Detection of Cannabis in samples of different sources

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Cannabis OR Hashish :

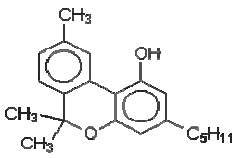
Cannabis or hemp is an annual plant . Known by the name : *cannabis sativa* (F. Cannabinaceae) .

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

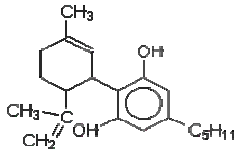
CHEMICAL COMPOSITION :

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

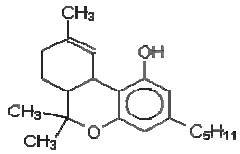
CBN is not present in the fresh plant and it is a measure of aging : as the sample ages , THC content declines and decompose to CBN , which means that fresh plant contains mainly $\triangle^9$ THC .



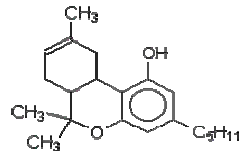
CANNABINOL (CBN)



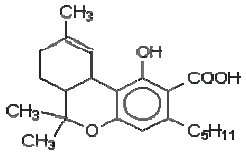
CANNABIDIOL (CBD)



Δ^9-TETRAHYDROCANNABINOL (Δ^9-THC)
(The primary psychoactive molecule)



Δ^8-TETRAHYDROCANNABINOL (Δ^8-THC)



Δ^9-TETRAHYDROCANNABINOLIC ACID

PHARMACOKINATIC:

D9-tetrahydrocannabinol (THC) accumulates in body fat because of its high lipid solubility. The amount stored in body fat is a function of the amount, frequency and potency of cannabis used. THC is slowly released from body stores over time and metabolised by the liver to produce a variety of inactive products (metabolites). The main metabolite is 11-nor-D9-carboxy THC (THC-COOH) which is conjugated with glucuronic acid and excreted in urine.

Screening for cannabis metabolites in urine (cannabinoids) is carried out by a specific immunoassay procedure. Such assays detect the presence of several cannabinoid substances in urine. Different assay cut-offs may be employed; most commonly 50 µg/L. The important aspects of these assays are listed in the following table:-

Assay cut-off (µg/L)	Likely cannabinoid detection time in days		Likely frequency of GC/MS confirmation of positive results	Situation where assay is most applicable
	Single	Chronic		
100	Up to 1	3 - 7	Greater than 99%	Clinical assessment
50	1 - 2	7 - 14	Greater than 95%	Assessment of Chronic and recent single abuse
15	2 - 4	7 - 28	> 50%	Employment and pre-employment screening

In exceptional cases confirmation of positive results can be carried out by the specific analysis (gas chromatography-mass spectrometry) of the major metabolite of cannabis in urine (THC-COOH). 'Positive' results will be reported for those specimens with a THC-COOH concentration > 15 µg/L. The current Laboratory policy is to only report those samples as being positive for cannabis if above the immunoassay 50 µg/L cut-off.

LAB WORK :

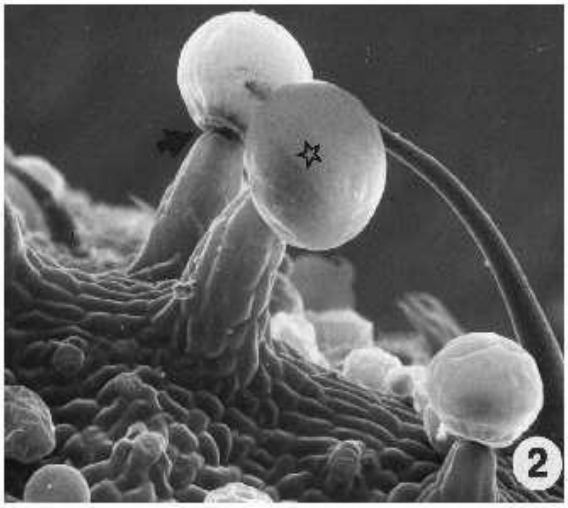
Screening of the plant including : Microscopically , chemical and chromatographic identification .

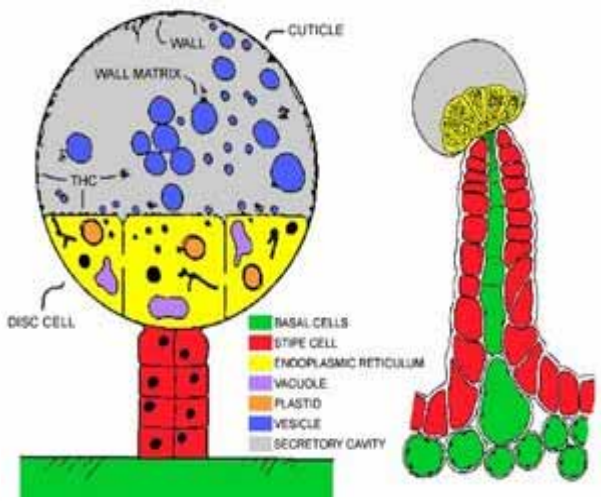
❖ Microscopy :

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

Cluster crystals of calcium oxalate are abundant.

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





❖ Chemical Identification :

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

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

a. Furfural test :

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

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

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

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

◎ *Duquenois – Levine* test:

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

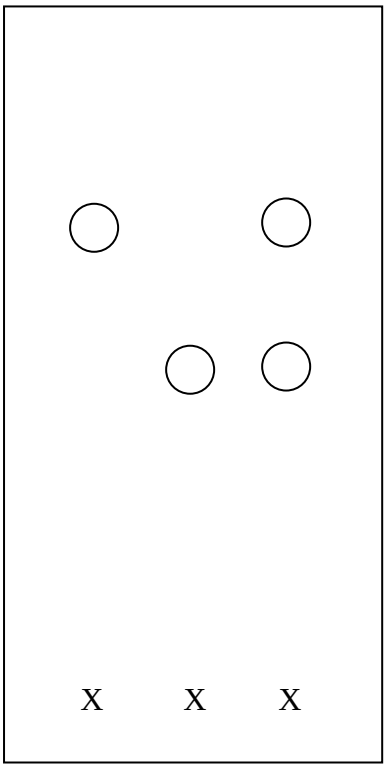
Cannabinoids rapidly form a blue/purple complex into the chloroform layer

❖ Chromatographic method (TLC)

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

Condition :

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



Tetrahydrocannabinonol
[THC (orange)]

Cannabinol
[CBN (Violet)]

What is an additional value of TLC in cannabis analysis ?

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

Cannabinoids

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

The Basics

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

Sample

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

Method and Instrument

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

Stability

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

Purpose

To detect recent usage.

Normal Results

Negative.

Abnormal Results

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

Interferences

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