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Recommended methods **for the identification and analysis of cannabis and cannabis products**

MANUAL FOR USE BY NATIONAL DRUG ANALYSIS LABORATORIES

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Laboratory and Scientific Section
UNITED NATIONS OFFICE ON DRUGS AND CRIME
Vienna

Recommended Methods for the Identification and Analysis of Cannabis and Cannabis Products

(Revised and updated)

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UNITED NATIONS
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Note

Operating and experimental conditions are reproduced from the original reference materials, including unpublished methods, validated and used in selected national laboratories as per the list of references. A number of alternative conditions and substitution of named commercial products may provide comparable results in many cases, but any modification has to be validated before it is integrated into laboratory routines.

Mention of names of firms and commercial products does not imply the endorsement of the United Nations.

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Contents

1. Introduction

1.1 Background

Cannabis products are the most widely trafficked drugs worldwide, accounting for 65 per cent of all global seizure cases (1.65 million cases) in 2006. 5,200 metric tons of herb and 1,000 metric tons of resin were seized in 2006. Practically all countries in the world are affected by cannabis trafficking. Similarly, cannabis also remains the most widely used drug worldwide, with an estimated 166 million people having used cannabis in 2006, equivalent to some 4 per cent of the global population aged 15-64.

At the same time, especially since the end of the last century, production methods have become increasingly sophisticated, resulting in the availability in illicit markets of a wide range of cannabis products with widely varying levels of the main psychoactive ingredient, delta-9-tetrahydrocannabinol (THC). Most recently, there has also been a renewed debate about increasing THC content (frequently referred to as "potency") in illicit cannabis products.

All of this requires analytical data which are comparable between laboratories and over time. However, most countries do not require by law the detailed analysis of the THC content of the different products, and where such analyses are carried out, they use a variety of approaches and experimental designs, reducing the comparability of results. For example, the conversion of natural constituents, such as tetrahydrocannabinolic acid (THCA), by both smoking and under certain analytical conditions into THC, and how this should be reflected in the analytical report, are issues which are not yet standardized worldwide. On the technological side, the analysis of cannabis products is further complicated by the relatively restricted availability of pure or well defined reference material of THC and other cannabinoids.*

The present manual is an updated and significantly revised version of the manual on "Recommended methods for testing cannabis" (ST/NAR/8), which was published in 1987. It has been prepared taking into account both developments in analytical technology and advances in the science of cannabis, and with a view to providing the analytical basis for an objective discussion about changes in THC content over time, and differences between regions and products.

*In this connection, it is important to be aware that THC was fully characterized only in the mid-1960s, and was only available as pure reference standard from the late 1960s. Results obtained before that time therefore should not be compared with today's results, and must be considered approximate.

1.2 Purpose and use of the manual

The present manual is one in a series of similar publications dealing with the identification and analysis of various types of drugs under international control. These manuals are the outcome of a programme pursued by UNODC since the early 1980s, aimed at the harmonization and establishment of recommended methods of analysis for national drug analysis laboratories.

In line with the overall objective of the series, the present manual suggests approaches that may assist drug analysts in the selection of methods appropriate to the sample under examination and provide data suitable for the purpose at hand, leaving room also for adaptation to the level of sophistication of different laboratories and the various legal needs. The majority of methods included in the present manual are validated methods, which have been used for a number of years in reputable laboratories and as part of inter-laboratory studies, collaborative exercises and proficiency tests. The reader should be aware, however, that there are a number of other methods, including those published in the forensic science literature, which may also produce acceptable results. **Any new method that is about to be used in the reader's laboratory must be validated and/or verified prior to routine use.**

In addition, there are a number of more sophisticated approaches, but they may not be necessary for routine operational applications. Therefore, the methods described here should be understood as guidance, that is, minor modifications to suit local circumstances should not normally change the validity of the results. The choice of the methodology and approach to analysis as well as the decision whether or not additional methods are required remain with the analyst and may also be dependent on the availability of appropriate instrumentation and the level of legally acceptable proof in the jurisdiction within which the analyst works.

Attention is also drawn to the vital importance of the availability to drug analysts of reference materials and books on drugs of abuse and analytical techniques. Moreover, the analyst must of necessity keep abreast of current trends in drug analysis, consistently following current analytical and forensic science literature.

UNODC's Laboratory and Scientific Section would welcome observations on the contents and usefulness of the present manual. Comments and suggestions may be addressed to:

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All manuals, as well as guidelines and other scientific-technical publications may be requested by contacting the address above.

2. Illicit production of cannabis products

2.1 Cannabis market

Cannabis products are by far the most abused drugs on the illicit drug market. Cannabis can be grown in virtually any country, and is increasingly cultivated indoors in technically advanced countries.

Production of herbal cannabis (marihuana) is widely dispersed, existing in almost every country in the world. Cannabis resin (hashish) is produced in about 65 countries, with main sources being North Africa and countries in South-West Asia, particularly Afghanistan and Pakistan.

Africa is home to the world's leading producer of (outdoor) cannabis resin—Morocco, the site of the largest known cannabis cultivation area. Most of the cannabis resin seized in Europe continues to be trafficked from Morocco. The resin from that country shares characteristics with resin from other, southern and eastern Mediterranean countries (see section 3.13.2.1).

Afghanistan is the world's second largest producer of resin from cannabis, grown alongside opium poppy fields. Resin from that country shares characteristics with resin from other parts of the Indian subcontinent (see section 3.13.2.2). Lebanon was once one of the world's leading resin suppliers and might still be if it were not for continued eradication efforts.

With regard to herbal cannabis, the American continent accounted for some 55 per cent of global production in 2006, followed by Africa (about 22 per cent). Most herbal cannabis is produced for domestic markets and for export to neighbouring countries, i.e. international trafficking in herbal cannabis is rather limited.

Since the 1970s, cannabis growers in North America and Europe have been working to create more potent cannabis, and the market for high-potency, indoor-produced sinsemilla (see section 3.6.1) is growing in many key consumption countries. Sinsemilla potency has increased dramatically in the last decade in the United States, Canada, and The Netherlands—the three countries at the vanguard of cannabis breeding and production technology—and there are indications that its market share is growing in many other countries.

However, there is no evidence that the effective potency* of cannabis on the European market has increased significantly. This is because in most European countries imported cannabis (herbal and resin) continues to dominate the market and the potency of these imported products has remained stable over many years at around 6-8 per cent. The increase in cannabis potency observed in some countries since the end of the 1990s is the result of the increased availability of home-produced herbal cannabis, which is cultivated using high THC breeds and intensive hydroponic techniques. Indoor cultivation of herbal cannabis now occurs in most, if not all, European countries. However, despite this trend towards home-grown (indoor) cultivation in Europe, import of cannabis products from outdoor cultivation, mainly cannabis resin, is still observed, especially in Central Europe [1, 2].

Limited time-series data on cannabis potency suggest that the mean Δ^9-THC concentration in home-produced herbal cannabis seizures increased from around 1.5 per cent in the 1980s to around 4 per cent in the late 1990s and around 10 per cent in the last five years [3, 4]. Recent reports from some European countries suggest mean THC concentrations (potency) of up to 15-20 per cent in certain herbal materials, but there is significant variation between samples even within a given year [5, 6, 7, 8].

Although the high-potency indoor herbal cannabis has a higher THC content than the cannabis resin from Morocco, the latter is still being sold in Europe and considered by experienced cannabis users to produce a good high.

A more detailed and up-to-date overview of the worldwide production, trafficking and use of cannabis can be found in the annual World Drug Reports published by the United Nations Office on Drugs and Crime [9].

*The term "effective potency" refers to the weighted mean potency of all cannabis products, taking into account their relative availability.

3. Description of the cannabis plant and illicit cannabis products

3.1 Name

Cannabis sativa L. (Linnaeus)

3.2 Synonyms

There are many local and street names and synonyms used for cannabis and it is beyond the scope of this manual to list them all. They include: hemp, marihuana, marijuana, pot, gandia, grass, chanvre and many more [10].

3.3 Taxonomy

The genera *Cannabis* and *Humulus* (hops) belong to the same family (*Cannabaceae*, sometimes known as *Cannabinaceae*). Generally, cannabis is considered to be monospecific (*Cannabis sativa* L.) which is divided into several subspecies (*C. sativa* subsp. *sativa*, *C. sativa* subsp. *indica*, *C. sativa* subsp. *ruderalis*, *C. sativa* subsp. *spontanea*, *C. sativa* subsp. *kafiristanca*) [11]. However, the chemical and morphological distinctions by which cannabis has been split into these subspecies are often not readily discernible, appear to be environmentally modifiable, and vary in a continuous fashion. For most purposes, it will suffice to apply the name *Cannabis sativa* to all cannabis plants encountered [12].

3.4 Physical appearance

Cannabis is an annual, dioecious,* flowering herb. Staminate (male) plants are usually taller but less robust than pistillate (female) plants. Stems are erect and can vary from 0.2-6 m. However, most of the plants reach heights of 1-3 m. The extent of branching, like the plant height, depends on environmental and hereditary factors as well as the method of cultivation (see also section 5.3.1).

*The majority of plants is dioecious (i.e. male and female flowers are found on separate plants), although monoecious plants (i.e. bearing both male and female flowers) may also be encountered.

Figure 1. Morphological aspects of *Cannabis sativa* L. [13]

A Inflorescence of male (staminate) plant
B Fruiting female (pistillate) plant
1 Staminate flower
2 Stamen (anther and short filament)
3 Stamen
4 Pollen grains
5 Pistillate flower with bract
6 Pistillate flower without bract
7 Pistillate flower showing ovary (longitudinal section)
8 Seed (achene*) with bract
9 Seed without bract
10 Seed (side view)
11 Seed (cross section)
12 Seed (longitudinal section)
13 Seed without pericarp (peeled)

*The seed is actually a fruit, or technically, an achene. It contains a single seed with a hard shell.

3.5 Similarities

Several plant species bear morphological characteristics that show more or less resemblance to *Cannabis sativa*. Some of them are illustrated below. However, a closer look at their macroscopic and/or microscopic characteristics makes confusion very unlikely [12]. In addition, there are also simple presumptive tests available to differentiate *Cannabis sativa* from other plant materials (see section 5.4.3).

Figure 2. Some plant species which bear morphological characteristics with some resemblance to *Cannabis sativa* L.

Hibiscus cannabinus

Acer palmatum

(Picture: [14])

Urtica cannabina

(Picture: [15])

Dizygotheca elegantissima

Figure 2. Some plant species which bear morphological characteristics with some resemblance to *Cannabis sativa* L. *(continued)*

Potentilla recta

Datisca cannabina *(Picture: [16])*

Seeds of the common hop (*Humulus lupulus*) and Japanese hop (*Humulus japonicus*) might be confused with the seeds of *Cannabis sativa*. However, the presence of a characteristic reticulate ("tortoise shell") pattern on the surface of cannabis seeds enables them to be identified readily.

Figure 3. Seeds which bear morphological characteristics with some resemblance to the seeds of *Cannabis sativa* L.

Cannabis sativa

Humulus lupulus

Humulus japonicus

3.6 Breeding

The plant is best suited to well structured neutral to alkaline clay and loam soils, with good water-holding capacity, which are not subject to water logging.

Among many trials of breeding, crossing *sativa* and *indica* strains led to the development of "skunk", a hybrid said to be 75 per cent *sativa* and 25 per cent *indica*.

This strain is said to be one of the first which combines the high THC content of *C. sativa* subsp. *sativa* with the rapid growth cycle and yield of *C. sativa* subsp. *indica*. In some countries, cannabis with a high THC content is generally referred to as "skunk" today.

3.6.1 Sinsemilla (Spanish for: "no seed")

The term sinsemilla refers to a cultivation technique rather than a genetic strain. Cannabis with the highest level of THC is comprised exclusively of the female flower heads ("buds") that remain unfertilized throughout maturity and which, consequently, contain no seeds. The production of sinsemilla requires identifying the female plants and ensuring that they are not exposed to pollen.

3.6.2 Cloning

The first and most obvious boost to sinsemilla production was the use of clones. Cloning simply means propagating from a successful "mother" plant. This cutting is rooted and transplanted. It is a genetic duplicate of its mother and thus can be used to create even more cuttings. A square metre of mother plants can provide numerous clones a week.

3.6.3 Artificially induced hermaphrodites

Although genetics disposes a plant to become male or female, environmental factors, including the diurnal light cycle, can alter the sex (hermaphrodites). Natural hermaphrodites with both male and female parts are usually sterile, but artificially induced hermaphrodites can have fully functional reproductive organs. "Feminized" seeds sold by many commercial seed suppliers are gained from artificially hermaphroditic females that lack the male chromosome or by treating the seeds with hormones or silver thiosulfate. Thus, production of only pistillate (female) plants can be achieved by seed as well [17,18].

3.6.4 Outdoor production

The main production of cannabis worldwide is still outdoors and these plants are generally but not necessarily grown from seeds.

Outdoor sinsemilla production is realized by identifying and destroying male plants before pollination or by the use of artificially induced hermaphroditic females (see section 3.6.3).

3.6.5 Indoor production

Growing cannabis from seed means that half of the crop might be unwanted male plants. For cost-intensive greenhouse production this is usually avoided, which can be achieved easily by cloning. Cloning and indoor production go hand in hand. Indoor production is mainly encountered in technologically advanced countries, where big basements or closed factories are usually used. One or more rooms in houses or other dwellings are also frequently converted into grow rooms often using hydroponic techniques, i.e. growing plants in nutrient solutions instead of soil.

In soil, the optimum pH for the plant is 6.5 to 7.2. In hydroponic growing, the nutrient solution is best at 5.2 to 5.8, making cannabis well-suited to hydroponics, and thus indoor production, because this pH range is hostile to most bacteria and fungi [19].

An example and overview of trends in illicit cannabis cultivation in the United Kingdom, including relevant legal and forensic implications, can be found in [20].

3.7 Industrial cannabis

Industrial cannabis (industrial hemp) comprises a number of varieties of *Cannabis sativa* L. that are intended for agricultural and industrial purposes. They are grown for their seeds and fibres. Industrial cannabis is characterized by low THC content and high cannabidiol (CBD) content. In most European countries the current upper legal limit for cultivation is 0.2 per cent THC (Canada: 0.3 per cent). The ratio of CBD to THC is greater than one.

In many countries, “lists of approved cultivars” exist. Varieties which are consistently found to exceed the legally acceptable levels for THC may be removed from these lists.

Harvesting for fibres occurs at the end of flowering of the female plants and before seed formation.

3.8 Flowering

Flowering usually starts when darkness exceeds eleven hours per day. The flowering cycle can last anywhere between four and twelve weeks, depending on the strain and environmental conditions. Flowering times given by seed companies usually refer to the time taken to flower when grown from seed. Plants grown from cuttings can take a week or so longer to finish flowering.

3.9 Harvesting

A good sign of ripeness is the colour of the hair-like structures (stigmas). As each flower ripens, these usually shrivel and turn brown. When about 75 per cent of the stigmas are brown, the plants are ready to harvest.

3.10 Yield

Mean and/or minimal yield estimates are of forensic and legal interest. However, yield estimates are difficult, strongly dependent on cultivar/breed, cultivation technique, nutrition, light intensity, duration and rhythm, etc. Studies undertaken in Australia and New Zealand have shown that yields from indoor and outdoor grown plants are so variable that it is not meaningful to apply a set formula for wet : dry : saleable material or grams per plant or square metre.*

Nevertheless, some empirical studies are available and summarized below. Variations due to different cultivation factors as mentioned above have to be considered.

Studies in Germany, The Netherlands and from EUROPOL are reported as follows:

Table I. Indicative minimum and/or average yields for flowering tops per indoor cannabis plant

Minimum yield (g/plant)	*Average yield (g/plant)*	*Reference*
	22	21
25	40	22
	33.7	24
28		25

Table II. Indicative yields of dried herbal cannabis per unit cultivation area

Outdoor cultivation (g/m2)	*Indoor cultivation (g/m2)*	*Reference*
75		23
	505	24
	400	25

Reference 23 also suggests that about 100kg of herbal cannabis ("kif") are required to obtain 1-3 kg of resin.

* Unpublished data.

3.11 Δ^9-THC distribution in cannabis plants and products [26]

The THC content* varies depending on the plant part:

10-12 per cent	in pistillate flowers
1-2 per cent	in leaves
0.1-0.3 per cent	in stalks
< 0.03 per cent	in the roots

The THC content of the different cannabis products (herb, resin and oil) is the result of the ratio of the different plant parts used in their production. A study in Switzerland in 2006 showed, for example, that two thirds of seizures of herbal cannabis ranged between 2 per cent and 12 per cent THC. Two thirds of the resin seizures ranged between 4 per cent and 21 per cent, depending on details of the cultivation and production method (see also chapter 3.13.2), while extraction of resin and/or flowering tops can result in cannabis oil with a THC content of up to 60 per cent [27].

For more information on THC contents of cannabis products seized worldwide see also UNODC's annual World Drug Reports [9].

3.12 Biosynthesis

It was until recently presumed that tetrahydrocannabinolic acid (THCA, the precursor of THC) is formed by cyclization of cannabidiolic acid (CBDA). Newer studies give evidence that it is actually formed from cannabigerolic acid (CBGA) through oxidocyclization by the enzyme THCA-synthase [28, 29, 30, 31].

CBGA is the precursor for THCA as well as for CBDA and for cannabichromenic acid (CBCA). The corresponding THC, CBD and cannabichromene (CBC) are generated by decarboxylation.

Cannabinol (CBN) is a degradation product of THC, i.e. it does not occur naturally, but is an artefact. (see also chapter 3.14)

3.13 Cannabis products

Cannabis has been used as an agricultural crop for textile fibres for centuries. Other legitimate cannabis products include cannabis seed, cannabis seed oil and the essential oil of cannabis.

*Figures about THC content refer to "total content" (see section 5.4.1).

Illicit cannabis products fall into three main categories: herbal cannabis, cannabis resin and liquid cannabis (cannabis oil). It must be stressed that no two illicit cannabis products have identical physical appearances. Produced from a highly variable natural product using a batch process capable of wide variation, and subsequently subjected to processing and transformation for trafficking purposes, cannabis products appear in illicit markets in a multitude of forms.

3.13.1 Herbal cannabis

It is still the traditional belief that only the fruiting and flowering tops and leaves next to the flowering tops contain significant quantities of the psychoactive constituent (THC); they are known as the "drug-containing parts", and generally it is only these parts of the plant that are sold in the illicit market (B in figure 1, page 8).

Indeed, these parts contain the highest amount of THC. However, illicitly consumed herbal cannabis also includes bigger leaves located at greater distance from the flowering tops.

Also the leaves next to the male flowering tops of potent cannabis plants contain consumable amounts of THC. However, the content is much lower than that for female plants and they are therefore not material of first choice. The central stem and main side stems contain little THC but they may still be used in the production of cannabis oil.

The dried leaves and flowers of the cannabis plant are known as "marihuana", and a plethora of other regional names exist [10]. "Marihuana" is found in the illegal market unchanged, i.e. raw from the plant (also called "dried flower"), processed as compressed slabs or coins, or as ground up material. The presentation of the herbal material in illicit markets varies widely, from region to region as well as within the countries of each region.

High quality product can be made by sieving crushed herbal cannabis to remove those parts of the plant which contain relatively low levels of, or no, cannabinoids. Essentially, this removes seeds and all but the most insignificant stem material. All that passes through the sieving process has been derived from the flowering and fruiting tops' herbal material, therefore a relative enrichment of THC occurs. In the illicit traffic, the product is known as "Kif". It is a characteristic product of North Africa. Such material has high cannabis resin content and can be compressed into slabs, which bear some physical resemblance to cannabis resin slabs (hashish). However, when subjected to microscopic examination, such slabs are found to have retained essential herbal characteristics (see also section 5.3.2), and are considered a sort of "purified marihuana".

A third, and in some western European countries dominant, way of producing high quality herbal cannabis is indoor production. Very potent hybrids such as "skunk",

"white widow", etc. are generally used and cultivation conditions are optimized. Propagation occurs mainly by cloning of the mother plants (see section 3.6.2); seedlings are seldom encountered anymore. Premises used for indoor cultivation include basements, factories, warehouses and unused portions of commercial or industrial facilities. They are often equipped with automated nutrition and water supply, air conditioning, systems to filter and deodorize outlet air and automated illumination to mimic day and night phases. The combination of ideal growing conditions and high THC cultivars generates products with a maximum THC content which is often two to ten times higher than that observed in the late eighties. Herbal cannabis with a total THC content of more than 10 per cent, cannabis resin with 25 per cent THC and cannabis oil with 60 per cent THC are not unusual today.

The drying process is simple. Either the drug-containing parts are cut off or the entire plant is suspended upside down and air-dried. Drying is complete when the leaves next to flowering tops are brittle. Depending on the humidity and ambient temperature, this takes approximately 24 to 72 hours. The residual water-content in this material is about 8-13 per cent. This material is directly suitable for smoking in a joint and can be stored for many months, although THC degrades with time, when exposed to air, light and humidity.

3.13.2 Cannabis resin (hashish)

The resinous secretions of the plant, produced in the glandular trichomes (see section 5.3.2) can be collected, thus obtaining a higher THC-containing product from which most recognizable plant material is removed. In addition to the secretions, it consists of finer plant material and appears as loose or pressed sticky powder, depending on the method of production.

Worldwide, the production of cannabis resin is centred in two main regions. The countries around the southern and the eastern part of the Mediterranean form one region, and the countries in South and South-West Asia form another. A variety of processes have been used in both regions to produce cannabis resin. However, in general, the countries of one region use similar techniques. Sieving is an important part of the process in both regions.

3.13.2.1 Cannabis resin from Mediterranean countries

In this region, the dried herbal material is typically threshed. Threshing, which is often carried out against a wall, is done to separate the resin-producing parts of the plant. Particles of cannabis resin and fragments of cannabis leaves, as well as cannabis seeds, become detached from the more fibrous parts of the plant. The latter are discarded. The material is then sieved to remove seeds and major fibrous parts. The resulting product is now enriched in resin content and therefore in THC. At this stage, macroscopic botanical characteristics are virtually absent, but microscopically the material still exhibits many botanical traits. Physically, it resembles a fine

sticky powder and, at this stage, it is usually compressed into slabs. Sometimes a logo, which can be used for characterization and comparison, is stamped into the slabs. In some countries (eastern Mediterranean) the material is placed in cloth bags prior to compression, while in other locations (North Africa) cellulose wrapping is added before compression. In the north-eastern Mediterranean and Central Europe, the fine sticky powder is occasionally trafficked without having been compressed into slabs.

3.13.2.2 Cannabis resin from South and South-West Asia

A different approach to the production of cannabis resin is known to be used in South and South-West Asian countries. The fruiting and flowering tops of the cannabis plants grown there contain high levels of resin to an extent that makes these parts of the plant very sticky to the touch. When the fruiting and flowering tops of a fresh plant are rubbed between the palms of the hand, the resin is transferred from the plant to the palm. An alternative approach is to rub the sticky parts against rubber sheeting or to walk through a field of cannabis plants wearing rubber sheeting or leather. Resin accumulates on the surface as it brushes against the fruiting and flowering tops of the plant; when sufficient material has been collected, the sheeting or leather may be scraped clean, and the material is compressed into slabs. The above described technique can be applied to the uncut plants in the fields. Alternatively, the flowering and fruiting tops may be collected in a similar way to that used in herbal cannabis production, allowed to dry, and then be broken and crushed between the hands into a coarse powder. This powder is then passed through sieves so that it attains fineness similar to that obtained in the Mediterranean region. The fine powder, which is still green, is stored in leather bags for four to five months. The powder is then exposed to the sun for a short time—sufficient for the resin to melt. It is put back into the leather bags for a few days, after which it is removed and kneaded well with wooden rods so that a certain amount of oily material appears on its surface. Kneading is continued until a material suitable for pressing into slabs has been produced.

A fundamentally different method, also known to have been used in some South and South-West Asian localities, involves immersion of the plant material, apart from the main stems, in boiling water. This removes the resin from the fruiting and flowering tops. The plant material which has been extracted is discarded and when the extracting liquid cools, a layer of solidified resin forms on its surface. The resin is removed and formed into slabs or whatever shape is favoured. The problem with this method is that water is introduced into the resin. This results in the slabs of resin frequently turning mouldy as they age. By quantity, little cannabis resin is made in this more elaborate way.

3.13.2.3 Cannabis resin from "pollinators"/"ice-o-lators"

Together with the indoor cultivation, an efficient method of separating the resin has been developed. A device comparable to a tumble-dryer lined with a finely woven

net is placed in a box, lined with plastic. This so-called "pollinator" is partly filled with dried and deep-frozen flowering and fruiting tops of the cannabis plant. Low temperature reduces the stickiness of the resin. During rotation of the pollinator, the THC-bearing parts of the leaves and flowering tops break and pass through the net. They stick to the plastic walls and floor and can be collected as a fine powder. Compared to the starting dried material, an up to 8-fold enrichment in THC can be achieved with this procedure.

Figure 4. "Pollinator" and powdered sticky resin (product) [32]

A similar method is used to produce so called "ice hash", in which the dried plant material is put in a coarse sieve with ice cubes and then agitated using a mechanical paint stirrer. The ice causes the resin balls to freeze and drop off the plant. The process is repeated for a series of progressively more finely-meshed sieves until a powdered product similar to the above is achieved.

3.13.3 Liquid cannabis (hashish oil)

Liquid cannabis is a concentrated liquid extract of either herbal cannabis material or of cannabis resin. The reason for the production of liquid cannabis is to concentrate the psychoactive ingredient, THC. This may help traffickers evade interdiction, because more psychoactive material can be contained in a smaller quantity of product. Of equal value to the trafficker is the ability to insert the liquid cannabis into any cavity and to use concealments which cannot easily accommodate herbal or resin cannabis, thereby reducing the possibility of detection by the form or odour of the material.

Extraction is performed in a suitable vessel with an organic solvent (e.g. petroleum ether, ethanol, methanol, acetone) at room temperature with stirring, by passive extraction or under reflux.

When the batch of cannabis or cannabis resin is thought to be fully extracted, the suspension is filtered and the extracted material is discarded. If necessary, a second fresh batch of cannabis material may be placed into the vessel and extracted with the same batch of solvent used for the initial extraction. This process can be repeated as often as required, using a number of batches of cannabis or cannabis resin with a single batch of extracting solvent. After the final batch has been extracted, the solvent is evaporated to obtain the required consistency of the oil. In some clandestine laboratories, especially in those countries where organic solvents are expensive or difficult to purchase, the excess solvent may be recovered for future use.

In general, liquid cannabis, whether made from cannabis or cannabis resin, is dark brown or dark green in colour and has the consistency of thick oil or a paste.

3.13.4 Cannabis seeds and cannabis seed oil

Cannabis seeds are a less well known though potent source of Ω-3-fatty acids. Cannabis seed oil is a clear yellow liquid. The seed contain approximately 29 per cent to 34 per cent oil by weight [33]. 100 g of cannabis seed oil contains about 19 g α-linolenic acid. A ratio of about 3:1 of Ω-6- to Ω-3-fatty acids makes cannabis seed oil a high quality nutrient. However, due to its high proportion of unsaturated fatty acids, this oil tends to get rancid rapidly if not stored in a cool and dark place.

Although the seed is enclosed by the bracteole, which is the part of the plant with the highest density of glandular trichomes and thus the highest THC concentration, the seeds themselves do not contain THC. However, they may be contaminated with cannabis materials (e.g. flowering tops, husks, resin), resulting in detectable amounts of THC. Similarly, if THC is detected in cannabis seed oil, it most likely originated from a poor separation of the seeds from the bract [34].

3.13.5 Cannabis essential oil

The essential oil of cannabis is a clear and slightly yellow-coloured liquid. It is obtained by steam distillation of the freshly cut cannabis plants. A great demand for this essential oil does not exist and it seems that it is rather a side product from seed oil or hashish-oil production. The essential oil does not contain THC, but is responsible for the characteristic smell of cannabis products, and is also the basis for their identification by sniffer dogs.

3.14 Estimation of the age of cannabis samples

CBN does not exist in freshly and carefully dried marihuana. If it is present, the sample is understood to have started to degrade and should not be used for

comparative purposes. It is feasible to estimate the age of a given marihuana sample on the basis of its THC and CBN content, assuming storage was carried out at room temperature. It is for this reason that analysis for comparative purposes is generally not carried out more than three months after sample seizure [35].

THC appears to degrade at a higher rate for the first year than for subsequent years. One study suggests that samples with a ratio of CBN to THC of less than 0.013 are less than six months old, and those with a ratio between 0.04 and 0.08 are between one and two years old. However variations from experimental conditions should be considered when using this approach to estimate the age of cannabis samples [36].

3.15 Drug-type versus fibre-type cannabis

As has been described in section 3.7, the total THC content is used to define fibre-type cannabis (cf. the current upper legal limit for industrial hemp of 0.2 per cent THC and 0.3 per cent THC, respectively, in Europe and Canada). Another simple way of distinguishing between drug-type and fibre-type cannabis is by using the ratio of the main cannabinoids THC, CBN and CBD [37].

As described above in section 3.12, both CBD and THC, via their acids CBDA and THCA, are derived biosynthetically from CBGA. If the peak area ratio* of [THC+CBN] : [CBD] is <1, then the cannabis plant is considered to be a fibre-type. If the ratio is >1, it is considered a drug-type. Because THC is oxidized partly to CBN after cutting and drying the plant material, the sum of the peak area of THC and CBN is used and divided by the area of CBD.

$$X = \frac{[THC]+[CBN]}{[CBD]}$$

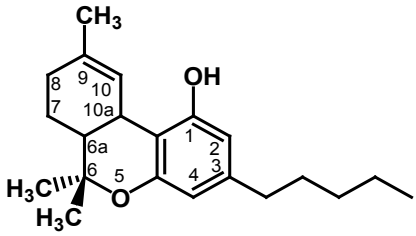
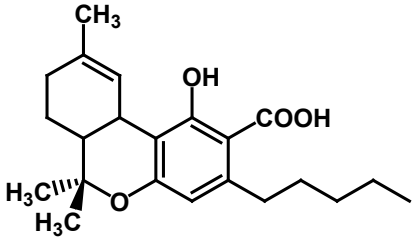
$[THC]$ Area of THC in the chromatogram

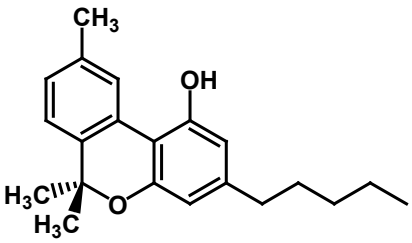
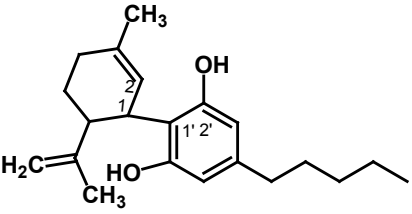
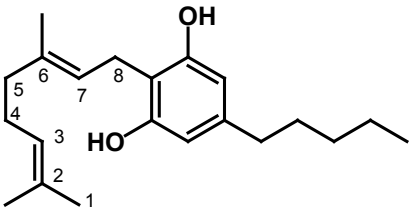
$X > 1$ Drug-type cannabis

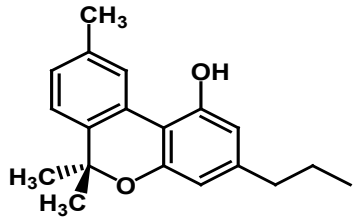
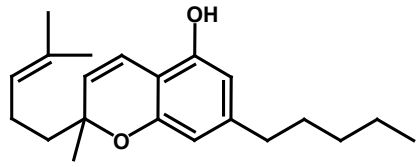
$X < 1$ Fibre-type cannabis

*Refers to the peak area ratio in the gas chromatogram (GC-FID).

4. Chemical constituents of forensic significance

(-)-Δ^9-trans-Tetrahydrocannabinol Tetrahydrocannabinol, THC  Main pharmacological characteristics: - Euphoriant - Anti-inflammatory - Analgesic - Anti-emetic	CAS: 1972-08-3 Empirical formula: $C_{21}H_{30}O_2$ Molecular weight: 314.46 g/mol Melting point viscous oil pKa 10.6 log P 6.99 (octanol/water) Solubilities: Water insoluble (2.8 mg/L 23°C) Ethanol soluble Chloroform soluble Hexane soluble
(-)-Δ^9-trans-Tetrahydrocannabinolic Acid THCA  Main pharmacological characteristics: - Antibacterial - Antibiotic	CAS: 23978-85-0 Empirical formula: $C_{22}H_{30}O_4$ Molecular weight: 358 g/mol Melting point n/a (decomposition/decarboxylation of THCA to THC at about 125-150°C) Solubilities: Water insoluble Ethanol soluble Chloroform soluble Hexane soluble

Cannabinol CBN  Main pharmacological characteristics: - Sedative - Anticonvulsant - Antibiotic - Anti-inflammatory	CAS: 521-35-7 Empirical Formula: $C_{21}H_{26}O_2$ Molecular Weight: 310.43 g/mol Melting Point 76–77 °C log P 6.23 (octanol/water) Solubilities: Water insoluble Ethanol soluble Chloroform soluble Hexane soluble
Cannabidiol CBD  Main pharmacological characteristics: - Anxiolytic - Anti-inflammatory - Antipsychotic - Antispasmodic - Analgesic	CAS: 13956-29-1 Empirical formula: $C_{21}H_{30}O_2$ Molecular weight: 314.46 g/mol Melting point 66–67 °C log P 5.79 (octanol/water) Solubilities: Water insoluble Ethanol soluble Chloroform soluble Hexane soluble
Cannabigerol CBG  Main pharmacological characteristics: - Antibiotic - Anti-inflammatory - Antifungal - Analgesic	CAS: [25654-31-3] (E); [95001-70-0] (E/Z) Empirical formula: $C_{21}H_{32}O_2$ Molecular weight: 316.48 g/mol

Cannabivarin CBV  	CAS: 33745-21-0 Empirical formula: $C_{19}H_{22}O_2$ Molecular weight: 282.38 g/mol
Cannabichromene CBC  Main pharmacological characteristics: - Anti-inflammatory - Antifungal - Antibiotic - Analgesic	CAS: 20675-51-8 Empirical formula: $C_{21}H_{30}O_2$ Molecular weight: 314.46 g/mol

5. Qualitative and quantitative analysis of cannabis products

5.1 Sampling

The principal reason for a sampling procedure is to permit an accurate and meaningful chemical analysis. Because most methods—qualitative and quantitative—used in forensic science laboratories for the analysis of drugs require very small aliquots of material, it is vital that these small aliquots be representative of the bulk from which they have been drawn. Sampling should conform to the principles of analytical chemistry, as laid down, for example, in national pharmacopoeias or by regional or international organizations [38].

There may be situations where, for legal reasons, the normal rules of sampling and homogenization cannot be followed. This may happen if, for example, the analyst wishes to preserve some part of an exhibit as visual evidence in court. For compressed slabs, it is also important to ensure that the entire block is composed of cannabis. This is achieved by prising open the block and examining the material closely.

To preserve valuable resources and time, forensic laboratories 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 procedures below are recommended. They are based on the sampling procedure recommended by the European Union for outdoor cannabis plantations for industrial hemp [39] and have been adapted to take into account the practical aspects and variety of cannabis products in the illicit market.

5.1.1 Sampling of plants (indoor and outdoor plantations)

For each cannabis field—visually considered to be of the same species—30 fruiting or flowering tops, one per plant, randomly chosen, not from the border of the field, are cut to a length of about 20 cm and stored in a paper bag. For identification purposes (qualitative analysis), the sampling of one representative plant in the described manner is usually considered sufficient.*

*See example of a hemp field given in reference 38 in relation to the comparison of the hypergeometric and Bayesian methods.

Figure 5. Sampling fruiting tops of the *Cannabis* plant

Wherever possible, the sample should be dried before sending to the laboratory. If it has to be stored for any reason before being analysed, it should be kept in the dark and cool.

Once dried, the degradation of the main cannabinoids is stopped. However, at this stage THC is still sensitive to air (oxygen) and UV light, which oxidize THC to CBN. Therefore, the preferred storage conditions are in the dark and cool.

5.1.2 Sampling of seized cannabis products

For general aspects of qualitative sampling of multi-unit samples, reference 38 can be consulted. For material with obvious external characteristics, i.e. material all recognizable as cannabis, a sampling method based on the Bayes' model may be preferred over the hypergeometric approach.

5.1.2.1 Herbal cannabis

In the illegal market, a huge variety of herbal cannabis products is encountered, including loose plant material, or in the form of "dry flowers", "sachets", or "herbal tea". As described in the previous section, 30 pieces considered to belong to the same phenotype are taken as one sample. If there is less material, all is taken. Coarse stem material is cut off. Seeds in the fruiting tops remain in the exhibit.

Moist material has to be packaged in paper bags. For dried material plastic bags are suitable.

5.1.2.2 Cannabis resin

The cannabis resin can be taken as it is. The required amount per sample (see section 5.4) can be taken with a grater from different areas of the slab. However, since

the surfaces of slabs are usually oxidized, samples should be taken from a freshly broken inner surface of the slab.

5.1.2.3 Liquid cannabis (oil)

The required amount of cannabis oil (see section 5.4) can be taken as it is.

5.2 Minimum criteria for positive identification of cannabis

The following sections describe a number of methods for the examination and analysis of cannabis products. The choice of the methodology and approach to analysis as well as the decision whether or not additional methods are required remain with the analyst and will also depend on the availability of appropriate instrumentation and the level of legally acceptable proof in the jurisdiction within which the analyst works. For cannabis products that exhibit characteristic botanical features, a combination of colour test, thin-layer chromatography and physical (macroscopic and microscopic) examination is considered an acceptable minimum analytical approach for positive identification. General rules for method selection have been formulated by the Scientific Working Group on Drugs (SWGDRUG) [40].

5.3 Physical examination

The methods used to identify cannabis products depend upon the nature of the product. Herbal material can be identified based on its morphological characteristics alone, provided that the required ones are present.

Where there are no morphological characteristics, as in the case of resin and hashish oil, the identification is based on chemical analysis, demonstrating the presence of cannabinoids, such as tetrahydrocannabinol (THC), its degradation product cannabinol (CBN) and/or cannabidiol (CBD).

5.3.1 Macroscopic characteristics

Morphological characteristics and variation in colour of cannabis plants are influenced by the seed strain as well as by environmental factors such as light, water, nutrients and space.

As a dioecious herb, the flowers on individual plants are unisexual, however, there are often transitional flowers and flowers of the opposite sex which develop later. Male plants are usually taller but less robust than female plants. Stems are green,

erect, hollow and longitudinally grooved (figure 6). They can vary from 0.2-6 m, although most of the plants reach heights of 1-3 m.

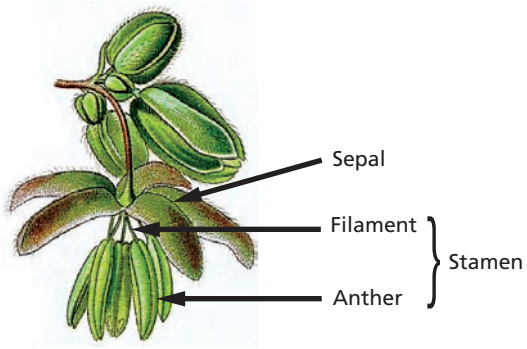
The extent of branching, like plant height, depends on environmental and hereditary factors as well as the method of cultivation. The side branches vary from opposite to alternate at any part of the main stem. The leaf arrangement changes from decussate (oppositely arranged) to alternate on the extremities of the plant. Leaf stalks (petioles) are 2-7 cm long with a narrow groove along the upper side. The leaf is palmate and consists of 3-9 linear-lanceolate leaflet blades of 3-15 x 0.2-1.7 cm. The margins are coarsely serrated, the teeth pointing towards the tips; the veins run out obliquely from the midrib to the tips of the teeth. The lower (abaxial) surfaces are pale green with scattered, white to yellowish brown, resinous glands (figure 7).

Figure 6. Grooved stem of *Cannabis sativa*

Figure 7. Abaxial (left) and adaxial (right) surfaces of *Cannabis sativa* leaves

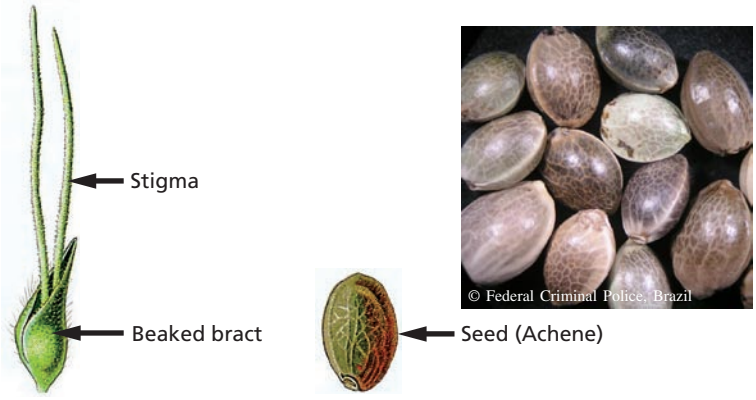
Each staminate (male) flower consists of five whitish-green minutely hairy sepals about 2.5-4 mm long and five pendulous stamens, with slender filaments and stamen.

Figure 8. Morphological characteristics of male flowers



The pistillate (female) flowers are more or less sessile and are borne in pairs. Each flower has a small green bract enclosing the ovary with two long, slender stigmas projecting well above the bract.

Figure 9. Morphological characteristics of female flower and fruit



The fruit, an achene, contains a single seed with a hard shell tightly covered by the thin wall of the ovary, ellipsoid, slightly compressed, smooth, about 2-5 mm long, generally brownish and mottled. The fruit is commonly regarded as a seed.

5.3.2 Microscopic characteristics

Cannabis sativa can be identified by microscopic structures on the surface of the plant, namely, by trichomes (i.e. hair-like projections from a plant epidermal cell). Two types of trichomes occur and can be observed with a binocular microscope with a magnification factor of 40 as shown in figures 10 and 11:

(a) Non-glandular trichomes are numerous, unicellular, rigid and curved hairs, with a slender pointed apex:

- Cystolithic trichomes found on the upper surface of the cannabis leaves have a characteristic bear claw shape and may have calcium carbonate crystals (cystoliths) visible at their bases. Frequently, the trichome is broken and the cystolith freed;
- Non-cystolithic trichomes occur mainly on the lower side of the leaves, bracts and bracteoles and lack the enlarged base;
- The simultaneous presence of these bear claw-shaped trichomes on the upper surface and the fine, slender non-cystolithic trichomes on the lower surface of the leaves is a characteristic of cannabis.

Figure 10. Microscopic view of non-glandular trichomes [41]

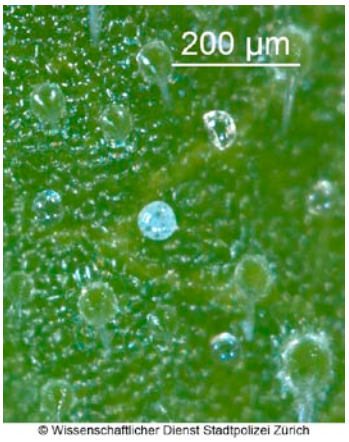
Cystolithic trichomes

Non-cystolithic trichomes

(b) Glandular trichomes. They occur as:

- Sessile glands, i.e. trichomes without stalk, which are generally found on the lower epidermis;
- Small bulbous glandular trichomes with one-celled stalks;
- Long multicellular stalks on the bracteoles surrounding the female flowers (multicellular stalked glandular trichomes).

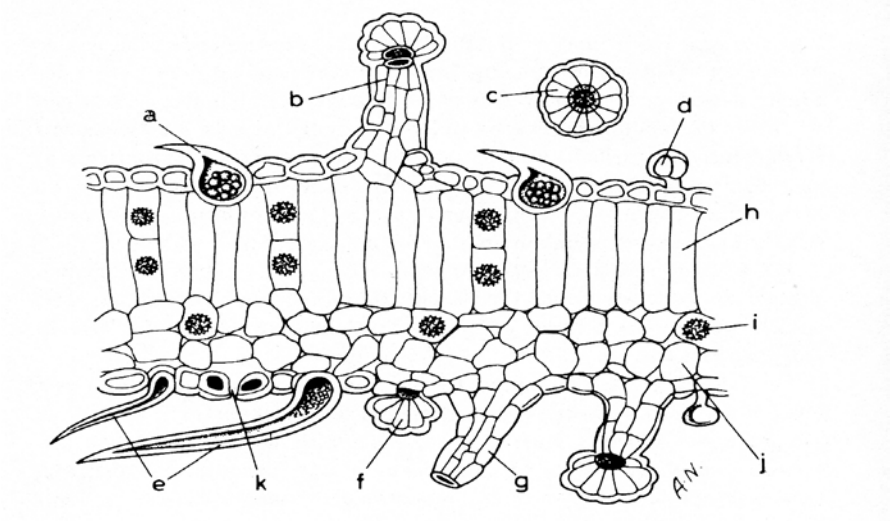
Figure 11. Microscopic view of glandular trichomes [41]



Sessile glands

Stalked glandular trichomes

Figure 12. Cross section of a bract from the fruiting plant [42]



a: cystolithic trichome; **b**: large glandular trichome with several cells in head and stalk; **c**: head of one of the large glandular trichomes; **d**: small glandular trichome with bicellular head and unicellular stalk; **e**: thick walled conical trichomes; **f**: large developing glandular trichome; **g**: stalk of a large glandular trichome; **h**: palisade cell; **i**: cluster crystal; **j**: parenchymal cell; **k**: stoma

The glandular trichomes are the structures where the cannabis resin is produced and stored. These are mainly associated with the flower structures (pistillate plants being particularly rich in these structures) but they can also be found on the underside of the leaves and occasionally on the stems of young plants.

Some plants possess trichomes that may be confused with those present on *Cannabis sativa* and care should be taken in definitive identification. However, the combination of cystolithic hairs on the leaf upper surface and longer trichomes and sessile glands on the lower surface, which is unique to *Cannabis sativa*, enables positive identification of even fragmented material.

It should be noted, however, that very immature seedlings and stems with no leaf attached cannot be definitively identified as *Cannabis sativa* by botanical examination.

For details on cannabis identification and more sophisticated microscopy techniques, the following literature can be consulted [43, 44, 45, 46].

5.4 Chemical examination

5.4.1 General aspects

THC is usually present at a quite low level in fresh plant material and is considered to be derived artificially from THCA by non-enzymatic decarboxylation during storage and consumption (e.g. smoking) [47].

In terms of analytical approach, it is a choice whether THCA and THC are measured separately or whether "Total-THC" (i.e. the combined amount of THC and THCA) is measured. This choice is sometimes made by national legislation. If there is no legal requirement for either approach, it is common practice to measure Total-THC since that best represents the pharmacological activity of the material.

Total-THC can be obtained by decarboxylation of THCA into THC. This can be during or prior to analysis. For practical reasons the latter is recommended.

The sample extract can be put into a heating block at 150°C in an open glass vial. After the evaporation of the solvent, decarboxylation is completed within five minutes. However, it is recommended that this step be validated in each forensic laboratory.

Complete THCA decarboxylation may occur during injection in some gas chromatography injector systems, whereas other injector systems show a very poor decarboxylation at the same temperature. This is presumably due to different injector geometries. A higher injection temperature may also cause THC decomposition in

the liner. Therefore, if decarboxylation is not performed prior to analysis, the specific gas chromatograph system and analysis conditions must be validated to ensure that they yield complete decarboxylation of THCA and do not cause decomposition of THC [48].

5.4.2 Sample preparation for chemical examination

5.4.2.1 Preparation of herbal cannabis

Fresh (wet) plant material is either air dried at room temperature for several days or dried at 70°C until the leaves become brittle. At this stage, the water content of the plant material is typically 8-13 per cent.

The dried material is then coarsely selected (only flowers and leaves are used), pulverized (preferably by a cutter with a high revolution speed, i.e. 100 rps) and sieved (mesh size 1 mm).*

5.4.2.2 Preparation of cannabis resin

Cannabis resin is reduced to small pieces by a grater. Alternatively for sticky material, the sample is cooled down with liquid nitrogen and immediately pulverized as described above.

5.4.2.3 Preparation of cannabis oil

Cannabis oil can be used directly for analysis.

5.4.3 Presumptive tests

5.4.3.1 Colour tests

Colour tests for cannabis are among the most specific colour tests available (only a few plants such as henna, nutmeg, mace and agrimony give false-positive results) [49]. However, a positive colour test only provides an indication of the possible presence of cannabis-containing material and not a definitive identification of cannabis. It is therefore mandatory for the analyst to confirm such results by the use of additional, typically more discriminative techniques. For example, a laboratory may allow a combination of a colour test, thin-layer chromatography and microscopy for cannabis plant material for positive identification, provided that at least three cannabinoids are identified by TLC [50].

*Note that both drying and sieving are part of the validated methods described in this manual. Sieving ensures homogeneity of the samples. Should the sieving process be skipped, the laboratory has to demonstrate that the homogeneity is within the accepted tolerance.

The analyst is also strongly advised to co-analyse a cannabis control sample (e.g. a reference material containing a mixture of cannabinoid reference standards) and a blind sample to verify the test results and the functionality and reliability of all test reagents.

5.4.3.1.1 Fast Corinth V salt test

On a filter paper		
Reagent A:	Petroleum ether	
Reagent B:	Fast Corinth V salt*	1% w/w in anhydrous sodium sulphate
Reagent C:	Sodium bicarbonate	1% w/w aqueous solution

Method

Fold two filter papers laid on top of each other into quarters and open them partly to form a funnel, place a small amount of pulverized sample into the centre of the upper paper. Add two drops of reagent A allowing the liquid to penetrate to the lower filter paper. Discard the upper filter paper and allow the lower filter paper to dry. Add a very small amount of reagent B to the centre of the filter paper and then add two drops of reagent C.

Results

A purple red coloured stain at the centre of the filter paper is indicative of a cannabis containing product. THC, CBN and CBD yield the same hue.

This is a practical advantage in a field test reagent for samples of different age or origin.

* Fast Corinth V salt = Dichlorozinc; 2-methoxy-5-methyl-4-(4-methyl-2-nitrophenyl)diazenyl-benzenediazonium; dichloride
= Azoic diazo component 39
= $C_{15}H_{14}N_5O_3 \cdot 0.5\ ZnCl_4$

5.4.3.1.2 Fast Blue B salt test

On a filter paper		
Reagent A:	Petroleum ether	
Reagent B:	Fast Blue B salt**	1% w/w diluted with anhydrous sodium sulphate
Reagent C:	Sodium bicarbonate.	10% w/w aqueous solution

Method

Same procedure as with Fast Corinth V Salt.

Results

A purple red coloured stain at the centre of the filter paper is indicative of a cannabis containing product.

This colour is a combination of the colours of the different cannabinoids which are the major components of cannabis: THC = red, CBN = purple, CBD = orange.

Note

Fast Blue B Salt keeps very well when stored in a refrigerator, but when kept at room temperature, it tends to deteriorate with time and the powder becomes a solid rock (especially in warm regions).

** Fast Blue B salt = Di-*o*-anisidinetetrazolium chloride

5.4.3.1.3 Rapid Duquenois test (Duquenois-Levine test)

In a test tube		
Reagent A:	Acetaldehyde (A1) Vanillin (A2)	0.5 ml (A1) and 0.4 g (A2) in 20 ml ethanol Solution must be stored at a cool dark place and discarded if it assumes a deep yellow colour.
Reagent B:	Concentrated hydrochloric acid	
Reagent C:	Chloroform	

Method

Place a small amount of the suspect material in a test tube and shake with 2 ml reagent A for one minute. Add 2 ml of reagent B and shake the mixture. Allow to stand for ten minutes. If a colour develops, add 2 ml of reagent C, mix gently

Results

If the lower (chloroform) layer becomes violet coloured this indicates the presence of a cannabis product.

Notes

This test is not as sensitive as the two filter paper tests above.

5.4.3.2 Immunoassays

Immunoassays can be performed not only on biological samples, but also on minute traces of the drug substance itself. However, as these analyses are costly and do not add much power of proof, they are rarely used for presumptive identification.

5.4.4 Ion mobility spectrometry (IMS)

THC screening can be performed by using an ion mobility spectrometer. Problems with the separation from heroin signals and humidity have been noted [51]. It is therefore not the method of choice.

5.4.5 Thin-layer chromatography (TLC)

There are a number of TLC methods for the qualitative and semi-quantitative analysis of cannabis, using a variety of different stationary phases (TLC plates) and solvent systems, and slightly different sample preparation and spot visualization techniques. Many of those methods also produce acceptable results but each method that is newly introduced to a laboratory must be validated and/or verified prior to routine use. The following method has been field-tested and is considered fit-for-purpose.

Plate: HPTLC 10 x 10 cm Silica gel		
System A:	Petroleum ether 60/90 Diethyl ether	80% v/v 20% v/v
System B:	Cyclohexane Di-isopropyl ether Diethylamine	52% v/v 40% v/v 8% v/v
System C: (for cannabinoid acids)	n-Hexane Dioxane Methanol	70% v/v 20% v/v 10% v/v
Tank conditioning: 30 min. with filter paper on one side.		

Sample preparation

If the sole purpose of the THC examination is qualitative (i.e. to confirm the micro- or macroscopic evidence that the suspect material is cannabis), homogenization of the herbal material is not necessary (refer to section 5.4.2 for details of sample preparation for chemical examination). Those parts of the cannabis plant known to contain the highest levels of cannabinoids (i.e. the flowering tops and upper leaves) should be selected for extraction.

Suitable quantities for extraction are about 500 mg of herbal cannabis, 100 mg of cannabis resin and 50 mg of liquid cannabis (cannabis oil). The extraction scheme should be designed to produce final solutions with THC concentrations of about 0.5 mg/ml. Typical levels of THC in cannabis materials are listed in section 3.11.

The sample is extracted with 10 ml of solvent for 15 minutes at room temperature by shaking or in an ultrasonic bath. The extract is filtered and is now ready for chromatography.*

Since cannabinoids are easily soluble in most organic solvents, methanol, petroleum ether, n-hexane, toluene, chloroform and solvent combinations, such as methanol : chloroform (9:1) are equally suitable for their extraction. It should, however, be noted, that non-polar solvents such as n-hexane and petroleum ether give a relatively clean extract but will only extract the neutral/free cannabinoids quantitatively, while the other solvents and their combinations give quantitative extractions of the cannabinoid acids as well.

For identification, the simplest clean extraction with petroleum ether is enough, while for the purposes of quantitation and total THC determinations other solvents have to be used.

Standard solutions

The standard solutions should be prepared at a concentration of approximately 0.5 mg cannabinoid per ml in methanol and should be stored in a cool, dark place.

Visualization

The plates must be dried prior to visualization. This can be done at room temperature or by use of a drying box, oven or hot air. In the latter cases, care must be taken that no component of interest is decomposed.

Spray reagent: (must be freshly prepared prior to use, preferably once per day)**

Method 1:	Fast Blue B salt	50 mg in 20 ml of NaOH (0.1 N)
Method 2:	Fast Blue B salt	50 mg in 1 ml of water, then 20 ml of methanol is added.

*It should be noted that the procedure described is part of a method that has been field-tested and was found fit-for purpose. Passive extraction, with the sample/solvent mixture allowed to stand, can also be employed. Filtration can be done but is not required; use of the supernatant liquid should produce reliable results. For identification purposes, smaller amounts of solvents and sample quantities may be sufficient. Any modification to the method described needs to be evaluated in the analyst's laboratory.

**Daily preparation of the spray reagent may not be required when Fast Blue BB or Fast Blue RR are used (0.2 per cent w/v solution of Fast Blue BB or Fast Blue RR in methanol or methanol/water 1:1).

Note

It is important for proper colour development that the TLC plate be made alkaline. One way of achieving this is by using visualization method 1. Alternatively, diethylamine may be sprayed on the TLC plate before the Fast Blue B solution. In all cases the plates should not be over wetted as spot diffusion may occur.

Fixation

To provide a permanent record, the analysis results need to be preserved. Preservation is best achieved by a succession of sprayings. Thus the spraying sequence is:

Diethylamine – Fast blue B solution – Diethylamine

The plates are then dried with hot air, or overnight at room temperature.

For storage, the plates are sealed inside clear plastic bags. Such plates have a long lifetime without darkening. As an alternative, plates can be scanned or photographed to provide a permanent record of the analysis results.

Note

Fast Blue B is claimed to be a potential carcinogen, so appropriate precautions must be taken with it.

Results

Rf x 100 values are subject to variation depending on laboratory conditions (temperature, humidity, etc.) as well as other parameters (e.g. age and quality of cannabis materials used). It is therefore good practice to run cannabinoid standards along with the sample on the same TLC plate.

Compound	Developing system, Rf x 100 values*		
	A	B	C**
CBN	33	26	47
THC	37	38	49
CBD	42	42	47
THCA	6	–	36

*Results refer to employment of method using HPTLC plates, as described in this section. Traditional 20x20 plates with a 0.25 mm thick layer of silica gel provide comparable separations, but the corresponding Rf values will have to be determined.

**System C is only recommended for the separation and identification of cannabinoid acids. It does not provide adequate separation of CBN, THC and CBD.

5.4.6 Gas chromatography-flame ionization detection (GC-FID), without and with derivatization

Whether or not derivatization is required depends on the purpose of the analysis. Without prior derivatization (i.e. silylation) of THC and THCA, GC analysis will decarboxylate the latter and produce the total THC content of the cannabis sample, which is the sum of free THC and THC generated from THCA. As the total THC content represents the maximum potency of the usually smoked (and therefore also decarboxylated) cannabis, most legal systems consider total THC content as the relevant parameter. However, if both contents have to be reported, prior derivatization is required (see also section 5.4.1).

*5.4.6.1 Capillary column technique**

The method below is a validated method [52]. The validation encompasses the entire process from sample preparation to GC analysis. Other methods may also produce acceptable results but must be validated and/or verified prior to routine use.

Column:	15 m x 0.25 mm, 0.25 µm;
Phase:	5% Diphenyl – 95% Dimethylpolysiloxane
Carrier:	Hydrogen, 1.1 ml/min, constant flow
Injector:	Split/splitless, 280°C
Split ratio:	20:1
Oven:	2 min at 200°C, 10°C/min 200-240°C, 2 min at 240°C
Detector:	FID 300°C, H_2 35 ml/min, Air 350 ml/min
Internal standard:	Tribenzylamine (TBA) in ethanol (0.5mg/ml)
Injection:	1.5 µl, Split
Elution order:	CBD, THC, CBN

Sample preparation

Two hundred mg of dry and homogenized herbal cannabis (see section 5.4.2) are extracted with 20 ml internal standard (ISTD) solution (see below) for 15 minutes in an ultrasonic bath. Due to the higher THC concentration in cannabis resin, only 100 mg resin is needed. If the sample is liquid cannabis (cannabis oil), a weight of about 50 mg is sufficient.

As, depending on the GC system, it has not been established that the decarboxylation of THCA in the GC liner is quantitative, it is strongly recommended to carry

*The packed column technique is no longer included in this manual as GC systems are now typically equipped with capillary columns (narrow-bore and mega-bore columns). Laboratories that are using GC systems with packed columns are encouraged to continue to use their established (validated) methods. Information about packed column techniques is available on request from lab@unodc.org.

out a decarboxylation step prior to the GC analysis.* To this end, 500 µl of the solution is transferred to a 2 ml GC vial. The vial is put into a heating unit (150°C) for 12 minutes where the solvent is evaporated and the THCA is decarboxylated. The residue is dissolved in 1.5 ml ethanol, the vial is shaken well and the resulting solution is then analyzed by GC.

Calibration

As THC reference material degrades rapidly and is not easily available in an acceptable quality, the quantification of THC can be performed with CBN reference material. The calibration with CBN instead of THC is known and widely accepted. In theory the correlation factor is 1.00 [53]. For validation purposes, showing the validity of the theoretical factor in the given gas chromatograph, it is good policy to measure and monitor CBN ratio with a similar compound like CBD.

Solutions for calibration

CBN standard solutions are prepared in 2 ml GC vials according to the table below:

Stock solution (SS):	1 mg CBN/ml ethanol
Intermediate dilution (ID):	100 µl stock solution + 900 µl ethanol
Internal standard solution (ISTD):	0.5 mg tribenzylamine (TBA)/ml ethanol

Std 1	50 µl ID	+ 500 µl ISTD-solution	+ ~ 950 µl ethanol	0.1%
Std 2	250 µl ID	+ 500 µl ISTD-solution	+ ~ 750 µl ethanol	0.5%
Std 3	50 µl SS	+ 500 µl ISTD-solution	+ ~ 950 µl ethanol	1%
Std 4	150 µl SS	+ 500 µl ISTD-solution	+ ~ 850 µl ethanol	3%
Std 5	250 µl SS	+ 500 µl ISTD-solution	+ ~ 750 µl ethanol	5%
Std 6	500 µl SS	+ 500 µl ISTD-solution	+ ~ 500 µl ethanol	10%
Std 7	800 µl SS	+ 500 µl ISTD-solution	+ ~ 200 µl ethanol	16%

Standard solutions must be stored in a cool, dark place, for a maximum of four months.

*If decarboxylation *prior* to analysis is not performed, the specific gas chromatograph system and analysis conditions must be validated to ensure that they yield complete decarboxylation of THCA and do not cause decomposition of THC.

Silylation

If THCA has to be analysed separately, i.e. without decarboxylation, 1.5 ml aliquots of the above (non-thermally decarboxylated) extract has to be derivatized before GC analysis. Derivatizing agents frequently used are:

MSTFA:	N-methyl-N-trimethylsilyltrifluoroacetamide
BSTFA/TMCS:	N,O-bis(trimethylsilyl)trifluoroacetamide/Trimethylchlorosilane (1 per cent)

Silylizable solvents such as ethanol have to be removed, usually by a gentle stream of nitrogen. The residue is taken up in 1.5 ml chloroform. 100 µl MSTFA are added and heated for 30 min at 70°C. The resulting solution can be analysed directly.

5.4.7 Gas chromatography-mass spectrometry (GC-MS)

The GC-MS analysis can be performed analogous to the GC-FID analysis.

Reference spectra of the most common cannabinoids, in derivatized or underivatized form, are available in common commercial MS databases.

5.4.8 High-performance liquid chromatography (HPLC)

The method below is a validated method for the analysis of total THC content (THC + THCOOH) in herbal cannabis after extraction with methanol/chloroform and subsequent decarboxylation [54, 55]. The validation encompasses the entire process from sample preparation to HPLC analysis. Other methods may also produce acceptable results but must be validated and/or verified prior to routine use. With adequate verification, the same method can also be applied to other cannabis products.

Column type:	250x4mm RP-8 (5 µm); pre-column 4x4mm RP-8 (5 µm)
Column temperature:	30°C
Mobile phase:	Acetonitrile : water (8:2 v/v), isocratic, stop time 8 min.
Flow:	1 ml/min
Detection:	Photodiode array (PDA), 220 nm and 240 nm
Injection:	10 µl
Elution order:	CBD, CBN, THC, THCA (if decarboxylation is not performed or is incomplete)

Sample preparation

500 mg of dry and homogenized herbal cannabis (see section 5.4.2) are extracted with 5 ml methanol : chloroform (9:1 v/v) by the following procedure: 10 seconds on a vortex, 15 min. ultrasonic bath including again vortexing after 5, 10 and 15 minutes, then centrifugation.

Decarboxylation

200 µl of the above extract are transferred into a derivatization vessel. The solvent is evaporated under nitrogen gas to dryness. The sample is decarboxylated for 15 minutes at 210°C. The residue is dissolved in 200 µl methanol : chloroform (9:1 v/v).

Preparation of the final solution

The above decarboxylation solution is diluted with methanol by a factor of 100 (in two steps, each 100 µl + 900 µl) and is then used for the analysis.

For lower THC contents (< 0.5 per cent), a dilution factor of 10 instead of 100 is sufficient.

Calibration

Stock solution: Standard solution 1 mg (-)-Δ^9-THC/ml methanol

Dilution 1: 100 µl (stock solution) + 900 µl methanol = 0.1 mg THC/ml methanol

Dilution 2: 100 µl (dilution 1) + 900 µl methanol = 0.01 mg THC/ml methanol

No.	*Concentration (mg/ml)*	*STD (vol. of standard)*	*Methanol (vol. of methanol)*
1	0.001	10 µl 0.01 mg/ml	90 µl
2	0.005	50 µl 0.01 mg/ml	50 µl
3	0.01	10 µl 0.1 mg/ml	90 µl
4	0.05	50 µl 0.1 mg/ml	50 µl
5	0.1	100 µl 0.1 mg/ml	0 µl

Standard solutions must be stored in a dark, cool place for up to four months.

Results

For a qualitative identification, the retention time as well as the DAD spectrum of the cannabinoid have to match.

Substance	*Retention time (min)**	*Relative retention time**
Cannabidiol	4.9	0.69
Cannabinol	6.0	0.85
(-)-Δ^9-THC	7.1	1.00
(-)-Δ^9-THC acid	7.4	1.04

* Carried out on a 250-4mm LiChrospher® 60 RP-select B (5µm) with a pre-column 4-4 LiChrospher® 60 RP-select B (5µm)

The calculation for the quantitative results is carried out at the wavelengths of 220 and 240 nm.

6. Additional analytical techniques and approaches for the analysis of cannabis products

This section gives a brief overview of some additional techniques and approaches that can be applied to the analysis of cannabis products.

6.1 GC-FID profiling of seizures of cannabis products

For a chemometric classification, standardized GC profiles are used. The analysis can be performed on a standard column. For cluster analysis, the terpenoid range, which mainly consists of sesquiterpenes, is used. GC profiles of cannabis specimens with the same origin show a similar peak pattern, thus allowing samples to be linked. Correlation studies indicate that it might be feasible to determine the geographic origin of a cannabis specimen on the basis of its chemical signature [56].

However, because of the high natural variability of cannabis, the need for authentic cannabis reference material (i.e. of known origin), and the use of likelihood ratios (probabilities) to describe regions of origin, the forensic value of GC profiles for the purposes of origin determinations may be limited.

By contrast, this approach could be used for batch-to-batch analysis. This could provide the opportunity to link samples of the same age, phenotype and production facility. The feasibility would have to be proven using a large data set.

6.2 Solid phase-micro extraction (SPME)

SPME is a solvent-free sample preparation technique, which can be used for the sampling and analysis of volatile chemical markers in the headspace over solutions, directly over the suspected material, or it can be used for the analysis of aqueous solutions containing the target analytes. For cannabis products the SPME analysis of both volatile constituents and the cannabinoids have been reported [57, 58].

Headspace-SPME of has also been performed in hemp food using alkaline hydrolysis (NaOH) and on-fibre derivatization (MSTFA) followed by gas chromatographic-mass spectrometric (GC-MS) detection. Using deuterated standards, the method proved to be robust for the analysis of the main cannabinoids THC, CBN and CBD and, compared to liquid-liquid extraction, it is substantially faster [59].

6.3 Stable isotope ratio-mass spectrometry (IRMS)

The variation of stable isotope ratios of carbon and nitrogen is most useful for sourcing the geographical origin of plant materials. Unlike other drugs such as heroin and cocaine, cannabis is not chemically processed for illicit supply and therefore maintains its original elemental and isotopic profiles. Thus, these parameters could be used as an indication of geographical origin [60].

However, different growing conditions (e.g. amount of watering, grown without or with soil, i.e. indoor or outdoor grown, type of soil and fertilizer, etc.) can affect the isotopic composition of the plants and thus discrimination may be limited [61]. In addition, meaningful results are only possible when authentic cannabis reference material (of known origin) is available.

6.4 DNA profiling

This technique provides the opportunity to link products on the basis of their genetic profiles, which could be useful from an investigative point of view, e.g. to link producers, traffickers and consumers.

However, unlike human DNA, such a fingerprint may not necessarily be unique, as cloning of cannabis strains is quite common. Matching DNA profiles of two samples does therefore not by itself prove that they come from the same plant, let alone the same grower. Due to the fact that growers also sell their cuttings, the forensic value of a match obtained with this relatively expensive technique is sometimes questionable.

For an overview and description of the different DNA testing methods, see reference 62.

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