RECOMMENDED METHODS FOR TESTING PEYOTE CACTUS (MESCAL BUTTONS)/ MESCALINE AND PSILOCYBE MUSHROOMS/ PSILOCYBIN

MANUAL
FOR USE BY NATIONAL
NARCOTICS LABORATORIES

UNITED NATIONS

DIVISION OF NARCOTIC DRUGS
Vienna

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INTRODUCTION

Background

Over the past few years there has been a considerable increase in the number of substances newly placed under international control. At the same time, seized quantities of drugs already under control have also shown an alarming and unprecedented increase in certain regions. This new situation, involving an increase both in the frequency and volume of seizures, presents a challenge not only to national law enforcement authorities, but also to the technical and scientific staff of forensic laboratories.

Analysts have to be able to deal with more substances and preparations and to use faster, more accurate and more specific methods of identification and analysis. In addition, the international character of drug trafficking requires the timely exchange of analytical data between laboratories and law enforcement authorities both on the national and the international levels.

The Commission on Narcotic Drugs, at its tenth special session in February 1988, reviewed the technical and scientific assistance programme of the Division of Narcotic Drugs with special emphasis on the development of laboratory methodologies. It noted with satisfaction that the harmonization of laboratory methods and the programme on establishment of recommended methods of testing for national forensic laboratories was pursued vigorously and that such methods had already been developed for heroin, cocaine, cannabis products, opium/crude morphine, amphetamine/methamphetamine, ring-substituted amphetamine derivatives, methaqualone/mecloqualone, LSD, and benzodiazepine derivatives.

In emphasizing the importance of the expert group meetings organized by the Division on various scientific and technical aspects of drug control and the high practical value for national law enforcement and laboratory services of the technical manuals as the outcome of the expert meetings, the Commission at its 33rd regular session strongly recommended that such meetings and the publication of laboratory manuals continue on a regular basis. It proposed that a manual for the analysis of hallucinogenic plant products be prepared.

Purpose of the manual

In accordance with the recommendation of the Commission on Narcotic Drugs, a group of fifteen experts was convened in June 1989 in Wiesbaden, Federal Republic of Germany, by the Division of Narcotic Drugs in cooperation and with the financial support of the Government of the Federal Republic of Germany through UNFDAC. The present manual published by the United Nations Division of Narcotic Drugs reflects the conclusions of the group of experts and has been designed to provide practical assistance to national authorities by describing recommended methods to be used in forensic laboratories for the identification and analysis of Peyote Cactus (Mescal Buttons)/Mescaline as well as for Psilocybe Mushrooms/Psilocybin. The manual may also serve as a guide to national authorities in assessing existing methods used within their own government and university laboratories.

This manual is one in a series of similar publications dealing with the identification and analysis of various groups of drugs under international control; it was preceded by manuals on heroin (ST/NAR/6), cocaine (ST/NAR/7), cannabis (ST/NAR/8), amphetamine/methamphetamine ((ST/NAR/9), opium/crude morphine (ST/NAR/11), ring-substituted amphetamine derivatives (ST/NAR/12), methaqualone/mecloqualone

(ST/NAR/15), benzodiazepine derivatives (ST/NAR/16), LSD (ST/NAR/17), and barbiturate derivatives (ST/NAR/18).

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

Use of the Manual

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

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

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

Attention is also drawn to the vital importance of the availability of textbooks on drugs of abuse and analytical techniques. Furthermore, the analyst must continually keep abreast of current trends in analysis, consistently following current analytical and forensic science literature. For this purpose, attention is drawn to the Multilingual Dictionary of Narcotic Drugs and Psychotropic Substances under International Control (ST/NAR/1), and to the Manual of Staff Skill Requirements and Basic Equipment for Narcotics Laboratories (ST/NAR/2), both published by the Division of Narcotic Drugs. The latter publication lists bibliographic references as well as a selection of well-known journals in the field. Analysts should refer to these and to previous manuals in this series for general description of the analytical techniques included in this manual.

It is equally important that the latest information on changes in drugs available in the illicit traffic be quickly disseminated. This may often need to be done prior to publication in specialized periodicals dealing with forensic and other chemical analyses, since these publications are available to the forensic community some

two to three years after the changes become known. The value of frequently published national reports on the latest information on such changes in drugs and on work being undertaken and analytical results obtained within individual laboratories cannot be over-emphasized.

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

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PEYOTE CACTUS (MESCAL BUTTONS) / MESCALINE

I. DESCRIPTION OF PEYOTE CACTUS AND MESCAL BUTTONS

Lophophora williamsii (LEM. ex SALM-DYCK) COULT. (Cactaceae) was important to the ancient Aztecs as a ritual hallucinogen. They called this cactus "Peyotl", meaning "furry thing", a reference to the tufts of hairs crowning the mature plant (see Figure 1). The cactus is used today under the name of "Peyote" by several Indian tribes of northern Mexico. The use of Peyote has also spread to the United States and Canada, where native American groups, mainly members of the American Native Church, still utilize the cactus in rituals.

More than 25 synonyms, like Anhalonium williamsii and Echinocactus lewinii, have been used for this cactus, which is indigenous to the western hemisphere. It grows in deserts from central Mexico north to southern Texas and New Mexico (United States), especially in the Rio Grande valley. The Peyote cactus occurs isolated or in groups usually on limestone soils, on rocky slopes, dried river beds and flatlands.

Mescal Buttons (Peyote Buttons), the form most commonly found in the illicit traffic are the dried, brown, disk-shaped tops of the cactus.

The principal psychotropic alkaloid of Peyote is mescaline, a phenylethylamine derivative. Up to 30% of the total Peyote alkaloids is present as mescaline with a content varying between 0.5 and 1.5%.

Mescaline can easily be synthesized in clandestine laboratories, for example by starting from 3,4,5-trimethoxybenzaldehyde. It appears mostly as the sulfate or hydrochloride salt from time to time on the illicit drug markets in the United States and Europe.

Other minor Peyote alkaloids are the phenylethylamines N-methylmescaline, 3-O-demethylmescaline and hordenine. About 30 additional alkaloids, for example pellotine, anhalonidine, anhalamine and lophophorine, belong to the group of the structurally related tetrahydroisoquinoline derivatives. These minor alkaloids do not show any psychotropic activity.

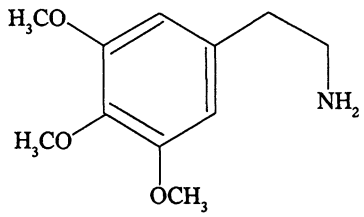
Lophophora diffusa (CROIZAT) BRAVO contains pellotine as the main alkaloid and only traces of mescaline.

The mescaline-containing cactus species Trichocereus pachanoi BRITT. et ROSE and Trichocereus peruvianus BRITT. et ROSE (Cactaceae) are known to grow in subtropical and temperate areas of South America, especially in the Andean regions. They are called "San Pedro" in Peru and "Aguacolla" in Ecuador.

Several other species of the Cactaceae family also contain phenylethylamine derivatives, but no mescaline or only traces of it. They are called "False Peyotes" and belong to the genera of Ariocarpus, Coryphantha, Echinocereus and Mamillaria.

II. CHEMICAL CONSTITUENTS OF FORENSIC INTEREST

MESCALINE



$C_{11}H_{17}NO_3$

M.Wt. = 211.3

Melting points (°C)

Base (crystals)	*Sulfate (dihydrate) (prisms)*	*HCl (needles)*
35-36	**183-186**	**181**

Solubilities

	Base	*Sulfate (dihydrate)*	*HCl*
Water	**moderately soluble**	**sparingly soluble**	**soluble**
Water, boiling	**-**	**soluble**	**soluble**
Ethanol	**soluble**	**sparingly soluble**	**solube**
Methanol	**soluble**	**soluble**	**soluble**
Chloroform	**soluble**	**-**	**-**

III. THE ANALYSIS OF PEYOTE CACTUS, MESCAL BUTTONS AND MESCALINE

A. Sampling

The principal reason for a sampling procedure is to produce a correct and meaningful chemical analysis. Because most methods, qualitative and quantitative, used in forensic laboratories for the examination of drugs require very small aliquots of material, it is vital that these small aliquots are 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 in publications such as "Official Methods of Analysis" published by 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 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 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 sulfate 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°C 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°C to constant weight as described in various pharmacopoeias in order to determine the moisture content. In quantifying the

mescaline content, care should be taken 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 all items and the content of all packages by eye, and possibly by TLC to determine:

1. **If all specimens and the content of all packages are the same suspect material, and/or**

2. **If one or more specimens or if the content of one or more packages are different from those of the majority of the specimens and packages. The simplest indicator is the physical appearance of the material. If one or more specimens or if the content of packages obviously differ, these specimens or packages 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 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 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 packages should be segregated and subjected to separate analysis.

The compositing of multiple container items is as follows:

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

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

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

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

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

Sampling errors for quantitative methods are reduced if large aliquots of material are subjected to sequential dilution with the dissolving solvent. If the total sample size is large, this approach may be adopted. However, when large amounts of material are used for the first dissolution, it may be necessary to add the solvents by pipette to avoid error due to insoluble materials.

3. Mescaline tablets and capsules

(a) Single container

1. 1-50 dosage units - randomly select 1/2 of 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 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, sieving through a 20-mesh sieve and thoroughly mixing. Perform the analysis on the composite.

B. Extraction techniques

1. Peyote cactus and Mescal Buttons

Mescaline is quantitatively extracted from dried and pulverized plant material using methanol-concentrated ammonia solution 99:1 (4 x 0.5 ml per 10 mg plant material; shaking or sonication).

For TLC it is not necessary to perform a further clean-up of the extracts. For GC and HPLC analysis it is recommended to eliminate possibly interfering lipids by a simple diethylether pre-extraction (4 x 0.5 ml per 10 mg plant material; shaking or sonication).

For infrared spectroscopy mescaline has to be isolated from the plant material in as pure a form as possible by preparative TLC or HPLC using the chromatographic systems described under "TLC" (see Chapter III. E.) and "HPLC" (see Chapter III. G.).

2. Mescaline powders

Both the free base and the salts (sulfate, hydrochloride) are soluble in methanol and this is the solvent of choice for sample and standard preparation for qualitative and quantitative analysis.

A representative sample of finely powdered mescaline as determined by the sampling procedure above is dissolved in an appropriate amount of methanol to obtain a solution of about 1mg/ml.

3. Mescaline tablets and capsules

A representative sample of finely powdered tablets or the content of the representative number of capsules as determined by the sampling procedure above is extracted with methanol (shaking or sonicating) to obtain, after filtration, a solution of about 1 mg/ml.

C. Physical examination

1. Macroscopic characteristics (see Figure 1)

Lophophora williamsii (Peyote) is a simple, spineless, very succulent, dull bluish or greyish-green plant. The cactus attain a size of about 2-7 cm (height) x 4-12 cm (diameter). The roots are napiform, usually 8-11 cm long. The spineless crowns are globular, top-shaped, or somewhat flattened, 2-8 cm in diameter, with 8-10 well defined ribs and furrows. Tufts of hair are usually equally spaced on the ribs of mature plants. The pale pink, rarely whitish flowers with a size of 1.5-2.5 cm are located at the centre of the crown. The fruits are club-shaped, red to pinkish, 2 cm long or shorter and with 10-30 black, verrucose seeds.

2. Microscopic characteristics

The microscopic examination of ground cactus material does not show enough characteristics and is therefore inappropriate for forensic purposes.

For a more detailed morphological and microscopic description of the Peyote cactus, the reader is referred to the following books and review papers:

References:

1. *Schultes, R. E. and Hofmann, A.: "The Botany and Chemistry of Hallucinogens", 2nd edition., C.C. Thomas, Springfield, IL, U.S.A. (1980).*

2. *Boke, N. H. and Anderson, E. F., Amer. J. Bot. 57 (1970) 569-578.*

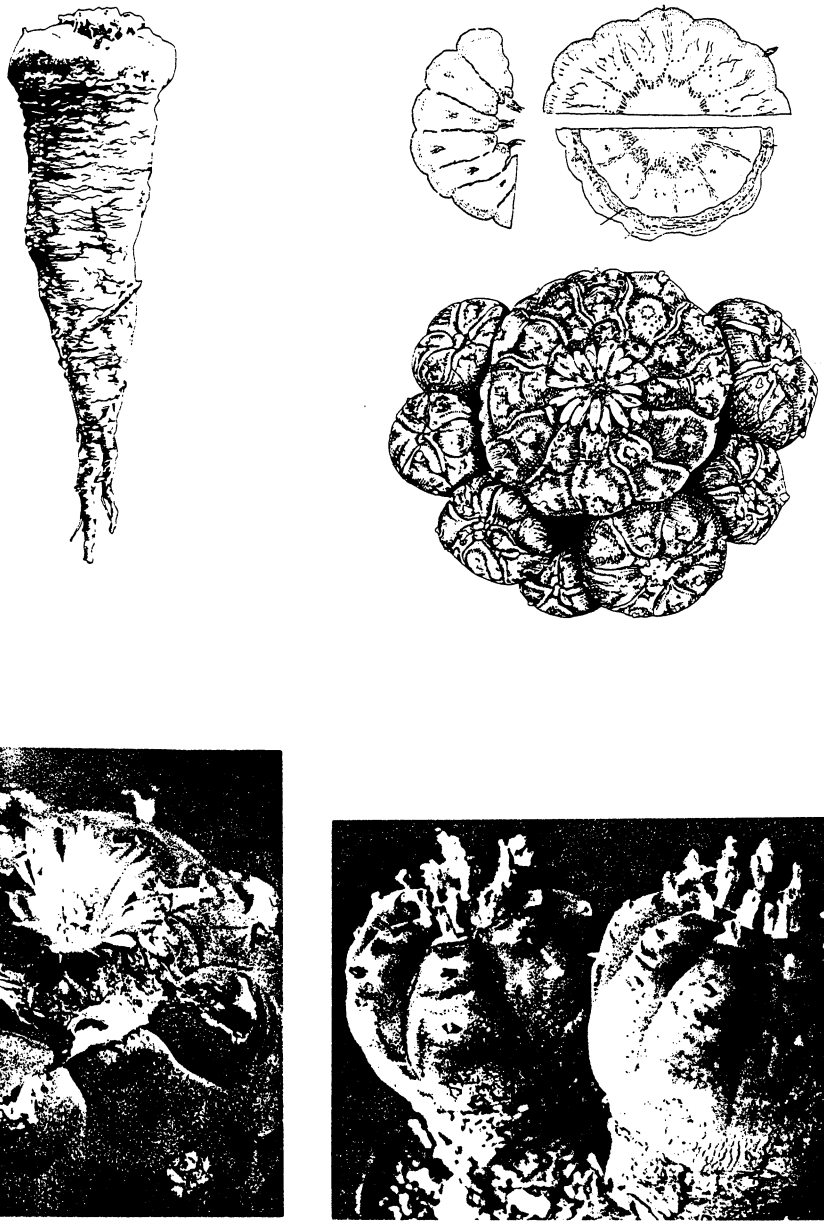


Figure 1 *Lophophora williamsii (LEM. ex SALM-DYCK) COULT.*

- Peyote Cactus, Peyotl -

D. Presumptive tests

1. Colour tests

It must be stressed that positive results to colour tests are only presumptive indications of the possible presence of mescaline. Many other materials, for example other primary phenylethylamines and amphetamines as well as those which are harmless and which are not controlled under national legislation or international treaties, may give similar colours with the test reagent. It is mandatory for analysts to confirm such results by using alternative techniques.

Because the positive colour reaction for mescaline in Peyote cactus using for example Marquis' reagent may be obscured by the vegetable matter, colour tests are not recommended for the plant material.

Marquis' Reagent

A_1 : Add 8-18 drops of 40% formaldehyde solution to 10 ml glacial acetic acid.
A_2 : Concentrated sulfuric acid.

Method:

Place a small amount of the suspected mescaline material (from powders, tablets or capsules) on a spot plate. Add one drop of reagent A_1 and two drops of reagent A_2. An orange to orange-red colour indicates the possible presence of mescaline. The detection limit of this method is about 10 µg.

Reference:

1. *"Rapid Testing Methods of Drugs of Abuse". A manual for use by national narcotics laboratories, ST/NAR/13, United Nations (New York) 1988.*

E. Thin-layer chromatography

Plates:

Activated silica gel G on glass backed plates; the coating (0.25 mm thickness) contains a fluorescing additive which fluoresces at 254 nm.

Developing solvents:

System A:	**Chloroform**	**82**
	Methanol	**17**
	Concentrated ammonia solution	**1**
System B:	**Methanol**	**100**
	Concentrated ammonia solution	**1.5**

Preparation of solutions to be applied to the TLC plate :

a) **Peyote cactus, Mescal Buttons:**

About 10 mg of a representative sample of the suspected dried and pulverized plant material is extracted using the method outlined in Chapter III. B. If using ninhydrin reagent for visualization the extract should be concentrated to approxymately 200 ul under a stream of nitrogen. and 10 ul of the filtered extract are spotted onto the TLC plate.

b) **Mescaline powder:**

Prepare a solution at a concentration of approximately 1 mg/ml methanol as outlined in Chapter III. B. and spot 1 ul of this solution onto the TLC plate.

c) **Mescaline tablets and capsules:**

Extract the material using the method outlined in Chapter III. B. and spot 10 ul of this extract onto the TLC plate.

d) **Standard solution:**

Prepare a solution of 1 mg/ml mescaline sulfate or hydrochloride in methanol and spot 1 ul of this standard solution onto the TLC plate.

Visualization:

The plates must be dried prior to visualization. This can be done by air-drying at room temperature or by short use of a hot air blower.

Visualization methods:

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

Spray reagents:

(1) Fluorescamine reagent: Prepare a solution of 10 mg fluorescamine in 50 ml dried acetone.

(2) 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 detection limit is about 10 ng mescaline. 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°C for at least 15 minutes. A violet spot is given by mescaline. The detection limit is about 1 ug mescaline.

RESULTS

With developing systems A and B, mescaline gives spots at R_f x 100 value approximating 36 and 24, respectively.

F. Gas liquid chromatography

1. Packed column technique

(A) Method 1

Operating conditions:

Detector	:	**FID**
Column	:	**6 ft (or 2 m), 2 to 4 mm ID glass**
Packing	:	**5% SE-30 on 100-120 mesh Gas Chrom Q**
Carrier gas	:	**Nitrogen at 30 ml/min**
Column temperature	:	**150°C**
Internal standard	:	**n-alkanes.**

METHOD

Preparation of internal standard solution:

Dissolve an appropriate n-alkane in methanol to give a concentration of 1 mg/ml.

Preparation of the standard solution:

To an accurately weighed amount of the mescaline (sulfate or hydrochloride) standard, add internal standard solution to give a concentration of 1 mg/ml.

Preparation of sample solution:

Add internal standard solution to an accurately weighed portion of the sample extract (Peyote, Mescal Buttons, mescaline tablets or capsules, mescaline powder) described under Chapter III. B. above.

Inject 1 ul of each solution into the gas chromatograph. The content (%) of mescaline can be calculated using the general formula:

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

where:

C_x %	=	**content of component x in the sample (w/w %).**
$C_{r.std.}$	=	**concentration of substance x in the standard reference solution (w/v %).**
$C_{sam.}$	=	**concentration of the sample (w/v %).**
A_x	=	**peak area for substance x during the sample chromatography.**
$A_{r.std.}$	=	**peak area for standard obtained during the standard chromatography.**
$A_{int.std.\ in\ sam.\ chr}$	=	**peak area of the internal standard obtained during the sample chromatography.**

The amount of mescaline in the plant material should be expressed as the percent mescaline base calculated per fresh or dry weight.

(B) Method 2

Operating conditions:

Detector	:	**FID**
Column	:	**1.5 m, 3.2 mm ID stainless steel**
Packing	:	**1.5% OV-101 on 100-200 mesh Chromosorb G**
Carrier gas	:	**Nitrogen at 30 ml/min**
Column temperature	:	**150°C**
Internal standard	:	**n-alkanes.**

METHOD

The preparation of the solutions of internal standard, standard and sample is carried out as described above. Inject 1 ul of each solution into the gas chromatograph.

RESULTS

Mescaline has a retention index RI of 1688.

For alternative GC techniques see:

References:

1. *Phytochem. 14 (1975) 2509.*

2. *J. Chromatogr. 189 (1980) 79.*

2. Capillary column technique

Operating conditions:

Detector	:	**FID (and/or NPD)**
Column	:	**Fused silica, chemically bonded and cross-linked methyl phenylsilicone, such as DB-5 or equivalent; 20 m, 0.174 mm ID**
Film thickness	:	**0.40 um**
Carrier gas	:	**Helium**
Split ratio	:	**Splitless**
Column temperature	:	**150°C (1.5 min) to 280°C, 10°C/min**
Injector/detector temp.	:	**250/310°C**
Internal standard	:	**n-alkanes.**

METHOD

The preparation of the solutions of internal standard, standard and sample is carried out as described above. Inject 1 ul of each solution into the gas chromatograph.

RESULTS

Mescaline has a retention time of 7.5 min.

G. High performance liquid chromatography

Reverse phase

Operating conditions:

Column	:	**150 mm by 4.6 mm ID**
Packing material	:	**Octadecyl-silica (Spherisorb ODS-1 or equivalent), 3 um particle size**
Mobile phase	:	**Water 892 ml (892 g)**
		Acetonitrile 108 ml (84 g)
		o-Phosphoric acid 5.0 ml (8.5 g)
		Hexylamine 280 ul (0.22 g)
Flow rate	:	**1.0 ml/min**
Detection	:	**UV at 205 nm (the detection limit for mescaline is about 500 pg)**
Injection volume	:	**5 ul by syringe or loop.**
Quantitation	:	**by peak area, internal standard method.**

Internal standard solution:

Dissolve methoxamine hydrochloride in methanol-concentrated ammonia solution 99:1 to give a concentration of 150 mg/L.

Standard solution:

Dissolve mescaline standard (sulfate or hydrochloride) in the internal standard solution to give a concentration of 1 mg/10 ml.

Sample solution:

The preparation of the sample solutions is carried out as described under Chapter III. F.

RESULTS

The capacity ratios (k' values) of mescaline and methoxamine (internal standard) are 2.53 and 3.16, respectively.*

(* These values will vary depending upon laboratory conditions and instrumental parameters).

H. Spectroscopic techniques

In some countries, confirmation of identity by spectroscopic means is required. Theoretically each substance has an unique infrared and mass spectrum and these methods would permit the unequivocable identification of isolated or synthesized mescaline base or salt. Depending upon the purity of mescaline, an extraction step as described under Chapter III. B. may be required prior to spectroscopic analysis.

The following sections provide references dealing with spectroscopic techniques for those laboratories which require such confirmation.

1. Ultraviolet spectroscopy

Because other phenylethylamines yield similar results, this method is not specific for the analysis of mescaline and therefore is not recommended.

2. Infrared spectroscopy

For description of the standard methods (halide disk, microhalide, nujol mull and thin-film techniques) see previous manuals in the series. Both the free base and salts (sulfate, hydrochloride) can be used.

Major peaks in the IR spectrum of mescaline base, sulfate and hydrochloride occur at the following wavenumbers (cm^{-1}): 1591, 1513, 1245, 1130, 995, 835, 670.

3. Mass spectroscopy

Characteristic ions of mescaline are: m/z 211 (molecular ion), 182 (base peak), 167, 151.

Reference:

1. *"Clarke's Isolation and Identification of Drugs", 2nd edition, The Pharmaceutical Press (London) 1986.*

PSILOCYBE MUSHROOMS / PSILOCYBIN

I. DESCRIPTION OF PSILOCYBE MUSHROOMS

Psilocybe mushrooms played a major role in the divinatory and magic rites of the ancient inhabitants of the Aztec empire. Important religious cults based on the sacramental consumption of these "sacred" mushrooms also called "teonanacatl" ("divine flesh"). Still today the hallucinogenic mushroom cult, namely the use of the species Psilocybe mexicana, is deeply rooted in the native tradition of Mexican Indians.

All hallucinogenic mushrooms so far recognized, exept for one, belong to a single group within the Basidiomycotina, the so called agarics (Agaricales). The term agaric is the general name applied to those fungi which basically possess an umbrella-shape, consisting of a cap (or pileus) on a centrally placed stem (or stipe), and radiating plate-like structures, called gills (or lamellae), and on the underside of that cap the basidia, those reproductive cells on which the spores develop (see Figures 2 and 3). It should be noted that some agarics which may be mistaken for Psilocybe species contain toxic material, such as for example gastroenteric irritants, cytolytic and/or haemolytic compounds.

The genus Psilocybe belonging to the family of Strophariaceae, is undoubtedly the most important, being an almost cosmopolitan genus of the hallucinogenic mushrooms. More than 140 different species are known, 80 of them are known to contain psychotropic substances. They are found from the arctic to the tropics, although their main distribution is in the temperate areas. The species grow on the soil and on a variety of organic substrates such as humus, dung, rotting wood, peat, and also on clumps of mosses. Other psilocybin containing mushrooms belong to the genera of Panaeolus (Coprinaceae), Conocybe (Bolbitiaceae), Inocybe (Cortinariaceae), and Pluteus (Pluteaceae). The most important two species, from the aspect of drug abuse, are Psilocybe semilanceata and Psilocybe cubensis. Since a few years ago, a marked increase has been observed in the abuse - by ingestion of fresh or dried fruit-bodies - of these potent psychoactive mushrooms which are controlled in many countries.

Psilocybe semilanceata (FR.) QUEL., "Liberty Caps" (see Figure 2), is the most widespread psilocybin-containing mushroom occuring for example in North and Middle Europe, North America, Russia, and Australia. It grows very scattered to gregarious on rich soil, among grass, in fields near farmyards, and in well-manured pastures or meadows.

Psilocybe cubensis (EARLE) SINGER (synonym: Stropharia cubensis EARLE) (see Figure 3) is known to grow in South America, Central America, southern Mexico, West Indies, Florida, and southeastern Asia. It grows singly or in small groups, usually on dung or rich pasture soil.

Psilocybe mexicana HEIM (see Figure 4) is known to grow in southern Mexico and Guatemala between 4,500 and 5,500 feet, especially in limestone regions, growing isolated or sparsely in moss or herbs along roadsides, humid meadows, and cornfields, and in the neighbourhood of pine and oak forests.

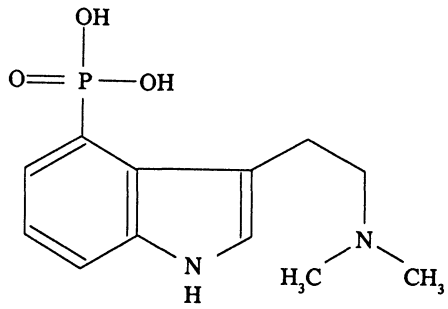
The main alkaloids present in these species are the phosphorylated indoleamines *psilocybin* (4-phosphoryloxy-N,N-dimethyltryptamine) and *baeocystin* (4-phosphoryloxy-N-methyltryptamine, norpsilocybin), the latter being possibly the direct biochemical precursor of psilocybin. *Psilocin* (4-hydroxy-N,N-dimethyltryptamine), the dephosphorylation product and hallucinogenic metabolite of psilocybin, is usually present only in trace amounts. It is formed enzymatically or more often as a result of inappropriate drying and storage. It is presently still not known

whether baeocystin produces psilocybin-like psychotropic effects. The content of psilocybin and baeocystin varies between 0.2-2% and 0.05-0.7%, respectively.

Psilocybin, legally or illegally synthesized or isolated from mushrooms, is from time to time encountered in the illicit drug traffic.

II. CHEMICAL CONSTITUENTS OF FORENSIC INTEREST

PSILOCYBIN
(4-Phosphoryloxy-N,N-dimethyltryptamine)



$C_{12}H_{17}N_2O_4P$

M.Wt. = 284.3

Melting point (°C)

Base
(crystals/MeOH)

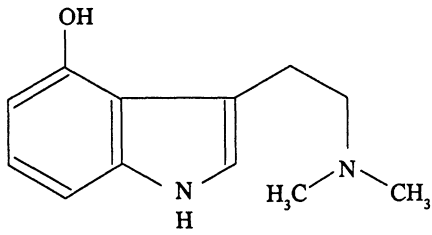
185-195 (decompos.)

Solubility

Base

Water	**insoluble**
Water, boiling	**1:20**
Diluted acetic acid	**soluble**
Ethanol	**insoluble**
Methanol, boiling	**1:120**
Chloroform	**insoluble**

PSILOCIN
(4-Hydroxy-N,N-dimethyltryptamine)



$C_{12}H_{16}N_2O$

M.Wt = 204.3

Melting point (°C)

Base
(crystals/MeOH)

173-176

Solubility
(sparingly stable in solution, especially when exposed to light and air!)

Base

Water	**insoluble**
Water, boiling	**1:120**
Diluted acetic acid	**soluble**
Ethanol	**insoluble**
Methanol	**1:120**
Chloroform	**insoluble**

III. THE ANALYSIS OF PSILOCYBE MUSHROOMS AND PSILOCYBIN

A. Sampling

The principal reason for a sampling procedure is to produce a correct and meaningful chemical analysis. Because most methods, qualitative and quantitative, used in forensic laboratories for the examination of drugs require very small aliquots of material, it is vital that these small aliquots are 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 in publications such as "Official Methods of Analysis" published by 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 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 Psilocybe mushrooms may consist of several fresh, dried or pulverized fruit-bodies. Psilocybin exhibits, mostly in its brown-yellow coloured base form, 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. Psilocybe mushrooms

(a) *Sampling of single package items*

The simplest sampling situation is where the submitted item consists of a single package of Psilocybe mushrooms. The material should be removed from its container or wrappings, placed in a clean clear plastic bag and the net weight recorded. Fresh mushroom specimens should be dried at 40°C.

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

Because of the variable moisture content of Psilocybe mushrooms specimens, it is essential for quantitative analysis that a portion of the representative sample be dried at 110°C to constant weight as described in various pharmacopoeias in order to determine the moisture content. In quantifying the psilocybin content, care should be taken 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 all items and the content of all packages by eye, and possibly by TLC to determine:

1. **If all specimens and the content of all packages are the same suspect material, and/or**

2. **If one or more specimens or if the content of one or more packages are different from those of the majority of the specimens and packages. The simplest indicator is the physical appearance of the material. If one or more specimens or if the content of packages obviously differ, these specimens or packages 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 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 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. Psilocybin 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 packages should be segregated and subjected to separate analysis.**

The compositing of multiple container items is as follows:

a) **If there are less than 10 packages, all packages should be sampled.**

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

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

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

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

Sampling errors for quantitative methods are reduced if large aliquots of material are subjected to sequential dilution with the dissolving solvent. If the total sample size is large, this approach may be adopted. However, when large amounts of material are used for the first dissolution, it may be necessary to add the solvents by pipette to avoid error due to insoluble materials.

3. Psilocybin tablets and capsules

(a) Single container

1. **1-50 dosage units - randomly select 1/2 of 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 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, sieving through a 20-mesh sieve and thoroughly mixing. Perform the analysis on the composite.

B. Extraction techniques

1. Psilocybe mushrooms

Psilocybin is quantitatively extracted from dried and pulverized mushrooms using methanol (by shaking or sonicating).

A pre-extraction step is necessary for presumptive testing of Psilocybe mushrooms, as the vegetable matter interferes with the colour reaction. One dried Psilocybe mushroom specimen is ground to a powder in a mortar with a pestle, transferred to a glass-stoppered tube, and, after addition of 1-2 ml methanol, shaken for 5 minutes. Place a plug of glass wool on top of the mushroom/methanol mixture in the tube and, using a disposable pipette, draw a few drops of the methanol solution through the glass wool. Two drops of this solution are used for the colour test.

For infrared spectroscopy, like for Peyote, psilocybin has to be isolated from the mushroom material in as pure a form as possible by a chromatographic technique, for example preparative TLC, using the system described under Chapter III. E.

2. Psilocybin powders

The free base is sufficiently soluble in methanol and this is the solvent of choice for the preparation of sample and standard solutions for qualitative and quantitative analysis. It should be noted, that psilocin is sparingly stable in methanol, especially when exposed to light and air.

A representative sample of finely powdered psilocybin as determined by the sampling procedure described under Chapter III. A. is dissolved (if necessary, by shaking or sonicating) in an appropriate amount of methanol to obtain a solution of about 1 mg/ml.

3. Psilocybin tablets and capsules

A representative sample of finely powdered tablets or the content of the representative number of capsules as determined by the sampling procedure described under Chapter III. A. is extracted with methanol (by shaking or sonicating) to obtain, after filtration, a solution of about 1 mg/ml.

C. Physical examination

1. Macroscopic characteristics (see Figures 2-4)

Agarics traditionally have been identified after a careful recording of only field characters such as colour and texture, a method, however, which relies heavily not only on the availability of fresh material but also the presence of the entire fruit-body. In addition, many of the characters used are very subjective and variable. Therefore the identification of Psilocybe mushrooms basing on a macroscopic examination needs some experience and should only be performed by trained mycologists.

Psilocybe semilanceata **mushrooms have a 40-70 x 2-3 mm, whitish to yellowish stipe (stem), sometimes staining blue or greenish blue at the base. The pileus (cap) has a size of (0.5-) 10-20 (-40) mm (diameter) x 0.5-25 mm (height), is first conic to obtusely conic or sharply conic, bell-shaped pointed ("liberty helmet shape"), long and slender, striat when moist, opaque when faded, reddish brown at first to greyish brown, fading to pale ochraceous or yellowish brown to clear colour. The gills (lamellae) are adnate or adnexed, pale greyish violaceous to dark violaceous brown. The dark brown spores have a size of 12-14 x 6-8 x 5 um, are subellipsoid or ellipsoid, and show a broad, up to 1.5 um wide germ pore.**

Psilocybe cubensis **(Stropharia cubensis) mushrooms have a 40-70 (-150) x 4-20 mm, hollow, usually thickened near base, stiff, often bent, white, becoming yellowish or ashy red stipe (stem). The pileus (cap) is 25-70 mm in diameter, conic to convex, becoming campanulate to gradually expanded-umbonate, surface fulvous when young, changing to ochraceous and cremous or whitish in older fungi, frequently bluing in age or when injured. The gills (lamellae) are adnate or adnexed, at first dark greyish, becoming deep violet grey or dark purplish brown. The dark brownish violet or blackish violet, ellipsoid, smooth spores have a size of 11-13 x 7-9 x 6.5-7 um.**

Psilocybe mexicana **mushrooms have a 20-60 (-80) x 1-2 mm, hollow, ochreous to lightly yellowish pink stipe (stem). The pileus (cap) is 10-15 mm in diameter, conic to campanulate or subumbonate, brownish or deep ochraceous to fulvous or yellowish grey. The gills (lamellae) are sinuate, adnate or adnexed, pale grey to dark violet brown. The dark violaceous brown or blackish purple brown, ellipsoid or subellipsoid spores have a size of 9-10 x 6.5-7.5 x 6-7 um.**

2. Microscopic characteristics (see Figures 2-4)

The microscopic examination of fragments or powders of Psilocybe mushroom specimens should only be performed by trained mycologists. The characters used are the spores (size and colour), 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.

For a more detailed morphological and microscopic description of Psilocybe species, the reader is referred to the following references:

References:

1. *Schultes, R. E. and Hofmann, A.: "The Botany and Chemistry of Hallucinogens, 2nd edition, C. C. Thomas, Springfield, IL, U.S.A., (1980).*

2. *Watling, R., J. Forens. Sci. Soc. 23 (1983) 53.*

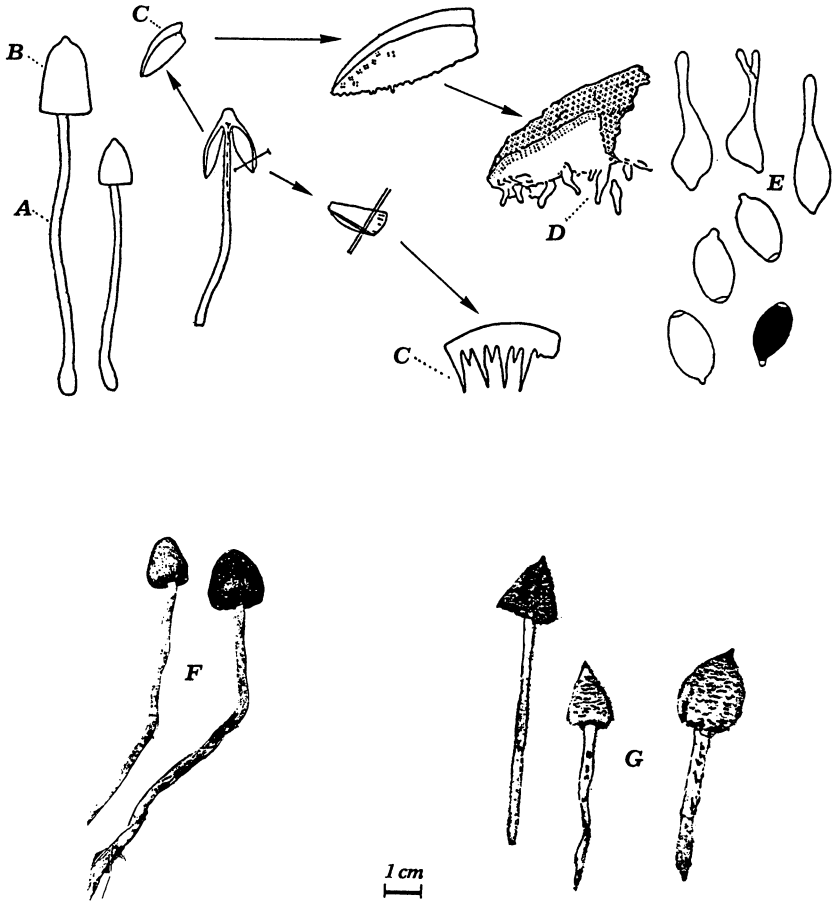


Figure 2 *Psilocybe semilanceata (FR.) QUEL.*

A *Stipe (stem)*
B *Pileus (cap)*
C *Gills (lamellae)*
D *Cystidia*
E *Spores*
F *Fruit-bodies, fresh*
G *Fruit-bodies, dried*

Figure 3 *Psilocybe cubensis (EARLE) SINGER*

A *Fruit-body, dried (cut)*
B *Fruit-body, dried, young mushroom*
C *Fruit-body, dried, old mushroom*
D *Spores*
E *Fruit-bodies, fresh*

A B

Figure 4 *Psilocybe mexicana HEIM*

A *Fruit-bodies*
B *Cultivated mushrooms*

D. Presumptive tests

1. Colour tests

It must be stressed that positive results to colour tests are only presumptive indications of the possible presence of psilocybin and psilocin. Many other C-2 unsubstituted indoles, for example DMT and LSD as well as those which are harmless and uncontrolled by national legislation or international treaties, may give similar colours with the test reagent. It is mandatory for analysts to confirm such results by the use of alternative techniques.

a) **Ehrlich Reagent**

Dissolve 1 g of p-dimethylaminobenzaldehyde in 10 ml methanol, then add 10 ml concentrated ortho-phosphoric acid.

Method:

Place a small amount of the suspected material (from powders, tablets and capsules) on a spot plate. Add two drops of Ehrlich reagent. After a few minutes a violet to grey violet colour indicates the possible presence of psilocybin or psilocin. The detection limit of this method is about 1 ug.

b) **Ehrlich Reagent - modified for mushroom specimens**

Since the positive colour reaction for psilocybin in mushrooms using Ehrlich reagent can be obscured by the colour contributed by vegetable matter, the following modification of the usual procedure is both simple and effective:

Method:

Extract one Psilocybe mushroom specimen as described under Chapter III. B. Transfer two drops of the methanolic extract to a spotting plate. Evaporate to dryness by placing the spotting plate on a flat heating surface. Redissolve the residue by adding two drops of Ehrlich reagent. A violet to grey violet colour appearing after a few minutes indicates the possible presence of a psilocybin-containing mushroom.

c) **Marquis' Reagent**

A_1 : Add 8-10 drops of 40% formaldehyde solution to 10 ml glacial acid.
A_2 : Concentrated sulfuric acid.

Method:

Place a small amount of the suspected material (from powders, tablets and capsules) on a spotting plate. Add one drop of reagent A_1 and two drops of reagent A_2. An orange colour indicates the possible presence of psilocybin, a green-brown colour the presence of psilocin. The detection limit of this method is about 10 ug.

Because the positive colour reaction for psilocybin in Psilocybe mushroom specimens using Marquis' reagent is obscured by vegetable matter and because many other natural products develop a yellow colour with concentrated sulfuric acid, this colour test is not recommended for the mushroom material.

E. Thin-layer chromatography

Plates:

Activated silica gel G on glass backed plates; the coating (0.25 mm thickness) contains a fluorescing additive which fluoresces at 254 nm.

Developing solvents:

System A:	**n-Butanol**	**20**
	Acetic acid	**10**
	Water	**10**
System B :	**Methanol**	**100**
	Concentrated ammonia solution	**1.5**

Preparation of the solutions to be applied to the TLC plate :

a) **Psilocybe mushroom:**

About 20 mg of a representative sample of the suspected dried and pulverized mushroom material is extracted with 2 ml of methanol using the method outlined in Chapter III. B. The filtered extract is concentrated to about 0.5 ml under a stream of nitrogen and again filtered if necessary, and 2 ul of this extract are spotted onto the TLC plate.

b) **Psilocybin powder:**

Prepare a solution at a concentration of approximately 1 mg/ml methanol as outlined in Chapter III. B. and spot 2 ul of this solution onto the TLC plate.

c) **Psilocybin tablets and capsules:**

Extract the material using the method outlined in Chapter III. B. and spot 2 ul of this extract onto the TLC plate.

d) **Standard solution:**

Prepare a solution of 1 mg/ml psilocybin and psilocin in methanol and spot 2 ul of this standard solution onto the TLC plate. It should be noted that psilocin is sparingly stable in methanol, especially when exposed to light and air.

Visualization:

The plates must be dried prior to visualization. This can be done by air-drying at room temperature or by short use of a hot air blower.

Visualization methods:

1. **UV light at 254 and 365 nm.**
2. **Ehrlich reagent.**
3. **para-Dimethylaminocinnamaldehyde.**

Spray reagents:

(1) **Ehrlich reagent: Prepare a solution as described in Chapter III. C.**

(2) **p-Dimethylaminocinnamaldehyde: Dissolve 0.5 g of para-dimethylaminocinnamaldehyde in 50 ml methanol, then add 10 ml concentrated hydrochloric acid.**

Method:

Observe the plate under UV light at 254 and 365 nm. Up to seven dark blue spots appear at 254 nm on the fluorescent background, two of these spots are fluorescent at 365 nm. Then spray with reagent (1) or (2). With spray reagents (1) and (2), psilocybin and baeocystin give grey-violet to violet colours while psilocin gives a blue colour. Reagent (2) is more sensitive than (1) and yields better colour stability. The detection limit for (2) is 20 ng for psilocybin and 10 ng for psilocin.

RESULTS

R_f x 100 values:

Compound	Developing system A	B
Psilocybin	34	5
Baeocystin	45	5
Psilocin	59	39

F. Gas liquid chromatography

1. Packed column technique

(A) Method 1: without derivatization

It should be noted that direct injection of Psilocybe mushroom extracts or psilocybin sample solutions on packed columns will convert psilocybin to psilocin by thermal dephosphorylation and only psilocin will be detectable. Thus prior derivatization is necessary if psilocybin is to be detected.

Operating conditions:

Detector	:	**FID**
Column	:	**6 ft (or 2 m), 4 mm ID glass**
Packing		**2.5% SE-30 on 80-100 mesh Chromosorb G**
Carrier gas	:	**Nitrogen at 45 ml/min**
Column temperature	:	**200°C**
Internal standard	:	**n-alkanes.**

METHOD

Preparation of internal standard solution :

Dissolve an appropriate n-alkane in methanol to give a concentration of 1mg/ml.

Preparation of the standard solution:

To an accurately weighed amount of the psilocin standard, add internal standard solution to give a concentration of 1 mg/ml.

Preparation of sample solution:

An accurately weighed amount (about 20 mg) of the representative sample of dried and pulverized mushroom material is extracted with 2 ml of internal standard solution by shaking or sonicating for 15 min. The filtered extract is then concentrated to about 0.5 ml under a stream of nitrogen and again filtered (if necessary). Prepare the solutions of a representative sample of psilocybin powder, tablets and capsules as outlined under Chapter III. B.

Inject 1 ul of each solution into the gas chromatograph. The content (%) of psilocin can be calculated using the general formula:

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

where:

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

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

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

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

$A_{r.std.}$ = peak area for standard obtained during the standard chromatography.

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

The amount of psilocin in Psilocybe mushroom specimens should be expressed as the percent psilocin base calculated per dry or fresh weight.

RESULT

Psilocin has a retention index RI of 1980.

(B) Method 2: with derivatization

Operating conditions:

Detector	:	**FID**
Column	:	**1.6 m, 2.8 mm ID glass**
Packing		**1.5% SE-30 on 100-120 mesh Chromosorb W**
Carrier gas	:	**Helium, 50 ml/min**
Column temperature	:	**150-250°C, 7.5°/min.**
Injector/detector	:	**220/250°C**
Internal standard	:	**n-alkanes.**
Derivatizing reagent	:	**bis(trimethylsilyl)trifluoroacetamid (BSTFA) and trimethylchlorosilane (TMCS).**

METHOD

The preparation of mushroom sample is done as described above but with the following modification:

To eliminate sugars which may interfere with the derivatization, 1 ml of acetone is added to the methanolic solutions. Let the mixture stand for about 30 min at room temperature or for 10 min in a freezer and centrifuge or filter the precipitate. The solution is evaporated to about 0.5 ml under a stream of nitrogen.

For TMS derivatization, the solution is then transferred to a 1-ml derivatization vial and dried completely under a stream of nitrogen. The vial is sealed and 15 ul of dry pyridine, 15 ul of TMCS and 100 ul of BSTFA are added. The vial is then heated at 100°C for 30 min. It is important that the reaction vial is agitated periodically for the first 10 min of heating to assist dissolution.

The preparation of the solutions of internal standard, psilocybin and psilocin standard, and samples of psilocybin powder, tablets and capsules is done as described under Chapter III. F. 1. a. The TMS derivatization of the standard and sample solutions is done as described above for mushroom samples.

Inject 1-2 ul of each solution into the gas chromatograph.

RESULTS

Psilocybin tri-TMS and psilocin di-TMS have retention times of 13.1 and 8.5 min, respectively.

2. Capillary column technique

(A) Method 1: without derivatization

As mentionned earlier, direct injection of Psilocybe mushroom extracts or psilocybin sample solutions on capillary columns will convert psilocybin to psilocin by thermal dephosphorylation and only psilocin will be detectable. Thus prior derivatization is necessary if psilocybin is to be detected.

Operating conditions:

Detector	:	**FID**
Column	:	**Fused silica, chemically bonded and cross-linked methyl phenylsilicone, such as DB-5 or equivalent; 20 m, 0.174 mm ID**
Film thickness	:	**0.40 um**
Carrier gas	:	**Helium**
Split ratio	:	**Splitless**
Column temperature	:	**150°C (1.5 min) to 280°C, 10°C/min**
Injector/detector temp.	:	**250/310°C**
Internal standard	:	**n-alkanes.**

METHOD

Prepare the solutions of internal standard, psilocin standard and samples as described under Chapter III. F. 1. a.

Inject 1 ul of each solution into the gas chromatograph.

RESULTS

Psilocin has a retention time of 11.2 min.

(B) Method 2: with derivatization

Operating conditions: **as described under Chapter III. F. 2. A.**

METHOD

Prepare the solutions of internal standard, standards and samples as well as the TMS derivatives as described under Chapter III. F. 1. B.

Inject 1-2 ul of each solution into the gas chromatograph.

RESULTS

Compound	Retention time (min)
Psilocin (underivatized)	**11.2**
Psilocin di-TMS	**11.9**
Psilocybin tri-TMS	**15.3**
Baeocystin tri-TMS	**15.7**

For alternative GC and derivatization techniques see:

References:

1. *"Clarke's Isolation and Identification of Drugs", 2nd edition, The Pharmaceutical Press (London) 1986.*

2. *J. Pharm. Sci. 66 (1977) 743.*

3. *Microgram 17 (1984) 28.*

G. High performance liquid chromatography

1. Normal phase

Operating conditions:

Column	:	**250 mm by 4.6 mm ID**	
Packing material	:	**Partisil-5, 5 um particle size**	
Mobile phase	:	**Methanol**	**220**
		Water	**70**
		1 M Ammonium nitrate, buffered to pH 9.6 with 2 M ammonia	**10**

containing the disodium salt of EDTA (1 mM).

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 (detection limit for psilocybin and psilocin: 10 and 7.5 ng.**
		(b) Fluorimetric detection with excitation at 267 nm and 320-nm emission filter (detection limit for psilocybin and psilocin: 5 and 20 ng).
		(c) Electrochemical detection at a potential of + 0.65 V versus Ag-AgCl reference electrode (detection limit for psilocybin and psilocin: 5 ng and 75 pg).
Injection volume	:	**10 ul by syringe or loop.**
Quantitation	:	**by peak height.**

Preparation of solutions of samples and standards for chromatography:

The solutions are prepared as described under Chapter III. F. 1. A., but without using the internal standard.

RESULTS

The capacity ratios (k' values) are as follows*:

Compound	**k' values**
Baeocystin	**1.93**
Psilocybin	**2.43**
Psilocin	**3.10**

(* These values will vary depending upon laboratory conditions and instrumental parameters).

Reference:

1. J. Chromatogr. 270 (1983) 293.

2. Reverse phase (gradient technique)

Operating conditions:

Column	:	**250 mm by 4.6 mm ID**
Packing material	:	**Octadecyl-silica (Spherisorb ODS-1 or equivalent), 5 um particle size.**
Mobile phase	:	**(A) Water, containing 0.3 M ammonium acetate and buffered to pH 8 with ammonia** **(B) Methanol, containing 0.3 M 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	:	**2.0 ml/min.**
Detection	:	**UV at 269 nm (detection limit for psilocybin and psilocin: 10 ng).**
Injection volume	:	**10 ul by syringe or loop.**
Quantitation	:	**by peak area, IS method.**

Preparation of solutions of samples and standards for chromatography:

Prepare the solutions as described under Chapter III. F. 1. A., but with adding bufotenin as internal standard at a concentration of 80 ug/ml methanol.

RESULTS

The capacity ratios (k' values) are as follows*:

Compound	**k' values**
Baeocystin	**1.53**
Psilocybin	**3.20**
Bufotenin (IS)	**6.27**
Psilocin	**6.80**

(* These values will vary depending upon laboratory conditions and instrumental parameters).

Reference:

1. J. Chromatogr. 408 (1987) 402.

H. Spectroscopic techniques

In some countries, confirmation of identity by spectroscopic means is required. Theoretically each substance has an unique infrared and mass spectrum and these methods would permit the unequivocable identification of isolated or synthesized psilocybin or psilocin. Depending upon the purity of psilocybin and psilocin, an extraction step as described under Chapter III. B. may be required prior to spectroscopic analysis.

The following sections provide references dealing with spectroscopic techniques for those laboratories which require such confirmation.

1. Ultraviolet spectroscopy

Because other (dimethyl) tryptamine derivatives yield similar results, this method is not specific for the analysis of psilocybin and psilocin and therefore is not recommended.

2. Infrared spectroscopy

For description of the standard methods (halide disk, microhalide, nujol mull and thin-film techniques), see previous manuals in the series.

Major peaks in the IR spectrum (KBr) of psilocybin occur at the following wavenumbers (cm^{-1}): 1620, 1585, 1505, 1360, 1065.

Major peaks in the IR spectrum (KBr) of psilocin occur at the following wavenumbers (cm^{-1}): 1620, 1585, 1261, 1236, 1061, 1042.

3. Mass spectroscopy

Psilocin (underivatized)	:	m/z 204 (molecular ion), 146, 130, 77, 58 (base peak), 42.
Psilocin di-TMS	:	m/z 348 (molecular ion), 290, 73, 58 (base peak).
Psilocybin tri-TMS	:	m/z 500 (molecular ion), 485, 455, 442, 73, 58 (base peak).

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