Cannabis sativa: A systematic review of plant analysis

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Background: Cannabis has been the most widely used illicit drug worldwide throughout many years. Reports from different countries indicate that the potency of cannabis preparation has been increasing, as well as the ratio of tetrahydrocannabinol/cannabidiol has been changing. The high consumption couple with the variable chemical profile of the drug is increasing gradually the interest in researching the cannabis plant. **Methods:** This article reviews available literature on the analytical methods currently used for the detection and quantification of cannabinoids in cannabis plant. The papers were screened by two independently researchers and following a pre-specified protocol. **Results and Discussion:** The systematic review of the literature allowed to include 42 citations on cannabis plant analysis and botanical aspects of cannabis. **Conclusions:** The analytical methods for cannabis material published in the included articles of this systematic review showed lack of relevant information of the development of methods on GC and LC analysis and the limits of detection and quantification of mass detectors. These information, on the methods of analysis, are essential and extremely important, since in the current scenario the analytical approach should consider the action of modulation CBD with THC, which alters the disruptive effects of the drug and also presents important pharmacological activity.

Keywords: *Cannabis sativa*, marijuana, cannabinoids detection, plant analysis and analytical methods.

Introduction

Cannabis sativa L. (cannabis) remains the most widely cultivated, produced, trafficked and consumed drug worldwide (1, 2). At the same time, the plant has been used over the centuries for medicinal purposes (2-5), which results in controversial perception and opinion on its use. According to the "World Drug Report" published by UNODC (1), in the previous year, cannabis market development yielded an increased in the global seizures of 2% on cannabis resin and 4% on cannabis herb, which led to seizures of 1.433 and 5.834 tons, respectively.

Cannabis has over 500 identified chemicals in plant and around 100 of them are classified as (phyto) cannabinoids (5, 6). Analyses of the cannabinoids content and chemical profile in cannabis plants are extremely relevant, because both the medicinal effects and the adverse health effects may be associated with the potency and/or interplay of certain cannabinoids and other compounds (such as terpenoids) due to cannabis consumption (4, 5). In relation to forensic interest, the cannabinoids data may also assist in developing classification models to chemotypes, on distinction of the varieties, in establishing the growth period of the plant and in drug trafficking restraint (5, 7, 8).

Although there are currently several well-established methods available for chemical analyses of cannabinoids (9-11), the high variability of cannabis samples become the chemical profile interpretation very difficult. There is a need for adaptations of traditional methods of cannabis analysis in light of new scientific evidence regarding the plant and its plant metabolites, taking into account the pharmacological activity as a potential drug and as a drug of abuse for recreational use. The instrumental analysis that have been commonly used to analyze cannabinoids are gas chromatography (GC) and liquid chromatography (LC) (3, 12). The use of GC, generally coupled to flame ionization detection (FID) (9, 11) or mass spectrometry (MS) detection, allows the analysis of a large variety of cannabinoids with high resolution (8, 10, 13). For analyzing cannabinoids in their acid forms, LC is the preferred method. In addition, the compounds can be efficiently screened using ultraviolet (UV) or photodiode-array (PDA) detector (11, 14). Other methods have been used, as the genetic profile analysis (12). A genetic analysis, e. g. real-time polymerase chain reation (PCR), is an alternative method to chemical analysis, to examine forensic samples of cannabis in order to determine the tetrahydrocannabinol (THC) content (15, 16).

A systematic review becomes important during the process of realization of a survey, since, before starting the practical part, it is fundamental to design the analysis based on already performed methods for that type of research. Thus, this study aims to conduct a systematic review to summarize published results concerning the methods available for cannabis plant analysis over the period 2010-2016.

Methods

The systematic review produced was based on relevancy to the topics of cannabis plant analysis, profile of cannabis and methodology of cannabis analysis. The source used for guidance and to performe the present paper was the PRISMA guidelines (17).

Search strategy

A systematic literature search was carried out by consulting six electronic scientific databases: PubMed (MEDLINE), LILACS, Scopus, SciELO and Google Scholar, through July 2016 to August 2016.

A combination of the following search terms was used: "cannabis", "marijuana", "cannabinoids", phytocannabinoids", "drug detection", "analytical methods", "plant" and "herbal". The publication date was imposed as restriction to the retrieved articles: from 2010 through August 2016. Manuscripts were limited to English, Portuguese and Spanish languages.

Selection criteria

The follow inclusion criteria were established: 1) original research papers published since 2010 until August 2016; and 2) papers which evaluate analysis in cannabis plant material as the population of interest.

There were no limits for cannabis plants: preparations (herbal form - the leaves, flowering tops, and resin form – hashish, hash oil), chemotypes (fiber, intermediate or drug), phenotypes (genetic factors - alleles B_T, B_D, B_C and B_0, a mutant form of the B_D locus), varieties (*indica*, *sativa* or *ruderalis*), gender (male, female or monoecious), geographic location and origin, cultivation methods (outdoor, indoor, cloning or pollination), grown conditions (soil, humidity, temperature and photoperiod), growth period at harvesting, sample conservation at the time of analysis, or modes of supplying (seized, purchased or cultivated).

Search articles

The selection process concerning the articles to perform the paper (Figure 1) was accomplished uniformly by two independent researchers. Thus, both researchers conducted the screening, the determination of eligibility and the inclusion or exclusion of the papers related to methodology analysis for cannabis plant to attende this systematic review.

In the initial screening it was evaluated all titles and abstracts which researchers consider relevant. Articles that completely or partially lacked clear data to the information within the topic headings, such as analysis concerning synthetic cannabinoids or papers that showed solely the cannabis seizures data were rejected. The review papers and monographic scientific publications were also excluded of this study. There were no divergent opinions between the reviewers in including or excluding studies to eligible the articles for the systematic review.

Data set

After the selection performed at all databases involved in the study, repeated files were deleted and the remaining papers were placed in a single folder. The papers identified as potentially relevant were thoroughly reviewed and accepted or excluded from the study through consensus, by the reviewers.

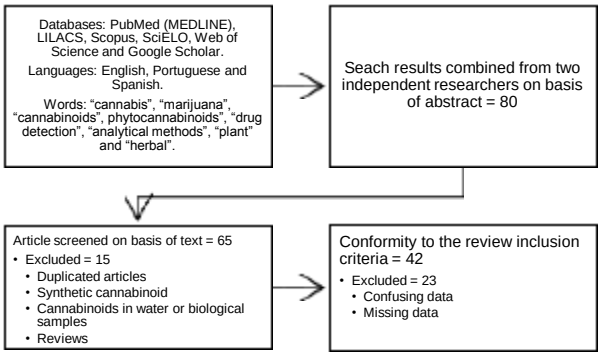

Figure 1 Selection process of the articles.

Results and Discussion

Cannabis sativa L.: botanical aspects forensic view

Although there is constant discussion regarding the botanical classification of cannabis (18-20) since it was first classified in 1753, by the Swedish botanist Carolus Linnaeus (Carl Von Linné), the "Recommended methods for the identification and analysis of cannabis and cannabis products" of the United Nations Office on Drugs and Crime considers that the plant has only one recognized specie, it is the *Cannabis sativa* L. (Linnaeus) (9, 18, 19). Other species reported for the genus (*C. sativa* subsp. *sativa*, *C. sativa* subsp. *indica*, *C. sativa* subsp. *ruderalis*, *C. sativa* subsp. *spontanea*, *C. sativa* subsp. *kafiristanca*) currently are recognized as subspecies of the *C. sativa* L. (9, 18-20).

Furthermore, due to the difficulty of distinguishing the cannabis subspecies either in chemical terms or morphologically, given that cannabis presents continuous changes according to the environment and conditions in which it was planted, the designation *C. sativa* is considered suitable for all plants for the genus (9, 20).

Cannabis is an annual plant, dicotyledonous, angiosperm, usually dioecious, with male and female flowers on separate plants, but can also be monoecious, comprising flowers of both sexes in a single plant. The stamens (male) are generally higher, but less robust than the pistils (female). Before the occurrence of the flowering, the gender of the cannabis plant is

indistinguishable, however, throughout the plant development each gender varies widely, and the difference among the male and female plant becomes evident. The roots are straight and can range from 0.2 to 6 meters, though the majority of plants reach heights from 1-3 meters. Both the branching degrees, such as plant height, depends on hereditary and environmental factors and the manner of cultivation (9, 21, 22).

The fruits of cannabis, usually referred to as seeds, are small dried nuts, botanically named achene. The fruit contains one seed consists of two cotyledons and the major part of its mass is rich in reserve substances. The weight of achenes is quite variable, from 2 to 70 grams per 1,000 seeds. Typically, the seeds in monoecious varieties are smaller than in dioecious variety (21). The plant spreads from these seeds, which grow vigorously in sunny environments, with neutral to alkaline soils requiring nutrients and water in abundance. The pollen grains produced by male flowers require air currents to carry them to the female flowers, resulting in fertilization and consequent production of seeds (9, 21).

The cannabis growth cycle can be divided into four phases: germination and emergence; vegetative stage; flowering and seed formation; and senescence. The vegetative phase can be divided into three phases: juvenile stage; photosensitive phase; and flower development phase (21, 23). Male plants cease the dissemination after producing millions of pollens and then died (9, 21).

Because it is a short-day plant, the critical photoperiod of cannabis is the time of day which the seed is induced to flower in time when the juvenile stage be ready, corresponding to approximately 14 hours (21, 23). Flowering plant usually begins when the darkness exceeds 11 hours a day and this flowering cycle ranges between 4 and 12 weeks, depending on the strain and environmental conditions (9, 23). Shorter days (longest nights) induce early flowering and consequently the plant to complete its life cycle. Thus, cannabis starts flowering when exposed to short days - 12 to 14 hours (nights from 10 to 12 hours or more). However, a single interrupted night of darkness can disrupt and delay the maturation of flowering. Moreover, maintain one or two short days may induce flowering, which may be irreversible in early maturing varieties (23).

After ripening seeds, they can be harvested, eaten by birds or rodents, or fall to the ground where they can germinate the following spring (9). The female plants produce several individual bunches of flowers, a large cluster on the upper torso and various small in each branch. Instead of setting the seeds in the first flowers, the female plants continue to produce additional flowers and these are covered by glands named trichomes containing a rich resin cannabinoids and terpenoids (22).

Although the genetics of the plant determines that it becomes male or female, environmental factors including the diurnal light cycle, can change the gender of the plant (hermaphrodites). Natural hermaphrodite with both genders are generally sterile, but induced hermaphrodites can artificially have fully functional reproductive organs. Feminized seeds of cannabis are obtained from artificially hermaphroditic females lacking the male chromosome or by seed treatment with hormones or silver thiosulphate. Thus, the production of pistils (female) can also be obtained by seeds (9, 21, 23).

Over the years a wide variety of chemical constituents that are part of the various classes of natural products have been identified in *C. sativa* (24, 25). Currently, more than 750 chemical constituents have been identified in the plant (25). Among these, the natural products are mono- and sesquiterpenes, flavonoids, steroids, nitrogen compounds, besides the cannabinoids, the class of metabolites with toxicological significance (24, 25). Of the total compounds identified to date, more than 100 are classified as cannabinoids (25), which are encountered only in cannabis plants (24, 25).

The term "cannabinoids" refers to a group of C_{21} or C_{22} terpenophenolic compounds, including analogues and metabolites (24, 25). They are secondary metabolites with a predominantly nonpolar character and therefore poorly soluble in water. They are synthesized in secreting cells which are inside glandular trichomes (26, 27). These structures are present in greater proportion in the flowers and inflorescences (buds) female unfertilized prior to senescence (26, 28). Smaller amounts of cannabinoids are found in leaves, petioles and stems, and they are absent in the roots and seeds, since the seeds of cannabis are protected by bracts, forming structures called achenes. As a result, the latter plant organs do not contain cannabinoids (27, 28).

Cannabis material analyzed

According to UNODC (1), cannabis is opposite in relation to other drugs, because although the number of being stable seizures, the number of users and dependence reported increases each year (1, 29). It suggests that in 23 of the 50 north american states in which the medicinal and/or recreational use cannabis moves a business equivalent to the tobacco industry, being treated as a commodity (30). In the last two decades, the ratio of THC/CBD (cannabidiol) increased in the seized marijuana and this is linked to increase of neurotoxicity and cases of drug dependence (31). Functional neuroimaging studies have reported increases in neural activity in regions that may be related to cannabis intoxication or change in mood and reduction in activity of regions related to cognitive functions impaired during acute intoxication (32). Studies showed that frequent use is associated with greater severity of dependence, triplicates the chance of developing psychotic episodes and increased risk of cardiovascular disease and lung cancer (33-35). Therefore, the knowledge and understanding of

cannabis and its compounds are very important to provide data for further researches and corroborate with the clinical findings about pontency of the drug. Furthermore, the results of analysis can provide similarities between samples, sources of interconnecting production and trafficking. So, the data set acquired from cannabis samples analysis may also provide informations which can trace ways to assist forensic experts and control the cannabis use.

Cannabis is a complex plant that naturally contains cannabinoids groups, closely related terpenophenolic compounds, which can occur a huge variation in their quantitative ratios. When study cannabis samples, understanding how the cannabinoids are chemically related to each other is substantial, since changes in the cannabinoid profile might occur not only in the different chemotypes. The conditions during growing and storage, such as environmental factors of cultivation (climates and elevation of cultivated area), the development stage of the plant at harvest time as well as genetic characteristics of seed-stocks are important factors that influence in the high variability and chemical composition of cannabinoids contents in cannabis plants (6, 36).

The progress in cannabis cultivation techniques have shown that stress conditions also increases the production of cannabinoids, besides have led to an increase in the potency and yield of cannabis. Advances including plantation using automated indoor lighting, ventilation, automated irrigation and fertilization and using selective breeding of certain strains of cannabis are some examples of the main techniques that have been used through of the last decade (36, 37).

Chemical types of cannabinoids can be divided in three groups: I) cannabinoids produced by biosynthesis of the plant (acid cannabinoids); II) cannabinoids present in the plant resulting from natural decarboxylation of acid cannabinoids (neutral cannabinoids) under the influence of storage, light and/or heat, by losing the relatively unstable carboxyl-group in the form of CO_2; and III) cannabinoids occurring as artefacts by degradation products resulting from various influences, such as UV-light, oxidation or isomerization (38).

The most common types of acid cannabinoids found in cannabis plant are tetrahydrocannabinolic acid (THCA), cannabidiolic acid (CBDA), cannabigerolic acid (CBGA) and cannabichromenic acid (CBCA) (5, 6, 38). These acids can be converted to their neutral counterparts by decarboxylation to form Δ9-tetrahydrocannabinol (Δ9-THC), cannabidiol (CBD), cannabigerol (CBG) and cannabichromene (CBC), respectively (6, 11, 36, 38). Degradation of Δ9-THC results in formation of cannabinoids breakdown products as cannabinol (CBN), produced by oxidative degradation (36-38) and Δ8-tetrahydrocannabinol (Δ8-THC) transformed by isomerization (6, 38), while THCA can further degrade into cannabinolic acid (CBNA) and this to CBN (5, 38). The cannabinoids cannabicyclolic acid (CBLA) and cannabicyclol (CBLA) arise, respectively, by exposure of CBCA and CBC to UV-radiation, leading to crosslinking of two double bonds in the molecule (38). Figure 2 shows the relationships between the main cannabinoid types that are usually detected in cannabis plant.

Methods of analysis

Although the most usual instrumental methods for analysis of cannabinoids are still GC/FID and LC/UV, and even the use of thin layer chromatography (TLC) is accepted as a confirmatory method for the cannabinoid profile by UNODC (9), the requirements for an acceptable cannabis assay and the knowledge of cannabinoids present in plant have changed dramatically over the years resulting in a large number of laboratories using a diverse array of analytical methodologies.

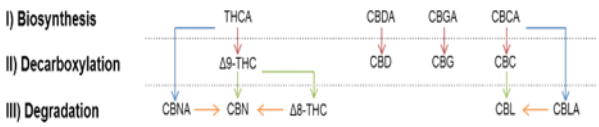

Figure 2 Relationships between the main cannabinoid types that are usually detected in cannabis plant as cannabinoids produced by biosynthesis, cannabinoids resulting from natural decarboxylation and cannabinoids as artefacts by degradation. CBC: cannabichromene; CBCA: cannabichromenic acid; CBD: cannabidiol; CBDA: cannabidiolic acid; CBG: cannabigerol; CBGA: cannabigerolic acid; CBL: cannabicyclol; CBLA: cannabicyclolic acid; CBN: cannabidiol; CBNA: cannabinolic acid; Δ8-THC: Δ8-tetrahydrocannabinol; Δ9-THC: Δ9-tetrahydrocannabinol; THCA: tetrahydrocannabinolic acid.

Gas Chromatography analysis

GC is an appropriate method for cannabis profiles and chemical fingerprints, as it allows the identification of a large variety of cannabinoids with very high resolution, especially when coupled with MS (GC/MS). However, during the analysis the high temperatures required for sample vaporization before injection can result in decarboxylation of the acid cannabinoids to their corresponding neutral forms and the thermal degradation of some cannabinoids (13). Therefore, to quantifying cannabinoids by GC analysis is required to determine the total content of each cannabinoid (the sum of its acid and neutral form), because the thermal conversion of acid cannabinoids may be not complete, resulting in a nonrepresentative analysis of the cannabis samples (6, 13).

To avoid the decarboxylation of acid forms, a time-consuming derivatization before GC analysis must be performed (39), e.g. by silylation as the trimethylsilyl ethers. However, an effective derivatization yield for all components in a complex mixture is difficult to achieve (13) and may also occur the thermo-degradation of

derivatized cannabinoids in injector and/or column system (40). Whereas the cannabis plant mainly contains the acid forms of cannabinoids, GC analysis presents a limited value to establish the metabolic profile of a cannabinoid sample. Table 1 lists all the GC methods described in the articles included in this systematic review.

Liquid Chromatography analysis

An accurate manner to assay the cannabis composition is to use a method that does not involve thermal stress, such as LC (40). This technique allows the simultaneous detection of both acid and neutral cannabinoids with no need of derivatization. However, the complex composition of the cannabis material leads to an arduous perform to achieve the separation of major cannabinoids and significant peak overlap occurs. The use of LC coupled with MS (LC/MS) may assist to resolve cannabinoids of interest though LC/MS but does not allow characterization of an entire cannabis sample, merely the determination of specific analytes (13). Table 2 lists all the LC methods described in the articles included in this systematic review.

Mass spectrometry detector and analysis

MS is a technique that can be used as a detector, coupled to a chromatography system or the sample may be analyzed directly in MS, lacking the separation of compounds contained in the sample. The main MS objective, as a detector or a method of analysis is to identify an analyte, especially in the presence of other analytes, based on the measurement of the analyte ion mass, according to their different mass ratios/charge (*m/z*) (41, 42). Table 3 lists all the MS parameters used to identify cannabinoids, described in the articles included in this systematic review.

Although the full analysis of a complete cannabis extract with a single HPLC method is hard, resulting in chromatographic overlap, the HPLC method may be routinely combined with a secondary analysis by GC. Similarly, the difficulty to analyze both the acid cannabinoids and neutral with a GC method due to the necessity of derivatizing the acid forms, the GC method may also be combined with a secondary analysis by HPLC. By combining these two techniques of analysis, all major cannabinoids could be effectively identified and quantified.

Simultaneously to the methods presented in Table 1, Table 2 and Table 3, there are some additional techniques and approaches that can be applied to the analysis of cannabis products. Methods for the identification of marijuana include: botanical identification, microscopical examination of leaves (58), chemical screening tests (57-59), THC identification through biochemical methods (60), and the use of molecular sequencing to identify DNA sequence homology to reference marijuana samples (61-63). Besides that, there are some unusual techniques used for this proposital, such as Nuclear Magnetic Resonance and electronic nose systems (51, 54)

The genetic analysis provides the opportunity to link products on the basis of their genetic profiles, which could be useful from an investigative point of view, e.g. to link producers, traffickers and consumers (9). The main technique used is the PCR for analysis of SNPs (15, 28), Inter-Simple Sequence Repeat (ISSR) (49, 61), STR (45, 62) or specific genes (16, 63). However, unlike humans, the DNA fingerprint may not necessarily be unique, as cloning of cannabis strains is quite common. Matching DNA profiles of two samples does not by itself prove that they come from the same plant, let alone the same grower (9). Moreover, the diferent subspecies, as all other environmental and nutritional parameters affect the genetic analysis (45), along with the fact that genetic analysis of cannabis samples is a relatively expensive technique and sometimes questionable (9). It is not recommended to only perform genetic testing for forensic purposes.

Table 1 GC methods described in the articles included in this systematic review.

Reference	Extraction method	Solvent extraction	Column	GC conditions	Cannabinoids	Detector
(3)	liquid-liquid	hexane / ethyl acetate (6:4)	HP-5MS (30 m, 0.25 mm i.d., 0.25 µm film thickness) with a stationary phase of 5% phenyl and 95% dimethyl polysiloxane	Temperature program starts at 100 °C (hold for 1 min), increases to 290 °C (at 20 °C/min) and holds for 10 min.	CBD CBN THC	MS
(7)	liquid-liquid	hexane	HP-5ms (30 m, 0.25 mm i.d., 0.25 µm film thickness)	Temperature program starts at 100 °C, increases to 260 °C (at 10 °C/min) and holds for 10 min	cannabinoid 1 THV cannabinoid 2 CBL CBD THC CBG CBN	MS
(8)	liquid-liquid	hexane	HP-5MS (30 m, 0.25 mm i.d., 0.25 µm), 5% phenylmethylpolysiloxane	Temperature program starts at 100 °C (hold for 1 min), increases to 260 °C (at 10 °C min/min) and holds for 10 min	CBC CBD THC CBG CBN	MS
(10)	liquid-liquid	methanol	HP–5MS (30 m, 0.25 mm i.d., 0.25 µm film thickness)	Temperature program starts at 150 °C (hold for 1 min), increases to 280 °C (at 10 °C/min) and hold for 5 min	Δ8-THC THCV CBC CBD THC CBG CBN	FID and MS
(12)	liquid-liquid	hexane	HP-5MS (30 m, 0.25 mm i.d., 0.25 µm film thickness)	Temperature program starts at 100 °C, increases to 260 °C (at 10 °C/min) and holds for 10 min	CBC cannabivarin CBD THC CBG CBN	MS

(36)	liquid-liquid	methanol	DB-1 (30 m, 0.32 mm i.d., 0.25 µm film thickness)	Temperature program starts at 230 °C (hold for 7 min), increases to 260 °C (at 10 °C/min) and holds for 2 min	CBD	FID
					THC	
					CBN	
(37)	liquid-liquid	0.5 mg/mL tribenzylamine in ethanol	HP 1 (25 m, 0.32 mm i.d., 0.52 mm film thickness)	Temperature program starts at 250 °C (hold 9.50 min)	THC	FID and MS
					CBD	
					CBN	
(43)	liquid-liquid	100 mg of 4-androstene-3,17-dione + 10 mL chloroform + 90 mL methanol	DB-1 (15 m, 0.25 mm i.d., 0.25 µm film)	Temperature program starts at 170 °C (hold for 1 min), increases to 250 °C (at 10 °C/min) and holds for 3 min	THC	FID
					THCV	
					CBD	
					CBC	
					CBG	
					CBN	
(44)	liquid-liquid	ethanol	DB5 (30 m, 0.25 mm i.d., 0.25 µm film thickness)	Temperature program starts at 60 °C, increases to 240 °C (at 3 °C/min) and holds for 5 min	CBDV	FID
					THCV	
					CBD	
					CBC	
					CB(1)	
					CBGM	
					Δ8-THC	
					THC	
					CBG	
(44)	liquid-liquid	ethanol	HP5 (30 m, 0.25 mm i.d., 0.25 µm film thickness)	Temperature program starts at 60 °C, increases to 240 °C (at 3 °C/min) and holds for 5 min	CBDV	MS
					THCV	
					CBD	
					CBC	
					CB(1)	
					CBGM	
					Δ8-THC	
					THC	
					CBG	

(45)	liquid-liquid	ethanol	HP5ms (30 m)	Temperature program starts at 80 °C (hold 1 min), increases to 300 °C (at 50 °C/min) and holds for 9.6 min	THC	MS
(46)	-	-	DB-1 (15 m, 0.25 mm i.d., 0.25 µm film)	Temperature program starts at 170 °C (hold for 1 min), increases to 250 °C (at 10 °C/min)	THCV	FID
					CBD	
					CBC	
					THC	
					CBG	
					CBN	
(47)	liquid-liquid	100 mg of 4-androstene-3,17-dione + 10 mL chloroform + 90 mL methanol	DB-1MS (15 m, 0.25 mm i.d., 0.25 µm film)	Temperature program starts at 170 °C (hold for 1 min), increases to 250 °C (at 10 °C/min) and hold for 3 min	THC	FID
					THCV	
					CBC	
					CBD	
					CBG	
					CBN	
(48)	liquid-liquid	ethanol	DB5 (30 m, 0.25 mm i.d., 0.25 µm film thickness)	Temperature program starts at 60 °C, increases to 240 °C (at 3 °C/min) and holds for 5 min	THCV	FID
					CBD	
					CBC	
					unknown cannabinoid	
					CBGM	
					THC	
					CBG	
					CBN	
(48)	liquid-liquid	ethanol	HP5 (30 m, 0.25 mm i.d., 0.25 µm film thickness)	Temperature program starts at 60 °C, increases to 240 °C (at 3 °C/min) and holds for 5 min	THCV	MS
					CBD	
					CBC	
					unknown cannabinoid	
					CBGM	
					THC	
					CBG	
					CBN	
(49)	-	-	DB-1 (15 m, 0.25 mm i.d., 0.25 µm film)	Temperature program starts at 170 °C (hold	THCV	FID
					CBD	

				for 1 min), increases to 250 °C (at 10 °C/min)	CBC	
					THC	
					CBG	
					CBN	
(50)	liquid-liquid	methanol	DB-1 (30 m, 0.32 mm i.d., 0.25 µm film thickness)	Temperature program starts at 230 °C (hold for 7 min), increases to 260 °C (at 10 °C/min) and holds for 2 min	CBD	FID
					THC	
					CBN	

CB(1): unknown cannabinoid; CBC: cannabichromene; CBD: cannabidiol; CBDV: cannabidivarin; CBG: cannabigerol; CBGM: cannabigerol monomethyl ether; CBL: cannabicyclol; CBN: cannabidiol; Δ8-THC: Δ8-tetrahydrocannabinol; THC: tetrahydrocannabinol; THCV: tetrahydrocannabivarin; FID: flame ionization detector; MS: mass spectrometry;

Table 2 LC methods described in the articles included in this systematic review.

Reference	Extraction method	Solvent extraction	Column	Mobile phase	Flow (mL/min)	Cannabinoids	Detector
(4)	liquid-liquid	methanol / chloroform (9:1)	C18 (3.5 µm, 150 mm×4.6 mm i.d.) with a 1 mm opti-guard C18 precolumn	solvent A: 50 mM ammonium formate buffer pH 3.75 with 10% acetonitrile, solvent B: 90% acetronitrile; The gradient program: 0 min, 70% B; 15 min, 90% B; 30 min, 90% B; 31 min, 70% B and 40 min 70%.	1.0	CBDA	DAD (272 nm)
						CBGA	
						CBG	
						CBD	
						THCV	
						CBN	
						THCA	
						THC	
						CBC	
(5)	liquid-liquid	ethanol / chloroform (9:1)	C8 (3 µm, 125 mm×4 mm i.d.) with a guard column (3 µm depth filter × 4 mm)	solvent A: methanol and solvent B: water with 0.1% of acid acetic). The gradient program: 50% A, increased to 90% A over 20 min, maintained at 90% A over the next 1.5 min, decreased to 50% A over the next 0.5 min, and held at 50%	0.7	CBGA	DAD (230 nm)
						THCA	
						CBDA	
						CBG	
						THC	
						CBD	
						CBC	
						CBN	
(6)	liquid-liquid	methanol / chloroform (9:1)	C18 (5 µm, 250×2.1 mm i.d.) protected by a C18 guard column (5 µm, 10×2.1 mm i.d.)	methanol / water 50mM of ammonium formate (pH 5.19). The gradient program: 68% methanol, increased to 90.5% methanol over 25 min, then increased to 95% in 1min and maintaining for 3 min	0.3	CBDA	DAD (211 nm) neutral
						CBGA	
						CBD	
						CBG	
						THCA	DAD (220 nm) acids
						CBN	
						THC	
						Δ8-THC	
(11)	liquid-liquid	hexane / ethyl acetate (9:1)	100 RP-18 (5 µm) LiChroCart 125-4	Triethylammoniumphosphate buffer pH 3.0 (25 mmol/L in nanopure water) and acetonitrile: 36:64, in isocratic mode	1.5	THC	UV (210nm)
						THCA	

(13)	liquid-liquid with soxhlet	ethyl acetate	CN 100Å (4.60 mm×150 mm i.d., 5 µm)	methanol and acid potassium permanganate chemiluminescence; 10% methanol in the initial mobile phase composition. The gradient program: 0 to 70% methanol over a gradient of 12 min followed by 3 min of 100% metanol	1.0	CBV	2D-HPLC: UV (220 nm) / CL
						CBCV	
						CBDV	
						CBLV	
			EC-C18 (4.60 mm×50 mm i.d., 2.7 µm)	methanol and acid potassium permanganate chemiluminescence; The gradient program: 0 to 100% methanol in 3 min followed by 3 min of 100% metanol		CBGV	
						CBN	
						CBD	
						CBL	
			-	Postcolumn acid potassium permanganate chemiluminescence was generated using custom-built manifold. The chemiluminescence reagent merged with the HPLC eluent at a T-piece junction and the light emitted from the reacting mixture was detected.	1.2	CBG	
						CBE	
						CBT	
						CBNA	
						CBCA	
						CBDA	
						CBLA	
						CBGA	
(14)	liquid-liquid	methanol / n-hexane (9:1)	LiChroCart 125-4, LiChrospher 60, RP-Select B, 5 µm, column holder: manu-CART "4" and pre column: LiChrospher 60, RP-Select B, 5 µm,	TEAP buffer 25 mmol/L in deionized water and acetonitrile: 36:64, in isocratic mode	1.0	CBD	DAD (210 nm)
						CBN	
						THC	
						THCA	
(39)	liquid-liquid	methanol / chloroform (9:1)	Onyx Monolithic (100 mm×4.6 mm i.d.)	methanol and water: 75:25, in isocratic mode	0.8	CBD	PDA (220 nm)
						CBN	
						THC	

(40)	HTH	ethanol	EC-C18 (150mm×2.1 mm i.d., 2.7 µm) with a EC-C18 guard column (5×2.1 mm i.d., 2.7 µm)	solvent A: water, 0.1% formic acid and solvent B: 0.1% formic acid. The gradient program: 8 min isocratic hold at 66% B, gradient to 95% B over 4 min; 95% B maintained for 1 min	0.5	CBDVA CBDV CBDA CBGA CBG CBD THCV THCVA CBN THC Δ8-THC CBC THCA	DAD (200 - 400 and 214 nm)
(51)	liquid-liquid	methanol	C18 (5 µm, 150 mm×2.1 mm i.d.)	solvent A: water, 0.1% formic acid and solvent B: methanol, 0.1% formic acid. The gradient program: 30% B, increased to 70% B in 1 min, then increased to 90% B in 30 min	0.25	THCA-D3 CBD CBG CBDA CBN THC Δ8-THC CBC THCA	MS
(52)	CPE	0.1 to 0.7 g Dowfax 20B102 + 0.2 g Na_2SO_4 diluted to 10 mL with deionized water	C18 (5 µm, 250×4.6 mm i.d.)	Acetonitrile and water, acidified with 2.5 M H_2SO_4 (pH=1.8): 83:17, in isocratic mode	1.0	THC	UV (DAD) (231 nm)

(53)	SFE	CO_2 as extraction solvent and ethanol (20%) as co-solvent.	C18 (2.6 µm, 150 mm×3 mm i.d.) with a guard column (0.5 µm depth filter×0.1 mm)	solvent A: deionized water, 0.1% formic acid, and solvent B: methanol, 0.1% formic acid. The gradient program: 50% B increased to 80% B over the first min, held at 80% B until 11 min, increased to 95% B over the next 2 min, held at 95% B until 16 min, decreased to 50% B over the next 2 min, and held at 50% B until 28 min	0.25	CBD	MS/MS
						THCV	
						CBG	
						CBN	
						THC	
						THCA	
				solvent A: water, 0.1% formic acid and solvent B: methanol, 0.1% formic acid. The gradient program: 50% B, increased to 100% B over 15 min, held at 100% B until 17 min, decreased to 50% B over the next 2 min, and held at 50 B until 22 min		cannabicoumaric acid	Q-ToF
						CBCA	
						10-EtO-9-OH-Δ^6a-THC	
						[(±)-4-AcO-CBC-C5	
						CBGA	
						CBGAM	
						THCA-C4	
(54)	liquid-liquid	ethyl acetate / ethanol 40%; and methanol / metanol 70%	5 phenyl (25 cm x 4.6 mm i.d.) and C8 guard column 3.9 mm×20 mm, 2/pkg	solvent A water (TFA 0.1%), solvent B water-acetonitrile (65:35, TFA 0.1%) and solvent C acetonitrile; The gradient program: solvent A 0 min 70%, 10 min 60%, 38 min 40%, 40 min 5%, 55 min 0%, 74 min 70%	0.9	CBDA	DAD-UV (210-400 nm) (257 nm) neutral (324 nm) acids
						CBGA	
						CBG	
			C18 (4.6 mm×250 mm i.d., 5 µm)	solvent B water-acetonitrile (65:35, TFA 0.1%) and solvent C acetonitrile; The gradient program: solvent B: 0 min 70%, 30 min 35%, 43 min 5%, 48 min 70%		CBD	
						CBN	
						THC	
						THCA	

(±)-4-AcO-CBC-C5: 4-acetoxycannabichrome; 10-EtO-9-OH-Δ^6a-THC: 10-ethoxy-9-hydroxy-Δ^6a-tetrahydrocannabinol; CBC: cannabichromene; CBCA: cannabichromenic acid; CBCV: cannabichromevarin; CBD: cannabidiol; CBDA: cannabidiolic acid; CBDV: cannabidivarin; CBDVA: cannabidivarinic acid; CBE: cannabielsoin; CBG: cannabigerol; CBGA: cannabigerolic acid; CBGAM: cannabigerolic acid A monomethyl ether; CBGV: cannabigerovarin; CBL: cannabicyclol; CBLA: cannabicyclolic acid; CBLV: cannabicyclolvarin; CBN: cannabidiol; CBNA: cannabinolic acid; CBT: cannabicitran; CBV: cannabivarin; CL: chemiluminescence; CPE: cloud point extraction; DAD: diode-array detector; Δ8-THC: Δ8-tetrahydrocannabinol; HTH: high throughput homogenization; MS/MS: tandem mass spectrometry; THC: tetrahydrocannabinol; THCA: tetrahydrocannabinolic acid; THCA-C4: tetrahydrocannabinolic acid-C4; THCA-D3: (±)-11-nor-Δ9-THC carboxylic acid-D3; THCV: tetrahydrocannabivarin; THCVA: tetrahydrocannabivarinic acid; PDA: photodiode-array detector; Q-ToF: quadrupole-time-of-flight; SFE: supercritical fluid extraction; TEAP: triethylammoniumphosphate 1 M; TOF: time-of-flight; UV: ultraviolet.

Table 3 MS detector parameters used to identify cannabinoids, described in the articles included in this systematic review.

Reference	Detector	Ionization mode	Cannabinoid	Quantifier ion (*m/z*)	Qualifier ions (*m/z*)	Limit of quantification (LOQ)	Limit of detection (LOD)
(3)	MS	EI (70 V)	CBD	231	174, 314	0.01% (w/w)	0.005% (w/w)
			CBN	295	238, 310	0.01% (w/w)	0.005% (w/w)
			THC	299	314, 231	0.01% (w/w)	0.005% (w/w)
(7)	MS	electron multiplier voltage (1976 V), ion source (230 °C), quadrupole (150 °C)	cannabinoid 1	231	314, 299, 271	-	-
			THV	271	286, 203, 243		
			cannabinoid 2	231	314, 174, 243		
			CBL	231	232, 274, 314		
			CBD	231	174, 314, 299		
			THC	299	314, 231, 271		
			CBG	193	231, 123, 316		
			CBN	295	238, 310, 223		
(8)	MS	-	CBC	-	-	-	-
			CBD				
			THC				
			CBG				
			CBN				
(10)	MS	EI (70 V)	Δ8-THC	-	-	-	-
			THCV				
			CBC				
			CBD				
			THC				
			CBG				
			CBN				
(12)	MS	electron multiplier voltage (1200 V), ion source (230 °C), quadrupole (150 °C)	CBC	231	174, 314, 299	-	-
			cannabivarin	267	282, 238, 223		
			CBD	231	174, 314, 246		
			THC	299	314, 231, 271		
			CBG	93	231, 123, 316		
			CBN	295	238, 310, 223		

(13)	TOF	ESI positive mode, nitrogen (as drying gas: 7 mL/min, 350 °C) and (as nebulizer gas: 16 psi), capillary voltage (4.0 kV), vaporizer temperature (350 °C), cone voltage (60 V)	CBV	-	282.38	-	-
			CBCV		286.41		
			CBDV		286.41		
			CBLV		286.41		
			CBGV		288.42		
			CBN		310.43		
			CBC		314.46		
			CBD		314.46		
			CBL		314.46		
			CBG		316.48		
			CBE		330.46		
			CBT		346.46		
			CBNA		354.44		
			CBCA		358.47		
			CBDA		358.47		
			CBLA		358.47		
			CBGA		360.49		
(37)	MS	-	THC	-	-	-	-
			CBD				
			CBN				
(45)	MS	-	THC	-	-	-	-
(51)	MS	ESI positive mode, ionization spray voltage (5.2 kV), turboIon spray (450 °C), nitrogen (as a turbo heating gas, nebulizing gas, and curtain gas)	CBD	-	315.2, 193.2	-	-
			CBG		317.2, 193.2		
			CBN		311.2, 223.2		
			THC		315.2, 193.2		
			Δ8-THC		315.2, 193.2		
			CBC		315.2, 193.2		
		ESI negative mode, ionization spray voltage (-4.5 kV), turboIon spray (450 °C), nitrogen (as a turbo heating gas, nebulizing gas, and curtain gas). For negative ionization, post-column addition of a 1% ammonia solution in the extract was utilized at a flow rate of 50 µL/min	THCA-D3		346.2, 302.1		
			CBDA		357.2, 339.2		
			THCA		357.2, 313.2		

(53)	MS/MS	APCI positive mode, capillary voltage (3500 V), vaporizer temperature (280 °C), nitrogen (7 L/min at 210 °C), nebulizer (32 psi)	CBD	315.1	192.8 259.0	0.5 ng/mL	0.2 ng/mL
			CBG	287.1	165.0 231.0	0.5 ng/mL	0.05 ng/mL
			CBDA	317.2	293.0 123.0	0.5 ng/mL	0.02 ng/mL
			CBN	311.0	222.9 293.0	0.5 ng/mL	0.05 ng/mL
			THC	315.0	193.0 259.0	0.5 ng/mL	0.05 ng/mL
			Δ8-THC	315.1	193.0 259.1	0.5 ng/mL	0.2 ng/mL
	Q-ToF	APCI positive mode, source (450 °C), capillary voltage (0.7 kV), corona discharge (5 μA), nitrogen (as the desolvation: 800 L/h) and (as cone gas: 20 L/h), source temperature (120 °C), desolvation temperature (300 °C)	CBC	-	373.2015	-	-
			THCA	-	359.2222	-	-
			10-ethoxy-9-hydroxy-Δ^6a-THC	-	375.2535	-	-
			4-acetoxycannabichrome	-	373.2380	-	-
			CBGA	-	361.2379	-	-
			CBGAM	-	375.2535	-	-
			THCA-C4	-	345.2066	-	-
(55)	MS/MS	LAESI positive, infrared laser (2940 nm), pulsed mode (10 Hz)	CBD	-	-	-	-
			THC				
(56)	MS	STELDI positive mode, laser power (20 μJ), 3 shots per step, collision-induced dissociation energy (30-50 eV)	CBD-C4	-	301, 259, 181	-	-
			CBND		311, 201, 193		
			CBN		311, 201, 193		
			THC		315, 297, 259, 193, 181		
			CBD		315, 297, 259, 193, 181		
			CBC		315, 297, 259, 193, 181		
			OTHC		329, 311, 193		
			CBCON-C5		329, 311, 193		
			CBGM		331, 201, 193		
			CBCVA-C3 A		331, 201, 193		
			CBDVA-C3		331, 201, 193		
			Δ9-THCA-C 4 A and/or B		345, 193, 299		

(57)	FT-ICR MS	ESI negative mode, infusion fow rate (5 mL/min), capillary voltage (3.0 kV), nebulizing temperature (250 °C), collision gas (nitrogen), ion accumulation (1 s), isolation window (1.0 - *m/z* units), collision energy (25-45%)	CBD-C1 or Δ9-THCO-C1	-	257.1547	-	-
			CBN-C3 or CBVD-C3		281.1547		
			CBE-C3, CBDV-C3, Δ9-THCV-C3 or 2-methyl-2-(4-methyl-2-pentenyl-7-propyl-2H-1-benzopyran-5-ol		285.1860		
			CBN-C4		295.1703		
			Δ9-THCOA-C1 A and/or B		301.1445		
			CBCN-C3		303.1602		
			DCBF-C5		307.1703		
			CBN-C5, CBF-C5 or CBND-C5		309.1860		
			Unknown		313.1809		
			Δ9-*trans*-THC-C5, CBD, Δ8-*trans*-THC, *cis*-Δ9-*trans*-THC-C5, CBL-C5 or CBC-C5		313.2173		
			OTHC		327.1966		
			(*E*)-CBGVA-C3, CBCON-C5 or CBE-C5		329.2122		
			CBEA-C3 B, CBDVA-C3 or Δ9-THCVA-C3 A		329.1758		
			CBCN-C5		331.1915		
			Unknown		331.2279		
			CBCVA-C3 A or Δ9-THCA-C4 A and/or B		343.1915		
			CBEA-C5 A and/or B		345.2071		
			$[CBNA - H]^-$		353.1758		
			$[CBDA\text{-}C5 - H]^-$, $[\Delta 9\text{-}THCA\text{-}C5\ A - H]^-$, $[\Delta 9\text{-}THCA\text{-}C5\ B - H]^-$ or $[CBLA\text{-}C5\ A - H]^-$		357.2071		
			Unknown		359.2228		
			(–)-6a,7,10a-trihydroxy-Δ9-tetrahydrocannabinol or (–)-cannabitetrol		361.2020		
			Unknown		367.1551		
			Unknown		369.1707		
			Δ9-THCA-A-8-one		371.1864		
			Unknown		375.1813		
			Unknown		377.1910		
			Unknown		385.1657		
			Δ9-THCA-A-COOH		387.1813		
			8β,11-*bis*-hydroxy-Δ9-THC-A		389.1970		
			Δ9-THCA + C_2H_2O		399.2177		
			Dimer: 328 Da + 310 Da		637.3899		

			Dimer: 314 Da + 328 Da		641.4212		
			Dimer: 326 Da + 328 Da		653.3848		
			Dimer: 346 Da + 328 Da		673.4110		
			Dimer: 354 Da + 328 Da		681.3797		
			Dimer: 358 Da + 328 Da		685.4114		
			Dimer: 390 Da + 328 Da		717.4008		

APCI: Atmospheric-pressure chemical ionization; C_2H_2O: ethenone; CBC: cannabichromene; CBC-C5: cannabichromene; CBCA: cannabichromenic acid; CBCN-C3: cannabichromanone-C3; CBCN-C5: cannabichromanone; CBCON-C5: cannabicoumaronone; CBCV: cannabichromevarin; CBCVA-C3 A: cannabichromevarinic acid A; CBD: cannabidiol; CBDA: cannabidiolic acid; CBDA-C5: cannabidiolic acid; CBD-C1: cannabidiorcol; CBDV: cannabidivarin; CBDV-C3: cannabidivarin; CBDVA-C3: cannabidivarinic acid; CBE-C3: (5a*S*,6*S*,9*R*,9a*R*)-C3-cannabielsoin; CBE-C5: (5a*S*,6*S*,9*R*,9a*R*)-cannabielsoin; CBEA-C3 B: (5a*S*,6*S*,9*RS*,9a*R*)-9,10-C3-cannabielsoic acid B; CBEA-C5 A and/or B: (5a*S*,6*S*,9*R*,9a*R*)-cannabielsoic acid A and/or B; CBF: cannabifuran; CBG: cannabigerol; CBGA: cannabigerolic acid; CBGAM: cannabigerolic acid monomethylether; CBGM: cannabigerol monomethyl ether; CBGV: cannabigerovarin; (*E*)-CBGVA-C3: cannabigerovarinic acid A; CBL: cannabicyclol; CBL-C5: cannabicyclol; CBLA: cannabicyclolic acid; CBLA-C5 A: cannabicyclolic acid A; CBLV: cannabicyclolvarin; CBN: cannabidiol; CBN-C5: cannabinol-C5; CBNA: cannabinolic acid; CBN-C3: cannabivarin; CBN-C4: cannabinol-C4; CBND: cannabinodiol; CBND-C5: cannabinodiol; CBT: cannabicitran; CBV: cannabivarin; CBVD-C3: cannabinodivarin; DCBF-C5: dehydrocannabifuran; Δ9-*trans*-THC-C5: (−)-Δ9-*trans*-(6a*R*,10a*R*)-tetrahydrocannabinol; Δ8-*trans*-THC: (−)-Δ8-*trans*-(6a*R*,10a*R*)-tetrahydrocannabinol; *cis*-Δ9-THC-C5: (−)-Δ9-*cis*-(6a*R*,10a*R*)-tetrahydrocannabinol; Δ9-THCA: Δ9-tetrahydrocannabinolic acid; Δ9-THCA-C4 A and/or B: Δ9-tetrahydrocannabinolic acid-C4 A and/or B; Δ9-THCA A and/or B: Δ9-tetrahydrocannabinolic acid A and/or B; Δ9-THCA-A-COOH: Δ9-THCOA-C1 A and/or B: Δ9-tetrahydrocannabiorcolic acid A and/or B; Δ9-THCA A-COOH: 11-nor-9-COOH-Δ9-tetrahydrocannabinolic acid A; Δ9-THCO-C1: tetrahydrocannabiorcol; Δ9-THCV-C3: Δ9-tetrahydrocannabivarin; Δ9-THCVA-C3 A: Δ9-tetrahydrocannabivarinic acid A; EI: electron-impact; 8β,11-*bis*-hydroxy-Δ9-THC-A: 8β,11-dihydroxy-Δ9-tetrahydrocannabinolic acid; Δ9-THCA-A-8-one: Δ9-tetrahydrocannabinolic acid A-8-one; ESI: electrospray ionization; FT-ICR MS: Fourier transform ion cyclotron resonance mass spectrometry; LAESI: laser ablation electrospray ionization; MS/MS: tandem mass spectrometry; *m/z*: mass-to-charge ratio; OTHC: 10-Oxo-Δ6*a*(10*a*)-tetrahydrocannabinol; Q-ToF: quadrupole-time-of-flight; STELDI: laser desorption ionization; THC: tetrahydrocannabinol; THCA: tetrahydrocannabinolic acid; THCV: tetrahydrocannabivarin; TOF: time-of-flight.

Conclusions

The analytical methods for cannabis material published in the articles included in this systematic review showed the need to update the methodologies regarding the new potency of the drug, whether for its pharmacological potential, improvement of clinical conduct or quantification in forensic science. Although the findings of the literature review refer to an increased difficulty in cannabinoid separation by LC analysis than by GC analysis, the mass detector provides unambiguous identification of different overlapping compounds according to those described in the systematic articles included in this systematic review. In addition, it was possible to show that GC analysis are more used.

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