, Manual of National Narcotics Laboratories, United Nations, New York, 1986.

2. Butler, William P, Methods of Analysis for alkaloids, opiates, marihuana, barbiturates & miscellaneous drugs, Bureau of Narcotics & Dangerous drugs, Department of Justice, US, Page 55 and 59.

3. Gunn, John W., In Analytical Manual of Bureau of Narcotics & Dangerous Drugs, Department of Justice, US (By Stanely P Sobol & Richard A Moore), Page No.136.

4. Krishnamurthy, R., and Srivastava, Ashwini K., J. of Ind. Acad. Foren. Sci., 33,1&2, 1994, p-29.

5. Varshney, Krishan Murari, HPTLC Study of the Stability of Heroin in Methanol, J. Planar Chromatogra. 15, 2003, p. 46-49.

6. Klemenc, S., Noscapine as an adulterant in illicit heroin samples, Foren. Sci. Int., 108, 2000, p 45-49.

Other References:

1. Krishnamurthy, Rukmani and Srivastava, Ashwini K., Simultaneous detection of adulterants and coextractants in illicit heroin by HPTLC with two successive mobile phases, J. Planar Chromatogra.,10, 1997, pp-388-390.

2. Krishnamurthy, R.; Malve, M. K.; and Shinde, B. M., Profiling of street narcotic drugs by High Performance liquid chromatography with photodiode array detector, J. Ind. Acad. Foren. Sci., pp – 66-67.

3. Krishnamurthy, R.; Ambulkar, S. V.; and Shinde, B. M., Case report: seizure of black brittle substance containing caffeine and paracetamol, the main adulterants in brown sugar., J. Ind. Acad. Foren. Sci., pp-56-58.

SECTION – 5

CANNABIS

5.1 Title: Analysis of Cannabis.

5.2 Scope: Various active constituents in cannabis plants & drugs.

5.3 Purpose: To analyze various cannabinoids.

5.4 Responsibility: Reporting officers and assisting scientific staff.

5.5 Sampling Procedure:

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 examinations 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 draw. Sampling should be undertaken to conform to the principles of analytical chemistry, as laid down, for example, in national pharmacopoeias or the methods prescribed in the book by the Association of Official Analytical Chemists.

1. Sampling of single package items

The simplest sampling situation is where the submitted item consists of a single package of material – for cannabis most often the material will be loose herb. The material should be removed from its container or wrappings, placed in a clean clear plastic bag and the net weight recorded. The analyst should then carefully use visual testing to ensure that the entire exhibit is material which is controlled under the legislation within which he or she works. The sequence of chemical tests can then follow. Homogenization of the material need only be applied in certain analytical situations, e.g. if the analyst wishes to quantify a particular cannabinoid. The simplest way of homogenizing cannabis (herbal or resin forms) is to pass the material through progressively finer sieves. In quantifying cannabionoids care should be taken to relate content found to the total amount of cannabis plant material which was originally taken for analysis i.e. the content should not be quoted as a percentage of the weight of the final sieved material which was subjected to extraction.

2. Sampling of items consisting of more than one package

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

1. If all packages contain suspect cannabis or cannabis-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 material. 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 material in all the packages is found by visual examination to be the same then the analyst may adopt one of two approaches:

(1) The contents of a number of packages may be combined and the combined bulk material may then be homogenised;

(2) alternatively, chemical testing may be applied to a number of the packages.

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

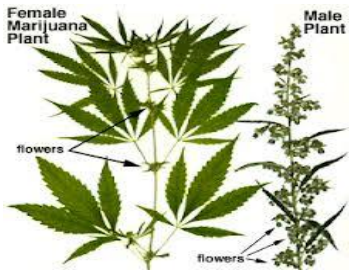
Sampling errors for quantitative methods are reduced if large aliquots of material are subjected to sequential dilution with the extraction solvent. If the cost of the solvent presents no problem and if the taking of a large aliquot will not significantly reduce the size of the exhibit to be taken to court, then this approach may be adopted. However, when large amounts of material are used for the first extraction, it may due to insoluble materials.

3. Sampling of materials containing large aggregates

If the aggregates can be easily reduced to small particles then this should be the approach, and sampling procedure followed as outlined previously. If the

material cannot be easily broken down, then random samples should be drawn from at least two different parts of the item. In the case of large compressed blocks of herbal material, the analyst should ensure that the block is entirely composed of cannabis. This is achieved by breaking open the block.

5.6 Major Chemical Constituents of the Cannabis:

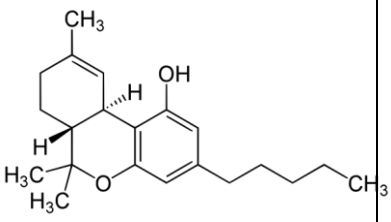


Cannabis Plant

Charas

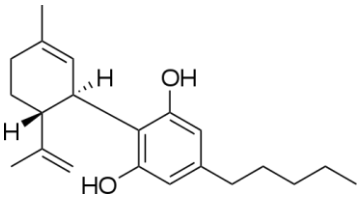
Ganja

Δ^9 – Tetrahydrocannabinol (THC)



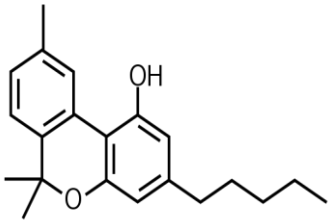
$C_{21}H_{30}O_2$
M.Wt-314.47

Cannabidiol



$C_{21}H_{30}O_2$
M.Wt-314.46

Cannabinol (CBN)



$C_{21}H_{26}O_2$
M.Wt-310.4319

5.7 Methods:

5.7.1 Microscope examination [1]

The following microscopic features (Trichomes) broken or as such are found on the surface of the fruiting and flowering tops of cannabis, which are the characteristic features of cannabis product.

1. Non glandular hair (trichomes), numerous, unicellular, rigid, curved, with a slender pointed apex and an enlarged base, usually containing a

cystolithic hair but frequently broken and the cystolith freed (especially in cannabis resin)

2. The glandular trichomes occur in three forms:

i. Sessile glands with one-celled stalk (generally on lower

epidermis).

ii. Long multicellular stalk form (generally on the bracteoles surrounding the female flowers). The head in both forms is globular consisting of eight to sixteen cells. It is frequently detached (especially in cannabis resin)

iii. Small glandular trichome, with one-celled stalk (For the diagram/figures please see "Recommended methods for testing CANNABIS, US, New York 1987")

5. 7.2 Colour tests:

Positive results of colour tests are only the presumptive indication of the possible presence of cannabis products. It is necessary for the analyst to confirm the presence of cannabis product by the use of an alternative technique.

a) Fast Blue B Salt Test

5. 7.2.1 Filter Paper Method [1]

Fold two filter papers to form fluted funnels. Keep these paper funnels on each other. Place a small amount of suspected sample into the corner of the upper funnel of the paper and add two drops of solution-1. Allow the liquid to penetrate to the lower filter paper funnel. Discard the upper filter paper and dry the lower filter paper. Now add a very small amount of the solid fast Blue B reagent to this lower paper and add two drops of solution 2. A purple–red coloured stain on the filter paper indicates the presence of cannabis product.

Reagents:

Solid reagent: Dilute & mix fast blue B salt with anhydrous sodium sulphate in the ratio of 1:100

Solution 1: Petroleum ether

Solution 2: A 10% w/w aqueous solution of sodium bicarbonate

5. 7.2.2 Test Tube method [1]

Take a small amount of suspected material in a test tube; add to it a very small amount of the solid reagent and 1 ml of solution 1. Shake well for one minute and add 1 ml of solution 2. Shake the test tube for two minutes, and allow this test tube to stand for 2 minutes. A purple red colour in the lower layer of chloroform indicates the positive result of the presence of cannabis product.

Reagents :

Solid reagent: Dilute & mix fast blue B salt with anhydrous sodium sulphate in the ratio of 2.5:100

Solution 1: Chloroform

Solution 2: 0.1N aqueous sodium hydroxide solution

5. 7.2.3 Duquenois-Levine Test [1]

Take a small amount of suspected material in a test tube and shake with 2 ml reagent for 1 minute, add 2 ml of conc. HCl and shake it well. Allow it to stand for 10 minutes and then add 2 ml of chloroform. Appearance of violet colour in chloroform layer (lower layer) indicates the presence of cannabis.

Reagent:- 5 drops of acetaldehyde and 0.4gms of vanillin are dissolved in 20 ml of 95% ethanol.

5. 7.2.4 Alternate test [2]

Extract the sample with petroleum ether. Filter and evaporate to dryness. Add 2 ml. of Duquenois reagent to dissolve the residue add 2ml. Conc. HCl. Shake and keep for 10min. Transfer the solution into a test tube add 2ml. of Chloroform and shake. Purple colour in the chloroform layer indicates the tetrahydrocannabinols.

Reagent:- 5 drops of acetaldehyde and 0.4gms of vanillin are dissolved in 20 ml of 95% ethanol.

5. 7.2.5 Test for differentiation between Bhang, Ganja and Charas [3]

Extract the suspected material of cannabis in ethanol. Take a drop of extract in a cavity of a spot tile or in a micro tube, add 2 drops of chromogenic reagent 1 and mix thoroughly followed by addition of 2 drops of reagent 2.

Bhang gives green colour, ganja gives blue colour while charas gives violet colour.

Reagent 1: p-Aminophenol (1 mg) in ethanol (10 ml)

Reagent 2: Caustic potash (1 g) in distilled water (10 ml)

5. 7.3Thin Layer Chromatography

a) Stationary Phase: Activated silica gel (with or without fluorescing substance) TLC plates of 0.25/0.20 mm thickness.

Mobile Phase/Solvent systems: [1]

System A : Petroleum ether & Diethyl ether (80:20)

System B : Cyclohexane, Di-isopropyl ether & Di-ethylamine (52:40:8)

System C : n-Hexane, Dioxane & Methanol (70:20:10)

Visualization : Fast blue B salt

b) Stationery Phase [4]: Silica gel G, TLC plate modified with 10% solution of silver nitrate by dipping or spraying and dried

System D : Toluene, using non- saturated condition (development in open chamber)

Silica gel G, TLC plate modified with 10% solution of diethylamine by dipping or spraying immediately before use.

System E : Xylene, hexane & diethylamine (25:10:1)

5.7.4 Gas Liquid Chromatography [1]

5.7.4.1 Packed Column Technique

Method 1

GC Conditions:

Detector : FID(Hydrogen 30ml/minute, Air 300 to 450 ml/min.)

Column size : 6 ft or 2m, 2-4mm ID

Column Packing material : 3% OV 17 or SE30 or OV1

Carrier gas : Nitrogen

Flow rate : 30ml/min.

Injector Temp. : 270°C

Oven Temp. : Isothermal between 240-260°C

Detector Temp. : 300°C

Internal std. : n-Tetradecane or n-docosane or other suitable n-alkane.

Method 2: with derivatization-

2 ml aliquots of the extracts prepared for TLC or GC analysis without derivatization (Method 1) can be used for silylation. Derivatizing agents frequently use are:

N,O-bis (trimethylsilyl)acetamide (BSA)

N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA)

N,O-bis (trimethylsilyl) trifluoroacetamide (BSTFA)

5. 7.4.2 Capillary Column Technique

Detector : FID

Column : OV-1 chemically bonded fused silica capillary

10m x 0.52mm id

Film Thickness : 1μm

Carrier gas : Helium

Flow rate : 2ml/min.

Inj.Technique : split/splitless

Inj.Temp. : 290°C

Oven Temp. : 240°C

Detector Temp. : 290°C

5. 7.5 High Performance Liquid Chromatography [2]

5. 7.5.1 Isocratic Technique

Method 1

HPLC conditions:

Column : 250mm x 4.6mm id

Packing material : Octadecyl-silica (medium load of C18 on partisil 5)

Mobile phase : 0.02 N sulphuric acid 20% v/v

Methanol 80% v/v

Flow rate : 2ml/min.

Detection : UV at 220nm or 254nm

Quantitation : by peak areas, internal standard method

Internal Std. : Di-n-octyl phthalate

Method 2

Operating Conditions:

Column : 150mm x 4.6mm id

Packing material : Octadecyl-silica (spherisorb S3 ODS 2) HPLC grade 3µm

Mobile phase : Methanol 85.0

Water 14.2

Acetic acid 0.8

Flow rate : 1.5ml/min.

Detection : UV at 230µm

Inj. Volume : 2-3 µl

Quantitation : by peak areas, internal or external standard methods

Operating Temp. : Ambient.

5. 7.5.2 Gradient Technique

Method 1

Column : 250 mm x 4.6mm id

Packing material : Ultrasil-Octyl HPLC grade 10um

Mobile phase : a. Acetonitrile

b. Water (deionised and filtered through 0.45µm filter)

Gradient Prog. : 25% A 75% B at the starting 36min.

linear gradient final composition 85% A, 15% B

Flow rate : 2ml/min.

Detection : UV at 254nm

Oven Temp. : 40°C

Inj. Volume : 20 µl

Quantitation : by peak areas, internal or external standard methods

Internal Std. : Di-n-octyl phthalate

Method - 2
Two columns are used in this method under identical operating conditions

Column 1 : 150 mm x 4.6mm id

Packing material : Spherisorb S 3 ODS 2 HPLC grade 3 um

Column 2 : 250mm x 5 mm id

Packing material : Spherisorb S 3 ODS 2 HPLC grade 5 um

Mobile phase : a. Methanol

b. 0.02N Sulfuric acid

Gradient Prog. : 80% A 20% B at the starting 20min. linear gradient final composition 90% A, 10% B

Flow rate : 1.5 ml/min.

Detection : UV at 230nm

Oven Temp. : Ambient

Detection : UV at 230nm

Operating Temp. : Ambient

Inj. Volume : 2-3 µl

5.7.6 Spectroscopic Techniques

5.7.6.1 Mass Spectrometry (GC-MS)

Principal peak at m/z

Δ^9 THC : 299, 231, 314, 43, 41, 295, 55, 271 **[4]**

Δ^8 THC : 221, 314, 248, 261, 193, 236, 222, 315 **[4]**

5. 7.6.2 Other method

UV – Vis Spectrophotometry [5]

[Details of the cannabis and its examinations may also be referred from **"Recommended methods for testing of cannabis"** described in the 'Manual for use by National Narcotics Laboratories, United Nations, New York, 1987']

References:

1. Recommended methods for testing Cannabis, Manual of National Narcotics Laboratories, United Nations, New York, 1987

2. Gunn, John W., In Analytical Manual of Bureau of Narcotics & Dangerous Drugs, Department of Justice, US (By Stanely P Sobol & Richard A Moore), Page No.167.

3. Tewari, Swaroop N., Liquor and Narcotic Drugs; A guide for the identification of drugs of abuse and recognition of drug abuses (P.86)

4. Moffat, A.C., Clarke's Isolation & Identification of Drugs, 2nd edition, The Pharmaceutical Press, London, 1986, P 423 and 172.

5. Lavanya, K. and Baggi, T.R., Quantitation of Δ^9 Tetrahydrocannabinol in Cannabis using 3-methyl benzthiazolinone 2-hydrazone, Microchemical J., 40, 1989, p 297-303.

SECTION – 6

COCAINE

6.1 Title : Analysis of Cocaine

6.2 Scope: Test exhibits containing various products of Erythroxylon coca

6.3 Purpose : Identification & estimation of cocaine and ecgonine

6.4 Responsibility: Reporting officers and assisting scientific staff.

6.5 Sampling Procedure:

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 examinations 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 draw. Sampling should be undertaken to conform to the principles of analytical chemistry, as laid down, for example, in national pharmacopoeias or the methods prescribed in the book by the Association of Official Analytical Chemists.

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 of homogenize to 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 powdering 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 into 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 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. Alternatively, 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.

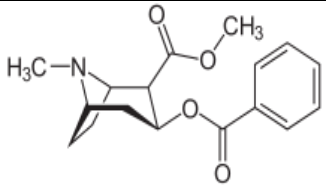
Sampling errors for quantitative methods are reduced if large aliquots of material are subjected to sequential dilution with the dissolving solvent. If the total sample size is large, this approach may be adopted. However, when large amounts of material are used for the first dissolution, it may be necessary 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 gram should be collected, weighed accurately and subjected to assay.

6.6 Major Chemical Constituents of the Cocaine



$C_{17}H_{21}O_4$
M.Wt-303.4

6.7 Methods:

6. 7.1 Colour Test

Positive results of colour tests are only the presumptive indication for the presence of cocaine. It is necessary for the analyst to confirm the presence of cocaine by an alternative technique.

6. 7.1.1 Scott's Test [1]:

Step-1: - Take appropriate amount of suspected material in a test tube, add 5 drops of soln-1 and shake well. A blue colour develops at once if cocaine is present. If blue colour is not appeared add more test sample. If blue colour is still not developing then it indicates that the sample does not contain cocaine.

Step-2: - Add one drop of soln.2, and shake. The Blue color will disappear and a clear pink colour solution will appear. If the blue Color does not disappear, add a second drop of solution 2.

Step-3: - Add several drops of solution 3 and shake. The appearance of blue color in Chloroform layer indicates positive test for the presence of cocaine.

Reagents:-

Solution-1: - 2% cobalt thiocyanate in water and diluted it with 96% glycerin in 1:1.

Solution-2: - Conc. Hydrochloric acid.

Solution-3: - Chloroform

6. 7.1.2 Ethyl Benzoate Test [2]

Take appropriate amount of suspected sample and moistened with nitric acid. Evaporate to dryness. Add few drops of alcoholic potash. Odor of ethyl benzoate indicates positive test for the presence of cocaine.

6. 7.2 Thin Layer Chromatography

Developing solvent systems: -

System A [1]: Chloroform, Dioxane, Ethyl acetate, Ammonia (29%) (25:60:10:5)

System B [1]: Methanol, Ammonia (29%) (100:1.5)

System C [1]: Cyclohexane, Toluene, Diethylamine (75:15:10)

System D [2]: Ethyl acetate, Benzene, Ammonium hydroxide (60:35:5)

System E [2]: Chloroform, Ethyl acetate, Ammonium hydroxide (40:10:10 drops)

Visualization

1. UV light 254
2. Acidified potassium iodo-platinate reagent.
3. Dragendorff's reagent

Preparation of solution for TLC

Appropriate sample of exhibits and standard are dissolved in methanol.

6. 7.3 Gas Liquid Chromatography

6. 7.3.1 Packed column technique [1]

Operating conditions

Detector : FID

Flame : Hydrogen at 30 ml 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

Flow rate : 30ml per minute.

Injector temperature : 220^{o}C

Oven temperature : 220^{o}C

Detector temperature : 300^{o}C

Internal standard : N-tetracosane or tetraphenylethylene

Derivatizing agent : N,O bis-trimethylsilylacetamide (BSA) or N-methyl – N-trimethylsilyltrifluoroacetamide (MSTFA)

6. 7.3.2 Packed column technique [3]

Operating conditions

Detector : Flame ionization

Column : 6 ft glass (1/4 inch i.d.)

Packing : 3% OV-1 on Chromosorb WHP (80/100 mesh)

Carrier gas : Nitrogen

Flow rate : 50ml per minute.

Injector temperature : 275^{o}C

Oven temperature : 210°C

Detector temperature : 275°C

6. 7.3.3 Capillary column technique [1]

Operating conditions

Column : OV-1 –chemically bonded fused silica capillary

Film thickness : 0.15 μm

Length : 25 m by 0.32 mm ID

Carrier gas : Hydrogen.

Spil rate : 2 ml per minute

Split ratio : 1:50

Detector : FID

Injector temp. : 250°C

Detector temp. : 280°C

Program starts at 150°C, immediate increase at 9°C per minute to 280°C.

6. 7.4 High Performance Liquid Chromatography Operating conditions [1]

Column : 160 mm by 5.0 mm ID

Packing material : Ocetadecyl-silica HPLC grade 5 um

Mobile phase : **Eluent A** - Methanol (300 ml), water (700 ml), 1%(v/v)phosphoric acid (1000 ml) and n-hexylamine (10.71 g; 14 ml) (pH 2.5)
Eluent B - Methanol (1000 ml), 1%(v/v) phosphoric acid (1000 ml) and n-hexylamine (10.71 g/14 ml) (pH 2.8)

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

Flow rate : 2.0 ml per minute for both eluents

Detector : UV at 230

Sample preparation :All materials are dissolved in the appropriate eluent.

Standard solutions :Dissolve approximately 1mg in 10ml eluent of any of the following substances:

Cocaine

Cis-cinnamoylcocaine

Trans-cinnamoylcocaine

Procaine

Lignocaine

Amylocaine

Butacaine

Benzocaine

Injector volume : 20 µl by loop injector

Quantitation : By peak areas, internal or external standard methods.

6. 7.5 Spectrophotometric techniques

6. 7.5.1 UV spectrophotometry

Aqueous acid – 233 nm (A^{1}_{1}=430 a), 275 nm **[4]**

Cocaine may be studied by scanning the samples in appropriate organic solvents with the help of UV-Vis spectrophotometry. The spectrum and the value of λ_{max} of the spectrum can be compared with the standard value given in the literature or with using the standard sample of Cocaine.

6. 7.5.2 IR/FT-IR Spectrophotometry:

Sample Preparation

1. Halide disc method
2. Micro Halide disc method
3. Nujol mull method (Liquid paraffin method)

Major peaks(cm^{1}) [1]

Cocaine base: 1275, 1700, 1106, 1728, 710, 1040, 1280.

Cocaine HCL: 1712, 1730, 1276, 1230(side peak), 732, 1106, 1075, 1025.

6. 7.5.3 GC-MS [4]

Principal peaks at m/z

Cocaine: 82,182,83,105,303,77,94,96

Ecognine: 82, 97, 42, 83, 96, 57, 94, 55.

Benzoylecognine: 124,82,168,77,105,42,94,83.

[Details of the cocaine and its examinations may also be referred from **"Recommended methods for testing of cocaine"** described in the 'Manual for use by National Narcotics Laboratories, United Nations, New York, 1986]

References:

1. Recommended methods for the identification and analysis of cocaine in seized material, United Nations, New York, 2012.
2. Butler, William P, Methods of Analysis for alkaloids, opiates, marihuana, barbiturates & miscellaneous drugs, Bureau of Narcotics & Dangerous drugs, Department of Justice, US, P 78.
3. Gunn, John W., In Analytical Manual of Bureau of Narcotics & Dangerous Drugs, Department of Justice, US (By Stanely P Sobol & Richard A Moore), Page No.116.
4. Moffat, A.C., Clarke's Isolation & Identification of Drugs, 2nd edition, The Pharmaceutical Press, London, 1986, P 489.

SECTION- 7

METHAQUALONE/MECLOQUALONE

7.1 Title: Analysis of Methaqualone/Mecloqualone

7.2 Scope: Test exhibits containing Methaqualone/ Mandrax and Mecloqualone.

7.3 Purpose: Identification and estimation of Methaqualone and Mecloqualone in crime exhibits.

7.4 Responsibility: Reporting officers and assisting scientific staff.

7.5 Sampling Procedure:

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 examinations 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 draw. Sampling should be undertaken to conform to the principles of analytical chemistry, as laid down, for example, in national pharmacopoeias or the methods prescribed in the book by the Association of Official Analytical Chemists.

(a). Sampling of single package items

The simplest sampling situation is where the submitted item consists of a single package of material – for Methaqualone/Mecloqualone most often the material will be loose Methaqualone/Mecloqualone. The material should be removed from its container or wrappings, placed in a clean clear plastic bag and the net weight recorded. The analyst should then carefully use visual testing to ensure that the entire exhibit is material which is controlled under the legislation within which he or she works. The sequence of chemical tests can then follow. Homogenization of the material need only be applied in certain analytical situations, e.g. if the analyst wishes to quantify a particular Methaqualone/Mecloqualone. The simplest way of homogenizing (Methaqualone/Mecloqualone or resin forms) is to pass the material through progressively finer sieves. In quantifying Methaqualone/Mecloqualone care should be taken to relate content found to the total amount of cannabis plant material which was originally taken for analysis i.e. the content should not be quoted as a

percentage of the weight of the final sieved material which was subjected to extraction.

(b). Sampling of items consisting of more than one package

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

1. If all packages contain suspect Methaqualone/Mecloqualone-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 material. 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. Alternatively, 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 the 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 solvents by pipette to avoid error due to insoluble materials. Insoluble adulterants are a frequent occurrence in 'street' samples seized within all countries.

(c). Sampling of materials containing gummy or large aggregates

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.

2. <u>Tablets and capsules – Commercial or licit preparations</u>

The preliminary determination of commercial origin is a subjective one. Clear-cut examples of products of commercial origin would be dosage units resembling descriptions as pictorial representations in national compendia of pharmaceutical preparations. Commercial preparations usually undergo quality control by the manufacturer; therefore, little useful information would be gained by screening a large number of units from each package. The amount of ingredient per tablet or capsule determined will be statistically valid for the entire lot.

(a) <u>Single container</u>

1. 1-50 dosage units – Randomly select ½ total number of units to a maximum of 20. Determine average weight, powder to pass through a 20-mesh sieve and mix thoroughly.

2. 51-100 dosage units – Randomly select 20 units, proceed as above.

3. 101-1,000 dosage units – Randomly select 30 units, proceed as above.

4. Greater than 1,000 dosage units – Randomly select a number of units equal to the square root of the total number present, rounded to the next higher integer; proceed as above.

(b) <u>Multiple containers</u>

Segregate containers by lot numbers and treat each group as described in 1 (b) above. Report results separately for each group.

Determine the square root of the total number of packages in each group. Randomly select a number of packages equivalent to the square root, rounded to the next highest integer.

From each of the selected packages, randomly select a number of dosage units equivalent to the square root of the total number of dosage units divided by the square root of the number of packages, rounded to the next higher integer.

Form a composite by grinding, seizing through a 20-mesh sieve and thoroughly mixing. Perform the analysis on the composite.

3. Tablets and capsules –Illicit origin

For illicit preparations, quality control may be regarded as non-existent. Wide variations may be suspected in tablet make-up, although in most instances, some of the active constituent will be present in each tablet. Some screening of individual units or containers is, therefore, necessary.

(a) Single container

Determine the total number of dosage units and the average weight per dosage unit (du).

For sample sizes up to 10 du – Screen all dosage units of all dosage units, rounding upward to the next higher integer.

For sample sizes from 8 du – Randomly select and screen /2 of all dosage units rounding upward to the next higher integer and selecting a minimum of 21 du and a maximum of 50 du.

Based on the results of the screening tests, proceed as follows:

1. If all dosage units appear to the identical, form a composite of screened dosage units as directed for licit preparations and analyze.
2. If the sample contains two dosage forms, subdivide the sample. If necessary, screen additional dosage units until both subsamples contain material for analysis, then form two composites and analyze.
3. If more than two dosage forms are present, the strategy is to make a composite of the most abundant dosage form, then to screen additional units until a sample of the same size is formed that contains only the less abundant dosage forms. This procedure is repeated until a composite is formed for each dosage form or until the sample is exhausted.

The percentage of dosage units containing a given controlled substance may be estimated by using the percent of units found to contain that substance out of the total number of units which were randomly selected and screened.

(b) <u>Multiple containers</u>

Randomly select a number of dosage units from each of a randomly selected number of containers, as determined in the compositing procedures for licit preparations, above. Screen each unit.

Based on the results of the screening test, proceed as follows:

1. If all screened units appear the same, combine screen units from all containers and form a composite.
2. If all screened units do not appear the same, each container should be treated as a separate exhibit or entity. Thus for each container, proceed according to the direction above for a single container.

7.6 Chemical Constituents of the Methaqualone/Mecloqualone

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 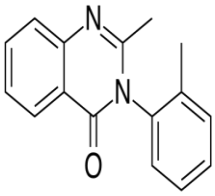
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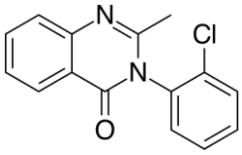
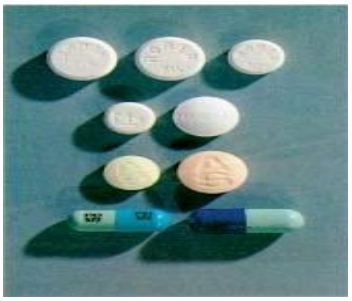
 
$C_{15}H_{11}ClN_2O$
M.Wt-270.71</td>
<td>  </td>
</tr>
<tr>
<td>  </td>
<td> 
Illicit Non Illicit</td>
<td></td>
</tr>
</table>

7.7 Methods:

7. 7.1 Presumptive Tests

7. 7.1.1 Color tests:

Positive results of colour tests are only the presumptive indication for the possibility of presence of methaqualone/mecloqualone. It is necessary for the analyst to confirm the presence of methaqualone/mecloqualone by the use of an alternative technique.

7. 7.1.1.1 Cobalt thiocyanate test [1]

Place a small/appropriate amount of suspected material in to test tube. Add one drop of Reagent A and one-drop reagent B.A blue colour indicates a positive test for the presence of methaqualone or mecloqualone.

Reagent A: 16% Hydrochloric acid solution.

Reagent B: 2.5 g cobalt (11) thiocyanate in 100 ml water.

7. 7.1.1.2 Fischer-Morris test [1]

Place a small/appropriate amount of suspected material in to test tube. Add seven drops of Reagent A and five drops reagent B. Allow it to stand for 1-2 minutes, then add 15 to 20 drops of chloroform. Shake it well and allow it to stand. Observe the colour of both layers.

In water layer Methaqualone and Mecloqualone do not give any colour while they give yellow colour in chloroform layer.

Reagent A: Conc. Formic acid (88%)

Reagent B: 5% aqueous sodium nitrite solution.

7. 7.2 Thin Layer Chromatography

Stationary Phase: Activated silica gel (with or without fluorescing substance) TLC plates of 0.25/0.20 mm thickness.

7. 7.2.1 Solvent/Mobile/Developing system [1]

System A: Cyclohexane, Toluene, Diethylamine

(75 : 15 : 10)

System B: Methanol and Cone. Ammonia

(100 : 1.5)

Sample preparation: Prepare a solution in methanol.

Visualization

The plate must be dried, prior to visualization at 120°C

1. UV light at 254 nm

2. Acidified potassium iodoplatinate reagent.

Preparation of Acidified Potassium Iodoplatinate Reagent:

Take 0.25g of Platinic Chloride and 5g of Potassium Iodide dissolved in sufficient amount of water to produce 100ml. Add 5ml of hydro-chloric acid to it.

7. 7.2.2 Solvent/Mobile/Developing system [2]

1. Chloroform and methanol.

(9 : 1)

2. Ethyl Acetate, Benzene and Ammonium Hydroxide

(60 : 35 : 5)

3. Ethanol, Dioxane, benzene and Ammonium Hydroxide

(5 : 40 : 50 : 5)

7. 7.3 Gas Chromatography

7. 7.3.1 Packed Column Technique [1]

Detector : FID

Column : 6 ft (or 2 m)2 to 4 mm ID glass

Packing : 3% OV-1, SE-30; OV-17 on 80 –100 chromosorb W HP

Carrier gas : Nitrogen.

Operating condition : Column temperature 240°C

Injector temperature : 280°C

Oven temperature : 240°C

Detector temperature : 280°C

Internal standard : Tetraphenylethylene or n-alkanes

Preparation of sample: Take a representative sample from the powder, tablet or capsules as outlined under sampling procedure given in the book [1]. Grind it to powder. Weigh about 10mg of sample and transfer to a 5ml Erlenmeyer flask quantitatively. Pipette 2.5ml of internal standard solution and add 1ml of 1M sodium bicarbonate solution. Heat on a steam bath for 5-8 min, cool it to room temperature, stopper and shake the flask. Allow the layer to separate and inject 1-2 micro liter of the chloroform layer (bottom layer) into gas chromatograph. Calculate the percentage of the drug as per the formula given in book [1].

Preparation of standard solution: Prepare a solution of methaqualone hydrochloride standard and dilute with internal standard solution to give a concentration of 4mg per ml.

Preparation of internal standard solution: Dissolve tetraphenylethylene or one of the n-alkanes in chloroform to give a concentration of 4 mg/ml.

7. 7.3.2 Capillary Column Technique [1]

Operating conditions:

Detector : FID

Column : Fused silica chemically bonded and cross linked methyl Silicone or methylphenylsilicone, such asOV-1 SE-54 BP-1, or DB-1

Film thickness : 0.25 μm

Length : 25 m ID 0.25mm

Carrier gas : nitrogen 1 ml/min

Spilt ratio : 20:1

Injector : 275°C

Detector : 275°C

Column temp. : 250°C

Internal standard : tetraphenylethylene or n-alkanes

Alternative Operating Conditions [2]

Detector : Flame ionization

Column : Glass (6ft. X 4mm. ID)

Packing : 5% OV-1 on Chrom WHP 80/100

Flow rate : 30 ml/min

Injector temperature : 270°C

Column temp. : 240°C

Detector temp. :270°C

7. 7.4 High Performance Liquid Chromatography [1]

7. 7.4.1 Normal Phase

Column : 125 mm by 4.9 mm ID

Packing material. : Silica HPLC grade, 5 um diameter

(Spherisorb S5W or equivalent)

Mobile phase. : A solution containing 1.17 g (0.01M) of ammonium perchlorate in 1000 ml of methanol. Adjust to pH 6.7 by addition of 1 ml of 0.1 M sodium hydroxide in methanol.

Flow rate : 2.0 ml/min

Detection : UV at 254 nm

Sample and standard

solution : In mobile phase [1mg per ml approx.]

Quantitation : By peak area, external standard method.

7. 7.4.2 Reverse Phase

Column : 250 mm by 4.6 mm ID.

Packing material : Octadecyl-silica HPLC 5 micron

(ODS-hypersil or equivalent)

Mobile phase. : Acetonitrile 40

1% aqueous ammonium acetate 45

2.5% aqueous diethylamine 15

The pH is adjusted to 8-9 by addition of ammonia or acetic acid

Flow rate : 1.5 ml/min

Detection. : UV at 254 nm

Sample and standard

solution : In mobile phase [1mg per ml approx.]

Injector volume : 1-5 micro liter

Quantitation : By peak area, external standard method.

7. 7.5 Spectroscopic Techniques

7. 7.5.1 Infra-red/FT-IR Spectrophotometry

Halide Disc Method:

Major peaks Cm^{-1}

Methaqualone : 1676, 1609, 778, 1469, 1271, 3012 **[1]**

Methaqualone hydrochloride : 1725, 762, 1649, 579, 1484, 1580. **[1]**

: 1682, 1599, 1565, 770, 1265, 697- KBr Disc **[3]**

Mecloqualone : 1683, 1607, 760, 1473, 1280, 3009.**[1]**

: 1682, 1605, 768, 782, 1282, 1583 **[3]**

7.7.5.2 UV –VIS Spectrophotometry

For Methaqualone:

Aqueous acid – 234 nm (A^{1}_{1}=1320 a), 269nm **[3]**

Aqueous alkali – 265 nm (A^{1}_{1}=347 a), 306nm **[3]**

Methaqualone and Mecloqualone may be studied by scanning the samples in appropriate organic solvents with the help of UV-Vis spectrophotometry. The spectrum and the value of λ_{max} of the spectrum can be compared with the standard value given in the literature or with using the standard sample of Methaqualone and Mecloqualone.

7. 7.5.3 Mass Spectrometry:

Principal peak at m/z

Methaqualone :	235, 250, 91, 233, 236, 65, 76, 132 **[3]**
4' hydroxy methaqualone :	251, 266, 249, 77, 143, 76, 253, 39 **[3]**
Mecloqualone:	270, 272, 255, 257, 152, 154, 111, 113.

[Details of the Methaqualone and Mecloqualone and their examinations may also be referred from "**Recommended methods for testing of Methaqualone and Mecloqualone"** described in the 'Manual for use by National Narcotics Laboratories, United Nations, New York, 1986']

References:

1. Recommended Methods for Testing Methaqualone/Mecloqualone, Manual of National Narcotics Laboratories, United Nations, New York, 1988.

3. Gunn, John W., In Analytical Manual of Bureau of Narcotics & Dangerous Drugs, Department of Justice, US (By Stanely P Sobol & Richard A Moore).

4. Moffat, A.C., Clarke's Isolation & Identification of Drugs, 2nd edition, The Pharmaceutical Press, London, 1986.

SECTION-8

AMPHETAMINES/METHAMPHETAMINES

8.1 Title: Analysis of amphetamines/methamphetamines

8.2 Scope: Test exhibits containing amphetamines/ methamphetamines or their derivatives.

8.3 Purpose: Identification and estimation of amphetamine/ methamphetamine drugs or their derivatives.

8.4 Responsibility: Reporting officers and assisting scientific staff.

8.5 Sampling Procedure:

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 examinations 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 reserve 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 who he works.

Amphetamine exhibits may be encountered as tablets and capsules both from the licit market by diversion and from illicit manufacture, and as fine or gummy powders. Methamphetamine is usually in the form of a powder or gummy

substance, although tablets and capsules from licit and illicit sources are available in some countries. Both amphetamine base and methamphetamine base are liquids and these, as well as solutions of the salt forms, are frequently encountered in the illicit market.

1. **Powders**

(a). 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 amphetamine or methamphetamine, 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 be thoroughly homogenized 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 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.

(b). Sampling of items consisting of more than one package

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

1. If all packages contain suspect amphetamine or methamphetamine 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. Alternatively, 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 the 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 solvents by pipette to avoid error due to insoluble materials. Insoluble adulterants are a frequent occurrence in 'street' samples seized within all countries.

(c). <u>Sampling of materials containing gummy or large aggregates</u>

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.

2. <u>Tablets and capsules – Commercial or licit preparations</u>

The preliminary determination of commercial origin is a subjective one. Clear-cut examples of products of commercial origin would be dosage units resembling descriptions as pictorial representations in national compendia of pharmaceutical preparations. Commercial preparations usually undergo quality control by the manufacturer; therefore, little useful information would be gained by screening a large number of units from each package. The amount of ingredient per tablet or capsule determined will be statistically valid for the entire lot.

(a) Single container

1. 1-50 dosage units – Randomly select ½ total number of units to a maximum of 20. Determine average weight, powder to pass through a 20-mesh sieve and mix thoroughly.

2. 51-100 dosage units – Randomly select 20 units, proceed as above.

3. 101-1,000 dosage units – Randomly select 30 units, proceed as above.

4. Greater than 1,000 dosage units – Randomly select a number of units equal to the square root of the total number present, rounded to the next higher integer; proceed as above.

(b) Multiple containers

Segregate containers by lot numbers and treat each group as described in 1 (b) above. Report results separately for each group.

Determine the square root of the total number of packages in each group. Randomly select a number of packages equivalent to the square root, rounded to the next highest integer.

From each of the selected packages, randomly select a number of dosage units equivalent to the square root of the total number of dosage units divided by the square root of the number of packages, rounded to the next higher integer.

Form a composite by grinding, seizing through a 20-mesh sieve and thoroughly mixing. Perform the analysis on the composite.

3. Tablets and capsules –Illicit origin

For illicit preparations, quality control may be regarded as non-existent. Wide variations may be suspected in tablet make-up, although in most instances, some of the active constituent will be present in each tablet. Some screening of individual units or containers is, therefore, necessary.

(a) Single container

Determine the total number of dosage units and the average weight per dosage unit (du).

For sample sizes up to 10 du – Screen all dosage units of all dosage units. , For sample sizes up to 11 du to 27 du – randomly select and screen ¾ of all doses units, rounding upward to the next higher integer.

For sample sizes 28 du - Randomly select and screen 1/2 of all dosage units rounding upward to the next higher integer and selecting a minimum of 21 du and a maximum of 50 du.

Based on the results of the screening tests, proceed as fol lows:

a. If all dosage units appear to the identical, form a composite of screened dosage units as directed for licit preparations and analyze.
b. If the sample contains two dosage forms, subdivide the sample. If necessary, screen additional dosage units until both subsamples contain material for analysis, then form two composites and analyze.
c. If more than two dosage forms are present, the strategy is to make a composite of the most abundant dosage form, then to screen additional units until a sample of the same size is formed that contains only the less abundant dosage forms. This procedure is repeated until a composite is formed for each dosage form or until the sample is exhausted.

The percentage of dosage units containing a given controlled substance may be estimated by using the percent of units found to contain that substance out of the total number of units which were randomly selected and screened.

(b) Multiple containers

Randomly select a number of dosage units from each of a randomly selected number of containers, as determined in the compositing procedures for licit preparations, above. Screen each unit.

Based on the results of the screening test, proceed as follows:

1. If all screened units appear the same, combine screen units from all containers and form a composite.

2. If all screened units do not appear the same, each container should be treated as a separate exhibit or entity. Thus for each container, proceed according to the direction above for a single container.

4. Aqueous solutions – Illicit origin

Aqueous solutions of methamphetamine HCl are illicitly available in solution by their very nature are homogeneous relatively small sample 10 ml represent the entire value.

(a) Single container

If sample size permits, pipet an amount for assay of at least 10 ml.

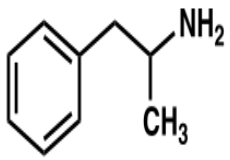
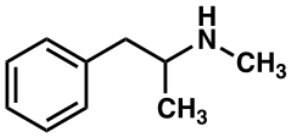
(b) Multiple container

Segregate containers by lot numbers or other characteristics and treat each group as described under 1.b above. Report results separately for each group.

5. Residues from syringes or clandestine laboratory glassware

Because of the trace amounts of amphetamine/methamphetamine usually present on hypodermic syringes seized from individuals or on glassware and other equipment found in clandestine laboratories. Wash the syringes or glassware with minimum amount of methanol and concentrate it for proceed with a selected test.

8.6 Major Chemical Constituents of the Amphetamines/ Methamphetamines

Amphetamine  $C_9H_{13}N$ M.Wt-135.20622	 	
Methamphetamine  $C_{10}H_{15}N$ M.Wt-149.24		

8.7 Methods:

8.7.1 Presumptive tests: -

Colour tests:

Positive results of colour tests are only the presumptive indication for the possibility of presence of amphetamines/methamphetamines. It is necessary for the analyst to confirm the presence of amphetamines/methamphetamines by the use of an alternative technique.

8. 7.1.1 Marquis reagent test [1]

Take appropriate amount of the exhibit (1-2mg of powder, one or two drops if liquid) on a spot plate; add not more than 3 drops of reagent drop wise. Appears of orange colour turning to brown indicates the positive test for the presence of amphetamine/methamphetamine.

Preparation of Marquis reagent:

Add 8 – 10 drops of 40% formaldehyde solution to 10 ml of conc. sulphuric acid.

8. 7.1.2 Simon's reagent test [1]

Take appropriate amount of sample on spot plate and add to it a drop of solution A followed by one drop of solution B. Now, add few drops of solution C. Appearance of blue colour indicates the positive tests for the presence of methamphetamine while appearance of slow pink to cherry red colour indicates the presence of amphetamine.

Preparation of the reagents

Solution A: 20% aqueous sodium carbonate solution.

Solution B: 50%ethanolic acetaldehyde solution.

Solution C: 1% aqueous sodium nitroprusside solution.

8. 7.1.3 Mandelin's Reagent Test [2]

Take appropriate amount of aqueous solution of amphetamine/as such, add few drops of Mandelin reagent, appearance of green, darkens rapidly indicates the positive test for the presence of amphetamines. On stirring the colour passes through several shades to emerald green and dark reddish brown, which changes to light red-brown on heating.

Preparation of Mandelin's Reagent

Dissolve 1 gram ammonium vanadate in 100ml. concentrated sulfuric acid.

8. 7.1.4 Colour test for MDA (3,4-methylenedioxyamphetamine) [3]

Marquis reagent : Brown to black to purple.

Meck's reagent : Green to blue to purple.

Mandelin's reagent : Purple to brown.

Froehde's reagent : Brown to purple.

8. 7.2 Thin Layer Chromatography

Stationary Phase: Activated silica gel (with or without fluorescing substance) TLC plates of 0.25/0.20 mm thickness.

Solvent/Mobile/Developing system:

System A Methanol and Conc. Ammonia **[1]**

100 : 1.5

System B Cyclohexane, Toluene and Diethylamine **[1]**

75 : 15 : 10

System C Methylethylketone, Dimethylformamide and Ammonium Hydroxide (13 : 1.9 : 0.1) **[3]**

System D Methanol and Chloroform **[3]**

1 : 1

System E Chloroform, methanol and acetic acid **[3]**

75 : 20 : 5

System F Acetonitrile, benzene, ethyl acetate and Ammonium hydroxide **[3]**

60 : 15 : 10 : 10

Visualization methods

(a) UV radiation at 254nm.

(b) Ninhydrin reagent spray

(c) Acidified potassium iodoplatinate reagent

(d) 1% aqueous solution of potassium permanganate

Preparation of Ninhydrin reagent

Dissolve 0.1% solution of ninhydrin in isopraponol.

Preparation of Acidified potassium iodoplatinate reagent

Dissolve 0.25 grams of platinic chloride and 5 gms of potassium iodide in water to 100 ml. and add 2 ml of concentrated hydrochloric acid to make it acidic.

Method:

The plate must be dried prior to visualization. This can be done at room temperature or by use of a hot air blower to remove traces of ammonia and other bases. Observe the plate under UV light, spray with the ninhydrin reagent. Heat it at 110°C for 5 minutes. Appearance of violet or pink spots are the indicative for the presence of amphetamines. Lighter spots indicates the presence of methamphetamines. The plate is over sprayed with the acidified iodoplatinate solution; dirty grey-violet-brown spots on a pink background indicate the positive tests for amphetamines and methamphetamines.

8. 7.3 Gas Chromatography

8. 7.3.1 Packed Column Technique [1]

Operating conditions:

Detector	: FID
Column	: 6 ft (or 2 M) 2-4 mm ID glass
Packing	: 3% SE-30 or OV-1
Carrier gas	: Nitrogen
Flow rate	: 30 ml per minute
Column temp.	: Programmed from 130°C to 260°C
Internal standard	: n-Tetradecane or other n-alkane with an even number of carbon atoms or propylamphetamine HCl.

8. 7.3.2 Column Technique [1]

Operating conditions:

Detector	: FID
Column	: Fused silica chemically bonded and cross linked methyl silicone or methyl phenyl silicone, such as SE-54, DB-1, DB-5 or equivalent.
Film thickness	: 0.25 μm
Length	: 10 to 30 m, ID 0.25mm

Carrier gas : Helium 40 cm/sec

Spilt ratio : 40:1

Column temp. : Programme 2 min at 75°C, increase to 280°C at 10°C/min.

Internal standard : n-Tetradecane or other n-alkane with an even number of carbon atoms or propylamphetamine HCl.

8. 7.4 High Performance Liquid Chromatography

8. 7.4.1 Isocratic Technique [1]

8. 7.4.1.1 Normal phase

Column : 125 mm by 4.9 mm ID

Packing material : Silica HPLC grade, 5μm diameter

(spherisorb-S 5μ or equivalent)

Mobile phase : **(a)** Methanol : Aqueous ammonium nitrate buffer solution (90:10, v/v).

To prepare a buffer solution add 94 ml of conc. ammonia and 21.5ml of conc. Nitric acid to 884 ml water and then adjust the pH to10 with ammonia.

(b) A solution containing 1.17 g (0.01M) of

ammonium perchlorate in 1000ml of methanol. Adjust to pH 6.7 by adding 0.1M sodium hydroxide in methanol (ca. 1ml).

Flow rate : 2.0 ml/min.

Detection : UV at 254nm

Sample preparation : dissolve in methanol.

Standard solution : Dissolve in methanol.

Injection volume : 1 to 5 microlitres by syringe or loop-injector.

Quantitation : By peak areas, external standard method

8. 7.4.1.1 Reverse Phase

Column : 250 mm by 4 mm ID

Packing material : Octadecyl-silica 5 μm diameter (LiChrosorb RP-18 or equivalent)

Mobile phase : Acetonitrile : 1% aqueous ammonium acetate : 2.5% aqueous diethylamine (40:45:15). The pH 8-9 adjusted with ammonia or acetic acid.

Flow rate : 1.5 ml per minute

Temperature : 35°C

Detector : UV at 254 nm

Sample preparation : All materials are dissolved in a mixture of 2 parts of water and 1 part acetonitrile.

Injection volume : 10-20 μl

Quantitation : By peak areas, internal standard method using lidocaine or procaine or external standard method.

8. 7.5 Spectroscopic techniques

8.5.5.1 Infra-red/FT-IR Spectroscopy

8. 7.5.1.1 Halide disc method

8. 7.5.1.2 Nujol mull method

8. 7.5.1.3 Thin film method: This method is useful particularly in case of liquid samples of free bases of amphetamine /methamphet- amine.

Major peaks (cm^{-1})

Amphetamine base:

(1) 504, 700 (a), 742 (c), 818, 1031, 1090, 1100, 1156, 1297, 1328, 1347, 1371 (f), 1453 (b), 1496 (e), 1581 (d) (film on KBr Disk) **[1]**

(2) 700, 740, 1495, 1090, 1605, 825 (thin film) **[4]**

Methamphetamine base:

(1) 525, 598, 700 (a), 741 (b), 790, 1031, 1071, 1085, 1117, 1126, 1156 (d), 1179, 1341, 1371 (e), 1446, 1453 (c), 1477, 1494, 1605 (f) (film on KBr disc) **[1]**

(2) 747, 698, 1060, 1491, 1590, 1085 (methylamphetamine hydrochloride, KBr Disc) **[4]**

8. 7.5.2 UV-Vis Spectrophotometry

Amphetamine:

Aqueous acid – 251nm, 257nm (A^1_1=14 a), 263nm **[4]**

Methamphetamine:

Aqueous acid – 252nm, 257nm (A^1_1=12.1 a), 263nm **[4]**

Amphetamine and Methamphetamine may be studied by scanning the samples in appropriate organic solvents with the help of UV-Vis spectrophotometry. The spectrum and the value of λ_{max} of the spectrum can be compared with the standard value given in the literature or with using the standard sample of amphetamine and methamphetamine.

8. 7.5.3 Mass Spectrometry:

For Amphetamine, the principal peaks at m/z are: 44, 91, 40, 42, 65, 45, 39, 43 **[4]**

For Methamphetamine, the principal peaks at m/z are: 58, 91, 59, 134, 65, 56, 42, 57 **[4]**

8. 7.5.4: Solid-phase microextraction-gas chromatography-mass spectrometry (SPME-GC-MS)[5]:

	SPME Parameters
Sample preparation	Dissolve seized material (e.g. ecstacy tablets) in 5 mL of 0.1 M aqueous acetate buffer followed by ultrasonication for 10 min and subject to SPME
SPME Mode	Head-Space SPME
Fiber	Polydimethylsiloxane-divinylbenzene (PDMS-DVB) 65 µm
Extraction Temperature	90 °C
Extraction time	30 min
Stirring speed	200 rpm
Desorption temperature	270 °C
Desorption time	2 min
	GC-MS Parameters
Column	Capillary (30 m×0.25 mm×0.25 µm), DB-1MS (100% PDMS)
Carrier gas	Helium (99.998%) @ 1 mL/min
Oven Temperature	Ramp-1: 80 °C for 3 min Ramp-2: @10 °C/min to 150 °C Ramp-3: @2 °C/min to 175 °C Ramp-4: @10°C/min to 300 °C (hold for 10 min)
Injection Mode	Split-less for SPME

[Details of the Amphetamine and methamphetamine and their examinations may also be referred from **"Recommended methods for testing of amphetamine and methamphetamine"** described in the 'Manual for use by National Narcotics Laboratories, United Nations, New York, 1986']

References

1. Recommended methods for testing amphetamine and methamphetamine, Manual of National Narcotics Laboratories, United Nations.

2. Butler, William P, Methods of Analysis for alkaloids, opiates, marihuana, barbiturates & miscellaneous drugs, Bureau of Narcotics & Dangerous drugs, Department of Justice, US.

3. Gunn, John W., In Analytical Manual of Bureau of Narcotics & Dangerous Drugs, Department of Justice, US (By Stanely P Sobol & Richard A Moore).

4. Moffat, A.C., Clarke's Isolation & Identification of Drugs, 2nd edition, The Pharmaceutical Press, London, 1986, P 349.

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SECTION – 9

BARBITURATES & DERIVATIVES

9.1 Title: Barbiturates & Derivatives

9.2 Scope: Barbiturate derivative drugs

9.3 Purpose: Identification and estimation of Barbiturates & derivatives.

9.4 Responsibility: Reporting Officers and supporting scientific staff.

9.5 Sampling Procedure:

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 examinations 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 reserve 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 who he works.

Barbiturate derivative exhibits may be encountered as tablets and capsules both from the licit market by diversion and from illicit market. In some countries bulk drug powder may be diverted from legitimate use.

1. **Powders**

(a). Sampling of single package items

The simplest sampling situation is where the submitted item consists of a single package of material. 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 be thoroughly homogenized 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 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.

(b). Sampling of items consisting of more than one package

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

1. If all packages contain suspect 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. Alternatively, 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 the 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 solvents by pipette to avoid error due to insoluble materials.

2. <u>Tablets and capsules – Commercial or licit preparations</u>

The preliminary determination of commercial origin is a subjective one. Clear-cut examples of products of commercial origin would be dosage units resembling descriptions as pictorial representations in national compendia of pharmaceutical preparations. Commercial preparations usually undergo quality control by the manufacturer; therefore, little useful information would be gained by screening a large number of units from each package. The amount of ingredient per tablet or capsule determined will be statistically valid for the entire lot.

(a) <u>Single container</u>

1. 1-50 dosage units – Randomly select ½ total number of units to a maximum of 20. Determine average weight, powder to pass through a 20-mesh sieve and mix thoroughly.

2. 51-100 dosage units – Randomly select 20 units, proceed as above.

3. 101-1,000 dosage units – Randomly select 30 units, proceed as above.

4. Greater than 1,000 dosage units – Randomly select a number of units equal to the square root of the total number present, rounded to the next higher integer; proceed as above.

(b) Multiple containers

Segregate containers by lot numbers and treat each group as described in 1 (b) above. Report results separately for each group.

Determine the square root of the total number of packages in each group. Randomly select a number of packages equivalent to the square root, rounded to the next highest integer.

From each of the selected packages, randomly select a number of dosage units equivalent to the square root of the total number of dosage units divided by the square root of the number of packages, rounded to the next higher integer.

Form a composite by grinding, seizing through a 20-mesh sieve and thoroughly mixing. Perform the analysis on the composite.

3. Residues from syringes

Because of the trace amounts of drug usually present on hypodermic syringes seized from individuals, the analyst should not attempt to perform presumptive tests but should proceed directly with conclusive analytical procedures.

Wash the syringe with a minimum amount of methanol and concentrate it for proceed with a selected test.

9.6 Major Chemical Constituents of the Barbiturates & Derivatives

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 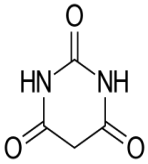
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 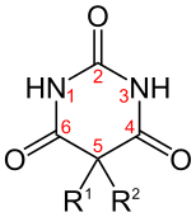
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 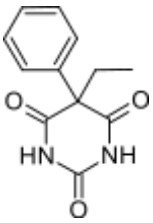
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 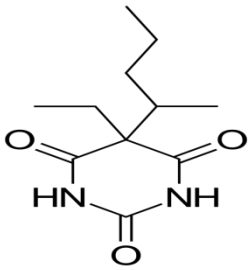
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 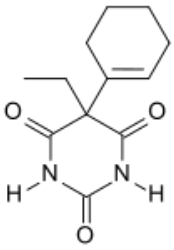
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 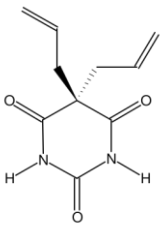
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M.Wt-208.214
  </td>
<td>Barbiturates
Tablets & Capsules
 
 

 
Injection</td>
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9.7 Method:

9. 7.1 Presumptive test

Colour tests

Positive results of colour tests are only the presumptive indication for the presence of barbiturate derivatives. It is necessary for the analyst to confirm the presence of barbiturate derivatives by an alternative technique.

9. 7.1.1 Dille-Koppanyi Test:

Method [1]:

Take an appropriate amount of suspected material in a depression spot plate. Add 3 drops of solution 1 followed by 3 drops of solution 2. Appearance of purple colour indicates the positive test for the presence of barbiturates.

Alternate Method [2]:

Take an appropriate amount of powdered sample (either from a capsule or pulverized tablet) in a test tube; add 2 ml of solution 1 and shake well. Now, add 1 ml of solution 2 and again shake well. Appearance of staple red violet colour indicates the presence of barbituric acid or one of its derivatives.

Solution 1: Dissolve 0.1gm of cobaltous acetate tetra hydarate in 100 ml of absolute methanol, then add 0.2 ml of glacial acetic acid.

Solution 2: Mix 5 ml isopropylamine with 95 ml of absolute methanol.

9. 7.2 Thin Layer Chromatography

Stationary Phase: Activated silica gel (with or without fluorescing substance) TLC plates of 0.25/0.20 mm thickness.

Solvent/Mobile/Developing system:

System 1 Ethyl acetate, Methanol and 25% Ammonia **[1]**

85 : 10 : 5

System 2 Chloroform and Acetone **[1]**

80 : 20

System 3 Benzene and acetic acid **[3]**

90 : 10

System 4 Dioxane, benzene and ammonium hydroxide **[3]**

20 : 75 : 5

System 5 Chloroform and Acetone **[3]**

90 : 10

The plates must be dried prior to visualization. This can be done at 120°C for 5 minutes in an oven or, more quickly, by using a hot air blower.

Visualization methods

1. UV light at 254 nm both before and after exposure to ammonia vapour **[1]**

2. Mercuric chloride-diphenylcarbazone reagent Spray reagent **[1]**

Dissolve 0.1 g of diphenylcarbazone in 50 ml of ethanol

Dissolve 1 g of mercuric chloride in 50ml of ethanol.

(Use fresh prepared solutions and mix (a) and (b) just before spraying).

First observe the plate under UV light at 254 nm. Expose the plate to concentrated ammonia vapours and observe again under UV light at 254nm. If necessary, spray with mercuric chloride-diphenylcarbazone reagent. Blue-violet spots on a pink background indicates the presence of barbiturates.

3. 0.2% aqueous potassium permanganate solution spray **[3]**

4. Saturated mercurous nitrate spray **[3]**

9. 7.3 Gas Liquid Chromatography [1]

9. 7.3.1 Packed Column Technique

a) Without Derivatization

The use of the packed column technique for the analysis of underivatized barbiturates is not recommended because of their decomposition at high temperature and the presence of mild steel component of the tube.

b) With Derivatization

Operating Conditions

Detector	: FID
Column	: 6 ft (or 2 m), 2 to 4 mm ID glass
Packing	: 3% SE-30 on 80-100 mesh chromosorb G HP
Carrier gas	: Nitrogen at 45-50 ml/min
Column temperature	: 190°C – 200°C
Injector temperature	: 220°C
Detector temperature	: 220°C
Internal standard	: n-alkanes
Derivatizing agent	: Trimethyl anilinium hydroxide 0.2 M in methanol (Meth Elute)

9. 7.3.2 Capillary Column Technique

a) Without derivatization

Operating Conditions

Detector	: FID
Column	: Fused silica, chemically bonded and cross-linked methylsilicone or methylphenylsilicone, such as OV-1, SE-30, SE-54 or equivalent
Film thickness	: 0.52 μm
Length	: 25m, 0.35 mm ID
Carrier gas	: Nitrogen at 1 ml/min

Split ratio : 20 : 1

Column temperature : Isothermal at 200°C or programmed from

200°C – 260°C at 4°C/min

Injector temperature : 275°C

Detector temperature : 275°C

Internal standard : n-alkanes

9. 7.4 High Performance Liquid Chromatography

9. 7.4.1 Reverse Phase

Method-1

Column : 250mm x 4.6 mm ID

Packing material : Octadecyl-silica HPLC grade, 5 μm (Sperisorb 5 ODS-2 or equivalent)

Mobile Phase : Acetonitrile 30

Water 70

Flow rate : 0.9 ml/min

Detection : UV at 220 nm

Injection volume : 1-5 μl by syringe or loop injector

Quantitation : By peak area, external standard method

Method – 2

Column : 150mm x 4.6 mm ID

Packing material : Octadecyl-silica HPLC grade, 5μm (Sperisorb 5 ODS- Hypersil or equivalent)

Mobile Phase A : 0.1 M sodium dihydrogen phosphate buffer 60

Methanol 40

pH 3.5 adjusted with phosphoric acid

Mobile Phase B : 0.1 M sodium dihydrogen phosphate buffer 60

Methanol 40

pH 8.5 adjusted with sodium hydroxide solution

Flow rate : 2.0 ml/min.

Detection : UV at 216 nm

Quantitation : By peak area, external standard method

9. 7.5 Spectroscopic Techniques [4]

9. 7.5.1 UV Vis Spectroscopy

Barbiturate derivatives may be studied by scanning the samples in appropriate organic solvents with the help of UV-Vis spectrophotometry. The value of λ_{max} can be compared with the standard value given in the literature or with using the standard sample of barbiturate derivatives.

9. 7.5.2 Mass Spectroscopy

Barbiturate derivatives may be studied by analyzing the samples with the help of Mass spectrometry (GC-MS). The value of principal peaks at m/z can be compared with the standard value given in the literature or with using the standard sample of barbiturate derivatives.

Allobarbitone:	41, 167, 124, 39, 80, 53, 68, 141.
Amylobarbitone:	156, 141, 157, 41, 55, 142, 98, 39.
3'-hydroxyamylobarbitone:	59, 157, 156, 141, 43, 41, 71, 69.
Aprobarbitone:	167, 41, 124, 168, 97, 39, 169, 45.
N'-hydroxyaprobarbitone:	41, 43, 183, 167, 140, 184, 124, 109.
Barbitone:	156, 141, 55, 155, 98, 39, 82, 43.
Brallobarbitone:	207, 41, 39, 124, 91, 165, 122, 44.
Butalbital:	41, 167, 168, 39, 124, 97, 141, 181.
Butobarbitone:	141, 156, 41, 55, 98, 39, 142, 155.
3'-hydroxybutobarbitone:	156, 141, 45, 157, 41, 29, 55, 27.
Cyclobarbitone:	207, 141, 81, 79, 67, 80, 41, 77.
Cyclopento barbitone:	67, 193, 66, 41, 169, 39, 65, 77.
Enallylpropymal:	181, 41, 182, 39, 124, 53, 138, 97.
Heptabarbitone:	121, 43, 78, 93, 80, 41, 141, 39.
Hexethal:	156, 141, 55, 41, 157, 43, 98, 39.
Hexobarbitone:	221, 81, 157, 80, 79, 155, 41, 77.
3'-Oxohexobarbitone:	250, 95, 39, 235, 66, 207, 41, 193.
Ibomal:	167, 209, 43, 124, 39, 41, 53, 140.

Idobutal:	167, 41, 168, 124, 39, 97, 141, 67.
Metharbitone:	155, 170, 112, 169, 55, 82, 41, 39.
Methohexitone:	41, 81, 53, 221, 79, 39, 178, 233.
Methylphenobarbitone:	218, 117, 118, 146, 103, 77, 91, 115.
Phenobarbitone:	204, 117, 146, 161, 77, 103, 115, 118.
Phenylmethylbarbituric acid:	104, 132, 218, 51, 103, 77, 78, 52.
Quinalbarbitone:	167, 168, 41, 43, 97, 124, 39, 55.
5-(2,3-dihydroxypropyl) quinal barbitone:	171, 43, 143, 41, 128, 55, 141, 159.
Secbutobarbitone:	141, 156, 41, 57, 39, 98, 157, 47.
Talbutal:	167, 168, 41, 97, 124, 39, 57, 53.
Thialbarbitone:	81, 223, 79, 41, 80, 157, 185, 77.
Thiopentone:	172, 157, 173, 43, 41, 55, 69, 71.
Vinbarbitone:	195, 41, 141, 69, 39, 152, 135, 196.

9. 7.5.3 IR/FTIR Spectroscopy

Barbiturate derivatives may be studied by scanning the samples with the help of IR Spectroscopy/FTIR. The value of principal peaks at wave numbers can be compared with the standard value given in the literature or with using the standard sample of barbiturate derivatives.

Allobarbitone:	1687, 1315, 925, 1219, 847, 1640 (KBr disk)
Amylobarbitone:	1725, 1696, 1758, 1317, 1240, 850 (KBr disk)
Aprobarbitone:	1693, 1720, 1745, 1316, 1255, 860 (KBr disk)
Barbitone:	1680, 1720, 1767, 1320, 1245, 875 (KBr disk)
Barbituric acid:	1710, 1734, 1750, 1225, 775, 1190.
Butalbital:	1690, 1720, 1740, 1310, 1290, 1200.
Butobarbitone:	1696, 1727, 1760, 1242, 850, 1215.
Cyclobarbitone:	1693, 1725, 1745, 1300, 1210, 830.
Cyclopentobarbitone:	1700, 1755, 1212, 815, 1319, 848.
Enallyl propymal:	1689, 1718, 1550, 1640, 1285, 672.
Heptabarbitone:	1673, 1761, 1718, 1237, 1292, 1303.
Hexethal:	1698, 1720, 1757, 1316, 1230, 850.
Hexobarbitone:	1720, 1665, 1748, 1200, 1275, 1045.
Ibomal:	1700, 1722, 1210, 1300, 830, 1622.
Idobutal:	1696, 1728, 1755, 835, 1290, 1207.

Metharbitone: 1655, 1699, 1755, 1275, 1205, 815.

Methohexitone: 1680, 1709, 1316, 1193, 1253, 1040.

Methyl phenobarbitone: 1707, 1684, 1754, 720, 1298, 1050.

Nealbarbitone: 1695, 1752, 1265, 833, 1205, 775.

Pentobarbitone: 1685, 1719, 1744, 1315, 1218, 845.

Phenobarbitone: 1712, 1684, 1670, 1770, 1310, 1300.

Phenylmethylbarbituric acid: 1702, 1720, 1751, 1240, 802, 716.

Quinalbarbitone: 1559, 1648, 1690, 1298, 1270, 925.

Secbutobarbitone: 1675, 1760, 1317, 1303, 1230, 853.

Talbutal: 1703, 1728, 1752, 1315, 1211, 829.

Thialbarbitone: 1685, 1610, 1300, 1130, 1270, 1000.

Thiopentone: 1670, 1540, 1300, 1170, 1735, 1220.

[Details of the barbiturates/barbiturate derivatives, extraction and their examinations may also be referred from **"Recommended methods for testing of barbiturates/barbiturate derivatives"** described in the 'Manual for use by National Narcotics Laboratories, United Nations, New York, 1989']

References

1. Recommended methods for testing barbiturate derivatives under international control, Manual of National Narcotics Laboratories, United Nations, 1989.

2. Butler, William P, Methods of Analysis for alkaloids, opiates, marihuana, barbiturates & miscellaneous drugs, Bureau of Narcotics & Dangerous drugs, Department of Justice, US.

3. Gunn, John W., In Analytical Manual of Bureau of Narcotics & Dangerous Drugs, Department of Justice, US (By Stanely P Sobol & Richard A Moore).

4. Moffat, A.C., Clarke's Isolation & Identification of Drugs, 2nd edition, The Pharmaceutical Press, London, 1986.

SECTION – 10

BENZODIAZEPINES

10.1 Title: Analysis of benzodiazepines

10.2 Scope: Benzodiazepine derivative drugs

10.3 Purpose: Identification and estimation of benzodiazepines

10.4 Responsibility: Reporting officers and supporting scientific staff.

10.5 Sampling Procedure:

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 examinations 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 reserve 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 who he works.

Benzodiazepine derivative exhibits are encountered predominantly as capsules and tablets as a result of diversion and from illicit market. In a few cases solutions for injection, syringes, syrups, suppositories and vials containing

powders to be made up with a suitable vehicle for injection may be submitted for analysis. In some countries, diazepam bulk drug powder may be diverted from legitimate veterniary use.

1. **Powders**

(a). Sampling of single package items

The simplest sampling situation is where the submitted item consists of a single package of material. 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 be thoroughly homogenized 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 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.

(b). Sampling of items consisting of more than one package

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

1. If all packages contain suspect 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. Alternatively, 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 the 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 solvents by pipette to avoid error due to insoluble materials.

2. <u>Tablets and capsules – Commercial or licit preparations</u>

The preliminary determination of commercial origin is a subjective one. Clear-cut examples of products of commercial origin would be dosage units resembling descriptions as pictorial representations in national compendia of pharmaceutical preparations. Commercial preparations usually undergo quality control by the manufacturer; therefore, little useful information would be gained by screening a large number of units from each package. The amount of ingredient per tablet or capsule determined will be statistically valid for the entire lot.

(a) Single container

1. 1-50 dosage units – Randomly select ½ total number of units to a maximum of 20. Determine average weight, powder to pass through a 20-mesh sieve and mix thoroughly.

2. 51-100 dosage units – Randomly select 20 units, proceed as above.

3. 101-1,000 dosage units – Randomly select 30 units, proceed as above.

4. Greater than 1,000 dosage units – Randomly select a number of units equal to the square root of the total number present, rounded to the next higher integer; proceed as above.

(b) Multiple containers

Segregate containers by lot numbers and treat each group as described in 1 (b) above. Report results separately for each group.

Determine the square root of the total number of packages in each group. Randomly select a number of packages equivalent to the square root, rounded to the next highest integer.

From each of the selected packages, randomly select a number of dosage units equivalent to the square root of the total number of dosage units divided by the square root of the number of packages, rounded to the next higher integer.

Form a composite by grinding, seizing through a 20-mesh sieve and thoroughly mixing. Perform the analysis on the composite.

3. Aqueous solutions

Aqueous solutions for injection and syrups are available in some countries. Since solutions by their very nature are homogeneous relatively small sample 10 ml represent the entire value.

(c) Single container

If sample size permits, pipet an amount for assay of at least 10 ml.

(d) Multiple container

Segregate containers by lot numbers or other characteristics and treat each group as described under 1.b above. Report results separately for each group.

Determine the square root of the total number of containers in each group. Randomly select a number of containers equivalent to the square root rounded to the next higher integer.

From each of the selected containers withdraw a 10 ml or larger sample (if size permits) for a composite.

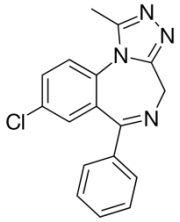
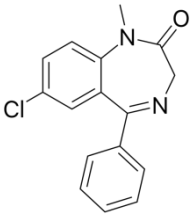
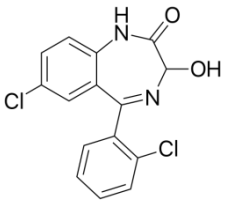
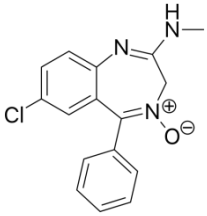
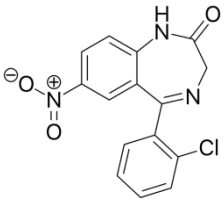
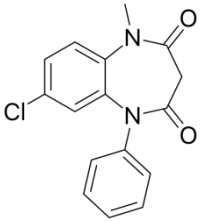
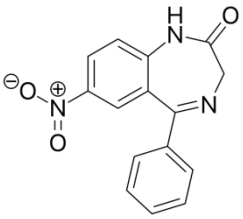
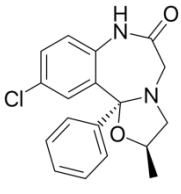
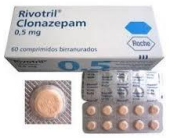
If size permits, pipit at least 10 ml of the composite for assay.

4. Residues from syringes

Because of the trace amounts of drug usually present on hypodermic syringes seized from individuals, the analyst should not attempt to perform presumptive tests but should proceed directly with conclusive analytical procedures.

Wash the syringe with a minimum amount of methanol and concentrate it to dryness under a stream of nitrogen. Proceed with selected tests.

10.6 Major Chemical Constituents of the Benzodiazepines are:

Alprazolam	Diazepam	Lorazepam	Chlordiazepoxide
			
$C_{17}H_{13}Cl N_4$ **M.Wt-308.8**	**$C_{16}H_{13}Cl N_2O$** **M.Wt-284.7**	**$C_{15}H_{10}Cl_2 N_2O_2$** **M.Wt-321.2**	**$C_{16}H_{14}Cl N_3O$** **M.Wt-299.8**
			
Clonazepam	**Clobazam**	**Nitrazepam**	**Oxazolam**
			
$C_{15}H_{10}Cl N_3O_3$ **M.Wt-315.7**	**$C_{16}H_{10\ 3}Cl N_2O_2$** **M.Wt-300.7**	**$C_{15}H_{11}N_{\ 3}O_3$** **M.Wt-281.3**	**$C_{18}H_{17}Cl N_2O_2$** **M.Wt-328.8**
			

10.7 Methods:

10. 7.1 Thin Layer Chromatography [1]

Stationary Phase: Activated silica gel (with or without fluorescing substance) TLC plates of 0.25/0.20 mm thickness.

Solvent/Mobile/Developing system:

Solvent System A: Chloroform and Acetone

80 : 20

Solvent System B: Chloroform and Methanol

90 : 10

Solvent System C: Cyclohexane, Toluene and Diethylamine

75 : 15 : 10

Sample preparation: The solutions of exhibit powder/tablet/ capsules and standard may be prepared in methanol.

Visualisation

The plates must be dried prior to visualization at 120°C for 5 minutes in an oven or, by using a hot air blower to remove all traces of diethylamine from the plate.

Visualization methods

1. UV light at 254 nm.
2. 2N Sulphuric acid/heat/UV light at 366 nm.
3. Acidified potassium iodoplatinate reagent.

Preparation of Acidified potassium iodoplatinate reagent: Dissolve 0.25 grams of platonic chloride and 5 g of potassium iodide in sufficient water to produce 100 ml. This is potassium iodoplatinate reagent; for the acidified version and 5 ml of concentrated hydrochloride acid to 100ml. of iodoplatinate solution.

Method: First observe the plate under UV light at 254 nm. Spray the plate with 2N sulphuric acid and heat it in a oven at 80oC for 5 minutes. Observe the fluorescent spot on plate under UV light at 366 nm. Spray the plate with acidified iodoplatinate reagent. Appearance of purple colour indicates the presence of benzodiazepines.

10. 7.2 Gas Liquid Chromatography [1]

10. 7.2.1 Packed Column Technique

Operating conditions:

Detector : FID

Column : 6ft (or 2 m),2 to 4 mm ID glass

Packing : 3% SE-30 or OV-1 on 80-100 Chromosorb W HP

Carrier gas : Nitrogen

Flow rate : 45 ml per minute.

Column Temp. : from 200°C to 280°C at 16°C/min

Injector temp. : 280°C

Detector temp. : 280°C

10. 7.2.2 Capillary Column Technique

Detector : FID

Column : Fused silica, chemically bonded and cross-linked methyl silicone such as BP-1, DB-1 or equivalent

Film thick : 0.25 μm

Length : 25 m, ID 0.25 mm

Carrier gas : Nitrogen 1 ml/min

Split ratio : 20 : 1

Column Temp. : 250°C

Injector Temp. : 275°C

Detector Temp. : 275°C

10. 7.3 High Performance Liquid Chromatography [1]

10. 7.3.1 Normal Phase

Column : 250 mm by 5 mm id

Packing material : Silica HPLC grade, 5μm diameter Spherisorb-S 5 W or equivalent

Mobile phase : **A**: Methanol (1000 ml) containing perchloric acid (100 μl)

B: Methanol-water-trifluoroacetic acid

(997 : 2 : 1, v/v/v)

Flow rate : 2.0 ml/min.

Detection : UV at 240 nm

Quantification : by peak areas, external standard method.

10. 7.3.2 Reverse Phase

Column : 250 mm by 5 mm ID

Packing material : Octadecyl-Silica 5 μm diameter ODS –

Hypersil or equivalent

Mobile Phase : **C**: Methanol:Water: Phosphate buffer (0.1 M)

55 : 25 : 20, v/v/v/, PH 7.25

D: Methanol: water: phosphate buffer (0.1M)

70 : 10 : 20, v/v/v), PH 7.67

The phosphate buffer (0.1M) is prepared by dissolving sodium dihydrogenphosphate didydrate (14.35g, 0.092 mol) and disodium hydrogenphosphate (1.14g, 0.008 mol) in 1000 ml water.

Flow Rate : 1.5 ml /min

Detector : UV at 240 nm

Quantitation : by peak areas, external standard method.

10. 7.4 Spectroscopic Techniques

10. 7.4.1 UV-Vis Spectroscopy

Benzodiazepine derivatives may be studied by scanning the samples in appropriate organic solvents with the help of UV-Vis spectrophotometry. The value of λ_{max} of the spectrum can be compared with the standard value given in the literature or with using the standard sample of benzodiazepine derivatives.

10. 7.4.2 Mass Spectroscopy

Benzodiazepine derivatives may be studied by analyzing the samples with the help of Mass spectrometry (GC-MS). The value of principal peaks at m/z can be compared with the standard value given in the literature or with using the standard sample of Benzodiazepine derivatives.

Bromazepam:	236, 317, 315, 288, 316, 286, 208, 78.
3-hydroxybromazepam:	79, 78, 52, 105, 304, 314, 316, 51.
Chlordiazepoxide:	382, 299, 284, 283, 241, 56, 301, 253.
Demoxepam:	285, 286, 269, 287, 241, 242, 77, 270.
Desmethylchlorideazepoxide:	285, 268, 284, 77, 286, 42, 287, 233.
Desmethyldiazepam:	242, 269, 270, 241, 243, 271, 244, 272.
Oxazepam:	257, 77, 268, 239, 205, 267, 233, 259.
Clobazam:	300, 258, 77, 259, 283, 302, 231, 256.
Desmethylclobazam:	286, 244, 77, 218, 51, 217, 288, 215.
Clonazepam:	280, 314, 315, 286, 234, 288, 316, 240.
7-acetamidoclonazepam:	43, 327, 299, 298, 292, 329, 328, 256.
7-aminoclonazepam:	285, 256, 257, 258, 44, 287, 110, 220.
Clorazepic acid:	242, 43, 270, 269, 241, 103, 243, 76.
Clozapine:	243, 256, 70, 245, 192, 227, 258, 326.
Demoxepam:	285, 286, 269, 287, 241, 242, 77, 270.
Diazepam:	256, 283, 284, 285, 257, 255, 258, 286.
Desmethyldiazepam:	242, 269, 270, 241, 243, 271, 244, 272.
Oxazepam:	257, 77, 268, 239, 205, 267, 233, 259.
Temazepam:	271, 273, 300, 272, 256, 77, 255, 257.
Flunitrazepam:	285, 312, 313, 286, 266, 238, 294, 284.
7-amino-1-desmethyl flunitrazepam:	269, 240, 241, 268, 270, 107, 121, 213.

7-aminoflunitrazepam: 283, 44, 255, 282, 254, 284, 264, 256.

Desmethylflunitrazepam: 298, 271, 299, 224, 272, 270, 252, 280.

Flurazepam: 86, 87, 99, 58, 84, 387, 315, 56.

N'-desalkylflurazepam: 260, 259, 288, 287, 261, 289, 262, 290.

Didesethylflurazepam: 30, 313, 246, 211, 273, 274, 302, 183.

N'-(2-hydroxyethyl)
flurazepam: 288, 273, 331, 287, 304, 290, 289, 275.

Ketazolam: 256, 284, 283, 285, 84, 257, 258, 255.

Lorazepam: 291, 239, 274, 293, 75, 302, 276, 138.

Lormetazepam: 305, 307, 306, 309, 308, 334, 102, 75.

Medazepam: 242, 207, 244, 270, 243, 271, 269, 165.

Desmethyldiazepam: 242, 207, 244, 270, 243, 271, 269, 165.

Desmethylmedazepam: 193, 255, 228, 256, 257, 165, 230, 258.

Nitrazepam: 280, 253, 281, 206, 234, 252, 254, 264.

7-acetamidonitrazepam: 293, 265, 264, 292, 43, 222, 223, 294.

7-amino nitrazepam: 251, 222, 223, 250, 252, 195, 110, 97.

2-amino-5-nitrobenzophenone: 241, 77, 242, 105, 44, 43, 195, 57.

Nordazepam: 242, 269, 270, 241, 243, 271, 244, 272.

Prazepam: 91, 269, 324, 55, 296, 295, 323, 297.

3-hydroxy Prazepam: 257, 55, 311, 77, 259, 313, 44, 312.

Triazolam: 313, 238, 342, 315, 75, 344, 239, 137.

10. 7.4.3 IR/FT-IR Spectrometry

Benzodiazepine derivatives may be studied by analyzing the samples with the help of IR Spectroscopy/FTIR. The value of principal peaks at wave numbers can be compared with the standard value given in the literature or with using the standard sample of barbiturate derivatives.

Bromazepam:	1685, 825, 750, 802, 1315, 1230.
Chlordiazepoxide:	1625, 760, 1260, 690, 1590, 850.
Clobazam:	1684, 1664, 704, 1490, 764, 845.
Clonazepam:	1685, 1610, 748, 1255, 1578, 1532.
Clorazepic acid:	1597, 1548, 1300, 702, 1230, 830.
Clozapine:	1600, 1560, 758, 1136, 1101, 1117.
Demoxepam:	1678, 690, 1240, 1265, 755, 717.
Diazepam:	1681, 1313, 705, 840, 1125, 740.
Flunitrazepam:	1697, 1620, 1490, 1528, 1107, 783.
Flurazepam:	1672, 1613, 1316, 1211, 1171, 1100.
Ketazolam:	1675, 820, 1105, 1195, 1308, 1242.
Lorazepam:	1685, 1149, 1317, 1120, 1605, 826.
Lormetazepam:	1682, 1153, 1121, 1315, 1610, 843.
Medazepam:	1610, 1178, 1298, 700, 1255, 815.
Nitrazepam:	1690, 1610, 698, 1536, 745, 784.
Nordazepam:	1680, 700, 1602, 820, 738, 790.
Oxazepam:	1687, 1706, 693, 830, 1136, 1123.
Prazepam:	1667, 1316, 740, 694, 1602, 704.
Temazepam:	1687, 1670, 1112, 1603, 705, 1150.
Triazolam:	761, 842, 1618, 1003, 1310, 827.

[Details of the Benzodiazepine derivatives, extraction and their examinations may also be referred from **"Recommended methods for testing of Benzodiazepine derivatives"** described in the 'Manual for use by National Narcotics Laboratories, United Nations, New York, 1988']

References:

1. Recommended methods for testing Benzodiazepine derivatives under international control, manual of National Narcotics Laboratories, United Nations, 1988.

Other references:

1. Sarin, R. K.; Sharma, G. P.; Varshney, K. M.; and Rasool, S. N., Determination of diazepam in cold drinks by High performance thin layer chromatography. J. Chromatogra. A, 822, 1998, p 332-335.

2. Krishnamurthy, R.; Kulkarni, J. P.; Malve, M. K.; and Shinde, B. M., Detection of depressant drugs in food articles used in duping cases, J. Ind. Acad. Foren. Sci., 35 (1&2), 1996 p-30-37.

3. Moffat, A.C., Clarke's Isolation & Identification of Drugs, 2nd edition, The Pharmaceutical Press, London, 1986.

SECTION – 11

MESCALINE

11.1 Title: Mescaline

11.2 Scope: Mescaline samples in crime exhibits

11.3 Purpose: Identification and estimation of mescaline drug

11.4 Responsibility: Reporting officers and supporting scientific staff.

11.5 Sampling Procedure:

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 examinations 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 who he works.

Seizures of Peyote may consist of one or more entire, living cactus specimens or fresh or dried parts of the cactus, for example Mescal Buttons. Mescaline exhibits, mostly in form of its sulphate or hydrochloride salts, are encountered

predominantly as powders, tablets or capsules in a single container or package or the material may be inside a number of packages.

1. **Peyote cactus and Mescal Buttons**

(a). Sampling of single package items

The simplest sampling situation is where the submitted item consists of a single Peyote cactus or Mescal Button specimen with or without package. The Peyote material should be removed from its container or wrappings, placed in a clean clear plastic bag and the net weight recorded. Entire living cactus specimens should be dried at 40^0C and then cut into two equal pieces, one of which is kept as evidence for court purposes.

The dried plant material should be thoroughly homogenized prior to the application of the sequence of chemical tests by pounding in a mortar with a pestle, or by use of an adapted commercial food –mixer or food-processor.

Because of the variable moisture content of Peyote cactus and Mescal Buttons specimens, it is essential for quantitative analysis that a portion of the representative sample be dried at 110^0C to constant weight as described in various pharmacopoeias in order to determine the moisture content. In quantifying the mescaline content, care should be tken to relate the content found to the total weight of material before drying.

(b). Sampling of items consisting of more than one package

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

1. If all packages contain suspect 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 material in all the packages is found by visual examination to be the same then the analyst may adopt one of two approaches:

1. The contents of a number of packages may be combined and the combined bulk material may then be homogenized;
2. Alternately, chemical testing may be applied to a number of the packages.

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

Sampling errors for quantitative methods are reduced if large aliquots of material are subjected to sequential dilution with the extraction solvent. If the cost of solvent presents no problem and if the taking of a large aliquot will not significantly reduce the size of the exhibit to be taken to court, then this approach may be adopted. However, when large amounts of material are used for the first extraction, it may be necessary that the solvent should be added by pipette to avoid error due to insoluble materials.

2. Mescaline powders

(a) Sampling of Single package items

The simplest sampling situation is where the submitted item consists of a single package of material. 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 be thoroughly homogenized 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 in a mortar with a 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.

(b) Sampling of items consisting of more than one package

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

1. If all packages contain suspect 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 ofa 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 first dissolution, it may be necessary to add the solvents by pipette to avoid error due to insoluble materials.

3.Mescaline tablets and capsules

1. 1-50 dosage units – Randomly select ½ total number of units to a maximum of 20. Determine average weight, powder to pass through a 20-mesh sieve and mix thoroughly.
2. 51-100 dosage units – Randomly select 20 units, proceed as above.
3. 101-1,000 dosage units – Randomly select 30 units, proceed as above.
4. Greater than 1,000 dosage units – Randomly select a number of units equal to the square root of the total number present, rounded to the next higher integer; proceed as above.

(b) Multiple containers

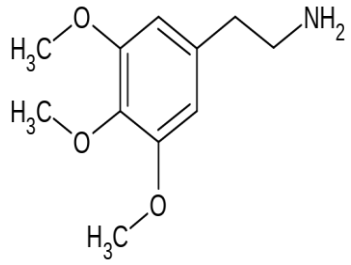
Segregate containers by lot numbers and treat each group as described in Chapter III. A. 1. b above. Report results separately for each group.

Determine the square root of the total number of packages in each group. Randomly select a number of packages equivalents to the square root, rounded to the next highest integer.

From each of the selected packages, randomly select a number of dosage units equivalent to the square root of the total number of dosage units divided by the square root of the number of packages, rounded to the next higher integer.

Form a composite by grinding, seizing through a 20-mesh sieve and thoroughly mixing. Perform the analysis on the composite.

11.6 Major Constituents of the Mescaline

Mescaline	Peyote cactus	Mescaline Peyote
 **$C_{11}H_{17}NO_3$** **M.Wt-211.3**		

11.7 Methods:

11.7.1 Presumptive Tests

Colour tests

Positive results of colour tests are only the presumptive indication for the presence of mescaline drugs. It is necessary for the analyst to confirm the presence of mescaline drug by an alternative technique.

Extraction Techniques: Dried and pulverized plant material can be extracted using methanol and Conc. ammonia solution (99:1). For Powders (Base or Salts) Extract in Methanol.

11. 7.1.1 Marquis test [1]

Take appropriate amount of the suspected material or exhibit on a spot plate add to it 1 drop of reagent A followed by 2 drops of reagent B. The appearance of orange to orange red colour indicates the presence of mescaline.

Solution A: Add 8 – 18 drops of 40% formaldehyde solution to 10 ml glacial acetic acid.

Solution B: Concentrated Sulphuric acid.

11. 7.1.2 Meck's reagent test [2]

Take appropriate amount of the suspected material or exhibit on a spot plate add to it few drop of Meck's reagent. The appearance of Orange changes to brown colour indicates the presence of mescaline.

Preparation of Meck's Reagent: 0.25 gms of selenious acid is dissolved in 25 ml of concentrated sulphuric acid.

11. 7.1.3 Frohde reagent Test [2]

Take appropriate amount of the suspected material or exhibit on a spot plate add to it few drop of Frohde reagent. The appearance of brown colour indicates the presence of mescaline.

Preparation of Frohde's Reagent: 50 mgs of molybdic acid or sodium molybdate is dissolved in 10 ml of hot concentrated sulphuric acid. The resulting solution should be colourless.

11. 7.1.4 Liebermann's Test [3]

Take appropriate amount of the suspected material or exhibit on a spot plate add to it 2 – 3 drops of Liebermann's reagent. Occasionally it is required to carry out the test in a test tube and heat it in a water bath at 100oC. The appearance of black colour indicates the presence of mescaline.

Preparation of Liebermann's Reagent: Add 5 grams of Sodium nitrite to 50 ml of sulphuric acid with cooling and swirling to absorb the brown fumes.

11. 7.2 Thin Layer Chromatography:

Stationary Phase: Activated silica gel (with or without fluorescing substance) TLC plates of 0.25/0.20 mm thicknesses.

Solvent/Mobile/Developing system:

System A: Chloroform, methanol and con. Ammonia **[1]**

82 : 17 : 1

System B: Methanol and Con. Ammonia **[1]**

100 : 1.5

System C: Methyl ethyl ketone, dimethylformamide and NH_4OH **[2]**

13 : 1.9 : 0.1

System D: Chloroform, methanol and acetic acid **[2]**

75 : 20 : 5

Visualization:

The plate must be dried prior to visualisation at room temperature or by using hot air blower, as per requirement.

1. UV light at 254 nm
2. Fluorescamine (Fluram) reagent
3. Ninhydrin reagent

Preparation of spray reagents

Fluorescamine (fluram) reagent: Dissolve 10mg fluorescamine in 50 ml dried acetone.

Ninhydrin reagent: Prepare a 10% solution of ninhydrin in ethanol.

Method:

Observe first the plate under UV light at 254 nm. Mescaline will absorb the light and appear as a dark spot on the fluorescent background. Then spray with reagent (1) or (2). When using reagent (1), the plate has to be dried after spraying by shortly using a hot air blower. Then observe the plate under UV light at 365 nm. Mescaline gives a bright yellow fluorescent spot. The fluorescence can be intensified by exposing the plate to ammonia vapour. When using reagent (2), the plate has to be heated after spraying in an oven at 120^{o}C for at least 15 minutes. A violet spot is given by mescaline.

Alternate method for visualisation [2]

Spray the developed TLC plate lightly with reagent A followed immediately with reagent B and place it in the iodine tank (reagent C). Mescaline gives yellow colour spot.

Reagent A: 10% aqueous sodium acetate;

Reagent B: 1% 2, 6 dibromo-p-benzoquinone-4-chlorimine in alcohol.

Reagent C: 2 grams of iodine crystals in TLC tank.

11. 7.3 Gas Chromatography:

11. 7.3.1 Packed Column Technique

Method 1 [1]

Operating conditions

Detector	: FID
Column	: 6ft (or 2 m), 2 to 4 mm id glass
Packing	: 5% SE-30 100-120 Mesh Gas Chrom Q
Carrier gas	: Nitrogen at 30 ml /min
Column Temp.	: 150°C
Internal Standard	: n-alkane.

Method 2 [1]

Operating conditions

Detector	: FID
Column	: 1.5m, 3.2 mm ID SS
Packing	: 1.5% OV-101 100-200 Mesh Chromosorb G
Carrier gas	: Nitrogen at 30 ml /min
Column Temp.	: 150°C
Internal Standard	: n-alkanes.

Method 3 [2]

Operating conditions

Detector	: FID
Column	: 5ft x 1/8" SS
Packing	: 5% SE-30 on 60/80 Mesh Chromosorb W
Carrier gas	: Nitrogen at 25 ml /min
Injector Temp.	: 200°C
Column Temp.	: (a) 180°C (b) 205°C
Detector Temp.	: 225°C

Method 4 [2]

Operating conditions

Detector	: FID
Column	: 5ft x 1/8" SS
Packing	: 3% XE-60 on 100/120 Mesh Aeropak 30
Carrier gas	: Nitrogen at 25 ml /min
Injector Temp.	: 200°C
Column Temp.	: (a) 150°C (b) 210°C
Detector Temp.	: 225°C

11. 7.3.2 Capillary Column Technique

Operating Conditions:

Detector	: FID (and/or NPD)
Column	: Fused silica, chemically bonded and cross-linked methyl phenyl silicone such as DB-5 or equivalent
Column length	: 20m x 0.174 mm ID
Film thick	: 0.40 μm

Carrier gas : Helium

Column Temp. : 150°C (1.5min) to 280 °C 10°c/min

Injector Temp. : 250°C

Detector Temp. : 310°C

Internal Std. : n –alkanes

Split ratio : Splitless

11. 7.4 High Performance Liquid Chromatography

Method A [1]

Operating Conditions

Column : Octadecyl-silica, (Spherisorb ODS-1 or equivalent), 3 µm particle size, 150 mm x 4.6mm ID.

Mobile phase : Water : 892ml (892 g)

Acetonitrile: 108ml (84 g)

o-phosphoric acid: 5.0ml (8.5 g)

Hexylamine : 280µl (0.22 g)

Flow rate : 1.0ml / min

Detection : UV at 205 nm

Injection volume : 5 µl

Quantitation : by peak area, internal standard method.

Internal standard : methoxamine hydrochloride

Method B [3]

Column : Silica (spherisorb S5W, 5µm, 12.5 cm x 4.9 mm ID) is used.

Eluent : A solution containing 1.175 g (0.01M) of ammonium perchlorate in 1000ml of methanol; adjust to pH 6.7 by

the addition of 1ml of 0.1M sodium hydroxide in methanol.

Method C [3]

Column : ODS-silica (ODS-Hypersil, 5µm, 25cm x 5mm ID)

Eluent : A solution containing 19.60 g (0.2M) of phosphoric acid and 7.314 g (0.1M) of diethylamine in 1000 ml of a 10% v/v solution of methanol; adjust the pH to 3.15 by the addition of sodium hydroxide solution.

Method D [3]

Column : silica (spherisorb, 5µm, 25cm x 5mm ID.

Eluent : Methanol : ammonium nitrate buffer solution (90:10).
To prepare the buffer solution add 94ml of strong ammonia solution and 21.5ml of nitric acid to 884ml of water and adjust to pH 10 by the addition of strong ammonia solution.

11. *7*.5 Spectroscopic Techniques

11. *7*.5.1 IR /FT-IR Spectroscopy

Major peaks of mescaline base, sulphate and hydrochloride (Cm^{-1}):

1591, 1513,1245, 1130, 995, 835, 670 (halide disk, nujol

mull and thin-film techniques) **[1]**

1127, 1242, 1592, 996, 1513, 834 (mescaline hydrochloride, KBr disk) **[3]**

11. *7*.5.2 UV Spectrophotometry

Aqueous acid 268nm (A^1_1 = 34c).

No alkaline shift.

Mescaline may be studied by scanning the samples in appropriate organic solvents with the help of UV-Vis spectrophotometry. The spectrum and the value of λ_{max} of the spectrum can be compared with the standard value given in the literature or with using the standard sample of mescaline.

11. 7.5.3 Mass Spectrometry

Principal peaks at m/z

Mescaline: a) 211 (Molecular ion), 182 (Base peak), 167,151 **[1]**

b)182, 30, 181, 167, 211, 183, 151, 148 **[3]**

[Details of the Mescaline, extraction and its examinations may also be referred from **"Recommended methods for testing of Peyote cactus (Mescal buttons)/Mescaline and psilocybe mushrooms/psilocybin"** described in the 'Manual for use by National Narcotics Laboratories, United Nations, New York, 1989']

References:

1. Recommended methods for testing peyote cactus (Mescal buttons)/ Mescaline and psilocybe mushrooms/psilocybin, Manual of national Narcotics Laboratories, United Nations, 1989.

2. Gunn, John W., In Analytical Manual of Bureau of Narcotics & Dangerous Drugs, Department of Justice, US (By Stanely P Sobol & Richard A Moore), p--

3. Moffat, A.C., Clarke's Isolation & Identification of Drugs, 2nd edition, The Pharmaceutical Press, London, 1986.

SECTION – 12

PSILOCIN/PSILOCYBIN

12.1 Title: Psilocin/Psilocybin

12.2 Scope: Psilocin/Psilocybin samples in crime exhibits

12.3 Purpose: Identification and estimation of Psilocin/ Psilocybin drug.

12.4 Responsibility: Reporting Officers and supporting scientific staff.

12.5 Sampling Procedure:

Sampling Procedure as follows at the Section-11 containing Mescaline and Psilocybin Mushrooms/Psilocybin are same.

12.6 Major Chemical Constituents of the Psilocin/Psilocybin

<table>
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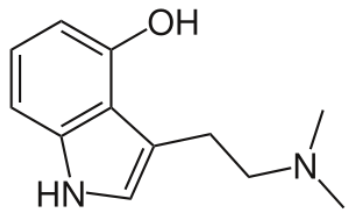
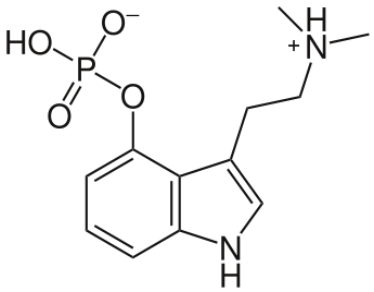
<td>Psilocin
 
$C_{12}H_{16}N_2O$
M.Wt-204.3</td>
<td>Psilocin
 
</td>
<td>Dried Psilocybin Mushroom
  </td>
</tr>
<tr>
<td>Psilocybin
 
$C_{12}H_{17}N_2O_4P$
M.Wt-284.25</td>
<td>Psilocybin Mushroom
  </td>
<td>Psilocybin Mushroom
  </td>
</tr>
</table>

12.7 Methods:

Extraction Techniques: Dried and pulverized plant material can be extracted using methanol for Psilocybin powders (base) is soluble in methanol.

12.7.1 Microscopic examination:

The microscopic examination of the fragments or powders of psilocybin mushroom specimens should only be performed by trained mycologists. The microscopic characteristics of fragments or powders of psilocybe mushroom are the spores, the cystidia (sterile cells in the fertile portions of the fruit-body), the basic structure of the outer layer of the cap and the basic structure of the tissue between the faces of the gills. When available gross-characters of the dried fruit-body are useful.

12. 7.2 Presumptive Tests:

Colour Tests

Positive results of colour tests are only the presumptive indication for the presence of Psilocin/Psilocybin drug. It is necessary for the analyst to confirm the presence of Psilocin/Psilocybin drug by an alternative technique.

12. 7.2.1 Ehrlich Reagent test [1]

Take an appropriate amount of the suspected sample on the spot plate and add 2 drops of Ehrlich reagent. Appearance of violet to grey – violet colour after few minutes indicates the presence of psilocin or psilocybin.

Preparation of Ehrlich reagent:

Dissolve 1 gram of p-dimethylaminobenzaldehyde in 10ml. methanol. Then add 10ml. conc. o-phosphoric acid.

12. 7.2.2 Marquis Test [1]

Take an appropriate amount of the suspected sample on the spot plate and add to it 1 drop of reagent A followed by 2 drops of reagent B. Appearance of orange colour indicates the presence of psilocybin. While appearance of green brown colour indicates the presence of psilocin.

Preparation of Reagent A:

8 – 10 drops of 40% formaldehyde in 10ml. in glacial acetic acid.

Preparation of Reagent B:

Conc. sulfuric acid.

12. 7.2.3 Froehde's reagent test [2]

Take an appropriate amount of the suspected sample on the spot plate and add to it few drops of Froehde's reagent. Appearance of green changes to blue grey colour indicates the presence of psilocin. While appearance of olive green changes to yellow indicates the presence of psilocybin.

Preparation of Froehde's Reagent: 50 mgs of molybdic acid or sodium molybdate is dissolved in 10 ml of hot concentrated sulphuric acid. The resulting solution should be colourless.

12. 7.2.4 Mecke's reagent test [2]

Take appropriate amount of the suspected material or exhibit on a spot plate add to it few drop of Mecke's reagent. The appearance of green changes to greenish black indicates the presence of psilocin. While the appearance of greenish yellow changes to brownish green indicates the presence of psilocybin.

Preparation of Mecke's Reagent : 0.25 gms of selenious acid is dissolved in 25 ml of concentrated sulphuric acid

12. 7.3 Thin Layer Chromatography :

Stationary Phase: Activated silica gel (with or without fluorescing substance) TLC plates of 0.25/0.20 mm thickness.

Solvent/Mobile/Developing system:

System 1: n-Butanol, Acetic acid and water

20 : 10 : 10

System 2: Methanol and Conc. ammonia solution

100 : 1.5

Visualization:

1. UV radiation at 254 nm and 365 nm
2. Ehrlich reagent
3. p- Dimethylaminocinnamaldehyde

Preparation of Ehrlich reagent:

Dissolve 1 gram of p-dimethylaminobenzaldehyde in 10ml. methanol. Then add 10ml. conc. o-phosphoric acid.

Preparation of p- Dimethylaminocinnamaldehyde reagent:

Disslove 0.5 grams of the said chemical in 50ml. of methanol. Then add 10ml. conc. HCl.

12. 7.4 Gas Chromatography

12. 7.4.1 Packed Column Technique

Method -1:

(With out Derivatization)

Detector : FID

Column : 6ft (or 2 m), 4 mm id glass

Packing : 2.5% SE-30 on 80-100 Mesh Chromosorb G/Q

Carrier gas : Nitrogen at 45 ml /min

Column Temp. : 200^{o}C

Internal Std. : n- alkanes

Method-2:

(with Derivatization)

Detector : FID

Column : 1.6m, 2.8 mm id glass

Packing : 1.5% SE-30 on 100-120 Mesh Chromosorb W

Carrier gas : Helium, 50 ml /min

Column Temp. : 150 -250°C, 7.5 °C/min

Inj/Det. Temp. : 220°C/250°C

Internal Std. : n- alkanes

Derivatizing reagent : Bis (trimethylsilyl) trifluoracetamid (BSTFA) and trimethylchlorosilane (TMCS)

12. 7.4.2 Capillary Column Technique

Method-1:

(without Derivatization)

Detector : FID

Column : Fused silica, chemically bonded and cross-linked methyl phenyl silicone such as DB-5 or equivalent

Film thickness : 0.40 μm

Carrier gas : Helium

Column Temp. : 150^0C (1.5min) to 280^0C, 10^0C/min

Injector Temp. : 250^oC

Detector Temp. : 310^oC

Internal Std. : n –alkanes

Split ratio : Splitless

Method-2:

(with Derivatization)

As mentioned in Method-1 (with derivatization of 12.5.4.2).

Derivatizing reagent: Trimethylchlorosilane (TMCS)

12. 7.5 High Performance Liquid Chromatography

12. 7.5.1 Normal phase

Operating conditions

Column : 250mm by 4.6 mm ID

Packing material : partisil-5, 5μm particle size

Mobile phase : methanol, water and 1M ammonium nitrate buffered to pH 9.6 with 2M ammonia (220:70:10) containing the disodium salt of EDTA (1mM)

The mobile phase is filtered under vacuum and sonicated for 10 min before use.

Flow rate : 1.0 ml/min.

Detection : (a) UV at 254 nm

(b) Fluorimetric detection with excitation at 267 nm and 320-nm emission filter.

Injection volume : 10 μl by syringe or loop.

Quantitation : by peak height.

12. 7.5.2 Reverse Phase

Column : 250nm by 4. mm ID

Packing material : Octadecyl-silica (spherisorb ODS-1 or equivalent), 5μm particle size.

Mobile phase : (A) Water, containing 0.3M ammonium acetate and buffered to pH 8 with ammonia.

(B) Methanol, containing 0.3M ammonium acetate.

The mobile phase is filtered under vacuum and sonicated for 10 min. before use.

Solvent programme : 0-2 min, 0% (B) in (A), isocratic; 2-14 min,

0-95% (B) in (A), linear gradient.

Flow rate : 2.0ml/min.

Detection : UV at 269 nm.

Injection volume : 10 μl by syringe or loop.

Quantitation : by peak area. Internal Standard method.

12. 7.6 Spectroscopic techniques

12. 7.6.1 UV Technique [3]

Psilocin: Aqueous acid – 266nm, 283nm, 292nm

Aqueous alkali – 270nm, 293nm.

Psilocybin: Aqueous acid – 268 nm,

Aqueous alkali – 269nm, 282nm, 292nm.

psilocin and psilocybin may be studied by scanning the samples in appropriate organic solvents with the help of UV-Vis spectrophotometry. The spectrum and the value of λ_{max} of the spectrum can be compared with the standard value given in the literature or with using the standard samples of psilocin and psilocybin.

12. 7.6.2 IR/FT-IR Technique:

Major peaks of psilocin and psilocybin are (Cm^{-1}):

psilocin (KBr disc)

1620, 1585, 1261, 1236, 1061, 1042 **[1]**

836, 1261, 1236, 1042, 1061, 733 **[3]**

psilocybin (KBr disc)

1620, 1585, 1505, 1360, 1065 **[1]**

1105, 1045, 1062, 1183, 1160, 932 **[3]**

12. 7.6.3 Mass Spectrometry:

psilocin underivatised:

Principal peaks: at m/z 204 (molecular ion), 146, 130, 77, 58 (base peak), 42 **[1]**

Principal peaks: at m/z 58, 204, 59, 42, 30, 146, 77, 44 **[3]**

psilocin di-TMS

Principal peaks at m/z 348(molecular ion), 290, 73, 58 (base peak) **[1]**

psilocybin

Principal peaks at m/z 58, 42, 30, 51, 204, 146, 77, 44 **[3]**

psilocybin tri-TMS

Principal peaks: at m/z 500 (molecular ion), 485, 455, 442, 73, 58 (base peak) **[3]**

[Details of the psilocin and psilocybin, extraction and their examinations may also be referred from **"Recommended methods for testing of Peyote cactus (Mescal buttons)/mescaline and psilocybe mushrooms/psilocybin"** described in the 'Manual for use by National Narcotics Laboratories, United Nations, New York, 1989']

References:

1. Recommended methods for testing peyote cactus (Mescal buttons)/ Mescaline and psilocybe mushrooms/psilocybin, Manual of national Narcotics Laboratories, United Nations, 1989.

2. Gunn, John W., In Analytical Manual of Bureau of Narcotics & Dangerous Drugs, Department of Justice, US (By Stanely P Sobol & Richard A Moore).

3. Moffat, A.C., Clarke's Isolation & Identification of Drugs, 2nd edition, The Pharmaceutical Press, London, 1986.

SECTION -13

Lysergic acid diethylamide

LYSERGIDE (LSD)

13.1 Title: **Lysergide (LSD)**

13.2 Scope: Crime exhibits containing the lysergide (LSD)

13.3 Purpose: Identification and estimation of lysergide (LSD)

13.4 Responsibility: Reporting officers and supporting scientific staff.

13.5 Sampling Procedure: Most LSD exhibits are either in paper, tablet or gelatin form. Powders are generally not encountered. The sampling procedure provided is applicable to these three forms. For the purposes of the sampling plan, one sheet of paper subdivided into smaller dosage units should be considered as one "Container".

In case of single container, determine the total number of dosage units and the average weight per dosage unit (du).

For sample sizes up to 10 du – screen all dosage units.

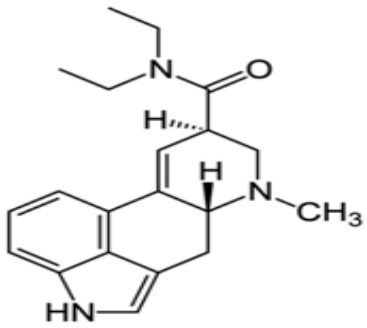
For sample sizes from 11 du to 27 du – randomly select and screen ¾ of all dosage units, rounding upward to the next higher integer.

For sample sizes from 28 dosage unit (du)– select randomly and screen ½ of all dosage units rounding upward to the next integer and selecting a minimum of 21 du and a maximum of 50 du.

In case of multiple containers having different contents, this should be segregated and subjected to separate analysis. Determine the square root of the total number of containers in each group. Randomly select a number of containers equivalent to the square root, rounded to the next highest integer.

13.6 Major Chemical Constituents of the Lysergide (LSD)

Lysergide (LSD)



$C_{20}H_{25}N_3O$

M.Wt-323.4

13.7 Methods:

Extraction techniques:

Mix the test samples for 30 seconds with a small amount of methanol sufficient to obtain a solution of approximately one microgram LSD per ml. After filtration, the extract can be used directly.

13. 7.1 Presumptive tests

Colour Tests

Positive results of colour tests are only the presumptive indication for the presence of LSD. It is necessary for the analyst to confirm the presence of LSD by an alternative technique.

Fluorescence [1]

Original sample/dosage unit shows blue fluorescence at 366 nm. Alternatively, take a drop of methanol extract on a filter paper and allow it to dry. Observe the spot under UV light at 366 nm. Appearance of blue fluorescence indicates the presence of LSD.

13. 7.1.1 Ehrlich reagent test [1]: Take appropriate amount of the sample or few drops of methanol extract of the sample in a depression spot plate and add two drops of Ehrlich reagent. Appearance of a blue to purple colour indicates the presence of LSD.

Preparation of Ehrlich reagent:

1g para-dimethylamine benzaldehyde (p-DMAB) in 10ml methanol. Add 10ml conc. ortho phosphoric acid.

13. 7.1.2 Marquis reagent test [2]: Orange colour changes to brown, which changes to purple.

Preparation of Marquis Reagent: 8-10 drops of 40% formaldehyde solution is added to 10 ml of Con. Sulphuric acid.

13. 7.1.3 Frohde's reagent test[2]: Olive green changes to blue, which changes to green.

Preparation of Frohde's Reagent: 50 mgs of molybdic acid or sodium molybdate is dissolved in 10 ml of hot concentrated sulphuric acid. The resulting solution should be colourless.

13. 7.1.4 Mecke's reagent test [2]: Olive green changes to blue-black.

Preparation of Mecke's Reagent: 0.25 gms of selenious acid is dissolved in 25 ml of concentrated sulphuric acid

13. 7.2 Thin Layer Chromatography

Stationary Phase: Activated silica gel (with or without fluorescing substance) TLC plates of 0.25/0.20 mm thickness.

Solvent/Mobile/Developing system:

Solvent System A: Chloroform: Methanol

90 : 10 **[1]**

Solvent System B: Chloroform: Acetone

20 : 80 **[1]**

Solvent System C: Chloroform: Acetone

1 : 2 **[2]**

Solvent System D: Chloroform saturated with NH_4OH & MeOH

18 : 1 **[2]**

Solvent System E: Benzene: dimethylformamide

13 : 2 **[2]**

Visualization:

Observe the plate under UV light at 254 nm. Appearance of dark spots on the fluorescent background on TLC plate. Now, observe the plate under UV light at 365 nm. A fluorescent spot (blue) on a dark background is shown by LSD. Now, spray the plate with Ehrlich reagent. Appearance of blue-purple colour spots indicates the presence of LSD.

Preparation of Ehrlich reagent:

1g para-dimethylamine benzaldehyde (p-DMAB) in 10ml methanol. Add 10ml conc. ortho phosphoric acid.

13. 7.3 Gas Liquid Chromatography [1]

13. 7.3.1 Packed Column Technique

With derivatization:

Detector : FID

Column : 3ft, 2.4mm id glass

Packing : 3%SE-30 on 80-100 mesh, chromosorb W

Carrier gas : Nitrogen at 30 ml/min

Column Temp. : 250°C

Injector temp. : 275°C

Detector Temp. : 275°C

Internal Std : n-alkanes in chloroform

Derivatizing Agent : N,O-bis-trimethysilylacetamide (BSA)

13. 7.3.2 Capillary Column Technique

Detector : FID

Column : BP1 fused silica

Film thickness : 0.25μm.

Length : 25m x 0.22 mm ID.

Carrier gas : Nitrogen at 1ml/min.

Split ratio : 20:1

Detector Temp. : 325°C

Injector Temp. : 300°C

Column Temp. : 275°C

Internal Std. : n-alkanes

13. 7.4 High Performance Liquid Chromatography [1]

13.5.4.1 Normal Phase

Column	: 125mm x 4.9 mm id
Packing material	: Silica HPLC grade 5 μm diameter
Mobile phase	: A sol. containing 1.17 g (0.01M) of ammonium perchlorate in 1000ml of methanol. Adjust to pH 6.7 by adding 0.1M sodium hydroxide in methanol.
Flow rate	: 2.0 ml/min.
Detection	: UV at 313 nm or fluorescence, excitation at 308 nm, emission of 370-700 nm.
Injection volume	: 1 – 5 μl by syringe or loop – injector.
Quantitation	: By peak area, external standard method.

Sample and standard solutions: All materials are dissolved in methanol to give an approximate concentration of 1mg/ml.

13. 7.4.2 Reverse phase

Column	: 10 cm x 4.6 mm id
Packing material	: Octadecyl-silica HPLC grade 5 μm
Mobile phase	: A sol. Containing of 65% methanol and 35% 0.025M Disodium hydrogen phosphate in water adjusted to ph 8.0 with 10% orthophosphoric acid.
Flow rate	: 1.0 ml/min
Detection	: UV at 280 nm or fluorescence excitation at 320nm, emission at 400nm.
Injection volume	: 1 – 5 μl by syringe or loop injector.

Sample and standard

Solutions : All materials are dissolved in methanol to give an approximate concentration of 0.5 to 1.0 mg per ml.

Quantitation : By peak area, external standard method.

13. 7.5 Spectroscopic Techniques

13. 7.5.1 UV/Fluorescence

LSD shows blue fluorescence under UV light at 365 nm **[1]**

Aqueous acid – 315 nm (A^{1}_{1}=225a) **[3]**

Aqueous alkali – 310 nm **[3]**

LSD may be studied by scanning the samples in appropriate organic solvents with the help of UV-Vis spectrophotometry. The spectrum and the value of λ_{max} of the spectrum can be compared with the standard value given in the literature or with using the standard sample of LSD.

13. 7.5.2 IR/FTIR Spectrophotometry

Principal peaks at wave numbers: 1626, 1307, 1136, 1066, 1212, 749 (KBr disk) **[3]**

13. 7.5.3 Mass Spectrometry

Principal peaks at m/z

LSD: 323, 221, 181, 222, 207, 72, 223, 324 **[3]**

[Details of the LSD, extraction and its examinations may also be referred from **"Recommended methods for testing lysergide (LSD)"** described in the 'Manual for use by National Narcotics Laboratories, United Nations, New York, 1989']

References:

1. Recommended methods for testing lysergide (LSD), Manual of national Narcotics Laboratories, United Nations, 1989.

2. Gunn, John W., In Analytical Manual of Bureau of Narcotics & Dangerous Drugs, Department of Justice, US (By Stanely P Sobol & Richard A Moore), p--

3. Moffat, A.C., Clarke's Isolation & Identification of Drugs, 2nd edition, The Pharmaceutical Press, London, 1986.

SECTION -14

DIPHENOXYLATE

14.1 Title: 1-(3-cyano-3,3-diphenylpropyl)-4 phenyl-4-piperidinecarboxylic acid ethyl ester.

14.2 Scope: Crime exhibits containing the diphenoxylate

14.3 Purpose: Identification of diphenoxylate

14.4 Responsibility: Reporting officers and supporting scientific staff.

14.5 Methods:

Extraction techniques: Tablets are extracted with acetonitrile-water (50 + 50) for liquid chromatographic analysis.*****

14.5.1 Presumptive tests

Colour Tests

Positive results of colour tests are only the presumptive indication for the presence of diphenoxylate It is necessary for the analyst to confirm the presence of diphenoxylate by an alternative technique.

14.5.1.2 Sodium Picrate test [1]: Mix the test samples with few drops of chloroform and sulfuric acid to hasten the reaction in a test tube. Hold a piece of filter paper, impregnated with reagent, expose to the vapor that come from the tube, and heating the contents to 30 degrees. The yellow colour filter paper changes from orange to brown –orange indicate the presence of diphenoxylate.

Preparation of sodium Picrate reagent:

Prepare the 5% solution of sodium bicarbonate and 0.5% of picric acid in distilled water.

14.5.1.3 Marquis reagent test [2]

Take appropriate amount of the exhibit (1-2mg of powder, one or two drops if liquid) on a spot plate; add not more than 3 drops of reagent drop wise. Appears of orange colour indicates the positive test for the presence of diphenoxylate.

Preparation of Marquis reagent:

Add 8 – 10 drops of 40% formaldehyde solution to 10 ml of conc. sulphuric acid.

14.5.2 Thin Layer Chromatography

Stationary Phase: Activated silica gel (with or without fluorescing substance) TLC plates of 0.25/0.20 mm thickness.

Solvent/Mobile/Developing system:

Solvent System A: Methanol : Ammonia

100 : 1.5

Solvent System B: Cyclohexane :Toluene : Diethylamine

75 : 15 : 10

Solvent System C: Chloroform: Methanol

9 : 1

Solvent System D: Methanol : n-Butanol & 0.1 Mol/L NaBr

6 : 4

Visualization:

Observe the plate under UV light. Appearance of dark orange colour spot corresponding to standard sample of diphenoxylate confirm the presence of diphenoxylate.

Spray reagents:

A. Dragendorffreagent : Orange colour spot

B. Acidified iodoplatinate reagent; Dark orange spot

14.5.3 Gas Liquid Chromatography

14.5.3.1 Capillary Column Technique

Detector : FID

Column diameters : 25 m x 0.32 id x 0.25 μm film thickness (DB1301)

Carrier gas : Helium at 1.5 ml/min.

Detector Temp. : 300 °C

Injector Temp. : 250 °C

Column Temp. : 100°C @ 35 °C / Min up to 235°C held 3.6 Min @ 8°C / Min up to 290°C final hold time 3 min.

14.5.4 High Performance Liquid Chromatography

Method 1.

Column : 125 mm x 4.9 mm id

Packing material : SilicaSpherisorb S5W HPLC grade 5 μm thickness

Mobile phase : A sol. 0.01 M Ammonium perchlorate pH 6.7 Adjust by adding 1 ml of 0.1 m Sodium Hydroxide in Methanol.

Flow rate : 2.0 ml/min.

Detection : UV at 258 nm

Injection volume : 1 – 5 μl by syringe or loop – injector.

Quantitation : By peak area, external standard method.

Sample and standard solutions: All materials are dissolved in methanol to give an approximate concentration of 1mg/ml.

Method 2

Column : C 18 Li Chrospher 100 RP (125x 4 mm id, 0.5 μm).

Mobile phase : Add 146 μl trimethylamine and about 750 μl phosphoric acid to 530 ml water adjust pH 3.3 using 10% potassium hydroxide and finally add 470 ml of acetonitrile.

Flow rate : 0.6 ml/min

Detection : UV diode array detector

Injection volume : 1 – 5 μl by syringe or loop injector.

Sample and standard

Solutions : All materials are dissolved in methanol to give an approximate concentration of 0.5 to 1.0 mg per ml.

Quantitation : By peak area, external standard method.

13.5.5 Spectroscopic Techniques

13.5.5.1 Ultra violet absorbance

Methanol – 252, 258 nm (A^{1}_{1}=14a) , 264 nm

Diphenoxylate may be studied by scanning the samples in appropriate organic solvents with the help of UV-Vis spectrophotometer. The spectrum and the value of λ_{max} of the spectrum can be compared with the standard value given in the literature or with using the standard sample of diphenoxylate.

13.5.5.2 IR/FTIR Spectrophotometry

Principal peaks at wave numbers: 1728, 1495, 1215, 1183, 1120, 697 cm-1 (KBr disk) **13.5.5.3 Mass Spectrometry**

Principal peaks at m/z

Diphenoxylate: 246,247,165, 115, 10391,56, 42,

***Reference**

Lehr G.J., "Determination of diphenoxylate hydrochloride and atropine sulphate in combination drug formulations by liquid chromatography" Journal of AOAC International 79 (1996) 1288-1293.

SECTION -15

TRAMADOL

15.1 Title: 2-[(Dimethyl amino)- Methyl]-1- 93-methoxyphenyl) cyclohexanol.

15.2 Scope: Crime exhibits containing the tramadol.

14.3 Purpose: Identification of tramadol.

14.4 Responsibility: Reporting officers and supporting scientific staff.

14.5 Methods:

Extraction techniques:

(1) The sample homogenized before taken in screw cap glass vail. The appropriate amount of the homogenized sample taken in glass vail then 2 ml of 3 M HCL added and supernatant removed following centrifugation at 3000 rpm for 10 min. The pH 9.0 of the solution adjusted by using potassium borate buffer prior extraction. The sample applied to the HCX column pretreated with 2 ml of methanol, 2 ml of deionized water and 2 ml of 0.1 M potassium borate buffer pH 9. After washing the column with 2 ml of 1% acetic acid followed with 2 ml acetonitrile, tramadol eluted with 2 ml of n-butyl chloride. The elution extract evaporated to dryness at 40 °C under a gentle stream of nitrogen and finally the residues dissolved in methanol for chemical tests as well as for further scientific instrumental analysis.

(2) Weigh the tablet and convert them into fine powder. Add appropriate amount of methanol and filter. Wash residues with methanol and add washings into the filtrate. Use this filtrate for analysis by HPLC or GC/GC-MS*.

14.5.1 Presumptive tests

Colour Tests

Positive results of colour tests are only the presumptive indication for the presence of tramadol It is necessary for the analyst to confirm the presence of tramadol by an alternative technique.

14.5.1.3 Marquis reagent test [1]

Take appropriate amount of the exhibit (1-2 mg of powder) on a spot plate; add not more than 3 drops of reagent drop wise. Appearance of violet to green change in colour indicates the positive test for the presence of tramadol.

Preparation of Marquis reagent:

Add 8 – 10 drops of 40% formaldehyde solution to 10 ml of conc. sulfuric acid.

14.5.2 Thin Layer Chromatography

Stationary Phase: Activated silica gel (with or without fluorescing substance) TLC plates of 0.25/0.20 mm thickness.

Solvent/Mobile/Developing system:

Solvent System A: Ethyl acetate :Methanol : Ammonia

85 : 10 : 5

Solvent System B: Chloroform: Methanol

9 : 1

Visualization:

Observe the plate under UV light. Appearance of dark orange/ violetcolour spot corresponding to standard sample of tramadol confirm the presence of tramadol.

14.5.3 Gas Liquid Chromatography

Method 1

Detector	: FID
Column	: RTX 10024. 25 m x 0.32 id (DB1301)
Column dimentions	: 30 m x 0.32 mm i.d. x 0.25μm film thickness.
Carrier gas	: Nitrogen at 1 ml/min.
Detector Temp.	: 280 °C
Injector Temp.	: 250 °C

Column Temp. : 100 °C @ 10 °C / Min up to 250 °C hold for 8 Min.

14.5.4 High Performance Liquid Chromatography

Method 1.

Column : RP-18 (Li Chrosorb, 250 x 4.6 mm id,5 μm thickness); guard RP-18 (Supelcosil), 4x 4 mm id, 5 μm

Mobile phase : 70% 0.01M phosphate buffer pH 5.9: 30% acetonitrile with 0.1 trimethylamine.

Flow rate : 0.75 ml/min.

Detection : UV at 218 nm

Injection volume : 1 – 5 μl by syringe or loop – injector.

Quantitation : By peak area, external standard method.

Method 2.

Column : SGX C18 (150 x 3.3 mm id,5 μm thickness) Compact Glass cartridge; ;guard (30x3.3 mm id) ,

Mobile phase : 4 g of hexanesulfonic acid sodium salt monohydrate dissolved in 400ml of 0.02 M phosphoric acid(1.28 ml 85% phosphoric acid in1000mL water) and mixed with 600mL acetonitrile

Flow rate : 0.9 ml/min.

Detection : UV at 275 nm

Injection volume : 1 – 5 μl by syringe or loop – injector.

Quantitation : By peak area, external standard method.

Method 3.

Column : OPd-50(Asahipack,125 x 4.0 mm id,5 μm thickness) Compact

Glass cartridge; ; guard (30x3.3 mm id) ,

Mobile phase : .01 M borate buffer (pH9): Methanol (40:60)ECD.

Flow rate : 0.7 ml/min.

RT : 10-11 minute

Sample and standard solutions: All materials are dissolved in methanol to give an approximate concentration of 1 mg/ml.

14.5.5 Gas Chromatograph Mass Spectrophotometer.

Method 1

Detector : MS (SIM m/z 263, 179)

Column : HP-5-MS Capillary

Column dimentions : 30 m × 0.25 mm i.d. × .25 μm film thickness

Carrier gas : Helium at 1 ml/min.

Detector Temp. : 280 °C

Injector Temp. : 250 °C

Column Temp. : 140 °C @ 24 °C / Min up to 260 °C hold 4 Min.

13.5.5 Spectroscopic Techniques

13.5.5.1 Ultra violet absorbance

Aq acid – 272, 258 nm (A^{1}_{1}=70a) , No alkaline shift occur.

Tramadol may be studied by scanning the samples in appropriate organic solvents with the help of UV-Vis spectrophotometer. The spectrum and the value of λ_{max} of the spectrum can be compared with the standard value given in the literature or with using the standard sample of tramadol.

13.5.5.2 IR/FTIR Spectrophotometry

Principal peaks at wave numbers: 1601, 1575, 1284, 1238, 1042, 702 cm-1 (KBr disk)

***Reference:**

Belal T, Awad T, Clark C.R, "Determination of paracetamol and tramadol hydrochloride in pharmaceutical mixure using HPLC and GC-MS" Journal of Chromatographic Science 47 (2009) 849-854.

SECTION – 16

MISCELLANEOUS DRUGS/SUBSTANCES

14.1 Title: Miscellaneous drugs

14.2 Scope: Crime exhibits containing miscellaneous drugs

14.3 Purpose: Identification and estimation of miscellaneous drugs

14.4 Responsibility: Reporting officers and supporting scientific staff.

14.5 Sampling Procedure: ---

14.6 Major Chemical Constituents of the Miscellaneous drugs/ Substances: ---

14.7 Methods: Since it is not possible to describe all chemical test methods/techniques for the chemical analysis of all types of crime exhibits pertaining to Narcotic Drugs and Psychotropic Substances and miscellaneous drugs/substances/chemical in connection with NDPS, some of the important techniques and references, which can be used for the examination of these substances to produce reliable results are given below:

Important techniques

1. Colour tests
2. Thin Layer Chromatography (TLC)
3. High Performance Thin Layer Chromatography (HPTLC)
4. High Performance Liquid Chromatography (HPLC)
5. Gas Liquid Chromatography (GLC)
6. Gas Chromatography – Mass Spectrometry (GC-MS)
7. X-Ray Diffractometer (XRD)
8. Ion-Mobility Spectroscopy
9. Microscopy
10. UV-Vis Spectrophotometry
11. IR/FTIR Spectroscopy, etc.

Precursor Chemical:

1. Acetic anhydride

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SECTION 17
LIST OF EQUIPMENTS

17.1 Major Equipments

a) Gas Chromatography – Mass Spectrometry (GC-MS)
b) Liquid chromatography-Mass Spectrometry (LC-MS)
c) High Performance Liquid Chromatography (HPLC)
d) Fourier transform infrared spectrophotometer (FTIR)
e) UV-Visible Spectrophotometer

17.2 Minor Equipments

a) Thin Layer Chromatography setup
b) Hot air oven
c) Tissue homogenizer
d) Centrifuge
e) Sonicator
f) Vortex shaker
g) UV-Cabinet (254 nm, 265nm and visible lights)
h) Hot plate cum magnetic stirrer
i) Fuming hood
j) Refrigerator
k) Water bath
l) Analytical balance

Formation of committees at National Level for formulation of SOPs and Manuals:

Background: In view of technological advancements in the scientific arena, the Standard Operating Procedures (SOPs) and Working Procedure Manuals, around which the technical and analytical exercise takes place in the laboratory in the examination of crime exhibits, needs periodical review to keep the laboratory updated.

For uniform SOP/Manuals and reporting pattern in all the CFSLs / State FSLs following committees were formed by JS (PM), MHA by including members from Central and State FSLs in the following areas:

Discipline	CFSL Member	Member
Biology/DNA	Dr. A. K. Sharma, Director, CFSL, Kolkata/Guwahati	1. Sh. Arun Sharma, Director, FSL, HP 2. Sh. Srikumar, Director, Chemical Examiner Lab, Thiruvananthapuram.
Chemistry/ Narcotics	Sh. K. M. Varshney, Coordinator, CFSL, Pune	1. Dr. R. K. Gupta, Director, FSL, Chhattisgarh. 2. Sh. B Shanmukham, Director, FSL, Puducherry. 3. Dr. Harsh Sharma, Director, FSL, Sagar (MP)
Explosives	Dr. Sukhminder Kaur, Coordinator CFSL, Pune	1. One officer from FSL, Delhi 2. One officer from FSL, Maharashtra
Toxicology	Dr. Vimukti Chauhan, SSO, CFSL, Chandigarh	1. Dr. K. V. Kulkarni, Director, DFSL, Maharashtra 2. One officer from FSL, Karnataka.
Ballistics	Sh. S. S. Baisoya, CFSL Chandigarh	1. Dr. D. K. Kaushal, Director, FSL, Haryana 2. Sh. N. P. Waghmare, Director, FSL, Goa 3. Dr. S. S. Das, Director, FSL, Odisha
Documents	Sh. M. C. Joshi, Dy. Director, CFSL, Chandigarh (Shimla Unit) and Dr. S. Ahmad, DFSS HQs., New Delhi	1. Ms. Deepa Verma, Director, FSL, Delhi
Psychology, Computer, Audio-Video	Dr. S. K. Jain, Director, CFSL, Chandigarh and Sh. M. Krishna, AD, CFSL, Hyderabad	Officers from FSL: HP, Delhi, Gujarat and Maharashtra
Crime Scene	Dr. M. Baskar, Dy. Director (Physics), CFSL, Chandigarh	1. Dr. Harsh Sharma, Director, FSL (MP) 2. Sh. R. K. Gupta, Jt. Director, FSL, Chhattisgarh

The officers of CFSLs will coordinate with the experts of State FSLs for convening of meeting(s) in the state and Central FSLs and finalization of SOPs and manuals.