UNODC
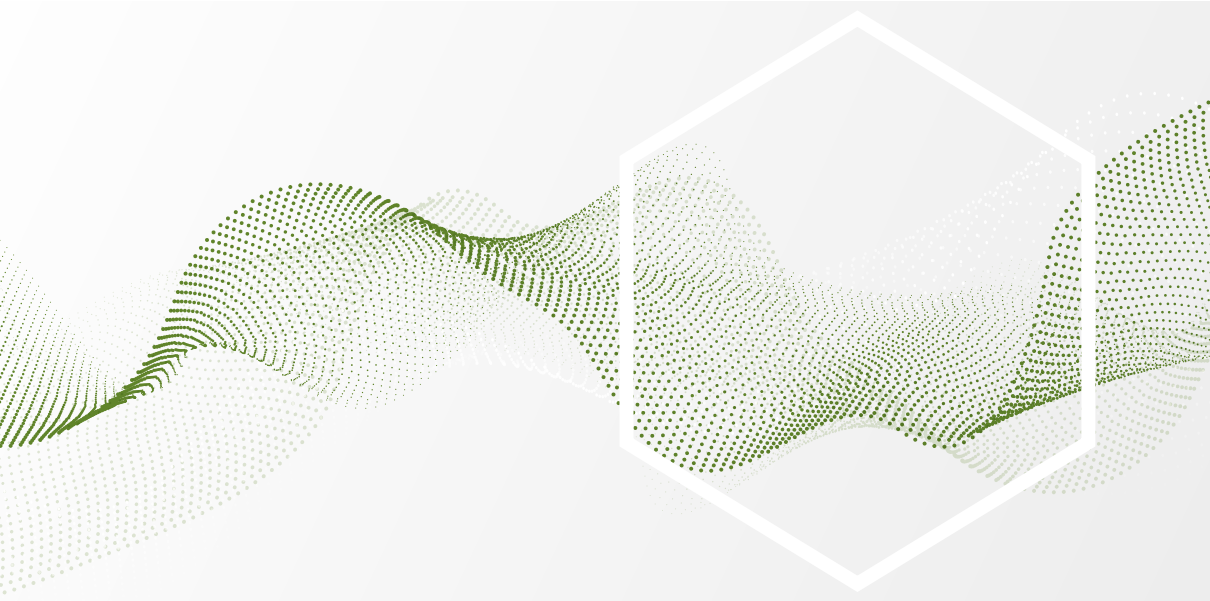United Nations Office on Drugs and Crime

Recommended Methods for the
IDENTIFICATION AND ANALYSIS OF CANNABIS AND CANNABIS PRODUCTS

Manual for use by
NATIONAL DRUG ANALYSIS LABORATORIES

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

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

(Revised and updated)

MANUAL FOR USE BY
NATIONAL DRUG ANALYSIS LABORATORIES

UNITED NATIONS
Vienna, 2022

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Operating and experimental conditions are reproduced from the original reference materials, including unpublished methods, validated, and used in selected national laboratories as per the list of references.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.

ST/NAR/40/REV.1

Acknowledgements

Contents

1. Introduction

1.1 Background

Unlike other plant-based drugs, for which cultivation and production are concentrated in only a few countries, cannabis is produced in almost all countries worldwide and cannabis products are the most widely trafficked drugs. Practically all countries in the world are affected by cannabis trafficking. In 2019, more than 5,000 tons of cannabis (herb and resin) seizures were reported globally. Similarly, cannabis also remains the most widely used drug worldwide, with an estimated 200 million people having used cannabis in 2019, nearly an 18 per cent increase over the past decade, and equivalent to some 4 per cent of the global population aged 15–64 [1].

Since the end of the last century, there have been rapid advances in cannabis plant cultivation techniques, and production methods have become increasingly sophisticated. Developments in legislation in some countries have resulted in changes in the dynamics of cultivation, production and markets of cannabis and cannabis products. These factors have resulted in the availability in illicit markets of a wide range of cannabis with increasing levels of tetrahydrocannabinol (THC), which refers to the psychoactive components of cannabis and comprises a number of isomers and stereochemical variants that are included in the international drug control conventions. There has been also an increase in the variety of products containing THC and the means of ingestion, including edibles, vapes and dabs, as well as an increased availability of cannabis products containing mainly cannabidiol (CBD), but which could also contain low levels of THC [2].

As a result, the analysis of cannabis and cannabis products has become more complex for forensic drug testing laboratories, with a need to identify and often quantitate low levels of THC, differentiate its isomers, in particular *delta*-9-THC (Δ^9-THC) and *delta*-8-THC (Δ^8-THC), and identify other cannabinoids that are present. This poses a variety of analytical challenges requiring reliable, reproducible, and sensitive analytical methods and techniques. In addition, the lack of availability of reference material of Δ^9-THC, its isomers and other cannabinoids is an increasing concern for forensic laboratories.

1.2   Purpose and use of the manual

The present manual is one in a series of similar UNODC 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 harmonizing and establishing recommended methods of analysis for national forensic drug analysis laboratories.

In 1987, UNODC prepared the first manual on *Recommended methods for testing cannabis* (ST/NAR/8), which was revised in 2009. The present manual is a revision of the UNODC manual on *Recommended Methods for the Identification and Analysis of Cannabis and Cannabis Products*, (ST/NAR/40), prepared as a response to the current challenges faced by national drug testing laboratories.

In line with the overall objective of this series of UNODC publications, the present manual suggests approaches that may assist drug analysts in the selection of methods appropriate to the sample under examination, and the range of technologies and resources that might be available in their laboratories, leaving room for adaptation to the level of sophistication of different laboratories and the various legal needs.

The manual includes analytical methodologies, using different techniques and instrumental modes of operation. The majority of methods included in the manual have been validated and many have been also published in the scientific literature. The reader should be aware that other published methods are available, which may also produce acceptable results. However, any new method that is to be used in a laboratory must be validated and/or verified prior to routine use.

While there are several more sophisticated approaches, they may not be necessary for routine operational applications. Therefore, the methods described here should be understood as guidance; 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 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, literature on drugs of abuse, and analytical techniques. Moreover, the analyst must keep abreast of current trends in drug analysis, consistently following current analytical and forensic science literature.

The Laboratory and Scientific Service of UNODC welcomes observations on the contents and usefulness of the present manual. Comments and suggestions may be addressed to:

Laboratory and Scientific Service
United Nations Office on Drugs and Crime
Vienna International Centre
P.O. Box 500
1400 Vienna Austria
Fax: (+43-1) 26060-5967
Email: lab-unodc@un.org

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

2. Cannabis production, market, and trends

Cannabis cultivation and production affects all regions worldwide and cannabis products continue to be the most widely used drugs. Cannabis can be grown in virtually any country and is also increasingly cultivated indoors. During the last decade, the cultivation of cannabis plant was reported to UNODC by 151 countries, with some countries reporting both indoor and outdoor cultivation. Outdoor cultivation of cannabis continues to be more widespread around the globe than indoor cultivation, although the increase in the latter is significantly larger. Most reported indoor cultivation of cannabis continues to be concentrated in countries of Europe and North America, with the main focus on achieving a high *delta*-9-THC content [1]. While production of herbal cannabis (marihuana) is widely dispersed throughout the world, cannabis resin (hashish) is produced mainly in a few countries in North Africa, the Middle East, and South-West Asia.

In addition to the major transformation of cannabis cultivation in recent years, the cannabis market has diversified to the extent that it now comprises a broad range of products with varying *delta*-9-THC content and means of ingestion, potency and effects [1]. Cannabis resin sold in Europe is now more potent than before, with a *delta*-9-THC content on average between 20 per cent and 28 per cent, almost twice that of herbal cannabis (8-13 per cent). Cannabis products available in Europe now include those with high *delta*-9-THC content as well as products containing cannabis extracts with low levels of *delta*-9-THC. New forms of cannabis have also appeared on the illicit market, which raises health concerns. Reports are also emerging of small-scale production of highly potent cannabis extracts. 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.

3. General introduction

3.1 Name of the cannabis plant

Cannabis sativa L. (Linnaeus)

3.2 Definitions

The definitions of cannabis and cannabis products included in article 1 of the Single Convention on Narcotic Drugs of 1961 as amended by the 1972 Protocol [3] are provided below.

Article 1. Definitions

(b) "Cannabis" means the flowering or fruiting tops of the cannabis plant (excluding the seeds and leaves when not accompanied by the tops) from which the resin has not been extracted, by whatever name they may be designated.

(c) "Cannabis plant" means any plant of the genus Cannabis.

(d) "Cannabis resin" means the separated resin, whether crude or purified, obtained from the cannabis plant.

"Cannabis and cannabis resin and extracts and tinctures of cannabis" are included in Schedule I of the Single Convention on Narcotic Drugs of 1961 as amended by the 1972 Protocol [3].

The chemical constituents of cannabis that are included in the Convention on Psychotropic Substances of 1971 are listed in section 4.

3.3 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 names such as marihuana, pot, ganja, grass, chanvre [4]. The term "cannabis" is also generally used to describe different products obtained from the cannabis plant [5].

3.4   Taxonomy

The genera *Cannabis* and *Humulus* (hops) belong to the same family of *Cannabaceae*, which also includes eight other genera [6] [7].

Notwithstanding the ongoing debate on whether the genus Cannabis is represented by one or more species, it is generally considered to be monospecific (*Cannabis sativa* L.) and includes subspecies such as *C. sativa* subsp. *sativa* and C. *sativa* subsp. *indica* [6] [8]. Varieties that have been reported include *Cannabis sativa* L. subsp. *sativa* var. *sativa*; *Cannabis sativa* L. subsp. *sativa* var. *spontanea* Vavilov (= *C. ruderalis*, Janishevsky); *Cannabis sativa* L. subsp. *indica* var. *indica* (Lam) Wehmer; *Cannabis sativa* L. subsp. *indica* var. *kafiristanica* (Vavilov) [9] [10].

The chemical and morphological distinctions of different subspecies are often not readily discernible, appear to be environmentally modifiable, and vary in a continuous fashion. However, DNA studies [6], [8], [11]–[14] have supported the perspective of cannabis being a monotypic genus with only one species, *Cannabis sativa* L., and for most purposes, it will suffice to apply this name to all cannabis plants encountered [15].

3.5   Physical appearance

Cannabis is an annual, flowering herb. The majority of plants are 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. Staminate (male) plants are usually taller but less robust than pistillate (female) plants. Stems are erect and their height 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. Figure I provides an overview of the morphological aspects of *Cannabis sativa* L. (Linnaeus). Details on the characteristics of the plant are provided in section 5.3.

3.6   Breeding and cultivation

In nature, the cannabis plant is propagated from seed and it 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. For several decades, clandestine cannabis breeders have produced types of drug plants, and hundreds of selections have been named and offered in the illicit trade. A wide range of cannabis varieties with different characteristics in terms of morphology and chemical composition are grown. Cross-breeding of cannabis 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*. Modern, selective breeding techniques propagate cannabis plants by cloning, or asexual reproduction, controlling plant genetics and the cannabinoid profile. THC, and more recently CBD, are the subject of breeding to increase or decrease their content in plants.

Figure I. Morphological aspects of *Cannabis sativa* L. [16]

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.

3.6.1   Sinsemilla

The term sinsemilla (Spanish for "no seed") 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 per 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 can be 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. The "feminization" of seeds helps ensure only female plants are grown from that seed. Eliminating the growth of male cannabis plants effectively achieves higher yields per square metre of growing space.

3.6.4   Outdoor production

The main illicit production of cannabis worldwide is still outdoors, and these plants are generally but not necessarily grown from seeds. The yield of outdoor cannabis cultivation greatly depends on the climatic and environmental conditions. Outdoor sinsemilla production is realized by identifying and destroying male plants before pollination or using artificially induced hermaphroditic females (see section 3.6.1 and 3.6.3).

3.6.5   Indoor production

Indoor production is typically standardized, with controlled environmental conditions such as light, temperature, air circulation, irrigation, plant nutrition and other factors that can influence the chemical and morphological profile of cannabis plants. Indoor cultivation often uses hydroponic techniques, that is, growing plants without soil in nutrient solutions or sand in an artificial environment. Cultivation under such controlled growing conditions allows continuous cultivation throughout the year and can result in four to six full harvests per annum. As a comparison, outdoor cultivation usually produces only one crop per year.

3.6.6   Flowering

Flowering usually starts when darkness exceeds 11 hours per day. The flowering cycle can last anywhere between 4 and 12 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 longer to finish flowering.

3.6.7   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. Typically, harvesting commences by cutting off the top half of the plant, which is then hung upside down on a drying cart or similar device.

3.6.8   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 in terms of grams per plant or square metre.* Nevertheless, some empirical studies are available and summarized below. Variations due to different cultivation factors as mentioned above must be considered. Studies in Germany, The Netherlands and from EUROPOL are reported in tables 1 and 2:

Table 1.   Indicative minimum and/or average yields for flowering tops per indoor cannabis plant

Minimum yield (g/plant)	Average yield (g/plant)	Reference
	22	[17]
25	40	[18]
	33.7	[19]
28		[20]

The authors of reference 18 have confirmed that the figures for the yield have not changed from 2009 to 2021.

Table 2. Indicative yields of dried herbal cannabis per unit cultivation area

Outdoor cultivation (g/m2)	Indoor cultivation (g/m2)	Reference
75		[21]
	505	[19]
	400	[20]

Reference 21 also suggests that about 100 kg of herbal cannabis ("kif") are required to obtain 1-3 kg of resin.

* Unpublished data.

3.7   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. Illicit cannabis products fall into three main categories: herbal cannabis, cannabis resin and cannabis oil (hashish oil). Cannabis products are derived from a highly variable natural material with batches of wide variation that are subsequently subjected to processing and transformation for trafficking purposes. Cannabis products appear in illicit markets in a multitude of forms.

3.7.1   Herbal cannabis

The fruiting and flowering tops and leaves next to the flowering tops contain the highest amount of THC. They are known as the "drug-containing parts", and generally only these parts of the plant are sold on the illicit market. However, illicitly consumed herbal cannabis also includes bigger leaves located at greater distance from the flowering tops. While the leaves next to the male flowering tops of potent cannabis plants contain THC, the content is much lower than that of female plants. The central stem and main side stems contain little THC but they may still be used in the production of cannabis oil (see section 3.7.3).

The dried leaves and flowers of the cannabis plant are commonly known as "marihuana"; however, a variety of other regional names exist [4]. "Marihuana" is found unchanged in the illegal market, that is, 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 countries.

A 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 – mainly seeds and parts of the stems. All material that passes through the sieving process is derived from

the flowering and fruiting tops of the herbal material resulting in a relative enrichment of THC. In the illicit trade, this product is known as "Kif", a characteristic product of North Africa. Such material has high cannabis resin content and can be compressed into slabs, which appear similar to cannabis resin slabs (see section 3.7.2). However, when subjected to microscopic examination, such slabs are found to have retained essential herbal characteristics (see section 5.3.2) and are considered a sort of "purified marihuana".

Another way of producing high-quality herbal cannabis is indoor production. Very potent hybrids, such as "skunk", "white widow", etc. are produced by optimized cultivation conditions. Propagation occurs mainly by cloning of the mother plants (see section 3.6.2). Places used for indoor cultivation 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 can generate herbal cannabis with a total THC content ranging from 10 per cent to 25 per cent, cannabis resin with 25 per cent THC and cannabis oil with 60 per cent THC.

The drying process of herbal cannabis is straightforward. 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 and can be stored for many months, although THC degrades with time, when exposed to air, light and humidity (see section 4.3).

3.7.2   Cannabis resin (hashish)

The resinous secretions of the plant, produced in the glandular trichomes (see section 5.3.2), can be collected to obtain a product with higher THC content than herbal cannabis, from which most recognizable plant material is removed. Cannabis resin consists of finer plant material and appears as loose or pressed sticky powder, depending on the method of production.

The production of cannabis resin is mainly carried out in two regions: in countries in the southern and the eastern part of the Mediterranean, and countries in South and South-West Asia. A variety of processes have been used in both regions to produce cannabis resin; however, sieving is an important part of the process in both regions.

Cannabis resin from Mediterranean countries

In this region, the dried herbal material is typically threshed out against a wall so that the resin-producing parts can detach from the more fibrous parts of the plant. 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

characteristics. 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.

Cannabis resin from South and South-west Asia

A common approach to produce cannabis resin in South and South-West Asian countries consists of rubbing between the palms of the hand the fruiting and flowering tops of the fresh cannabis plants so that the resin is transferred from the plant to the palm. The high levels of resin make these parts of the plant very sticky to the touch.

This can also be done by rubbing the sticky parts against rubber sheeting, or walking through a field of cannabis plants wearing rubber sheeting or leather. Resin accumulates on the surface, then the sheeting or leather may be scraped clean, and the material is compressed into slabs.

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 crushed between the hands into a coarse powder. This powder is then sieved so that it attains a fineness similar to that obtained in the Mediterranean region. The fine green powder is stored in leather bags for four to five months, then exposed to the sun for a short time sufficient for the resin to melt. After being put back into the leather bags for a few days, 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 different method involves immersion of the plant material, without the main stems, in boiling water to remove the resin from the fruiting and flowering tops. After 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. With this method, water is introduced into the resin, which frequently leads to mould formation over time. Little cannabis resin is made in this more elaborate way.

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

An efficient method for the separation of resin consists of a device similar to a tumble-dryer lined with a finely woven net 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 II.   "Pollinator" and powdered sticky resin (product) [22]

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 resin balls freeze and drop off the plant. The process is repeated for a series of progressively finer meshed sieves until a powdered product (figure II) is achieved.

3.7.3   Cannabis oil (hashish oil)

Cannabis oil is a concentrated liquid extract of either herbal cannabis material or of cannabis resin. In general, whether made from cannabis or cannabis resin, cannabis extracts are dark brown or dark green in colour and have the consistency of thick oil or a paste. The reason for their production is to concentrate the THC, thus also facilitating trafficking of smaller quantities of product with higher THC content.

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 herbal cannabis or cannabis resin is fully extracted, the suspension is filtered, and the extracted material is discarded. 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 several 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, the excess solvent may be recovered for future use.

3.7.4   Cannabis seeds and cannabis seed oil

Cannabis seeds are a potent source of Ω-3-fatty acids. Cannabis seed oil is a clear yellow liquid. The seeds contain approximately 29 per cent to 34 per cent oil by weight [23].

100 g of cannabis seed oil contains about 19 g *a*-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 becomes 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 is most likely to have originated from a poor separation of the seeds from the bract [24].

3.7.5 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. 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. This essential oil is not in great demand, and it seems that it is rather a side product from seed oil or hashish oil production. However, in some countries it is used as flavouring ingredient in hashish and in edibles.

3.7.6 Cannabis edibles and other products

Cannabis heated in an oil-based liquid (cannabis butter/oil) or herbal cannabis (after decarboxylation) immersed in high-proof alcohol (cannabis tinctures) are methods used to produce edible products. These products can come in many forms, including baked goods, candies, gummies, chocolates, lozenges, tea, coffee, and beverages. In recent years, chemical constituents of cannabis have also been identified in e-cigarettes and liquids for vaping.

Other products, such as hemp oil, are increasingly used as ingredients in a variety of cosmetics [25], including hair, skin, and oral care products. Generally, cannabis seeds and leaves (without the upper part of the plant, flowers, or fruits) of hemp varieties are used in the formulation of cosmetic products. Studies have shown that the THC content in such products is usually not more than 0.05 per cent [26]. The variety of products containing cannabis have a direct impact on the methods and procedures used by forensic laboratories for their analysis and identification.

3.8   Cannabis for industrial or horticultural purposes

There are a number of strains of *Cannabis sativa* L. with low THC content that are intended for industrial or horticultural purposes. They are grown mainly for their seeds and fibres. Harvesting for fibres occurs at the end of flowering of the female plants and before seed formation.

In most European countries the current upper legal limit for cultivation of industrial cannabis is 0.2 per cent THC and, for example, in both Canada and USA the legal limit is 0.3 per cent. In many countries, "lists of approved cultivars" exist and varieties which are consistently found to exceed the legally acceptable levels for THC may be removed from these lists. Cultivation of cannabis for industrial or horticultural purposes is increasing in several countries for the production of paper, textiles, rope, and construction materials based on fibre. The seeds are used in food products, cosmetics, plastics and fuel.

3.9   Cannabis for medical and scientific purposes
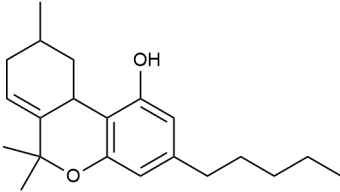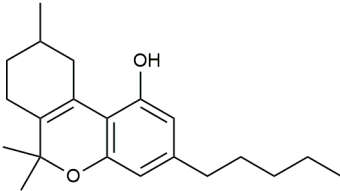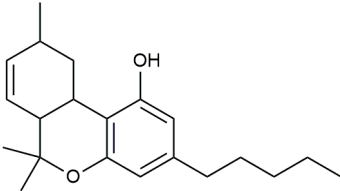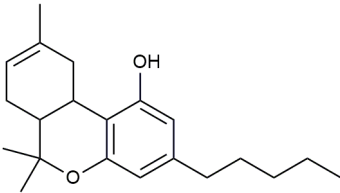
Cannabis is used for medical and scientific purposes in several countries, in line with the provisions of the international drug control conventions "to limit exclusively to medical and scientific purposes the production, manufacture, export, import, distribution of, trade in, use and possession of drugs" [3]. Examples of products that are medically approved for therapeutic use include:

- Nabiximol (Sativex®), a liquid cannabis extract of THC and cannabidiol, which is indicated for the treatment of pain and spasticity in multiple sclerosis
- Dronabinol (Marinol®), (-)-*trans*-Δ^9-tetrahydrocannabinol, a specific isomer of THC (under Schedule II of the Convention on Psychotropic Substances of 1971) [3], which is indicated for the treatment of loss of appetite in patients with AIDS and for severe nausea and vomiting associated with cancer chemotherapy

There are a variety of ongoing studies on other cannabinoid products for possible therapeutic uses.

4. Chemistry of cannabis

Tetrahydrocannabinol (THC) refers to the psychoactive components of cannabis and includes a number of isomers and stereochemical variants that are included in the international drug control conventions. THC, the following isomers and their stereochemical variants are listed in Schedule I of the Convention on Psychotropic Substances of 1971 [3].

Structure	Chemical names
	8,9,10,10*a*-tetrahydro-6,6,9-trimethyl-3-pentyl-6*H*-dibenzo[*b,d*]pyran-1-ol $\Delta^{6a(7)}$-tetrahydrocannabinol $\Delta^{6a(7)}$-THC *delta*-6*a*(7)-tetrahydrocannabinol, *delta*-6*a*(7)-THC
	7,8,9,10-tetrahydro-6,6,9-trimethyl-3-pentyl-6*H*-dibenzo[*b,d*]pyran-1-ol $\Delta^{6a(10a)}$-tetrahydrocannabinol $\Delta^{6a(10a)}$-THC *delta*-6*a*(10*a*)-tetrahydrocannabinol *delta*-6*a*(10*a*)-THC
	6*a*,9,10,10*a*-tetrahydro-6,6,9-trimethyl-3-pentyl-6*H*-dibenzo[*b,d*]pyran-1-ol Δ^{7}-tetrahydrocannabinol Δ^{7}-THC *delta*-7-tetrahydrocannabinol *delta*-7-THC
	6*a*,7,10,10*a*-tetrahydro-6,6,9-trimethyl-3-pentyl-6*H*-dibenzo[*b,d*]pyran-1-ol Δ^{8}-tetrahydrocannabinol Δ^{8}-THC *delta*-8-tetrahydrocannabinol *delta*-8-THC
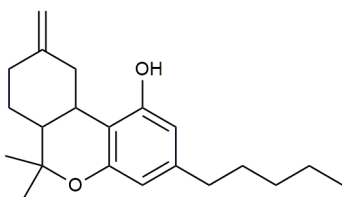
6a,7,8,9,10,10a-hexahydro-6,6-dimethyl-
9-methylene-3-pentyl-6H-dibenzo[b,d]pyran-1-ol
$\Delta^{9(11)}$-tetrahydrocannabinol
$\Delta^{9(11)}$-THC
delta-9,11-tetrahydrocannabinol
delta-9,11-THC
Exo-THC
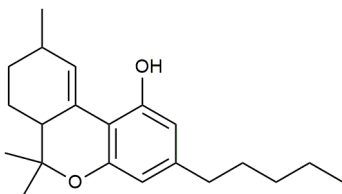
6a,7,8,9-tetrahydro-6,6,9-trimethyl-3-pentyl-
6H-dibenzo[b,d]pyran-1-ol
Δ^{10}-tetrahydrocannabinol
Δ^{10}-THC
delta-10-tetrahydrocannabinol
delta-10-THC
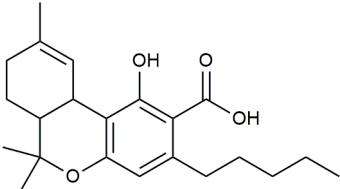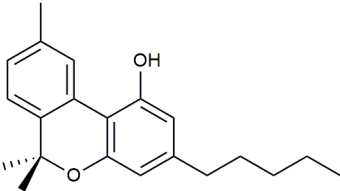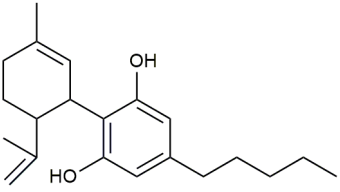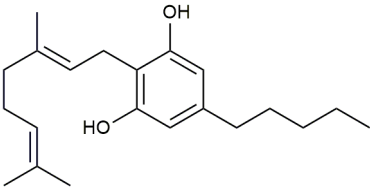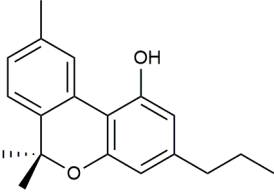
Dronabinol, a specific isomer of THC, and its stereochemical variants are listed in Schedule II of the Convention on Psychotropic Substances of 1971 [3].

Structure	Chemical names	Properties
	(6aR,10aR)-6a,7,8,10a-Tetrahydro-6,6,9-trimethyl-3-pentyl-6H-dibenzo[b,d]pyran-1-ol (-)-*trans*-Δ^9-tetrahydrocannabinol (-)-*trans*-Δ^9-THC (-)-*trans*-delta-9-THC	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)

The stereochemical variants of dronabinol are:
- (-)-*trans*-Δ^9-THC
- (+)-*trans*-Δ^9-THC
- (-)-*cis*-Δ^9-THC
- (+)-*cis*-Δ^9-THC

(-)-*trans*-Δ^9-THC is the only of these stereochemical variants that occurs naturally in thecannabis plant and is considered the primary psychoactive component of cannabis. Theterm *delta*-9-THC used in this manual encompasses all stereochemical variants, unlessotherwise specified. The chemical structures of selected constituents of cannabis which areof forensic significance are as follows.

Structure	Properties
delta-9-tetrahydrocannabinolic acid (THCA)	CAS: 23978-85-0 Empirical formula: $C_{22}H_{30}O_4$ Molecular weight: 358.21 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)	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)	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)	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)

CAS:20675-51-8
Empirical formula: $C_{21}H_{30}O_2$
Molecular weight: 314.46 g/mol
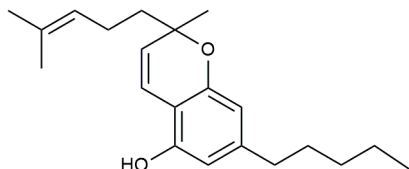
4.1 Biosynthesis

The main phytocannabinoid constituents of cannabis are *delta*-9-THC, CBD, and CBC. In fresh biomass, 95 per cent of these constituents exist as their acidic parents: *delta*-9-tetra-hydrocannabinolic acid (THCA), cannabidiolic acid (CBDA), and cannabichromenic acid (CBCA). These substances are formed via enzymatic catalysis of cannabigerolic acid (CBGA) using the respective synthase enzymes, namely, THCA synthase, CBDA synthase, and CBCA synthase (scheme 1). The corresponding *delta*-9-THC, CBD and CBC are then generated by decarboxylation induced by light/heat including during smoking or baking [27]. It should be noted that cannabinol (CBN) is a degradation product of THC (see section 4.3). It does not occur naturally.

Scheme 1.   Biosynthesis of main phytocannabinoids *delta*-9-tetrahydro-cannabinol (THC), cannabidiol (CBD) and cannabichromene (CBC)
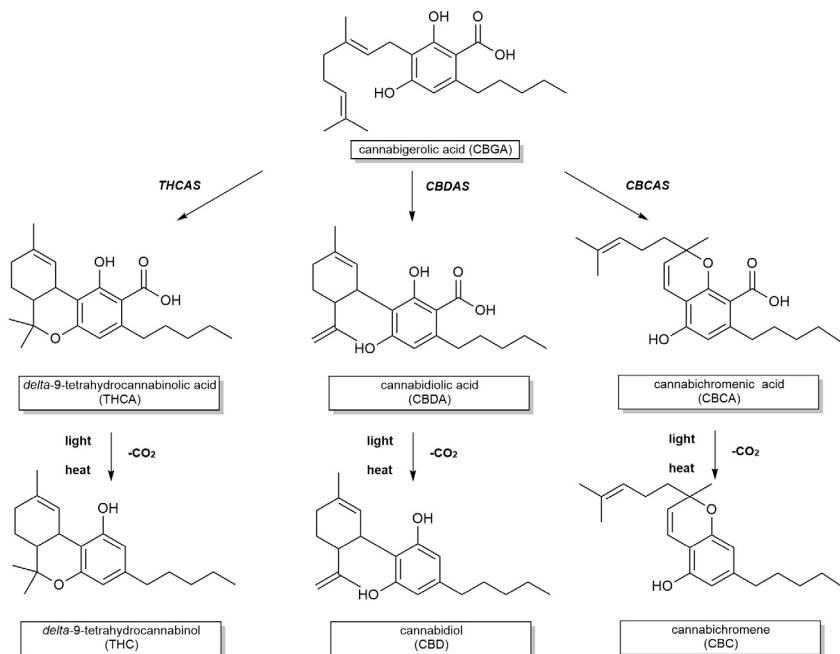
4.2   Chemical synthesis of THC

Delta-9-THC and (-)-CBD were isolated and structurally characterized by NMR [28] in 1964 and three years later, (-)-trans-*delta*-8-THC was synthesized by the Friedel-Crafts alkylation of olivetol with (-)-verbenol followed by boron trifluoride-mediated cyclization. Subsequent isomerization through chlorination and base-induced elimination led to the first synthesis of (-)-*trans-delta*-9-THC (scheme 2) [29][30]. A variety of other synthetic routes can be found in the literature and some are summarized in a recent review by Bloemendal *et.al.* [30].

Scheme 2.   Chemical synthesis of *(-)-trans-delta*-9-THC via reaction of olivetol with verbenol [29]
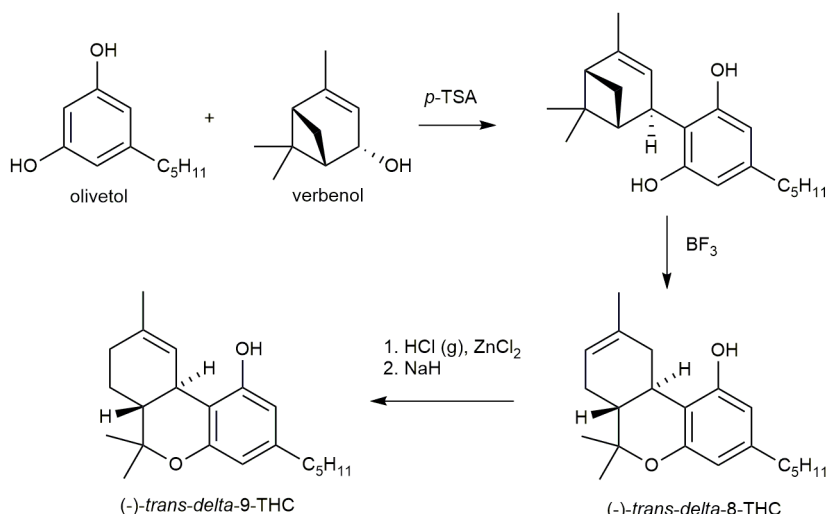
p-TSA=*para-toluenesulfonic acid, BF₃=boron trifluoride, NaH=sodium hydride.*

4.3   Stability of cannabinoids

In the handling of cannabis and cannabis products, there are four main chemical considerations with regard to the stability of cannabinoids and their implications for the storage of samples, the laboratory analysis and interpretation of results (see section 5.4), as follows:

- Decarboxylation of the THCA
- Degradation of THC to CBN through oxidation
- Conversion of the CBD to THC isomers
- Isomerism of Δ^9-THC to Δ^8-THC

4.3.1   Decarboxylation and degradation

Decarboxylation of THCA occurs when cannabis is harvested and dried, resulting in the formation of THC. Decarboxylation also occurs when a cannabis sample is heated, such as during smoking, exposure to light or during certain chemical analyses [31]. THC itself can also be converted to CBN under similar conditions. Therefore, the appropriate storage of cannabis samples and the selection of the appropriate methodologies for their chemical examination is crucial (see section 5.4) and the stability of cannabinoids is an important consideration in determining total THC content.

It is feasible to estimate the age of a given marihuana sample based on its THC and CBN content, assuming storage was at room temperature. Therefore, analysis for comparative purposes is generally not carried out more than three months after the sample is seized [32]. 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 [33]. Both the rate of decarboxylation of THCA to THC, and degradation of THC to CBN, are non-linear [34], [35].

4.3.2   Isomerism and conversion

In addition to the stereoselective syntheses mentioned previously, the conversion of CBD to Δ^9-THC/Δ^8-THC as well as other cannabinoids is well-documented in the literature [36]–[39]. In recent years, CBD and Δ^8-THC have gained increasing attention. In particular, Δ^8-THC, which is a minor component in naturally occurring cannabis plant, had been detected as a major component in products such as vape liquids, gummies and tinctures [40]–[42]. Besides Δ^8-THC, other THC isomers such as $\Delta^{6a,10a}$-THC and Δ^{10}-THC have also been detected in vape liquids.

The isomerization and conversion of Δ^9-THC to Δ^8-THC has also been reported in the literature. The reaction of Δ^9-THC with an acid results in a mixture of Δ^9-THC and Δ^8-THC. Depending on the reaction conditions (acidity, Lewis acid catalyst) different ratios of the isomers can be produced [43]. The conversion of CBD to Δ^9-THC, as well as the isomerization of Δ^9-THC to Δ^8-THC due to the use of acidic anhydride derivatizing agents has also been reported (see section 5.4.5). Unusually high levels of Δ^8-THC in cannabis samples indicate that they might be enriched with converted THC.

4.3.3   Stability of cannabinoids in cannabis resin

As the cannabis resin is commonly shaped into large, dense blocks, the degree of decarboxylation and degradation of cannabinoids and, consequently, the profile of cannabinoids vary in the different parts of the blocks, due to the different exposure of the resin to light and heat. Thus, for example, lower THCA and higher THC concentrations are observed in the exterior than in the inner part of the block due to decarboxylation [44] (see section 5.1.2). Other reactions such as polymerization and disproportionation can also occur [45] at higher temperature. Cannabis resin should also be stored in a cool, dark place.

4.3.4   Stability of cannabinoids in standard solutions

The stability of the standard solutions depends on the choice of solvent and storage conditions. Preparations of THC and THCA in methanol or methanol:chloroform (9:1) solutions are preferred over chloroform or petroleum ether as the cannabinoids are more stable in these solutions [43], [44]. The order of stability of the cannabinoids in methanol is CBN > Δ^9-THC > Δ^9-THCA.

Studies [46] indicated that methanolic stock solution of Δ^9-THC was stable for at least one year when stored at -20°C, while diluted working solutions were stable for at least one month when stored at +5°C. Stock solutions of Δ^9-THCA prepared in methanol are stable for at least three months when stored at -20°C, while diluted working solutions were stable for at least two weeks when stored at +5°C. In addition, Δ^9-THC tends to be more stable in basic solutions than acidic solutions [46].

Laboratories should verify the stability of their prepared standard solutions versus their laboratory acceptance criteria by determining the percentage difference of the concentration of the solutions on different days with respect to the concentration on day zero of the preparation, using an appropriate method (see section 5.4).

4.4   Extraction of cannabinoids in different solvents

For the chemical analysis of cannabis samples, the cannabinoids need to be extracted with a suitable solvent. A wide range of solvents can be used; however, they differ in their extraction efficiency and specificity. In general, non-polar solvents such as hexane and petroleum ether extract neutral cannabinoids well while acidic cannabinoids (such as THCA) are poorly extracted. These extracts are hence suitable for qualitative analysis and not for quantitative analysis of THCA or "total THC" content (the sum of THC and THCA). For the extraction of acidic cannabinoids, polar solvents such as isopropyl alcohol, ethanol and methanol can be used as well as solvent mixtures such as methanol:chloroform (9:1 v/v) and acetonitrile:methanol (8:2 v/v) [47], [48].

The selection of an appropriate solvent for the respective analysis is very important. Laboratories should verify the suitability of the chosen solvent for their intended purpose, and that the extraction efficiency and recovery of the chosen solvent meet the laboratory's requirements. In some cases, it may also be necessary to perform more than one extraction to achieve a satisfactory recovery [49].

4.5   THC distribution in cannabis plants and products

The THC content varies depending on the plant part [50]. The figures below refer to "total THC" content (see section 5.4.1).

10-12%	in pistillate flowers
1-2%	in leaves
0.1-0.3%	in stalks
< 0.03%	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 2020 showed, for example, that two thirds of seizures of herbal cannabis ranged between 3 per cent and 13 per cent THC. Two thirds of the resin seizures ranged between 7 per cent and 17 per cent, depending on details of the cultivation and production method (see also chapter 3.7.2), while extraction of resin and/or flowering tops can result in cannabis oil with a THC content of up to 80 per cent [51].

4.6 Drug-type cannabis versus cannabis for industrial purposes

As described in section 3.8, the total THC content is used to define cannabis for industrial purposes. Another simple way of distinguishing between drug-type cannabis and cannabis for industrial purposes is by using the ratio of the main cannabinoids THC, CBN and CBD [52].

If analysis is carried out using gas chromatography (GC) or liquid chromatography (LC) and the peak area ratio of [THC]+[CBN] : [CBD] in the chromatogram is <1, then the cannabis plant is considered to be for industrial purposes. If the ratio is >1, it is considered a drug-type cannabis. Because THC is oxidized partly to CBN after cutting and drying the plant material (see section 4.3), the sum of the peak area of THC and CBN is used and divided by the area of CBD.

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

$X>1$ indicates drug-type cannabis

$X<1$ indicates cannabis for industrial purposes

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 drug analysis laboratories 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. For seized material with obvious external characteristics, a sampling method based on the Bayesian approach may be preferred over the hypergeometric approach.

The use of a sampling approach recommended by international guidelines and adopted by the laboratory would help to preserve valuable resources and time by reducing the number of determinations needed. It is recognized that 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.

For general aspects of representative drug sampling of multi-unit samples, refer to the *Guidelines on Representative Drug Sampling* [53]. Sampling procedures for determination of purity have specific aspects that need to be considered, and the reader is directed to the *Guidelines on Sampling of Illicit Drugs for Quantitative Analysis* published by the European Network of Forensic Science Institutes (ENFSI) [54].

5.1.1 Sampling of plants (indoor and outdoor plantations)

The procedures below are based on the sampling procedure recommended by the European Union for outdoor cannabis plantations for industrial hemp [55] and have been adapted to take into account the practical aspects and variety of cannabis products in the illicit market.

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 (figure III) 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 [53]. An example of sampling a hemp field is given in reference 53 in relation to the comparison of the hypergeometric and Bayesian methods used for sampling of cannabis plants.

Figure III.   Sampling fruiting tops of the cannabis plant

Wherever possible, the sample should be dried before sending to the laboratory. If it must be stored before being analysed, it should be kept in a dark and cool place to avoid degradation of the main cannabinoids. At this stage, THC is still sensitive to air and ultraviolet (UV) light, which can oxidize THC to CBN and, therefore, precautions as to the storage conditions should be taken (see section 4.3).

5.1.2   Sampling of seized cannabis products

For general aspects of qualitative sampling of multi-unit samples, reference 53 can be consulted. For material with obvious external characteristics, that is, material all recognizable as cannabis, a sampling method based on the Bayesian approach may be preferred over the hypergeometric approach, as the former allows the use of other relevant, so-called prior information (e.g., external characteristics) [53].

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 for the sampling of plants (see section 5.1.1), 30 pieces of herbal cannabis considered to belong to the same phenotype are taken as one sample. If less material is available, all should be taken. Coarse stem material is cut off. Seeds in the fruiting tops remain in the exhibit. Moist material should be packaged in paper bags. For dried material plastic bags are suitable.

Cannabis resin

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 (see section 4.3).

Cannabis oil (hashish oil)

The amount of cannabis oil (see section 5.4) required can be taken directly from the bulk sample.

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. Other analytical techniques are acceptable if the analytical scheme and the level of selectivity is sufficient to scientifically support the conclusion while respecting the jurisdiction and laboratory protocols. General rules for method selection have been formulated by the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) [56].

5.3   Physical examination

The methods that can be used to identify cannabis and cannabis products depend on the nature of the material for examination. Herbal material can be identified based on its morphological characteristics. In the absence of morphological characteristics, as in the case of cannabis resin and hashish oil, the identification can only be based on chemical analysis, demonstrating the presence of cannabinoids, such as THC, its degradation product CBN, and/or CBD (see section 4.4).

The physical examination of cannabis based on its macroscopic and microscopic characteristics is described below.

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, temperature, and space.

Cannabis is an annual flowering herb [57]. The plants can vary in height from 0.2 m to 6 m, although most of the plants reach heights of approximately 1-3 m. Fresh plants contain 60-80 per cent of water by weight.

The plant is usually dioecious, where the male and female flowers occur on separate plants. Occasionally monoecious plants are encountered, where the male and female flowers occur on the same plant or even on the same branch. Growers prefer female plants over male plants for their higher potency and hence male plants are usually discarded as pollination of the female flowers will decrease resin production (see 3.6.1 and 3.6.4). Occasionally, hermaphroditism (see section 3.6.3) is also observed, where the formation of anthers (characteristic of the male flowers) occurs within the female flowers [58].

Male plants are usually taller but less robust than female plants while the female plants are leafier and with more branches. However, it is not possible to determine the sex of the plants until flowers are formed. Stems are green, angular, erect, sometimes hollow, and longitudinally grooved (figure IV). The extent of branching, like plant height, depends on environmental and hereditary factors as well as the method of cultivation.

The first pair of true leaves with single leaflets appears above the cotyledons, and subsequent pairs of leaves are oppositely arranged and at right angles to the previous pair (decussate arrangement). The number of leaflets increases, and the leaf arrangement changes from decussate (oppositely arranged) to alternate as the plant matures. The number of leaflets per leaf also decreases until single leaflet before flowering appears.

Figure IV. Grooved stem of *Cannabis sativa* L.

Figure V. Abaxial (left) and adaxial (right) surfaces of *Cannabis sativa* L. leaves
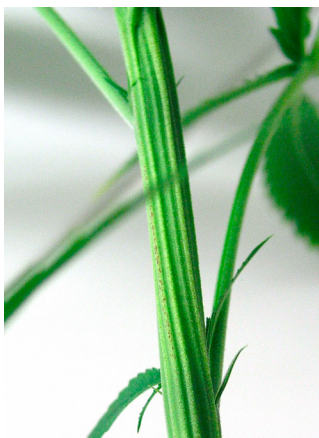
© Federal Criminal Police, Brazil

Leaf stalks (petioles) are 2-7 cm long with a narrow groove along the upper side. The leaf is palmate (also known digitate) and comprises a usually odd number of lanceolate leaflet blades, often in the range of 3 to 9 leaflets. Occasionally, an even number of leaflets are observed. The shape of the leaflets ranges from linear-lanceolate to oblanceolate or broad. The margins of the leaf are coarsely serrated, the teeth pointing towards the tips; the veins run out obliquely from the midrib to the tips of the teeth (pinnate venation). The lower (abaxial) surfaces are paler green in colour than the upper (adaxial) surface of the leaf (figure V).

The male inflorescence is loosely arranged, with many branching, and stands out from the leaves, with branches up to 20 cm long. 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 prominent anthers (figures VI and VIII).

Figure VI.   Morphological characteristics of male flowers
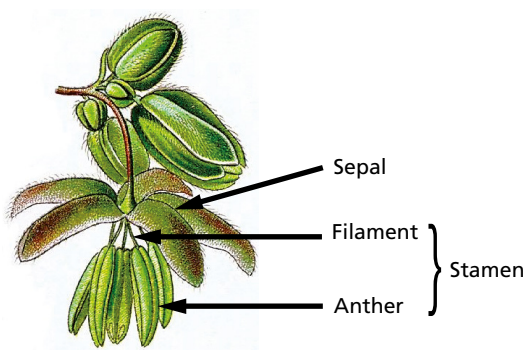
The female inflorescence is compact and shorter than the leaves. The pistillate (female) flowers are almost sessile and are borne in pairs. The female flowers are subtended by a pair of stipules at the node of the stem. Each flower has a small green bract enclosing a single ovary with two long, slender stigmas projecting well above the bract (figures VII and VIII).

Figure VII.   Morphological characteristics of female flower and seed (achene)

The fruit, an achene, contains a single seed with a hard shell tightly covered by the thin wall of the ovary. It is ellipsoid, slightly compressed, smooth, about 2-5 mm long, generally brownish and mottled. Its surface has a characteristic reticulate ("tortoise shell") pattern (figure VII). The fruit is commonly mistaken by the layperson as a seed.

Figure VIII.   Clusters of male (left) and female flowers (right)

5.3.2   Microscopic characteristics

Cannabis sativa L. has characteristic morphological features and can be identified by the presence of trichomes (i.e. hair-like projections from a plant epidermal cell) which are the microscopic structures on the surface of the plant [32], [59], [60]. Two types of trichomes, non-glandular trichomes and glandular trichomes, occur and can be observed with a magnification factor of 40 as shown in figures IX and X. A schematic presentation of a cross section of a bract from the fruiting plant with the different types of trichomes is shown in figure XI.

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

- Characteristic bear claw-shaped trichomes found only on the upper (adaxial) surface of the cannabis leaves. These trichomes may sometimes have calcium carbonate crystals (cystoliths) visible at their bases (cystolithic trichomes). Frequently, the trichome is broken and the cystolith freed.

- Non-cystolithic long and slender trichomes occur on the lower side (abaxial) of the leaves, bracts, stems, stipules and petioles, and lack the enlarged base. They are usually more profuse than cystolithic trichomes.

- 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 IX.   Microscopic view of non-glandular trichomes [61]
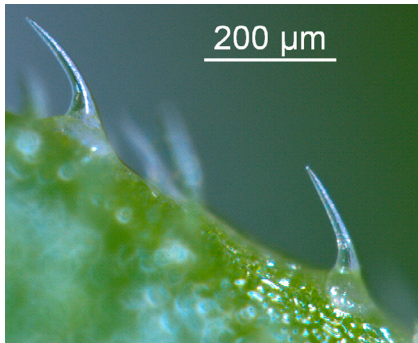
© Wissenschaftlicher Dienst Stadtpolizei Zürich

Cystolithic trichomes

© Wissenschaftlicher Dienst Stadtpolizei Zürich

Non-cystolithic trichomes

(b) Glandular trichomes are the structures where the cannabis resin is produced and stored. These glandular trichomes are mainly associated with the female flower but they can also be found on the leaves, on the veins, and occasionally on the stems of young plants. There are three main types of glandular trichomes:

- Bulbous: small swollen heads with one-celled stalks, present on all vegetative parts and flowers but difficult to observe due to their small size
- Capitate-sessile: large globular head without stalk, generally found on the lower surface of the leaves, and sometimes also observed on the upper surface of the leaves, veins and stems
- Capitate-stalked: most important glandular trichomes that appear as large globular heads on long multicellular stalks, present in large numbers on the bracts of the female flowers and sometimes observed on leaves and the veins of leaves

Figure X.   Microscopic view of glandular trichomes [61]
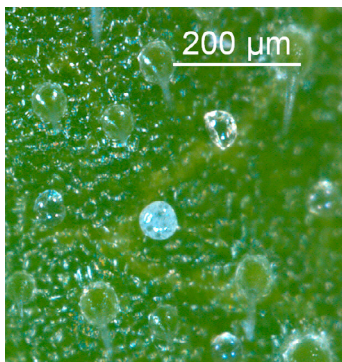
Sessile glands
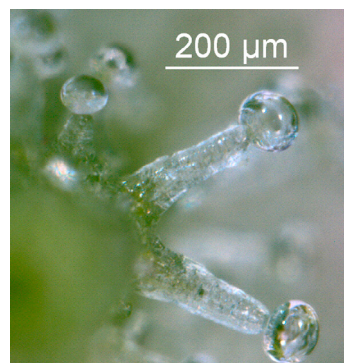
Stalked glandular trichomes

Figure XI.   Cross section of a bract from the fruiting plant [62]
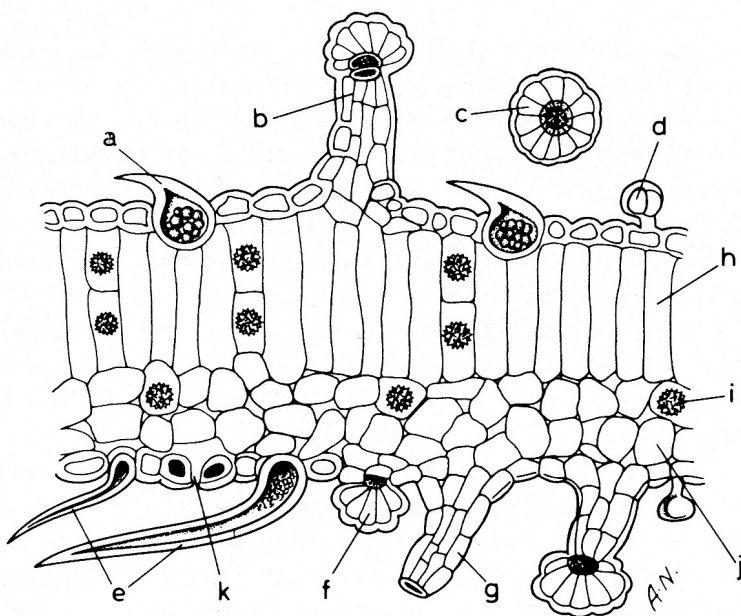
a: cystolithic trichome; b: large glandular trichome with several cells in head and stalk; c: head of one of the large glandular trichomes; d: small glandular trichome with bicellular head and unicellular stalk; e: thick-walled conical trichomes; f: large developing glandular trichome; g: stalk of a large glandular trichome; h: palisade cell; i: cluster crystal; j: parenchymal cell; k: stoma

The combination of cystolithic hairs on the leaf upper surface and longer trichomes and sessile glands on the lower surface is unique to *Cannabis sativa* L., thus enabling positive identification of even fragmented material. It should be noted that very immature seedlings and stems with no leaf attached cannot be definitively identified as cannabis by botanical examination.

For details on cannabis identification and other microscopy techniques, the literature can be consulted [63]–[66].

5.3.3   Differentiation of plants similar to cannabis

Several plant species may have a number of morphological characteristics that show some resemblance to *Cannabis sativa* L. Some of them are illustrated in figure XII. A closer look at their full macroscopic and/or microscopic characteristics enables differentiation, based on the distinctive morphological features of cannabis such as the appearance of leaf, stem, male and female flowers, as well as the presence of different types of trichomes on plant parts under the microscope [15] (see sections 5.3.1 and 5.3.2).

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

Hibiscus cannabinus

Hacer palmatum

Urtica cannabina

Dizygotheca elegantissima

Potentilla recta

Datisca cannabina

Some plants possess trichomes that may be confused with those present on *Cannabis sativa* L. and care should be taken in definitive identification. However, the combination of bear claw-shaped trichomes on the leaf upper surface and unicellular trichomes on the lower surface is unique to *Cannabis sativa* L. and enables the positive identification of even fragmented leaf material (see section 5.3.2).

In addition, there are also simple presumptive tests available to differentiate *Cannabis sativa* L. from other plant materials (see section 5.4.3), as well as confirmatory techniques to identify the cannabinoids that are found only in cannabis plant (see section 5.4).

Fruits (achenes) of the common hop (*Humulus lupulus*) and Japanese hop (*Humulus japonicus*) might be confused with the seeds of *Cannabis sativa* L. However, these can be effectively differentiated through the presence of a characteristic reticulate ("tortoise shell") pattern on the surface of cannabis fruits (figure XIII) [67].

Figure XIII.   Fruits (achenes) which bear morphological characteristics with some resemblance to the seeds of *Cannabis sativa* L.

Cannabis sativa   *Humulus lupulus*   *Humulus japonicus*

5.4   Chemical examination

5.4.1   General aspects

The chemical examination of cannabis plant material and cannabis products may be performed by using a range of different analytical techniques and methodologies such as simple colour tests, thin layer chromatography (TLC), gas chromatography-flame ionization detector (GC-FID), gas chromatography-mass spectrometry (GC-MS), liquid chromatography (LC) or liquid chromatography-mass spectroscopy (LC-MS or LC-MS/MS) and combinations thereof. The selection of the appropriate method and approach depends on the purpose of the analysis, and respective analytical requirements, for example qualitative and/or quantitative. Analysis may also be required to identify and often quantitate low levels of Δ^9-THC, differentiate its isomers, in particular Δ^9-THC and Δ^8-THC, and identify other cannabinoids present in the sample. Any method used in a laboratory should be validated and/or verified to demonstrate that the method and the analytical instrument provide accurate and precise results, and are fit-for-purpose. Quality controls and their frequency must be established according to the quality management policy of the laboratory.

Some important aspects related to the nature of cannabis and cannabis products should be considered prior to the analysis. The selection of sample should be appropriate and representative of the bulk material to yield meaningful results for interpretation. For example, herbal cannabis is a heterogeneous material and should be homogenized prior to sampling for quantitative analysis (see section 5.4.2). Appropriate selection of extraction solvents is important for the extraction of the neutral cannabinoids such as THC, CBN, CBD as well as the polar acidic cannabinoids, such as THCA (see section 4.4). In addition, the matrix of the different samples should be considered in the extraction procedure and preparation of sample to avoid any potential matrix interferences in the analysis.

The decarboxylation of THCA to THC at high temperatures during analysis should be considered, especially when GC analysis is performed [68]. In terms of quantitative analysis, it is a choice whether THCA and THC are quantitated separately or whether the total THC content of the cannabis sample, which is the combined amount (sum) of THC and THCA, is measured. Total THC is obtained by decarboxylation of THCA into THC and can occur during or be carried out prior to analysis (see section 5.4.5 and 5.4.7). A higher temperature may also cause THC decomposition to CBN; therefore, the analytical conditions must be validated to ensure that they yield complete decarboxylation of THCA and do not cause decomposition of THC [69].

The conversion of CBD to Δ^9-THC/Δ^8-THC and the isomerization of Δ^9-THC to Δ^8-THC under acidic conditions should also be considered for analysis (see section 4.3). Δ^8-THC is a minor naturally occurring THC isomer but might be present in some cannabis products. Methods with high resolution are required for the detection and differentiation of isomers, specifically Δ^9-THC and Δ^8-THC, such as GC-MS, LC-MS or LC-MS/MS. Sensitive methods are also required for the detection of low concentrations of THC.

5.4.2   Sample preparation for chemical examination

Preparation of herbal cannabis

The selection of cannabis plant materials for analysis should be performed with care, particularly for quantitative analysis, as cannabis is a heterogeneous material and different parts of the plant would give rise to variations of analytical results (see section 4.5). However, the selection of material and the procedures for their preparation depend on the analytical requirements.

For qualitative analysis, 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. For quantitative analysis, homogenization of the herbal material is necessary before taking samples for analysis. Bulk material of herbal cannabis consists of different parts (typically buds, leaves, stems, and small fragments). Homogenization is necessary to enable accurate quantitation of the sample as only a small portion is weighed for analysis [54].

For the drying of samples, different procedures are provided in the literature. For example, selected cannabis plant materials are dried by air at room temperature for several days, or at 35-40°C in a forced ventilation oven for 24 hours [31], [70], [71]. Comminution of the cannabis material can be carried out with a pestle and mortar (manually), or a laboratory mill (e.g., knife or ball mill) for different sample sizes. The selection of an appropriate set of milling or grinding parameters [72] depends on the type of sample encountered and should be verified by the laboratory before use (table 3). The use of dry ice to aid grinding of the plant materials has also been reported [49].

Table 3. Examples of milling/grinding parameters

Type of comminution technique	Container size (mL)	Milling/grinding parameters	Sample size (grams)
Knife mill	40	1 cycle of 2 mins at 25,000 rpm	1 to 4
	100		2 to 5
	1,000	15 cycles of 30 sec at 10,000 rpm	20 to 80

The particle size after milling or grinding can be determined by sieving the pulverized material through a sieve of known mesh size. The particle size should be appropriate for the type of analysis to be carried out.

Sieving ensures homogeneity of the samples. Should the sieving process be skipped, the laboratory must demonstrate that the homogeneity of the sample is within the accepted tolerance. Sample homogeneity of the pulverized plant material can be determined by taking an appropriate number of test samples across different sectors of the comminuted material and quantifying the cannabinoids (e.g., THC or CBN) present.

The following is an example of sample preparation of herbal cannabis as part of the validated GC and LC methods in this manual [73], [74].

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., 10,000 rpm) and sieved (mesh size 1 mm). It should be noted that both drying and sieving are part of the validated methods described in this manual.

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.

Preparation of cannabis oil (hashish oil)

Cannabis oil can be used directly for analysis or diluted appropriately to avoid overloading sensitive instrumentation.

5.4.3   Presumptive colour tests

Presumptive colour tests in general are non-specific tests that give a positive result simply by a colour change being observed by the addition of reagents to the sample of interest. However, 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 with the test used for cannabis [75]. However, a positive colour test only provides an indication of the possible presence of cannabis-containing material and not a definitive identification of cannabis. Confirmatory analysis is always required.

A negative control is required when undertaking presumptive testing to ensure that any colour change observed is due to the reaction between the substance(s) in the sample and the reagents, and not due to the reagents alone. It also ensures that the apparatus being used is thoroughly clean with no possibility of contamination. In addition, a positive control should be carried out on a reference material containing a mixture of cannabinoid reference standards or a known cannabis sample to verify the test results (colour change) and the functionality and reliability of all test reagents.

Fast Corinth V, Fast Blue B and Rapid Duquenois tests, commonly used for preliminary cannabis testing, are described below.

Fast Corinth V salt test

The test is performed on a filter paper.		
Reagent A:	Petroleum ether	
Reagent B:	Fast Corinth V salt*	1% w/w in anhydrous sodium sulfate
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 produce the same result.		

*Fast Corinth V salt ($C_{15}H_{14}N_5O_3 \cdot 0.5$ ZnCl$_4$):
   Dichlorozinc, 2-methoxy-5-methyl-4-(4-methyl-2-nitrophenyl)diazenyl-benzenediazonium dichloride;
   Azoic diazo component 39

Fast Blue B salt test

The test is performed on a filter paper.		
Reagent A:	Petroleum ether	
Reagent B:	Fast Blue B salt**	1% w/w diluted with anhydrous sodium sulfate
Reagent C:	Sodium bicarbonate	10% w/w aqueous solution
Method		
Same procedure as above 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 produced by the main cannabinoids: THC red, CBN purple, and CBD orange.		
Note		
Fast Blue B salt should be stored in the refrigerator. At room temperature, it tends to deteriorate over time and the powder becomes solid and hard (especially in warm regions).		

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

Rapid Duquenois-Levine test

The test is performed in a test tube.		
Reagent A:	Acetaldehyde (A1) Vanillin (A2)	0.5 mL (A1) and 0.4 g (A2) diluted in 20 mL ethanol
Reagent B:	Concentrated hydrochloric acid	
Reagent C:	Chloroform	
Method		
Place a small amount of the suspect material in a test tube. Add 2 mL of reagent A and shake the test tube for one minute. Add 2 mL of reagent B and shake the mixture again. Allow to stand for ten minutes. When a colour develops, add 2 mL of reagent C and mix gently.		
Results		
A violet colour of the lower (chloroform) layer is indicative of a cannabis-containing product.		
Notes		
The solution must be stored in a cool dark place and discarded if its colour turns to deep yellow. This test is not as sensitive as the two filter paper tests above.		

4-Aminophenol test (4-AP)

Recent studies have demonstrated the utility of 4-aminophenol (4-AP) colour test for the initial differentiation between drug-type and cannabis for industrial purposes [76].

The test is performed on a spot plate or in a test tube.		
Reagent A:	4-Aminophenol (4-AP)	300 mg 4-AP in 995 mL ethanol and 5 mL of 2 M hydrochloric acid
Reagent B:	Sodium hydroxide	30 g of sodium hydroxide in 300 mL of water and 700 mL ethanol

Method

Place a small amount (e.g., 5 mg) of plant material sample on a spot plate or in a test tube.

Add a sufficient amount of reagent A to cover the sample.

Add two to four drops of reagent B. The number of drops depends on the vessel used and the volume of reagent A needed to cover the sample.

Results

A blue colour is generated when the level of THC is approximately three times higher than that of CBD in the cannabis sample.

A pink colour is indicative of a THC level approximately three times lower than the level of CBD.

Notes

The colour change should be noted within the first 1–2 minutes after addition of Reagent B.

Inconclusive results (any colour other than blue and pink) are observed when the levels of THC and CBD are within a factor of three from each other, demonstrating the limitations of the test under those scenarios.

No significant reaction was noted for a number of household herbs except for sage and oregano.

Reagents A and B stored in the refrigerator (8°C) in amber containers are stable for at least six months.

5.4.4 Thin-layer chromatography

Thin-layer chromatography (TLC) is a commonly used technique for the separation and identification of drugs. It is inexpensive, rapid and flexible in the selection of both the stationary and mobile phase, and amenable to a wide variety of substances, ranging from the most polar to non-polar materials. TLC provides identification of the components when used in conjunction with a drug reference standard. It is used in combination with other techniques to confirm unequivocally the identity of the drug.

There are several TLC methods for the qualitative and semi-quantitative analysis of cannabis, using a variety of stationary phases (TLC plates) and solvent systems, and slightly different sample preparation and spot visualization/detection techniques. Many of these methods produce acceptable results. However, each method that is newly introduced to a laboratory must be validated and/or verified prior to routine use.

TLC plates (stationary phases)

Coating: Silica gel G with layer thickness of 0.25 mm and containing an inert indicator, which fluoresces under UV light wavelength 254 nm (Silica gel GF_{254}).

Typical plate sizes: 20 x 20 cm; 20 x 10 cm; 10 x 5 cm (the latter should be used with the 10 cm side vertical with the TLC tank).

HPTLC (High Performance TLC) plates can also be used. They have an optimized silica gel 60 sorbent with a particle size of only 5-6 μm, compared to the 10-12 μm used in conventional TLC plates, and offer quicker analysis and sensitivity (see TLC methods below).

Plates that are prepared by the analyst must be activated before use by placing them into an oven at 120ºC for at least 10 to 30 minutes. Plates are then stored in a grease-free desiccator over orange silica gel. Blue silica gel can also be used. However, due care should be taken as blue silica gel contains cobalt (II) chloride which is possibly carcinogenic to humans. Heat activation is not required for commercially available coated plates.

Methods

The methods described below have been field-tested and are considered fit-for-purpose.

TLC developing solvent systems

Prepare the selected developing solvent system (as described below) as accurately as possible by using pipettes, dispensers and measuring cylinders. Leave the solvent system in the TLC tank for a sufficient time to allow vapour phase saturation to be achieved prior to analysis (with adsorbent paper-lined tanks, this takes approximately 5 minutes).

Method 1

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

Method 2

Plate: Precoated TLC plastic plate of silica gel 60 F_{254}, 10 cm (height) × 20 cm (width), thickness 0.2 mm.		
System D: (only for neutral cannabinoids)	Toluene Diethylamine	97% v/v 3% v/v
Tank conditioning: 15 minutes with filter paper on one side		
Plate development: Approximately 10 minutes		

Sample preparation

If the purpose of the examination is qualitative (e.g., to confirm the micro- or macroscopic evidence that the suspect material is cannabis), homogenization of the herbal material is not necessary (see section 5.4.2). 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 should be designed to produce final solutions with THC concentrations of about 0.5 mg/mL. Typical levels of THC in cannabis materials are provided in section 4.5.

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 prior to chromatography. 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. However, any modification to the method described needs to be verified and approved in the analyst's laboratory.

Since cannabinoids are easily soluble in most organic solvents, methanol, petroleum ether, n-hexane, toluene, chloroform and solvent combinations, for example methanol:chloroform (9:1 v/v), are equally suitable for their extraction. However, 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 (see also section 4.4).

For qualitative analysis, the use of petroleum ether is sufficient for the extraction of the main cannabinoids, while for the purposes of quantitation and total THC determinations other solvents or combination of solvents should 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 (see section 4.3).

Spotting and developing

Apply, for example, 1 μL to 5 μL aliquots of sample solution, 2 μL of the standard solutions, and 2 μL of solvent (negative control) as separate spots on the TLC plate. It is also recommended to run a blank solvent at the same time to show that the solvent used to extract the sample does not contain any cannabinoids. Spotting must be done carefully to avoid damaging the surface of the plate.

Analytical notes

- The starting point of the run (the "spotting line") should be 2 cm from the bottom of the plate.

- The spacing between applications of sample (spotting points) should be at least 1 cm and spots should not be placed closer than 1.5 cm to the side edge of the plate.

- To avoid diffuse spots during development, the size of the sample spot should be as small as possible (2 mm) by applying solutions in aliquots rather than a single discharge.

- Allow spots to dry and place the plate into a solvent-saturated tank. (Saturation of the vapour phase is achieved by using solvent-saturated pads or filter paper as lining of the tank.)

- The solvent in the tank must be below the spotting line.

- Remove plate from the development tank as soon as possible when the solvent has reached the development line (10 cm from starting line) marked beforehand; otherwise, diffuse spots will occur.

Visualization/detection

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 subject to thermal decomposition. Use of a fume hood is recommended.

Visualization/detection methods

i. UV light at 254 nm
Dark spots against a green background are observed. The spots are marked and, if necessary, a digital photograph recorded.

ii. Spray reagent
Fast Blue B or Fast Blue BB salt as well as Fast Blue RR salt is used for the preparation of the spray reagent as described below (table 4).

Table 4.   Preparation of spray reagents

Reagent 1	Fast Blue B salt	50 mg in 20 mL of NaOH (0.1N)
Reagent 2	Fast Blue B salt	50 mg in 1 mL of water, then 20 mL of methanol is added.
Reagent 3	Fast Blue BB salt	20 mg in 25 mL of water, followed by addition of about 3 mL of 2.5M NaOH
Reagent 4	Fast Blue RR salt	50 mg in 25 mL of methanol or methanol:water (1:1)

Daily preparation of the spray reagent may not be required when Fast Blue BB or Fast Blue RR are used.

If the plate with the analysis results needs to be preserved, the following spraying sequence can be used: Diethylamine – Fast blue B solution – Diethylamine. After drying with hot air, or overnight at room temperature, the plate can be sealed in clear plastic bags and can be stored for a long time without darkening.

Analytical notes

- For proper colour development the TLC plate should be made alkaline. Therefore, diethylamine should be sprayed on the plate before using reagent 2 (table 4).

- The TLC plate should not be over-wetted with the spraying reagent as spot diffusion may occur.

- Appropriate precautions must be taken when using Fast Blue B as it is claimed to be carcinogenic.

- Fast Blue RR and Fast Blue BB are suitable alternatives, although both have a slower response time. Fast Blue BB gives spots with more intense and vivid colours.

Results (interpretation)

After visualization, mark spots (e.g., by pencil) and calculate retardation factor (R_f) values.

$$R_f \; = \; \frac{\text{Migration distance: from origin to centre of spot}}{\text{Development distance: from origin to solvent front}}$$

Table 5. R$_f$ x 100 values of the main cannabinoids using the above methods

Compound	*Developing system, R$_f$ × 100 values*			
	A	B	C	D
CBN	33	26	47	28
THC	37	38	49	35
CBD	42	42	47	41
THCA	6	–	36	-

Results in table 5 (R$_f$ × 100 values) for developing systems A, B and C refer to employment of methods using HPTLC plates, as described in this section. Conventional 20 cm x 20 cm plates with a 0.25 mm thick layer of silica gel provide comparable separations, but the corresponding R$_f$ values will have to be determined. Developing system C is only recommended for the separation and identification of cannabinoid acids. It does not provide adequate separation of CBN, THC and CBD.

Semi-quantitative analysis of cannabis using TLC

Analytical notes

- R$_f$ values are not always reproducible due to small changes in plate composition and activation, solvent systems, tank saturation or development distance. They are also subject to variations depending on laboratory conditions (temperature, humidity, etc.). Therefore, the R$_f$ values provided are indications of the chromatographic behaviour of the substances listed.

- It is essential that reference standards be run simultaneously on the same plate.

- For identification purposes, both the R$_f$ value and the colour of the spots after spraying with the appropriate visualization reagents should always be considered.

In some cases, it may be useful to quickly estimate the THC concentration of the cannabis sample. This can be done using the developed TLC plate and comparing the intensity of the TLC spot of a sample solution with TLC spots produced by adding increasing volumes of a THC reference standard solution of known concentration. For example:

Cannabis sample preparation

Weight of cannabis sampled	= 500 mg
Volume of solvent used for extraction	= 10 mL
Volume of sample spot applied on TLC plate	= 1 µL

THC standard preparation

Concentration of THC standard	= 0.5 mg/mL
Volume of standard spot applied on TLC plate	= 1, 2, 3 µL

Compare the intensity of the spot of the sample with the spots of the various concentrations of the THC standard solution. If the sample spot has a similar colour intensity as the 3 µL THC standard spot, the neutral THC content in the cannabis sample may be estimated using the following formula, using 3 µL of THC standard solution as an example:

$$\frac{Concentration\ of\ THC\ in\ sample\ spot\ x\ volume\ of\ solvent\ used\ for\ extraction\ x\ 100\%}{weight\ of\ sampled\ cannabis}$$

$$\approx \frac{Concentration\ of\ THC\ in\ standard\ spot\ x\ volume\ of\ solvent\ used\ for\ extraction\ x\ 100\%}{weight\ of\ sampled\ cannabis}$$

$$= \frac{(0.5\ \frac{mg}{ml}\ x\ 3)\ x\ 10\ ml\ x\ 100\%}{500\ mg} = 3.0\%$$

5.4.5   Gas chromatography-flame ionization detection

Gas chromatography-flame ionization detection (GC-FID) is a commonly used technique in seized drugs analysis, and can be employed for qualitative and quantitative analysis. An important consideration in the use of GC in the analysis of cannabis is decarboxylation of acidic cannabinoids, in particular THCA. The extent of the THCA decarboxylation to THC depends on instrument and method parameters and influences the determination of the total THC content.

Decarboxylation can be carried out prior to the analysis. Alternatively, derivatization, which prevents the decarboxylation, is an approach to quantitate THC and THCA separately. It should be also noted that THC can decompose to CBN at higher GC temperatures. Whatever approach is taken for the determination of THC, an appropriate method validation should be carried out.

Method

The parameters listed below are from a validated method [73]. 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.

GC-FID operational conditions	
Column:	15 m x 0.25 mm i.d., 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 (0.5 mg/mL)
Injection:	1.5 µL, Split
Elution order:	CBD, THC, CBN

Sample preparation

Extract 200 mg of dry and homogenized herbal cannabis (see section 5.4.2) 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 cannabis oil (hashish oil), an amount of about 50 mg is sufficient.

THCA decarboxylation prior to the analysis

It is strongly recommended to carry out a decarboxylation step prior to the GC analysis if the specific gas chromatograph system and analysis conditions do not yield complete decarboxylation of THCA.

Transfer 500 µL of the solution to a 2 mL GC vial. Put the vial into a heating unit (150°C) or 12 minutes for the THCA to be decarboxylated. When the solvent is evaporated dissolve the residue in 1.5 mL ethanol, shake the vial well and use the solution for analysis.

Calibration

As THC reference material may degrade, the quantification of THC using CBN reference material for the calibration is widely accepted. In theory the correlation factor CBN to THC is 1.00 and CBN can be used for the calculation of the THC content [74]. For validation purposes, it is good policy to measure and monitor CBN ratio with a similar compound like CBD to show the validity of the theoretical correlation factor in the given gas chromatograph.

Solutions for calibration

Prepare CBN standard solutions in 2 mL GC vials as described below:

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

No.	ID/SS	ISTD solution	Volume of ethanol	Concentration
Std 1	50 µL ID	+ 500 µL ISTD-solution	+ ~ 950 µL	0.1%
Std 2	250 µL ID	+ 500 µL ISTD-solution	+ ~ 750 µL	0.5%
Std 3	50 µL SS	+ 500 µL ISTD-solution	+ ~ 950 µL	1%
Std 4	150 µL SS	+ 500 µL ISTD-solution	+ ~ 850 µL	3%
Std 5	250 µL SS	+ 500 µL ISTD-solution	+ ~ 750 µL	5%
Std 6	500 µL SS	+ 500 µL ISTD-solution	+ ~ 500 µL	10%
Std 7	800 µL SS	+ 500 µL ISTD-solution	+ ~ 200 µL	16%

The range of concentrations of the standard solution used should be adjusted to the expected amount of THC in the sample to be analysed. Standard solutions must be stored in a cool, dark place, and can be used for a maximum of four months.

Derivatization

If THCA has to be analysed separately, that is, without decarboxylation, 1.5 mL aliquots of the above (non-thermally decarboxylated) extract have 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%)

Solvents such as ethanol should be removed, as they can also react with the derivatizing agent. They are usually removed by a gentle stream of nitrogen. The residue is taken up in 1.5 mL chloroform. Add 100 µL MSTFA and heat for 30 minutes at 70°C. The resulting solution can be analysed directly.

As mentioned in section 4.3., cyclization of CBD to THC, and isomerization of Δ^9-THC to Δ^8-THC can occur under acidic conditions. Therefore, acidic derivatizing reagents, such as trifluoroacetic anhydride-hexafluoroisopropyl alcohol (TFAA-HFIP) or pentafluoropropionic anhydride-pentafluoropropanol (PFPA-PFPOH) are not suitable reagents for derivatization of THC and CBD [77], [78].

5.4.6   Gas chromatography-mass spectrometry

Gas chromatography-mass spectrometry (GC-MS) is one of the most widely used techniques for the identification of drug samples in forensics. As a hyphenated technique, it combines the separation power and sensitivity of a GC with the analyte specificity of a spectrometric technique. It can provide highly specific spectral data on individual compounds in a complex mixture. However, this technique alone is not suitable for the

identification of thermally unstable cannabinoid acids, for example THCA and CBDA. For the identification of these compounds, derivatization before the GC-MS analysis or the use of a non-thermal method (e.g., FTIR or LC) would be required.

The GC-MS analysis can be performed analogous to the GC-FID analysis (see section 5.4.5). Parameters from a validated method for the qualitative analysis of common cannabinoids, including cannabinoid acids by derivatization, are given below.

Sample preparation with derivatization

Transfer 1 mL (or an appropriate amount) of sample extract (in a non-silylizable solvent such as petroleum ether) into a sample vial. Add 50 µL of MSTFA activated III (MSTFA activated with imidazole). Seal the sample vial and vortex to mix well. Allow the mixture to stand for 30 minutes at room temperature. Vortex and inject 1 µL of the sample into the GC-MS.

Column:	12.5 m x 0.20 mm i.d., 0.33 µm
Phase:	Agilent HP-5MS(5%-phenyl)–methylpolysiloxane
Carrier:	Helium, 1.6 mL/min, constant flow
Injector:	Split/splitless, 280°C
Split ratio:	70:1
Oven:	0.5 min at 80°C, 40°C/min 300°C, 1 min
Detector:	Mass detector, electron ionization mode tuned to perfluorotributylamine (PFTBA) Electron energy: 70 eV Interface temperature: 280°C Scan mode: positive Scan rate: 2^N, where N = 1 Ion source temperature: 230°C Quadrupole temperature: 150°C Gain factor: 1
Injection:	1.0 µl, split

Reference spectra of the most common cannabinoids including THC isomers, in derivatized or underivatized form, are available in common commercial MS databases and have been reported in the literature [79], [80].

The GC retention times of some THC isomers and other cannabinoids using the above method are shown in table 6 below.

Table 6.   GC retention times and GC-MS mass ions

Cannabinoid	Approx. retention time (min)	Mass ion (Normalized Abundance %)
CBD 2TMS*	5.05	458 (7), 390 (100), 351 (21), 337 (51), 324 (15), 319 (17), 301 (32)
CBD	5.37	314 (7), 246 (13), 231 (100), 193 (7), 174 (8), 121 (6)
(6aR,9S)-Δ^{10}-THC TMS	5.37	386 (89), 371 (100), 343 (34), 330 (18), 315 (39), 303 (31)
(6aR,9R)-Δ^{10}-THC TMS	5.45	386 (92), 371 (100), 343 (31), 330 (19), 315 (44), 303 (32)
CBN TMS	5.49	382 (10), 367 (100), 323 (2), 310 (5), 295 (3), 238 (3)
(-)-Δ^{8}-THC	5.53	314 (81), 299 (9), 271 (37), 258 (38), 243 (5), 231 (100)
(-)-Δ^{8}-THC TMS	5.54	386 (69), 371 (8), 343 (22), 330 (44), 315 (3), 303 (100)
(6aR,9R)-Δ^{10}-THC	5.54	314 (72), 299 (100), 271 (44), 258 (24), 243 (27), 231 (45)
(-)-Δ^{9}-THC	5.58	314 (85), 299 (100), 271 (46), 258 (23), 243 (30), 231 (70)
(-)-Δ^{9}-THC TMS	5.58	386 (98), 371 (100), 343 (29), 330 (16), 315 (53), 303 (45)
(6aR,9S)-Δ^{10}-THC	5.62	314 (64), 299 (100), 271 (48), 258 (22), 243 (25), 231 (41)
CBN	5.71	310 (11), 295 (100), 251 (4), 238 (13), 223 (4), 165 (3)

*TMS= trimethylsilyl

Using the above method, (-)-Δ^{9}-THC can be differentiated from (-)-Δ^{8}-THC, (6aR,9R)-Δ^{10}-THC and (6aR,9S)-Δ^{10}-THC by their retention times. Due to different placements of the double bond at the 8, 9 and 10 positions, the isomers have differing abundance ratios of the main mass ions as indicated in the table above. The isomers (-)-Δ^{9}-THC and (-)-Δ^{8}-THC have distinct base peaks, namely m/z 299 and 231 respectively. Although (-)-Δ^{9}-THC and the two Δ^{10}-THC isomers have the same base peak (i.e., m/z 299), they may be differentiated by comparing the relative ratio of m/z 299 and 231.

Figure XIV.   GC-MS spectra of THC isomers

Mass Spectrum of (-)-Δ^{9}-THC
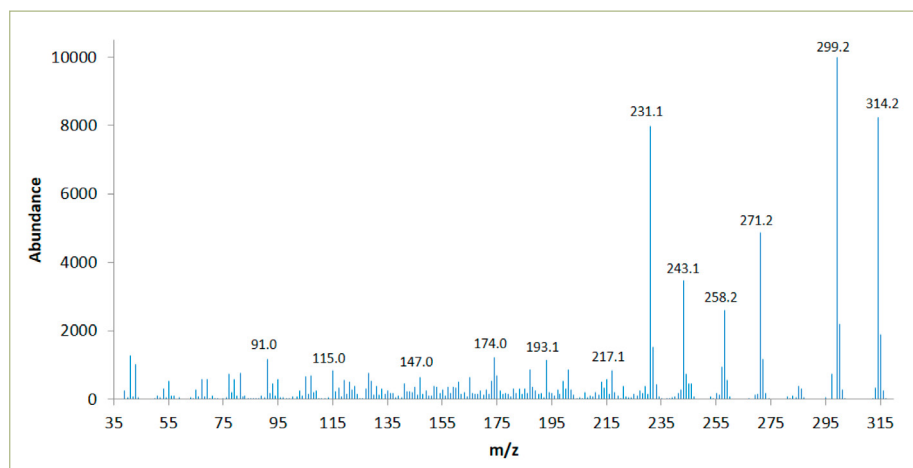
Mass Spectrum of (-)-Δ^8-THC
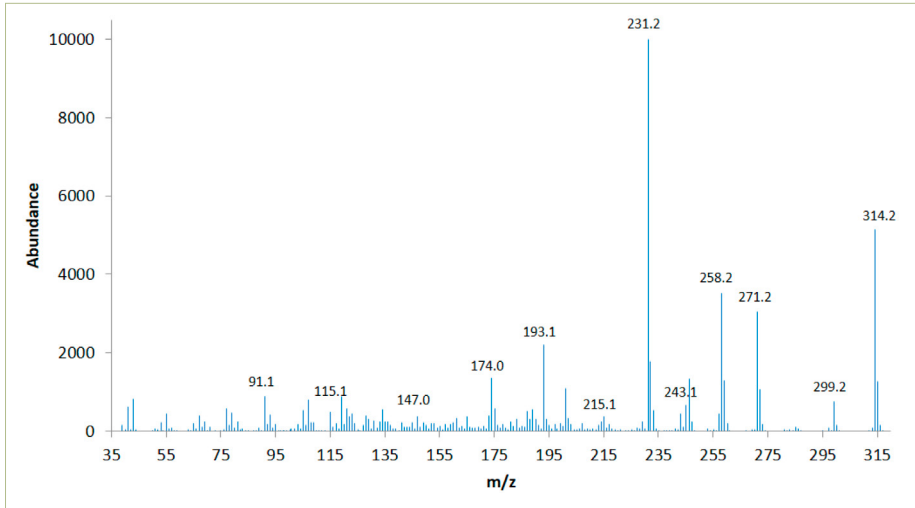
Mass Spectrum of $(6aR,9R)$-Δ^{10}-THC
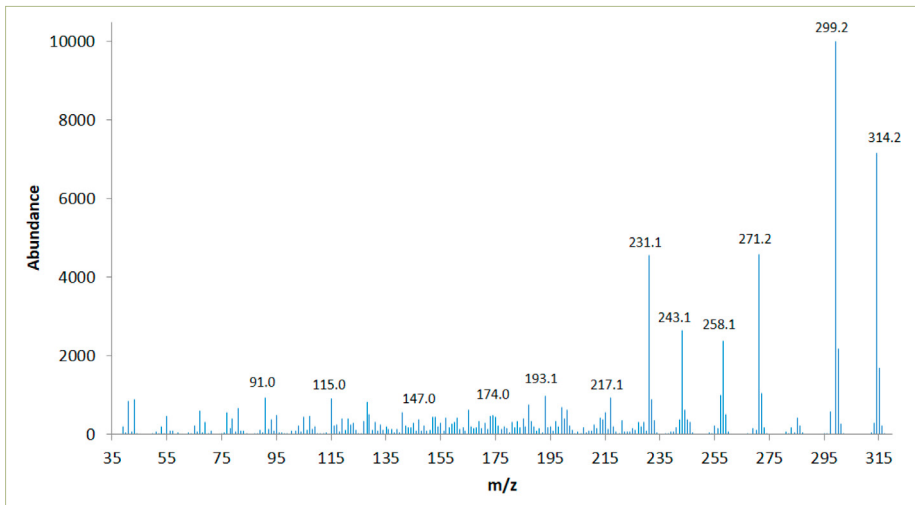
Mass Spectrum of (6aR,9S)-Δ^{10}-THC
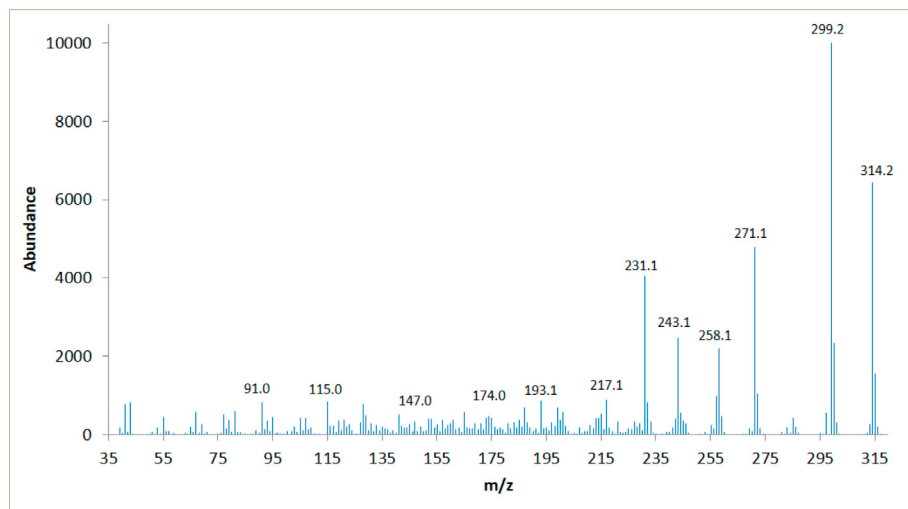
It should be noted however, that CBD and CBC co-elute using the above method. For analysis which requires the accurate determination of CBD either for qualitative or quantitative purposes, a GC column with a different selectivity would have to be used. A mid-polar column with (35%-phenyl)-methylpolysiloxane stationary phase provides a good resolution of CBD and CBC using the method below. The retention times of CBD and CBC acquired using this method are 11.06 and 10.79 minutes respectively.

Column:	15 m x 0.20 mm i.d., 0.33 μm
Phase:	Agilent HP-35MS (35%-phenyl–methylpolysiloxane
Carrier:	Helium, 12 psi, constant pressure
Injector:	Split/splitless, 280°C
Split ratio:	40:1
Oven:	3 min at 100°C, 40°C/min 260°C (7 min), 40°C/min 300°C (3 min)
Detector:	Mass detector, electron ionization mode tuned to perfluorotributylamine (PFTBA) Electron energy: 70 eV Interface temperature: 280°C Scan mode: positive Scan rate: 2^N, where N = 2 Ion source temperature: 230°C Quadrupole temperature: 150°C Gain factor: 1
Injection:	1.0 μl, split

5.4.7 Liquid chromatography

Liquid chromatography (LC) is a common and robust analytical technique which is not only used to separate components in a mixture but can also be used for quantitation. Due to the herbal nature of cannabis plant materials, sample clean-up is often necessary prior to analysis. It should be noted that the use of basic conditions should be avoided for the quantitation of acidic cannabinoids, as this can affect retention time of the analyte, peak shape, and selectivity. The peaks corresponding to the cannabinoids should be well-resolved as any co-elution with the target analyte will impact the accuracy of the quantitation results.

One advantage of using LC for quantitation of cannabinoids is the ease of quantifying the neutral and acidic cannabinoids without the need to perform decarboxylation. Hence, the total THC content of the sample is obtained by the summation of the amounts of THC and THCA from the two peaks in the chromatogram. Two methods are provided below with and without decarboxylation.

Method 1

The method below is a validated method for the analysis of total THC content (THC + THCA) in herbal cannabis after extraction with methanol/chloroform and subsequent decarboxylation [81], [82]. The validation encompasses the entire process from sample preparation to LC analysis. With adequate verification, the same method can also be applied to other cannabis products. Other methods may also produce acceptable results but must be validated and/or verified prior to routine use.

Column type:	250x4mm LiChrospher® 60 RP-8 (5 μm) pre-column 4x4mm RP-8 (5 μm)
Column temperature:	30°C
Mobile phase:	Acetonitrile:water (80:20 v/v), isocratic, stop time 8 min
Flow rate:	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

Extract 500 mg of dry and homogenized herbal cannabis (see section 5.4.2) with 5 mL methanol:chloroform (90:10 v/v) using the following procedure: 10 seconds on a vortex, 15 minutes in a ultrasonic bath including additional vortexing after 5, 10 and 15 minutes, then centrifugation.

Decarboxylation

Transfer 200 μL of the above extract into a derivatization vessel. Evaporate the solvent to dryness under nitrogen gas. Heat the sample for 15 minutes at 210°C. Dissolve the residue in 200 μL methanol:chloroform (90:10 v/v).

Preparation of the final solution for analysis

Dilute the above decarboxylation solution with methanol by a factor of 100 (in two steps, each 100 μL + 900 μL). For lower THC contents (< 0.5%), 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.	STD (vol. of standard)	Methanol (vol. of methanol)	Concentration (mg/mL)
1	10 μL 0.01 mg/mL	90 μL	0.001
2	50 μL 0.01 mg/mL	50 μL	0.005
3	10 μL 0.1 mg/mL	90 μL	0.01
4	50 μL 0.1 mg/mL	50 μL	0.05
5	100 μL 0.1 mg/mL	0 μL	0.1

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

Results

The retention time as well as the DAD (Diode-Array Detection) spectrum of the individual cannabinoids are used for their qualitative identification.

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-THCA	7.4	1.04

The calculation for the quantitative results can be carried out using either of the wavelengths at 220 or 240 nm.

Method 2

Studies have shown that the decarboxylation process might not yield full conversion of THCA to THC [69]. Degradation of THC to CBN can also occur if the temperature and duration of the decarboxylation are not optimized. The analysis of cannabis can also be carried out by LC without decarboxylation as described in the method below.

This method can be used for the analysis of CBD, CBDA, CBN, THC, CBC and THCA in cannabis as well as for the resolution of Δ^9-THC and Δ^8-THC after extracting with a suitable solvent, such as ethanol, methanol or mixture of solvents (see below). Two variations of the method (2A and 2B) are provided using the same instrumental parameters but with different mobile phase gradients. Method 2B allows separation of CBD from CBG if required.

Sample preparation

Extract 250 mg of dry and homogenized cannabis sample with 20 mL of methanol. Sonicate the mixture for 10 minutes and vortex. Filter the final sample solution through a 0.2 μm PTFE filter.

Volatile solvents, such as petroleum ether, are not suitable for sample extraction, since the higher rate of evaporation may cause inaccuracies in the results. It is also important to note that non-polar solvents, such as hexane, are not as effective in extracting the acidic forms of cannabinoids as compared to other more polar solvents and their mixtures (see section 4.4). Since the matrix of a herbal cannabis sample is complex, the use of internal standard may not be suitable. All methods must be validated and/or verified prior to routine use.

LC operating conditions

Column type:	Shimadzu Shim-pack XR-ODS II, 3.0 mm ID x 75 mm, 2.2 μm
Column temperature:	50°C
Mobile phase:	Mobile Phase A: 0.085% phosphoric acid in water Mobile Phase B: 0.085% phosphoric acid in methanol
Flow:	1 mL/min
Detection:	Photodiode array (PDA), 220 nm
Spectrum range:	210-350 nm
Injection:	0.5 μL
Elution order:	CBD, CBN, THC, THCA

The two different gradients of the mobile phase (method 2A and 2B) are described below:

Method 2A

Time (min)	% Mobile Phase A	% Mobile Phase B
0	25	75
7.50	10	90
7.51	5	95
9.50	5	95
9.51	25	75
13.00	25	75

Method 2B

Time (min)	% Mobile Phase A	% Mobile Phase B
0.00	40	60
5.00	40	60
16.00	28	72
24.00	15	85
25.00	15	85
25.01	40	60
29.00	40	60

Results

Method 2A is optimized for the quantitation of CBN, THC and THCA and allows for analysis within 13 minutes while method 2B gives peak resolution of CBD/CBG and is achieved within 29 minutes. Depending on whether CBD needs to be quantitated, the appropriate method can be selected for use. Examples of respective chromatograms from both methods are presented below.

Figure XV.   Examples of chromatograms from method 2A and 2B

Method 2A
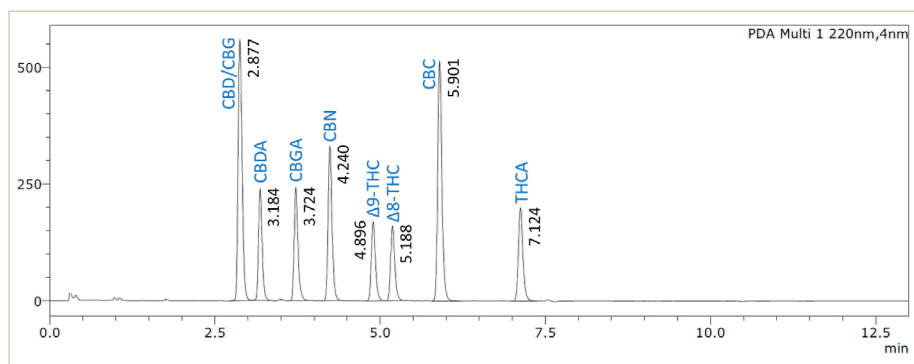
Method 2B
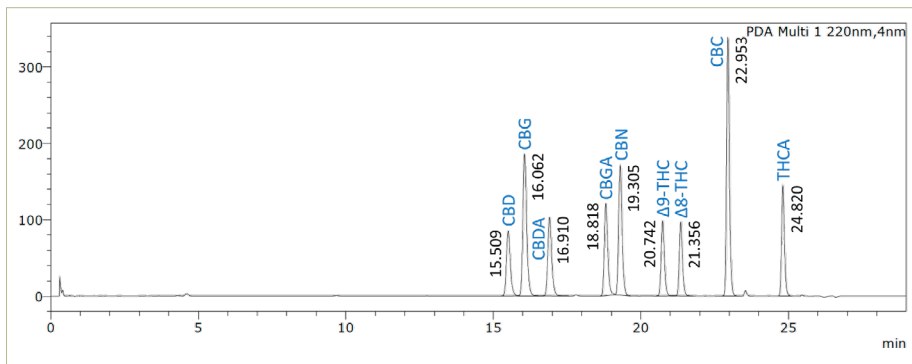
5.4.8 Liquid chromatography-tandem mass spectrometry

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is a powerful technique which combines the separation features of conventional LC or ultra-high-performance liquid chromatography (UHPLC) with the detection capabilities of a tandem mass spectrometer, resulting in significantly increased selectivity and reduced interference between active ingredients and matrix. With high sensitivity and selectivity, LC-MS/MS is suitable for qualitative and quantitative analysis of low concentrations of cannabinoids in complex herbal mixtures and matrices such as cannabis edibles [83]–[86]

Two methods are described below. The first method uses UHPLC-UV-MS for the qualitative and semi-quantitative analysis of cannabinoids in herbal mixtures. The second method uses LS-MS/MS for the qualitative analysis of cannabis-infused edible chocolate and could be adapted for the qualitative and quantitative analysis of cannabinoids in other type of samples [87].

Method 1

UHPLC coupled with ultraviolet detection and mass spectrometry (UHPLC-UV-MS) is a powerful technique which combines the separation features of UHPLC with the double detection capabilities of an ultraviolet (UV) detector and a mass spectrometer. With high sensitivity and selectivity, UHPLC-UV-MS is suitable for both qualitative and semi-quantitative analysis of cannabinoids in herbal mixtures.

The method below is suitable for the identification of cannabinoids and semi-quantification of total THC (THC and THCA) in herbal cannabis, cannabis resin and cannabis oil after extraction with methanol. The method allows the identification of cannabinoids using the retention time, UV profile and molecular mass of the compounds. The semi-quantification results are obtained by UV detection. Compounds are listed in table 7 and instrumentation parameters are described below.

Table 7. Summary of cannabinoids analysed

Compound name	Type of test
Cannabidivarinic acid (CBDVA)	Identification
Cannabidivarin (CBDV)	Identification
CBDA	Identification
CBGA	Identification
CBG	Identification
CBD	Identification
Tetrahydrocannabivarin (THCV)	Identification
Tetrahydrocannabivarinic acid (THCV)	Identification
CBNA	Identification
CBN	Identification
Δ^9-THC	Semi-quantitative
Δ^8-THC	Identification
Δ^9-THCA	Semi-quantitative
Cannabicyclol (CBL)	Identification
CBC	Identification
Cannabicyclolic acid (CBLA)	Identification

Preparation of solutions

The preparation of the solutions required for the analysis is described below including preparation of calibration standard solutions, mobile phase solutions, etc.

- 0.1% formic acid in water: mix 1 mL formic acid in 1 L purified water.
- Diluent solution: mix 250 mL of 0.1% formic acid in water and 750 mL of methanol.
- Mobile Phase A: mix 2 mL formic acid in 2 L of purified water, add 2.5 g of ammonium formate and dissolve.
- Mobile Phase B: mix 2 mL formic acid in 2 L of acetonitrile.
- Stock solution Δ^9-THC 50 μg/mL: from THC reference material, prepare a solution at a concentration of 50 μg/mL in the diluent solution.
- Calibration solution, Δ^9-THC 10 μg/mL: prepare a 10 μg/mL calibration solution from the Δ^9-THC 50 μg/mL stock solution.
- Calibration check solution: reference materials containing at least Δ^9-THC and Δ^9-THCA should be prepared. Dilute as needed in diluent to ensure that the final concentrations between 8-12 μg/mL of Δ^9-THC/Δ^9-THCA.
- Resolution check solution: prepare a solution for resolution verification that includes, at a minimum, the cannabinoids suspected to be present and requiring identification. Prepare this solution of cannabinoids by diluting appropriate amounts of reference materials with diluent solution.

Sample preparation

In a 15 mL centrifuge tube, precisely weigh an amount between 30-100 mg of representative homogenized sample. Add 10.0 mL of methanol. As needed and if the sample is very viscous, the extract can be heated for few minutes in a hot water bath.

Mechanically mix the tube for 30 minutes. Sonicate samples for 15 minutes. Centrifuge samples for approximately 5 minutes at 3000 rpm.

As needed, a dilution can be done in diluent solution (e.g., 1 mL of the extract in 10 mL). Filter samples as needed with a 0.2μm PTFE filter. Transfer an aliquot to a HPLC vial for analysis.

UHPLC-UV-MS operating parameters

UHPLC instrument parameters	
Column type:	Waters UHPLC HSS, 1.6 μm, 2.1 x 150 mm or equivalent
Column temperature:	40°C
Injection volume:	5 μL
Total run time:	16 min
Mobile phase A:	0.1% formic acid in water + 20 mM ammonium formate
Mobile phase B:	0.1% formic acid in acetonitrile
Column pressure:	8800 psi
Needle wash:	100% methanol
Seal wash:	acetonitrile:water (1:9 v/v)
UHPLC gradient:	see below
UV detection parameters	
Wavelength quantification:	220 nm
Wavelength identification:	scanning from 200 nm to 400 nm
Parameters and flow for isocratic pump for dilution post-column	
Mobile Phase Isocratic Solvent Manager (ISM):	0.1% formic acid in methanol (LCMS grade)
Seal wash:	methanol:water (1:1v/v)
Flow ISM:	0.5 mL/min
Split:	100
MS detection parameters	
Instrument:	Waters ACQUITY QDa Mass Detector
Gain:	10
Capillary:	1.5 kV positive

Probe:	600°C
MS detection mode:	Selected Ion Recording (SIR)
Ionization mode:	Electrospray Ionization (ESI)
Sampling rate:	10 points/second
Divert valve to MS detector:	0.50 min
Divert valve to waste:	11.50 min
SIR Programming:	See table 9

Mobile phase gradient

	Time (min)	Flow (mL/min)	% Mobile phase A	% Mobile phase B
1	0	0.400	35.0	65.0
2	2.50	0.400	23.0	77.0
3	8.50	0.400	23.0	77.0
4	10.50	0.400	10.0	90.0
5	11.00	0.400	10.0	90.0
6	12.50	0.400	35.0	65.0
7	16.00	0.400	35.0	65.0

Table 8.   Elution time (min) and λmax (nm) of the compounds

Analyte	Elution time (min)	λ max (nm)
CBDVA	3.20	223.5, 266.2, 304.3
CBDV	3.86	209.4, 275.7
CBDA	4.28	223.5, 266.2, 304.3
CBGA	4.48	223.5, 266.2, 304.3
CBG	4.81	204.7, 271.0
CBD	5.03	209.4, 275.7
THCV	5.21	209.4, 275.7
THCVA	5.68	223.5, 271.0, 304.3
CBNA	6.29	256.7
CBN	6.70	218.8, 285.3
Δ^9-THC	8.03	209.4, 275.7
Δ^8-THC	8.30	209.4, 275.7
Δ^9-THCA	9.02	223.5, 271.0, 304.3
CBL	9.25	209.4, 275.7
CBC	9.52	228.3, 280.5, 361.3
CBLA	10.44	228.3, 271.0, 304.3

Table 9.   Selected Ion Recording (SIR) programming

Segment	Analyte	Mass (Da) [M + H]	Cone voltage (V)	Suggested acquisition window Start (min)	End (min)
1	CBDVA	331.42	15	2.50	3.50
2	CBDV	287.41	15	3.10	4.10
3	CBDA	359.48	15	3.80	4.80
4	CBGA	343.23*	15	4.10	5.10
5	CBG	317.48	15	4.40	5.50
6	CBD	315.46	15	4.60	5.60
7	THCV	287.41	15	4.70	5.70
8	THCVA	331.42	15	4.80	5.80
9	CBNA	355.44	15	5.50	6.70
10	CBN	311.43	15	6.00	7.20
11	Δ^9-THC	315.46	15	7.20	8.40
12	Δ^8-THC	315.46	15	7.20	8.40
13	Δ^9-THCA	359.47	15	8.00	9.40
14	CBL	315.46	15	8.50	9.70
15	CBC	315.46	15	8.50	9.70
16	CBLA	359.47	15	9.60	10.60

*[M-OH]

MS detector parameters can be adjusted as needed to allow the acquisition of analytes in the appropriate segments, for example by changing the start and/or end of the acquisition windows or by changing the Events (Divert valve).

Qualitative analysis

Identification is accomplished by comparing the retention time of the analyte with the retention time of a reference standard. Furthermore, the UV spectrum and the mass of the analyte should be compared with a reference material.

Semi-quantitative analysis (calibration and calculations)

Δ^9-THC calculation

The semi-quantification of Δ^9-THC is done in external calibration with a single point calibration against the calibration solution Δ^9-THC 10 μg/mL:

Sa = Area analyte   Mech = Sample weight (mg)
Sstd = Area standard   FD = Dilution factor (mL)
Cstd = Standard concentration (µg/mL)

$$Amount\left(\frac{\mu g}{mL}\right) = \frac{Sa \times Cstd}{Sstd}$$

$$Amount\left(\frac{mg}{g}\right) = \frac{Sa \times Cstd}{Sstd} \times \frac{FD}{Mech} \times \frac{mg}{1000\ \mu g} \times \frac{1000\ mg}{g}$$

$$Amount\left(\%\frac{w}{w}\right) = \frac{Sa \times Cstd}{Sstd} \times \frac{FD}{Mech} \times \frac{mg}{1000\ \mu g} \times 100$$

Δ^9-THCA calculation by relative response factor (RRF)

The Δ^9-THCA is calculated by using the Δ^9-THC response using an appropriate relative response factor (RRF). The RRF value of Δ^9-THCA is determined experimentally following analysis of the Δ^9-THC and Δ^9-THCA calibration curves.

When a RRF is used for the semi-quantification of THCA by applying a correction factor to the THC calibration standard, the above equations are modified as follows: Sstd is replaced by Scorr.

Scorr = Area corrected with RRF   RRF = Relative response factor for THCA

$$Scorr = Sstd \times RRF$$

However, it is still possible to prepare a calibration solution for Δ^9-THCA and quantify it as for Δ^9-THC.

Total THC calculation

To report total THC values, the quantity obtained for THCA must be converted to THC equivalents as follows:

$$THC\ equivalent = \frac{molecular\ mass\ THC}{molecular\ mass\ THCA} \times Amount\ THCA$$

$$THC\ equivalent = \frac{314.46\ g/mole}{358.47\ g/mole} \times Amount\ THCA$$

$$Total\ THC = Amount\ THC + Amount\ THCA\ (in\ THC\ equivalent)$$

Method 2

Sample preparation

The solid edibles can be pulverized or reduced to smaller pieces, while liquid edibles can be used directly or after extraction. When the extraction method does not give satisfactory chromatographic results (e.g., interference with the matrix), QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method may be employed for the preparation of the

sample. QuEChERS was originally developed for pesticide analysis in food but has found widespread use, including cannabinoid analysis [83], [84].

Homogenize the material to obtain a representative sample. For example, freeze-dry the sample and grind to a powder. Hard or sticky candies, gummies and chocolates can be manually chopped or cut into small pieces. Weigh 2 g of homogenized sample or 0.5 g of oil product in 50 mL polypropylene tube.

Add 10 mL of purified water and a ceramic homogenizer, vortex briefly and leave to stand for 30 minutes. Add internal standard and 20 mL acetonitrile and shake at around 1700 rpm for 3 minutes in a vertical shaker/homogenizer.

Add QuEChERS salt mixture containing 4 g of magnesium sulfate, 1 g of sodium chloride, 1 g of trisodium citrate dihydrate and 0.5 g of disodium hydrogen citrate sesquihydrate. The extraction salts help to facilitate phase separation and partition the analytes from the aqueous layer into the acetonitrile layer. Shake the suspension at around 1700 rpm for 3-5 minutes. If the shaker/homogenizer does not have temperature control, the tubes may become slightly hot due to the reaction. Unscrew the cap intermittently to release the gas generated.

Centrifuge at around 4000 rpm for 5 minutes. For solid edibles, three distinct layers may be observed after centrifugation. The first layer (supernatant) is the acetonitrile phase containing cannabinoids and the organic-soluble matrix; the second layer is the insoluble matrix components and water-soluble matrix components, such as sugars; and the third layer is the undissolved excess extraction salts.

Transfer the supernatant to a snap-lock vial and dilute 50-fold with acetonitrile-water (1:1). Depending on the sensitivity of the instrument, less dilution may be required. Centrifuge the solution at 13000 rpm for 5 minutes to remove particulates, then transfer the supernatant to a LC vial for analysis.

Standard addition method may be performed by extracting the sample as described above together with another sample spiked with appropriately low concentrations of the cannabinoids. The spiking can be added together with the internal standard (see above). The concentration spiked can be at the regulatory limits of the specific cannabinoids, if applicable, or the detection limits of the instrument.

Analytical notes

- A standard addition approach is suitable under the following scenarios:
  a) when the absence of cannabinoids must be verified in a sample;
  b) if the amounts of cannabinoids are expected to be very low; or
  c) if the edible matrix is not common.

- Standard addition approach allows for the compensation of losses encountered during extraction and matrix effects arising from the less common edibles.

- It is a good practice to always extract a negative control to demonstrate that the analytical process does not introduce erroneous peaks in the chromatogram. This is performed by going through the same extraction process in the absence of the sample.

LC operating conditions [83]

Column type:	Waters ACQUITY UPLC BEH Shield RP18 Column 100 x 2.1 mm (1.7 μm)
Column temperature:	40°C
Mobile phase A:	aqueous 0.1% formic acid
Mobile phase B:	acetonitrile
Gradient:	See below (total run time 13 min).
Flow rate:	0.5 mL/min
Detection:	MS/MS (refer to table 10 for possible Multiple Reaction Monitoring (MRM transitions)
Injection:	5 μL
Elution order:	CBD, CBDA, CBN, Δ^9-THC, Δ^8-THC, THCA

Mobile phase gradient

Time (min)	% Mobile Phase A	% Mobile Phase B
0.00	50	50
1.00	50	50
9.00	0	100
11.00	0	100
13.00	50	50

Table 10. Retention times and MS/MS data

Cannabinoid	Ionization mode	Precursor ion > Possible daughter ions (MRM transitions)	Retention time (min)
CBD	positive	315 > 193, 259, 135	4.79
CBDA	negative	357 > 245, 339, 226	5.11
CBN	positive	311 > 223, 293, 195	5.47
Δ^9-THC	positive	315 > 193, 259, 123	5.73
Δ^8-THC	positive	315 > 193, 259, 123	5.83
THCA	negative	357 > 213, 245, 191	6.64
THC-COOH-d3 (IS)	negative	349 > 302, 248, 194	3.50

5.4.9 DNA-based identification of cannabis

The commonly used morphological examination (i.e. macroscopic and microscopic) and chemical analytical methods (e.g. TLC, GC, GC-MS, LC etc.) are generally sufficient for the identification of cannabis. However, a DNA-based approach for cannabis identification offers the advantage of species-level identification and can be particularly effective in situations where the sample lacks the morphologically distinct traits of cannabis plant material and/or contains a low THC level, for example highly fragmented form, young seedlings, seeds, roots or bare branches.

Currently, there are two well described approaches towards a DNA-based identification of cannabis – either using universal DNA barcodes or cannabis-specific DNA markers [88], [89]. Both approaches are based on the uniqueness of DNA sequences in the *Cannabis sativa* L. plant genome and share similar methodologies. However, the general conclusion from these studies indicates that a single DNA barcode is inadequate for providing the species resolution for the myriad of plant species.

The most direct approach to identify *Cannabis sativa* L. samples is to examine for cannabis-specific DNA markers, THCA synthase and CBDA synthase. These enzymes are involved in catalysing the oxidative cyclization of CBGA to THCA and CBDA, respectively (see section 4.1) [68], [90]. PCR amplification and DNA sequencing of either the DNA barcodes or cannabis-specific DNA markers would be followed by a comparison of the obtained DNA sequences against reference sequences stored in a repository to determine the species source of the sample. This has been performed via a Basic Local Alignment Search Tool (BLAST) [91], [92] search of the GenBank, which is a comprehensive international DNA public database that stores 9.9 trillion base pairs from over 2.1 billion nucleotide sequences accounting for almost half a million formally described species [93].

6. References

[1]   World Drug Report 2021, United Nations Office on Drugs and Crime. www.unodc.org/unodc/en/data-and-analysis/wdr2021.html (accessed Dec. 18, 2021).

[2]   European Monitoring Centre of Drug Addiction (EMCDDA), European Drug Report 2021, www.emcdda.europa.eu. www.emcdda.europa.eu/edr2021_en (accessed Dec. 18, 2021).

[3]   United Nations Office on Drugs and Crime, The_International_Drug_Control_Conventions, //www.unodc.org/unodc/en/commissions/CND/conventions.html (accessed Dec. 20, 2021).

[4]   United Nations Office on Drug and Crime, The Multilingual Dictionary of Narcotic Drugs and Psychotropic Substances under International Control (ST/NAR/1 Rev.2), New York, 2006. //www.unodc.org/unodc/en/scientists/multilingual-dictionary-of-narcotic-drugs-and-psychotropic-substances-under-international-control.html (accessed Dec. 20, 2021).

[5]   United Nations Office on Drugs and Crime, Terminology and Information on Drugs, Third Edition, 2016 (Sales No.E.16.16.XI.8). Accessed: Dec. 20, 2021. https://www.unodc.org/unodc/en/scientists/terminology-and-information-on-drugs_new.html

[6]   J.M. McPartland, *Cannabis and Cannabinoid Research*, vol. 3, no. 1, 203–212, 2018, doi: 10.1089/can.2018.0039.

[7]   S. Schilling, C. Dowling, J. Shi, L. Ryan and D. Hunt, The Cream of the Crop: Biology, Breeding and Applications of Cannabis sativa. 2020. doi: 10.22541/au.160139712.25104053.

[8]   Q. Zhang, X. Chen, H. Guo, L.M. Trindale and E.M.J. Salentijn, *Frontiers in Plant Science*, vol. 9, p. 1876, 2018, doi: 10.3389/fpls.2018.01876.

[9]   R.J. Hill, Marijuana, Cannabis sativa L., Regulatory Horticulture, Weed Circular No. 5, 9 (1-2), 57-66, p. 7, 1983.

[10]   E. Small and A. Cronquist, A Practical and Natural Taxonomy for Cannabis, *Taxon*, vol. 25, no. 4, 405–435, 1976, doi: 10.2307/1220524.

[11]   S. Gilmore, R. Peakall, and J. Robertson, *Forensic Science International*, vol. 172, no. 2, 179–190, 2007, doi: https://doi.org/10.1016/j.forsciint.2006.10.025.

[12]   J. Hey and C. Pinho, *Evolution*, vol. 66, no. 5, 1413–1429, May 2012, doi: 10.1111/j.1558-5646.2011.01542.x.

[13]   J. Sawler, J.M. Stout, K.M. Gardner, D. Hudson and J. Vidmar, *PLOS ONE*, vol. 10, no. 8, p. e0133292, Aug. 2015, doi: 10.1371/journal.pone.0133292.

[14]   R.C. Lynch, D. Vergara, S. Tittes, K. White and C.J. Schwartz, *Critical Reviews in Plant Sciences*, vol. 35, no. 5–6, 349–363, 2016, doi: 10.1080/07352689.2016.1265363.

[15]   "Flora of North America, Cannabis sativa (updated 2020)", http://floranorthamerica.org/ (retrieved November 2021). http://floranorthamerica.org/Cannabis_sativa (accessed Dec. 20, 2021).

[16]   W. Brandt, M. Gürke, G. Pabst, F.E. Köhler, G. Schellenberg and M. Vogtherr, Köhler's medizinal-pflanzen in naturgetreuen abbildungen mit kurz erläuterndem texte: Atlas zur Pharmacopoea germanica, austriaca, belgica, danica, helvetica, hungarica, rossica, suecica, neerlandica, British pharmacopoeia, zum Codex medicamentarius, sowie zur Pharmacopoeia of the United States of America. Gera-Untermhaus: Verlag von Fr. Eugen Köhler, 1883.

[17]   Europol Drugs Information Bulletin No. 3/2001, p.7.: https://op.europa.eu/en/publication-detail/-/publication/b082e4a4-3049-4013-91ea-b451ea5438d5

[18]   H. Mahler, "Moderne Methoden zur Anzucht von Cannabispflanzen - Berechnung des Ertrags von Indoor-Plantagen, Proceedings XV. GTFCh Symposium 2007, Mosbach, ISBN ISBN 978-3-00-023794-2", p. 14.

[19]   M. Toonen, S. Ribot, and J. Thissen, *Journal of Forensic Science*, vol. 51, no. 5, 1050–1054, 2006, doi: 10.1111/j.1556-4029.2006.00228.x.

[20]   Bureau Ontnemingswetgeving Openbaar Ministerie (BOOM) (2005), Wederrechtelijk verkregen voordeel hennepkwekrij bij binnenteelt onder kunstlicht: Standaardberekeningen en normen.

[21]   H. Stambouli, A.E. Bouri, M.A. Bellimam, T. Bouayoun, and N.E. Karni, *Bulletin on Narcotics*, vol. 57 1–2, 79–118, 2005.

[22]   With permission of Kantonspolizei Zürich, KTA-KF.

[23]   E.R. Service, Industrial Hemp in the United States: Status and Market Potential. www.ers.usda.gov/publications/pub-details/?pubid=41757 (accessed Dec. 20, 2021).

[24]   L.A. King, The Misuse of Drugs Act: A Guide for Forensic Scientists (RSC Publication), p. 82., 2003, https://1lib.at/book/1247461/bf0f71 (accessed Dec. 20, 2021).

[25]   G. Martinelli, A. Magnavacca, M. Fumagalli, M. Dell'Agli, S. Piazza, and E. Sangiovanni, *Planta Medica*, 2021, doi: 10.1055/a-1420-5780.

[26]   A.A. Jairoun, S.S. Al-Hemyari, M. Shahwan, B. Ibrahim, M.A. Hassali, and S.H. Zyoud, *Cosmetics*, vol. 8, no. 3, 2021, doi: 10.3390/cosmetics8030057.

[27]   B. Thomas and M. ElSohly, "The Botany of Cannabis sativa L.", 2016, 1–26. doi: 10.1016/B978-0-12-804646-3.00001-1.

[28]   Y. Gaoni and R. Mechoulam, *Journal of the American Chemical Society*, vol. 86, no. 8, 1646–1647, 1964, doi: 10.1021/ja01062a046.

[29]   R. Mechoulam, P. Braun, and Y. Gaoni, *Journal of the American Chemical Society*, vol. 89, no. 17, 4552–4554, 1967, doi: 10.1021/ja00993a072.

[30]   V.R.L.J. Bloemendal, J.C.M. van Hest, and F.P.J.T. Rutjes, *Organic Biomolecular Chemistry*, vol. 18, no. 17, 3203–3215, 2020, doi: 10.1039/D0OB00464B.

[31]   M. Taschwer and M. G. Schmid, *Forensic Science International*, vol. 254, 167–171, 2015, doi: https://doi.org/10.1016/j.forsciint.2015.07.019.

[32]   D. M. Cole, The Analysis of Controlled Substances | Analytical Techniques in the Sciences, 2003. https://onlinelibrary.wiley.com/doi/book/10.1002/0470868007 (accessed Dec. 20, 2021).

[33]   S.A. Ross and M.A. Elsohly, *Bulletin on Narcotics*, vol. Vol. XLIX and L, 139–147, 1997.

[34]   C. Lindholst, *Australian Journal of Forensic Sciences*, vol. 42, no. 3, 181–190, 2010, doi: 10.1080/00450610903258144.

[35]   J.W. Fairbairn, J.A. Liebmann, and M.G. Rowan, *Journal of Pharmacy and Pharmacology*, vol. 28, no. 1, 1–7, 2011, doi: 10.1111/j.2042-7158.1976.tb04014.x.

[36]   R. Mechoulam and L. Hanuš, *Chemistry and Physics of Lipids*, vol. 121, no. 1, 35–43, 2002, doi:10.1016/s0009-3084(02)00144-5.

[37]   Y. Gaoni and R. Mechoulam, *Tetrahedron*, vol. 22, no. 4, 1481–1488, 1966, doi: https://doi.org/10.1016/S0040-4020(01)99446-3.

[38]   G. R. Webster, L. Sarna, and R. Mechoulam, Conversion of cbd to delta8-thc and delta9-thc, US20040143126A1, 2004 Accessed: Dec. 20, 2021. https://patents.google.com/patent/US20040143126A1/en

[39]   P. Golombek, M. Müller, I. Barthlott, C. Sproll, and D.W. Lachenmeier, *Toxics*, vol. 8, no. 2, 2020, doi: 10.3390/toxics8020041.

[40]   B.E. Erickson, Delta-8-THC Craze Concerns Chemists | RealClearScience, Jul. 12, 2021. www.realclearscience.com/2021/07/12/delta-8-thc_craze_concerns_chemists_785049.html (accessed Dec. 20, 2021).

[41]   K. Tsujikawa, Y. Okada, H. Segawa, T. Yamamuro, K. Kuwayama, T Kanamori and Y.T. Iwata., *Forensic Toxicology*, vol. 40, pp. 125–131, 2022, doi: 10.1007/s11419-021-00592-9.

[42]   A. Chan-Hosokawa, L. Nguyen, N. Lattanzio, and W.R. Adams., *Journal of Analytical Toxicology*, p. bkab029, 2021, doi: 10.1093/jat/bkab029.

[43]   "World Health Organization (WHO), Expert Committee on Drug Dependence (ECDD), Critical review: Delta-9-tetrahydrocannabinol , 2018". Accessed: Dec. 20, 2021. www.who.int/medicines/access/controlled-substances/THCv1.pdf

[44]   R.N. Smith and C.G. Vaughan, *Journal of Pharmacy and Pharmacology*, vol. 29, no. 1, 286–290, 2011, doi: 10.1111/j.2042-7158.1977.tb11313.x.

[45]   K. Kovar and H. Linder, Investigation of Hashish: Content Uniformity of Different Samples by Coupled HPLC/PC-Analysis., 1992, doi: 10.1002/CHIN.199201294.

[46]   O. Zoller, P. Rhyn, and B. Zimmerli, *Journal of Chromatography A*, vol. 872, no. 1, 101–110, 2000, doi: 10.1016/s0021-9673(99)01287-x.

[47]   C. Citti, D. Braghiroli, M.A. Vandelli, and G. Cannazza, *Journal of Pharmaceutical and Biomedical Analysis*, vol. 147, 565–579, 2018, doi: 10.1016/j.jpba.2017.06.003.

[48]   M. Wang, Y.H. Wang, B. Avula, M.M. Radwan and S.A. Wanas, *Journal of Forensic Science*, vol. 62, no. 3, 602–611, 2017, doi: 10.1111/1556-4029.13341.

[49]   B. Patel, D. Wene, and Z. Fan, *Journal of Pharmaceutical and Biomedical Analysis*, vol. 146, 15–23, 2017, doi: https://doi.org/10.1016/j.jpba.2017.07.021.

[50]   G. Fritschi, B. Klein, and W. Szilluweit, Verteilung der THC-Gehalte in Marihuanapflanzen, p. 3.

[51]   THC Statistics, Swiss Federal Office of Public Health, 2020. Accessed: Dec. 18, 2021. [Online]. Available: https://sgrm.ch/inhalte/Forensische-Chemie-und-Toxikologie/Fachgruppe_Chemie/ Statistiken/THC/THC_2020_Neu.pdf

[52]   E. de Meijer, H.J. van der Kamp, and F. van Eeuwijk, *Euphytica*, vol. 62, 187–200, 2004.

[53]   Drugs Working Group of the European Network of Forensic Science Institutes (ENFSI) and UNODC, "Guidelines on representative sampling (ST/NAR/38)". 2009. www.unodc.org/ unodc/en/scientists/guidelines-on-representative-drug-sampling_new.html

[54]   European Network of Forensic Science Institutes (ENFSI), Guidelines on Sampling of Illicit Drugs for Quantitative Analysis. 2016. http://enfsi.eu/wp-content/uploads/2016/09/guide-lines_ quant_sampling_dwg_printing_vf4.pdf

[55]   Official Journal of the European Communities of 28 December 2000 (L 332/63), Commission Regulation (EC) No 2860/2000 of 27 December 2000, ANNEX VI, ANNEX XIII (article 7b(1)) (https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32000R2860&fro m=FR).

[56]   Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG), Recommendations, Version 8.0, 2019, www.SWGDRUG.org (retrieved November 2021).

[57]   R. Clarke, Marijuana Botany, Ronin Publishing, Inc, ISBN 0-914171-78-X. Ronin Publishing, Inc, 1981.

[58]   Z.K. Punja and J.E. Holmes, *Frontiers in Plant Science*, vol. 11, p. 718, 2020, doi: 10.3389/ fpls.2020.00718.

[59]   G.R. Nakamura, *Journal of Association of Official Analytical Chemists*, vol. 52, no. 1, 5–16, 1969, doi: 10.1093/jaoac/52.1.5.

[60]   G.T. Mitosinka, J.I. Thornton, and T.L. Hayes, *Journal of the Forensic Science Society*, vol. 12, no. 3, 521–529, 1972, doi: 10.1016/S0015-7368(72)70717-3.

[61]   Wissenschaftlicher Dienst, Stadtpolizei Zürich, Switzerland, with permission, 2007.

[62]   C.R.B. Joyce and S.H. Curry, The botany and chemistry of Cannabis, 1970.

[63]   B.P. Jackson and D.W. Snowdon, Powdered vegetable drugs: an atlas of microscopy for use in the identification and authentication of some plant materials employed as medicinal agents. London: Thornes, 2nd edition, 1974.

[64]   P. Dayanandan and P.B. Kaufman, *American Journal of Botany*, vol. 63, no. 5, 578–591, 1976, doi: 10.1002/j.1537-2197.1976.tb11846.x.

[65]   C.T. Hammond and P.G. Mahlberg, *American Journal of Botany*, vol. 60, no. 6, 524–528, 1973, doi: 10.1002/j.1537-2197.1973.tb05953.x.

[66]   A.B. Segelman, P.A. Babcock, B.L. Braun, and F.H. Segelman, *Journal of Pharmaceutical Sciences*, vol. 62, no. 3, 515–516, 1973, doi: https://doi.org/10.1002/jps.2600620344.

[67]   J.L. Fussell, J.I. Thornton and F.W. Whitehurst., *Journal of Forensic Identification*, 59, Accessed: Dec. 21, 2021. https://theiai.org/docs/JFI_2006-2009.pdf

[68]   S. Sirikantaramas, S. Morimoto, Y. Shoyama, Y. Ishikawa, Y. Wada, Y. Shoyama and F. Taura, *Journal of Biological Chemistry*, vol. 279, no. 38, 39767–39774,. 2004, doi: 10.1074/jbc. M403693200.

[69]   F.E. Dussy, C. Hamberg, M. Luginbühl, T. Schwerzmann, and T.A. Briellmann, Forensic Science International, vol. 149, no. 1, 3–10, 2005, doi: https://doi.org/10.1016/j. forsciint.2004.05.015.

[70]   B.D. Backer, B. Debrus, P. Lebrun, L. Theunis and N. Dubois, *Journal of Chromatography B*, vol. 877, no. 32, 4115–4124, 2009, doi: https://doi.org/10.1016/j.jchromb.2009.11.004.

[71]   L. Ambach, F. Penitschka, A. Broillet, S. König, W. Weinmann, and W. Bernhard, *Forensic Science International*, vol. 243, 107–111, 2014, doi: https://doi.org/10.1016/j.forsciint.2014.06.008.

[72]   M. Bovens, T. Csesztregi, A. Franc, J. Nagy, and L. Dujourdy, *Forensic Science International*, vol. 234, 174–180, 2014, doi: https://doi.org/10.1016/j.forsciint.2013.09.001.

[73]   Wissenschaftlicher Dienst, Stadtpolizei Zürich, Switzerland, validated method, with permission, 2007.

[74]   A.J. Poortman-van der Meer and H. Huizer, *Forensic Science International*, vol. 101, no. 1, 1–8, 1999, doi: 10.1016/s0379-0738(99)00004-3.

[75]   M.J. de Faubert Maunder, UNODC *Bulletin of Narcotics*, Issue 4, pp. 37-42, 1969, www.unodc. org/unodc/en/data-and-analysis/bulletin/bulletin_1969-01-01_4_page006.html

[76]   K. Lewis, R. Wagner, S.E. Rodriguez-Cruz, M.J. Weaver, and J.C. Dumke, *Journal of Forensic Sciences*, vol. 66, no. 1, 285–294, 2021, doi: 10.1111/1556-4029.14562.

[77]   J.M. Holler, M.L. Smith, S.N. Paul, M.R. Past, and B.D. Paul, *Journal of Mass Spectrometry*, vol. 43, no. 5, 674–679, 2008, doi: 10.1002/jms.1375.

[78]   R. Andrews and S. Paterson, *Journal of Analytical Toxicology*, vol. 36, no. 1, 61–65, 2012, doi: 10.1093/jat/bkr017.

[79]   T.B. Vree, *Journal of Pharmaceutical Sciences*, vol. 66, no. 10, 1444–1450, 1977, doi: 10.1002/ jps.2600661025.

[80]   D.J. Harvey, *Mass Spectrometry Reviews*, vol. 6, no. 1, 135–229, 1987, doi: 10.1002/ mas.1280060104.

[81]   Institute of Legal Medicine, St Gall, Switzerland, validated method, with permission, 2005.

[82]   R. Brenneisen, *Pharmaceutica Acta Helvetiae*, vol. 59, no. 9–10, 247–259, 1984.

[83]   N. Christinat, M.-C. Savoy, and P. Mottier, *Food Chemistry*, vol. 318, p. 126469, 2020, doi: 10.1016/j.foodchem.2020.126469.

[84]   I. Di Marco Pisciottano, G. Guadagnuolo, V. Soprano, M. De Crescenzo, and P. Gallo, *Rapid Communications in Mass Spectrometry*, vol. 32, no. 19, 1728–1736, 2018, doi: 10.1002/ rcm.8242.

[85]   R.F. Klein, *Microgram Journal.*, 14(1-4), 9-32, 2017.

[86]   G. McRae and J.E. Melanson, *Analytical and Bioanalytical Chemistry*, vol. 412, no. 27, 7381–7393, Nov. 2020, doi: 10.1007/s00216-020-02862-8.

[87]   AOAC SMPR® 2017.019 Standard Method Performance Requirements (SMPRs®) for Quantitation of Cannabinoids in Edible Chocolate. Accessed: Dec. 19, 2021. [Online]. Available: www.aoac.org/wp-content/uploads/2020/11/SMPR202017_019.pdf

[88]   A. J. Fazekas, K.S. Burgess, P.R. kesankurti, S.W. Graham and S.G. Newmaster, *PLoS One*, vol. 3, no. 7, p. e2802, 2008, doi: 10.1371/journal.pone.0002802.

[89]   CBOL Plant Working Group, "A DNA barcode for land plants", *Proceedings of the National Academy of Sciences U S A*, vol. 106, no. 31, 12794–12797, 2009, doi: 10.1073/pnas.0905845106.

[90]   F. Taura, S. Sirikantaramas, Y. Shoyama, K. Yoshikai, Y. Shoyama, and S. Morimoto, *Federation of European Biochemical Societies Letters*, vol. 581, no. 16, 2929–2934, 2007, doi: 10.1016/j.febslet.2007.05.043.

[91]   S.F. Altschul, W. Gish, W. Miller, E.W. Myers, and D.J. Lipman, *Journal of Molecular Biology*, vol. 215, no. 3, 403–410, 1990, doi: 10.1016/S0022-2836(05)80360-2.

[92]   S. McGinnis and T.L. Madden, *Nucleic Acids Research*, vol. 32, no. Web Server issue, W20-25, 2004, doi: 10.1093/nar/gkh435.

[93]   E.W. Sayers, M. Cavanaugh, K. Clark, K.D. Pruitt, C.L. Schoch, S.T. Sherry and I. Karsch-Mizrachi, "GenBank," *Nucleic Acids Research*, vol. 49, no. D1, D92–D96, 2021, doi: 10.1093/nar/gkaa1023.