Analytical Techniques Used for Analysis of Cannabinoids

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Cannabinoids can be analyzed using different techniques. The aim of this review was to identify and compare analytical methods used for the determination of cannabinoids in different matrices using liquid chromatography (LC)-based systems. A systematic literature review was carried out using the preferred reporting items for systematic reviews and meta-analyses (PRISMA) method. In the results, 41 relevant articles were identified. The most commonly used methods for the analysis of cannabinoids were high performance liquid chromatography-photodiode array (HPLC-DAD) (n= 8), ultrahigh-pressure liquid chromatography–mass spectrometry (UHPLC–MS) (n= 8), and HPLC–tandem mass spectrometry (HPLC–MS/MS) (n= 8). Matrices from which cannabinoids were extracted included plants, oil, hair, human biological fluids, resin, honey, wastewater, and commercial products (n= 41). The most commonly used stationary phases were C18 Poroshell (n= 9) and C18 Kinetex (n= 8). The identification and comparison of methods used for the determination of cannabinoids can help in the development of more efficient and effective methods of analysis.

CANNABIS IS PART of the plant family *Cannabacea* (1). *Cannabis sativa* is an annual dioecious flowering plant (2) known for its medicinal and textile uses since ancient times (1,3). *Cannabis sativa* contains chemically active compounds called cannabinoids, which have a wide range of therapeutic effects in humans (3). Medicinal uses of cannabinoids include management of spasticity related to multiple sclerosis (MS), chronic neuropathic and cancer pain, nausea and vomiting, sleep disorders, anxiety, epilepsy, and Tourette syndrome (4).

The principal cannabinoids known to have medicinal properties are Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD) (5). THC and CBD are synthesized and accumulate in their acidic form in *Cannabis sativa* (6). The alkylation of olivetolic acid (OLA) with geranyl-pyrophosphate (GPP) by olivatolate geranyltransferase produces cannabigerolic acid (CBGA) (3,4,7). The catalysis of CBGA by three oxidocyclases—Δ^9-tetrahydrocannabinolic acid synthase (THCAS), cannabidiolic acid synthase (CBDAS), and cannabichromenic acid synthase (CBCAS)—produces Δ^9-tetrahydrocannabinolic acid (THCA), cannabidiolic acid (CBDA), and cannabichromenic acid (CBCA), respectively (3,4,7). The decarboxylation of THCA, CBDA, and CBCA due to high temperatures (8) produces THC, CBD, and cannabichromene (CBC), respectively (4,8). Cannabinol (CBN) is produced as a result of oxidation of THC (4,8) and is a sign of deterioration of the plant (9).

THC is the main psychoactive component in cannabis and has been used in the management of chemotherapy-induced nausea and vomiting, for appetite stimulation in patients with acquired immunodeficiency syndrome (AIDS) (10), for suppressing spasticity related to MS (6), and in the treatment of migraines (1).

CBD is known to have the largest number of therapeutic properties (7) and is the main nonpsychoactive component in cannabis (11). CBD presents potent antioxidant and anti-inflammatory properties (6). CBD has anticonvulsive, neuroprotective, anxiolytic, antipsychotic, and antidepressant properties (12). CBD is used principally in children in the treatment of drug-resistant epilepsy, Dravet and Lennox-Gastaut syndromes (13,14).

The endocannabinoid system has two principal receptors CB1 (type-1) and CB2 (type-2) (15) connected to G-proteins, endogenous cannabinoids called arachidonoylethanolamine (anandamide) and 2-arachidonoyl glycerol (2-AG) and enzymes that are involved in synthesis and degradation of endocannabinoids (9,16). CB1 receptors are present in different regions of the human brain (17). Distribution of these receptors are in areas involved in cognitive function and mood (4,17). CB1 receptors can be also found in the liver, testes, and small intestine (16).

There are different analytical techniques for the determination and quantification of cannabinoids (10,18). Gas chromatography (GC) has been the method of choice for analysis of cannabinoids (10), but chemical derivarization is required to avoid decarboxylation of acid cannabinoids (18). Liquid chromatography (LC) allows determination of cannabinoids in neutral and acidic forms without the need for derivarization. LC has become more popular with the introduction of high performance liquid chromatography (HPLC) and ultra-high-pressure liquid chromatography (UHPLC) (7,18,19). LC, HPLC, and UHPLC can be coupled to different detectors: fluorescence, diode-array detection (DAD), mass spectrometry (MS), or an ultraviolet (UV) detector (20). The use of MS coupled to HPLC and UHPLC increases the selectivity and the sensitivity of analysis (7), but the cost is higher and requires more skilled expertise to operate (20). CBN does not have a fluorophore and therefore use of a fluorescence detector is unfavorable (2). HPLC and UHPLC coupled to a UV-visible detector is a method commonly used because it can be economic and more convenient than other methods of analysis. DAD offers a range of detection wavelengths but can be more expensive than UV (21). The aim of this study was to conduct a systematic literature search to compare and identify analytical methods and parameters used in the determination of naturally occurring cannabinoids in different matrices.

Experimental

A systematic literature review was carried out using the preferred reporting items for systematic reviews and meta-analyses (PRISMA) method. (Note: PRISMA. Transparent reporting of systematic review and meta-analysis. [Internet] PRISMA, 2021 [cited 2021, 23 April] Available from: http://prisma-statement.org/PRISMAStatement/FlowDiagram.) The systematic literature review included methods used for separation and determination of cannabinoids using LC. Sources included open access peer-reviewed journal articles published in English between the years 2015 and 2020. Databases used for the literature search were Pubmed and Scopus. Keywords used in the search were: analysis, cannabinoids, cannabis, tetrahydrocannabinol, cannabidiol, cannabinol, and LC. Data collected was presented in tables, according to the matrix in which the cannabinoids were presented. Data in each table compared the type of matrix, cannabinoids analyzed, sample preparation method, stationary phase, mobile phase, and detector.

Results and Discussion

In the study, 41 articles were identified. The articles were classified depending on the matrices used for the analysis: 18 articles analyzed cannabinoids from plant material and four articles in plant material and other matrices. Ten articles analyzed cannabinoids from biological fluids and hair, and

Table I: Analysis of cannabinoids from plants using HPLC coupled to UV or DAD

Method	Cannabinoids	Author and Date
HPLC-UV/DAD	CBD, CBDA, CBG, and CBGA	Brighenti et al, 2019
HPLC-DAD	THC, THCA, CBD, CBDA, and CBN	Ciolino et al, 2018
HPLC-UV	CBD, CBDV, and CBDB	Citti et al, 2019
HPLC-DAD	THC, THCA, CBDA, CBD, CBG, CBC, Δ^8-THC, and CBN	Giese et al, 2015
HPLC-UV	THC, CBD, CBN, CBDA, CBGA, THCA, THCV, CBG, and Δ^8-THC	Križman, 2019
HPLC-UV	Δ^9-THC, THCA, Δ^8-THC, CBD, CBDA, CBG, CBN, CBC, and THCV	Mudge et al, 2017
HPLC-DAD	THC, CBD, and CBN	Ribeiro Grijó et al, 2019

Table II: Analysis of cannabinoids from plants using UHPLC

Method	Cannabinoids	Author and Date
UHPLC-MS	THC, CBD, and THCA	Bala et al, 2019
UHPLC-DAD	THC, CBDA, CBG, CBGA, THCA, CBD, and CBN	Deville et al, 2020
UHPLC-DAD	THC, CBD, CBC, CBN, CBG, THCA, and CBDA	Elkins et al, 2019
UHPLC-DAD	THC, CBC, CBD, Δ^8-THC, THCA, CBDA, THCV, and CBDV	Fekete et al, 2018
UHPLC-UV	THC, CBD, CBN, THCA, CBDA, CBG, CBDVA, CBL, CBGA, CBDV, CBC, THCV, and Δ^8-THC	Mudge et al, 2018
UHPLC-UV UHPLC-MS/MS	THC, CBD, CBN, CBDA, CBGA, CBDV, THCA, CBG, and Δ^8-THC	Nemeškalová et al, 2020

one article from biological fluids and hair and other matrices. Four articles analyzed cannabinoids in oil, and four articles, in oil and other matrices. Ten articles analyzed cannabinoids from miscellaneous matrices.

Methods of Analysis of Cannabinoids from Plant Material

LC, HPLC, and UHPLC have been performed for the separation, determination and quantification of different cannabinoids in *Cannabis sativa*. The samples included aerial parts of the plant (n=1), male and female inflorescences (n=5), leaves (n=2), roots (n=1), colas (n=1), resins (n=1), buds (n=2), and flowers (n=8) (7,22–42). HPLC was the most popular analytical technique used for the analysis of cannabinoids in plants (n=13) (22–27,32,34,36,38–42). HPLC can be coupled to UV, DAD, MS, or fluoresence detectors (20).

The detectors most commonly used for the analysis of cannabinoids in plant material were UV or DAD (n=13) (23–27,32,34,36,40,41). For example, Križman completed a study using HPLC-UV (34). Križman carried out a simple isocratic HPLC method for the analysis of THC, CBD, CBN, cannabigerol (CBG), THCA, tetrahydrocannabivarin (THCV), CBGA, CBDA, and d^8-THC. The mobile phase consisted of water and actonitrile (ACN) in the ratio of 9:31 (v/v), with 0.1% formic acid (v/v) and 10 mM ammonium formate, using a Luna C18 (150 mm × 3 mm i.d., 3 µm) column and UV at 275 nm (34).

In recent years, UHPLC has become more popular (n=7) because of the small quantity of solvent needed in the mobile phase and a shorter analysis time (43). Bala and colleagues carried out a study using UHPLC coupled to MS to detect THC, CBD, and THCA which are present in large amounts in the cannabis plant and have therapeutic properties (22). The most commonly used detector to analyze cannabinoids from plants with UHPLC was DAD (n=3) (7,28,31). Elkins and colleagues analyzed THC, CBD, CBN, CBDA, CBC, and THCA using a simple method consisting of a mobile phase based in water containing 0.1% formic acid (HCOOH) and ACN containing 0.1% HCOOH (ranging between 40–100%) with gradient elution mode using a Phenomenex Luna Omega C18 (150 × 2.1 mm × 1.6 µm) column and DAD detection monitored at 280 nm (7).

While the use of DAD as a detector for HPLC and UHPLC is quite common, combined detectors such as UV-DAD, ESI-MS, and MS/MS have also increased in their popularity. One of the advantages of using ESI-MS or MS/MS is that the analysis can be performed in negative and positive ion mode. Neutral cannabinoids give a better signal in the positive ion-mode while acidic cannabinoids give better signal in the negative ion-mode (24). Brighenti and colleagues developed a method for the analysis of nonpsychoactive cannabinoids using the three combined detectors (24).

Other methods used for the analysis of cannabinoids were a fast-HPLC-DAD (25), UHPLC-travelling wave ion mobility (TWIM)-MS (30), and HPLC-Q-Exactive-Orbitrap-MS (39). Burnier and colleagues analyzed THC, CBD, CBN, and THC-A in a total run time of less than 5 min using a fast-HPLC-DAD method that could be an alternative to UHPLC but with a lower cost (25).

LC presents less sensitivity than HPLC and UHPLC. The use of LC requires less expensive and simpler equipment (33). Dong and colleagues developed a thermal desorption direct analysis in real time mass spectrometry method and compared the results with those obtained using a simple LC–MS (29).

One of the limiting factors in the analysis of compounds using an LC system is the solubility of cannabinoids prior to analysis. The analysis of cannabinoids from plant material using LC, HPLC, or UHPLC requires an extraction method to determine the presence of cannabinoids qualitatively and quantitatively (2).

Solvent extraction is the most commonly used analytical extraction method to extract cannabinoids from plant material. In our findings, 20 studies used solvent extraction as a part of the sample preparation method for the analysis of cannabinoids. The most commonly used solvents for the extraction of cannabinoids from plants are ethanol (EtOH) and methanol (MeOH), used in 7 out of 22 and 7 out of 22 studies, respectively. Ethanol is an organic solvent commonly used because of its higher eco-friendly behavior, even if it is more viscous than MeOH (31) and due to its high extraction efficacy because of its high affinity for the molecular structure of cannabinoids (44). MeOH is also commonly used because it presents a high extraction efficiency (24). Other extraction methods with solvents make use of ACN or a mix of solvents. Deville and colleagues performed the extraction technique with a mix of methanol/chloroform (90:10 v/v). The long-term use of chloroform by the analyst can cause liver and kidney injury to the operator of the method of analysis. Reducing the use of chloroform will increase safety in the laboratory and decrease costs of reagent disposal while improving the impact in the environment (36).

Sample preparation is usually accompanied by dynamic maceration (DM), which consists of extraction of analytes of interest from plant

Table III: Analysis of cannabinoids from plants using HPLC coupled to combined detectors

Method	Cannabinoids	Author and Date
HPLC-UV/DAD HPLC-ESI-MS	CBD, CBDA, CBG, and CBGA	Brighenti et al, 2017
HPLC-MS/MS	THC, CBD, CBC, CBG, CBN, CBDV, THCA CBGA, and CBDA	Palmieri et al, 2019
HPLC-ESI-MS HPLC-MS/MS	CBDA, CBGA, CBG, and CBD	Pellati et al, 2018
HPLC-MS/MS	THC, CBD, CBN, CBG, CBDA, and THCA	Zweigenbaum, 2020

Table IV: Analysis of cannabinoids from plants using different HPLC methods

Method	Cannabinoids	Author and Date
Fast-HPLC-DAD	THC, CBN, CBD, and THCA	Burnier et al,2019
UPLC-MS UPLC-TWIM-MS	Δ^9-THC, CBD, CBC, CBN, CBG, Δ^9-THCA, and CBDA	Dossantos et al, 2018
HPLC-Q-Exactive-Orbitrap-MS	THC, CBD, CBN, CBG, CBC, CBDV, THCV, CBDA, THCA, CBNA, CBCA, CBGA, CBDVA, and THCVA	Pavlovic et al, 2019

Table V: Analysis of cannabinoids from plants using LC

Method	Cannabinoids	Author and Date
LC–MS	THC, CBD, CBC, THCA, CBDA, THCV, CBDV, THCVA, CBDVA, CBCA, and CBL	Dong et al, 2019
LC–MS/MS	CBN	Hidayati et al, 2020

material using a solvent and vortex or stirring at ambient temperature (24). Brighenti and colleagues compared four different extraction techniques DM, ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), and supercritical fluid extraction (SFE). UAE and MAE are extraction techniques that use ultrasound waves and microwave energy for a faster and higher extraction of the secondary metabolites of cannabinoids (24). SFE is a more environmentally friendly technique than the extraction techniques of cannabinoids from plant material that use organic solvents (24). Elkins and colleagues extracted the resin from cannabis using a biobotanical SFE liquid CO_2 extractor (7). DM is the best method to extract acidic cannabinoids such as CBDA and MAE for CBD (24). Ribeiro Grijó and colleagues carried out the extraction process using solid phase extraction (SPE) with supercritical carbon dioxide ($scCO_2$) avoiding trace of organic solvents in the sample prepared (41).

The majority of the analysis of cannabinoids in plant material were carried out using an Agilent system (n=11) with different modular model systems (7,23,24,26,27,32,35–37,40,42). Among those studies using Agilent systems, the modular models 1100 and 1290 were the most popular and were used in three studies: (23–25,40,7,32,37) two studies used modular model 1200 system (35,36), one study used modular model 1260 system (42), and another study used modular model 1220 system (27). Ciolino and colleagues conducted the analysis using Agilent 1100, 1200, or 1260 HPLC-DAD systems (26). Another HPLC unit used was the Waters system, this unit was used in four studies (22,25,28,30). Other HPLC systems used were Thermo LTQ XL by Dong and colleagues, Finnigan Surveyor by Križman, and Nexera LC20AD XR system by Palmieri and colleagues (29,34,38).

The majority of the studies used C18 Poroshell (n=4), Kinetex (n=3), and Ascentis (n=3) columns. Gradient mode elution of the mobile phase was the most common method chosen for the analysis of cannabinoids from plants and only four studies out of 22 used an isocratic mode of elution. The majority of the mobile phases are composed of water and an organic solvent (n=16), usually MeOH and ACN. ACN was preferred because it decreases the total run time with respect to MeOH. The flow rate of the mobile phase ranged from 0.3 mL/min to 3 mL/min, with 0.4 mL/min (n=5) and 0.3 mL/min (n=5) being the most commonly used.

Methods of Analysis of Cannabinoids from Biological Fluids and Hair

Cannabis can be determined in biological fluids and hair (45). The analysis of cannabinoids in human fluids is important to understand their pharmacology in humans and to be able to establish the correct dosage (46). The availability of analytical techniques to detect and quantify THC in blood, saliva, hair, and urine is necessary to demonstrate consumption of illicit preparations (47).

Table VI: Analysis of cannabinoids from biological fluids using UHPLC

Method	Cannabinoids	Author and Date
UHPLC–MS/MS	THC-COOH	Cho et al, 2018
UHPLC–MS	CBD	Dybowski et al, 2020
UHPLC–MS/MS	THC, CBD, and CBN	Moorthy et al, 2019
UHPLC–MS/MS	THC, CBD, THCA-A, CBDA, THC-COOH, THC-COOH-gluc, 11-OH-THC, and THC-gluc	Pichini et al, 2019
UHPLC–MS/MS	THC, CBD, CBN, 11-OH-THC, and THC-COOH	Pires da Silva 2020
UHPLC–MS/MS	THC, COOH–THC, OH–THC, CBD, and CBN	Wei et al, 2015

Table VII: Analysis of cannabinoids from biological fluids using HPLC

Method	Cannabinoids	Author and Date
HPLC–MS/MS	THC, CBD, CBN, and THC-COOH	Chang et al, 2016
HPLC–MS/MS	THC, 11-OH-THC, and THC-COOH	Dziadosz et al, 2016
HPLC–MS/MS	THC, 11-OH-THC, THC-COOH, THC-C-gluc, CBD, CBN, CBG, CBDV, THCV, and THCV-COOH	Klawitter et al, 2017

Table VIII: Analysis of cannabinoids from biological fluids using LC

Method	Cannabinoids	Author and Date
LC-MS/MS	THC, THCOH, and THCCOOH	Toennes et al, 2014

The concentration of THC and its metabolites from blood and urine depends on the amount and route of administration and the time of analysis following consumption (48).

LC, HPLC, and UHPLC methods are used for the analysis of cannabinoids from different biological fluids (46,48–56). Six studies were carried out using UHPLC, three used HPLC, and one study by Toennes and colleagues used an Agilent 1290 Infinity LC system. Analysis was performed using a Kinetex XB-C18, 100 Å, (100 × 2.1 mm) column with a gradient mode mobile phase composed of 0.01% formic acid with 5 mM ammonium formate and ACN with 0.1 % formic acid ranging between 50–100%, the flow rate was 0.5 mL/min (53). The samples included urine (n=4), hair (n=1), human plasma (n=3), human serum (n=2), blood (n=1), and sweat (n=1) (46,48–55).

The detector most commonly used in the analysis of cannabinoids from biological fluids was MS/MS (n=9) because it presents higher selectivity and sensitivity allowing for the detection of major and minor cannabinoids in small quantities (2,7). THC and its metabolites (THC-OH and THC-COOH) are, in general, the cannabinoids analyzed in blood and urine because of the psychoactive effects of THC (2). Analysis of other cannabinoids such as CBD, CBN, CBG, CBDV, and CBDA were also identified in the literature (46,50–54,56).

Sample preparation is an important step in the analysis of compounds from biological fluids and has an effect on reproducibility, efficiency, and selectivity and eliminates interferences (2). Different techniques were performed to extract cannabinoids from biological fluids and hair. Protein precipitation (PP) is a popular technique used for the sample preparation in blood and can eliminate up to 98% of the protein (57). Dybowski and colleagues, Dziadosz and colleagues, and Klawitter and colleagues, carried out protein precipitation studies (46,48,51). Dybowski and colleagues analyzed CBD using an UHPLC–MS/MS system with a Gemini C18 column (4.6 x 100 mm, 3 µm) and an isocratic mode mobile phase consisting of 60% 25 mM formic acid with water and 40% 25 mM formic acid with ACN with a flow rate of 0.5 mL/min (46). Dziadosz and colleagues used an HPLC–MS/MS system with a gradient mode mobile phase and MeOH as organic solvent for the analysis of THC, 11-OH-THC, and THC-COOH (48). Klawitter and colleagues performed protein precipitation for plasma and urine and carried out analysis from both matrices using an HPLC–MS/MS (51). Moorthy and colleagues used volumetric absorptive microsampling (VAMS) devices in the sample preparation technique. VAMS is a relatively new microsampling tool used for obtaining dried biological matrices, which improves the accuracy of the sample volume (52,58). Pires de Silva and colleagues used salting-out assisted liquid-liquid extraction (SALLE), another recent extraction technique where the extraction solvent is a water miscible organic solvent (54,59). SALLE is cheaper and easier to use than SPE (59). Toennes and colleagues, and Weit and colleagues,

performed sample preparation using SPE (55,56).

Chang and colleagues performed hydrolysis of the urine specimen before the extraction method to improve sample accuracy (50). Pichini and colleagues carried out the study in oral fluid, serum, urine, and sweat patch samples. Sample preparation from oral fluid, serum, and urine were the same with further alkaline hydrolysis for urine samples for the quantification of CBD as it appears as glucuronide in urine. The extraction of cannabinoids from sweat patch samples was performed with MeOH as the extraction solvent (53).

Hair is also used as a matrix because traces of some compounds can be present in hair (49). Hair is a complex matrix that requires longer sample preparation times because washing and digestion steps are required (2,49). Cho and colleagues carried out the sample preparation washing the hair twice with MeOH to eliminate any external contaminants and performed the digestion with 1 M NaOH to free the cannabinoids from the matrix. Analysis was carried out using a system consisting of a binary pump, Agilent 1290 UHPLC pump (pump 1), and an additional Agilent 1260 pump (pump 2) (49).

Klawitter and colleagues, Chang and colleagues, and Toennes and colleaguesl also performed analysis with an Agilent HPLC unit. Ten studies used C18 columns. Three out of ten studies used Acquity and two out of ten used Kinetex. The majority of the studies used gradient mobile phase (n=9). Dybowski and colleagues performed an isocratic method of elution. The mobile phases were composed of ammonium formate or water and an organic solvent. ACN and MeOH are the organic solvents more commonly used for the mobile phase, with ACN being preferred (n=7) because of shorter elution times for cannabinoids (60). The flow rate ranged from 0.15 mL/min to 1 mL/min. A flow rate of 0.4 mL/min was the most commonly used (n=3).

Methods of Analysis of Cannabinoids from Oil

In recent years, CBD oil has become popular for use in different conditions (61). There is a lack of standardized extraction regulation (2,61). Different carrier oils on the market are olive oil, medium chain

Table IX: Analysis of cannabinoids from oil using HPLC

Method	Cannabinoids	Author and Date
HPLC-DAD	THC and CBD	Araneda et al, 2020
HPLC-DAD	THC, CBD, CBN, and THCA	Bettiol et al, 2019
HPLC-DAD	THC, THCA, CBD, CBDA, and CBN	Ciolino et al, 2018
HPLC-UV HPLC-MS	THC, CBD, THCA, CBDA, CBDV, CBG, and CBN	Citti et al, 2018
RP-HPLC/UV	THC and CBD	Deidda et al, 2019
HPLC-UV	Δ^9-THC, THCA, Δ^8-THC, CBD, CBDA, CBG, CBN, CBC, and THCV	Mudge et al,2017

Table X: Analysis of cannabinoids from oil using UHPLC

Method	Cannabinoids	Author and Date
UHPLC-UV-MS/MS	THC, CBD, CBN, CBDA, CBGA, CBDV, THCA, CBG, and Δ^8-THC	Nemeškalová et al, 2020
UHPLC-MS/MS	THC, CBD, THCA-A, CBDA, THC-COOH, THC-COOH-gluc, 11-OH-THC, and THC-gluc	Pichini et al, 2019

triglyceride (MCT), hemp seed oil, and black cumin seed oil.

HPLC is the method of analysis most commonly used (n=5) for the determination and quantification of cannabinoids in olive oil (n=2) and hemp seed oil (n=2) (26,27,36,60,66). The detectors most commonly used were UV (27,36,60) and DAD (26,64). Two studies carried out the analysis using UHPLC (37,53). Nemeškalová and colleagues carried out analysis in a wide variety of oils—paraffin oil, sunflower oil, castor oil, jojoba oil, shea oil, argan oil, almond oil, coconut oil, and aviril baby massage oil—using an UHPLC-UV-MS/MS method. UV-visible detection was used for the analysis of cannabis with a high amount of cannabinoids, while MS/MS was used for low quantities of major cannabinoids such as THC and CBD and for minor concentrations of cannabinoids (37).

Efficient extraction procedures are required for the analysis of cannabinoids in oil because oil cannot be injected directly in the HPLC due to its high viscosity (62). Bettiol and colleauges and Deidda and colleagues performed the same method to extract different cannabinoids from olive oil, consisting of 40 µL of sample in olive oil added to 960 µL of tetrahydrofuran (TFH) and vortex-mixed. Next, 50 µL of this solution was added to 950 µL of ACN in the study of Bettiol and colleagues, and in MeOH in the study by Deidda and colleagues (60,63). Mudge and colleagues carried out a solvent extraction with MeOH while Nemeškalová and colleagues used isopropanol/ethyl acetate (1:1, v/v). Ciolino and colleagues used EtOH or isopropyl alcohol (26,36,37). Araneda and colleagues performed analysis of cannabinoids using benchtop nuclear magnetic resonance (NMR) instruments to compare the results with the ones obtained using HPLC-UV. Analysis was carried out for five different concentrates of cannabinoids. The relative standard deviation for the samples analyzed with benchtop NMR was higher than that with the HPLC-UV. In the analyses performed with benchtop NMR, the amount of CBD in sample 1 and THC in sample 2 could not be quantified while in the analysis with HPLC both samples were quantified (64).

Three different brands of HPLC units were used among the articles published in the literature for the extraction of cannabinoids from oil. The HPLC unit most commonly used was Agilent (26,27,36,37). The second brand used was Thermo Fisher Surveyor and Pichini and colleagues used a Waters Xevo TQ-S. Bettiol and colleagues and Deidda and colleagues used a Thermo Fisher Surveyor Plus HPLC system using an Agilent PoroshellR 120 SB-C18 column, (2.1 mm × 150 mm; 2.7 µm) as a stationary phase and an isocratic mode mobile phase composed of ACN/5 mM phosphate buffer rate 75/25 v/v with a flow rate of 0.38 mL (60,63). Ciolino and colleagues also performed analysis using the isocratic mode for the mobile phase, but used two types of mobile phases 66:34 ACN: 0.5% acetic acid and 83:17 MeOH:50 mM citrate both using a flow rate of 1 mL/min. Analysis was carried out using an ACE column (26).

Seven studies used a C18 column as a stationary phase. The majority of the studies were performed using the brand Agilent Poroshell (n=4). The methods used for the mobile phase were gradient in four studies and isocratic in the other three.

The majority of the methods used ACN (n=5) as organic solvent and the amount ranged from 60–100% in mobile phase composition. The flow rate ranged from 0.38 mL/min to 1 mL/min.

HPLC Methods of Analysis of Cannabinoids from Miscellaneous Matrices

There is a need for quantitative analyses to determine cannabinoids such as CBD and THC in commercial products such as honey, capsules, and serum to calculate the amount of each cannabinoid and

evaluate the dosage and the exposure of the patient when the product is consumed (26). Studies were carried out in different matrices such as cannabis concentrates, honey (n=1), hemp nut (n=1), vaporized fluid (n=1), milk (n=1), liver (n=1), capsules (n=2), wastewater (n=1), cotton cloths (n=1), and gummies (n=1).

Ciolino and colleagues carried out analysis of cannabinoids in different commercial products (26). Methods of sample preparation were the same for all the matrices: the sample was weighed and MeOH (95% or 100%) was added as an extraction solvent. The sample was then vortex-mixed and filtered with nylon membrane filter of 0.45 µm. Depending on the quantity of cannabinoids, the sample was further diluted or directly injected in an Agilent 1100, 1200, or 1260 HPLC-DAD system with an ACE 5 C18-AR analytical column (5 µm, 4.6 mm i.d. x 250 mm) (26).

Jornet-Martínez and colleagues detected traces of cannabinoids in different matrices such as plastic bags, cotton tip, aluminium foil, office paper, piece of cotton cloth, and skin. Due to the complex nature of the matrices and the small quantity of cannabinoids, Jornet-Martínez and colleagues performed analysis using an in-tube solid-phase microextraction (IT-SPME) coupled on-line to nanoliquid chromatography (nanoLC), which improved the selectivity of the analysis. The study was carried out using a Zorbax 300SB C18 (50 × 0.075 mm i.d., 3.5 µm) column with a simple gradient mode mobile phase consisting of water and ACN ranging between 55–75%. Jornet-Martínez and colleagues performed an ultrasound assisted extraction for the preparation of the sample using just 1 mL of MeOH per sample making the sample preparation an eco-friendly technique (63).

Nemeškalová and colleagues performed analysis of cannabinoids in oils and plant materials as well as in cosmetics and gelatinous gummies. The large amount of therapeutic benefits of CBD has led to a varied market of CBD based-products such as candies and cosmetics, which contain smalls amounts of THC that need to be quantified due to its psychoactive effects. The method proposed by Nemeškalová and colleagues demonstrated its feasibility on 13 CBD-based products using an UHPLC-UV-MS/MS

with a Poroshell 120 EC-C18 (100 mm × 2.1 mm, 2.7 µm) column. The sample preparation was different for hydrophilic liquids, gummies, and hydrophobic cosmetics, but it consisted of dissolution and dilution (37).

Heo and colleagues performed analysis of different synthetic cannabinoids and THC in tablets, capsules, powders, liquids, cookies, and candy using an UHPLC-UV and UHPLC–MS/MS. Analysis with the UHPLC system was carried out using a Waters Acquity UPLC HSS C18 (2.1 mm × 150 mm, 1.8 µm) column with a gradient mode mobile phase. The column used for the analysis with UHPLC–MS/MS was a smaller one: Waters Acquity UPLC BEH C18 column (2.0 mm × 100 mm, 1.7 µm). Both methods can be used for adulterant in spection and sample analysis in food and dietary supplements (66). Analysis from wastewater was carried out to study the exposure of individuals living in a community to cannabinoids (67). Determination of cannabinoids in wastewater can give information about the use of cannabis in a determinate area. The extraction and separation of cannabinoids from wastewater is a difficult process because these compounds are hydrophobic in nature (67–71).

Jacox and colleagues developed a method for the analysis of THC and its metabolites THCCOOH and THCOOH-glucuronide and other licit and illicit drugs, using an UHPLC–MS/MS with a Kinetex C18 (2.1 mm x 100 mm, 1.7 µm) column and a gradient mode mobile phase consisting of 0.1% formic acid with water and 0.1% formic acid with ACN ranging between 40–95% at a flow rate of 0.5 mL/min (67).

Brighenti and colleagues carried out analysis in honey since apiary products are extensively consumed. Extraction of cannabinoids from honey was performed comparing two methods 1) ultrasonication in a water bath, and liquid–liquid (L/L) purification step and 2) SPE with QuEchERS. The use of L/L extraction can be time consuming and large amounts of solvent are required (71). Brighenti and colleagues reported reproducibility problems that occurred because of the emulsion formation. QuEchERS extraction has become more popular in the last year because is easier and quicker to use, and smaller amounts of solvent and samples are required (23, 72). QuEchERS consists of two steps: extraction and partition of the homogenized sample with an organic solvent and salt solution; and the use of the dispersive solid-phase extraction (dSPE) technique to extract and clean the supernatant (23,72). Brighenti and colleagues used the first step of this procedure for the extraction of cannabinoids from honey and analyzed them using an Agilent 1200 HPLC–MS/MS system with a Kinetex EVO C18 column (100 × 2.1 mm, 5 µm particle size). The mobile phase used consisted of 2.0 mM aqueous CH_3COONH_4 and ACN at a flow rate 0.35 mL/min (21).

Conclusion

HPLC is the most commonly used LC-based system for the analysis of cannabinoids. UHPLC is becoming popular because of its shorter analysis time and use of less solvent. The detector used can depend on the matrix from which cannabinoids are extracted from. MS/MS is used for matrices such as blood and urine, which are more complex and contain less quantities of cannabinoids while DAD and UV are used in plant material where the quantity of cannabinoids is higher. The most popular mobile phase is water and ACN in both with 0.1% HCOOH in gradient mode. C18 columns are the most commonly used. Identification and comparison of analytical methods for determination of cannabinoids in different matrices can help in the development of efficient and effective methods of analysis, which are useful for high throughput screening. Accurate and precise determination of concentrations of cannabinoids can help in better understanding the physiological effects and therapeutic properties of this class of compounds.

Supplemental Information

Please check the web version of this article for detailed supplemental tables listing data pulled from the authors literature review.

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