RECORDS OF PHARMACEUTICAL AND BIOMEDICAL SCIENCES

Recent Analytical Approaches in Quality Control of *Cannabis sativa* L. and Its Preparations

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Abstract

Cannabis sativa L. (family Cannabaceae) is gaining more and more attention all over the world, due to its long historical clinical practice and its recent legalization either for recreational or medicinal uses. The quality control of the plant is a critical for its legalization and globalization. This review deals with the recent analytical methods (years 2015-2018) in the quality control of Cannabis, including the traditional chromatographic methods like HPLC, LC/MS/MS, UPLC, GC/FID, and GC/MS; and spectrophotometric, including FT-IR, NIR and NMR techniques as well as the most recent advanced techniques. The comprehensive methods, such as fingerprint and multi-component quantification are emphasized; hyphenated techniques, like HPLC, LC/MS/MS, GC/FID, GC-MS, LC-NMR, chemometric methods.

Keywords: *Cannabis sativa*; Cannabaceae; Cannabinoids; Analysis; Quality Control

1. Introduction

Along the history, *Cannabis sativa*, family Cannabinaceae, has been recognized as an important medicinal plant. Much research has been carried out on the medical applications of cannabis, and several countries have regarded the plant as an important medicine (Murray et al., 2007). The current systematic classification of cannabis is listed in Table 1 (Hazekamp and Fischedick, 2012; Jiang et al., 2006).

Table 1 The systematic taxonomy of *Cannabis sativa* L.

Division	Angiosperms
Class	Dicotyledons
Subclass	Archichlamydeae
Order	Urticales
Family	Cannabaceae
Genus	*Cannabis*
Species	*sativa* L.

The chemistry of cannabis has been studied extensively, approximately 565 compounds have been identified (Pertwee, 2014). The most interesting among these constituents are the cannabinoids; Cannabinoids are a class of terpenophenolic compounds with a C-21 skeleton found exclusively in cannabis concentrated in a resinous secretion produced by the trichomes of the plant (Cerdá et al., 2012).

These trichomes are particularly concentrated at specific parts of the female inflorescence (Jiang et al., 2006). The cannabinoids form a group of related compounds of which about 120 are known (ElSohly and Slade, 2005). Of the major cannabinoids in *C. sativa* L., delta-9-tetrahydrocannabinol (Δ^9-THC) is considered to be the compound that possesses the psychoactive properties (ElSohly and Slade, 2005). In plant tissues, cannabinoids are biosynthesized in an acidic (carboxylated) form. The most common types of acidic cannabinoids found are delta-9-tetrahydrocannabinolic acid A (THCA-A), cannabidiolic acid (CBDA) and cannabigerolic acid (CBGA) and cannabichromenic acid (CBCA). THC acid exists under two forms: THCAA and THCAB. However, only traces of THCAB can be detected in cannabis samples (ElSohly and Slade, 2005), THCAA is the major form and will be further referred to as THCA. CBGA is the direct precursor of THCA, CBDA and cannabichromenic acid (CBCA).

Hillig and Mahlberg (Hillig and Mahlberg, 2004) identified three chemotypes (chemical phenotypes) of cannabis: drug-type plants (chemotype I) show a high [total THC/total CBD], intermediate type plants (chemotype II) have an intermediate ratio (1:1), and fibretype plants (chemotype III) exhibit a low [total THC/total CBD] (Clarke and Merlin, 2016).

Cannabis preparations are consumed by millions of people all over the world, both for medicinal and recreational purposes (Hazekamp and Fischedick, 2012). Pharmacological activities of the cannabinoids are very diverse, ranging from analgesic and antiemetic to the treatment of glaucoma and multiple sclerosis (Russo, 2013; Williamson and Evans, 2000). Δ^9-THC has been proven to be the most psychoactive ingredient in cannabis. It has many diverse pharmacological effects with possible therapeutic value in the treatment of different medical conditions. These include relief of nausea and vomiting associated with chemotherapy, appetite stimulation, reduction of symptoms of multiple sclerosis, and treatment of glaucoma, as well as spasticity from spinal cord injury (Fattore and Fratta, 2011; Lewis and Brett, 2010; Maurer et al., 1990; Naef et al., 2003).

Other non-psychotropic cannabinoids, mainly CBD and CBG, are increasingly investigated showing partially distinctive effects (Borrelli et al., 2013; Costa et al., 2007; Iannotti et al., 2014; Izzo et al., 2009).

Scientists all over the world are trying to explore other possible medical uses for cannabinoids. Cannabis analysis and understanding the chemistry and chemical profile of cannabis preparations is important in light of the fact that several states in the USA have legalized the medical use of cannabis and cannabis preparations (Bachhuber et al., 2014; Cerdá et al., 2012).

Although there are many review reports covered cannabis analysis (Radwan et al., 2017), (Brenneisen, 2007; Klein, 2015; Lucas and Eades, 2018; Radwan et al., 2017; Raharjo and Verpoorte, 2004; Thomas and ElSohly, 2016; Vollner et al., 1986) and the last report was done in 2015 by Drug Enforcement Administration (DEA) group (Leghissa et al., 2018) ; the need of analysis and quality control of cannabis is moving in outstanding pace. Here we sought to report a quick review covering the years (2015-2018) to provide an inclusive and comprehensive analysis of the different techniques used for the characterization of cannabis natural products through the years range 2015-2018 using most recent methods such as GC, LC, and MS, also there are a variety of other techniques ranging from TLC to new

spectroscopic techniques, which add to the picture through literature survey.

Literature Survey Details

Literature overview of proposals was selected on Web of Science, Science Direct, PubMed, and Scopus basis obtained with the key words of Cannabinoids, HPLC, LC/MS, UPLC/MS/ UHPLC/MS, GC/FID, and GC/MS in the publication period range of 2015-2018, including the new containing ones.

Results

The techniques were organized as liquid chromatography (LC), gas chromatography (GC), then the innovative techniques

Planar Techniques

By 2016-

The products of the colorimetric test were identified by positive-ion mode electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry (ESI(+)FT-ICR MS), collision-induced dissociation (CID) experiments or ESI(+)MS/MS, ultraviolet–visible (UV–Vis) spectroscopy and thin layer chromatography (TLC) (dos Santos et al., 2016)

By year 2015 –

 by TLC/MS, for determination of cannabinoids and pesticides in cannabis (Eike et al., 2015); Thin Layer Chromatographic Analysis of Psychoactive Plant *Cannabis Sativa* L.) (Goutam et al., 2015).

HPLC

Liquid chromatography is an important cannabis analysis technique. It is the most preferable analytical tool in cannabis analysis. Consequently, it is used by many Cannabis laboratories for determination of cannabinoids. In contrast to GC, it needs less sample preparation steps (no derivatization or decarboxylation of acid cannabinoids, due to GC heat). Reversed phase LC columns are commonly used (C18 or biphenyl alternates). Acetonitrile, 0.1 % formic in water or methanol acid are frequently considered as mobile phases.

By the year 2018-

A generic HPLC workflow was developed, validated, and successfully applied to the analysis of phyto-cannabinoids in *C. sativa*

extracts. (Fekete et al., 2018). Eight major cannabinoids were analyzed via HPLC-DAD was used for analysis of eight major cannabinoids, which helped in defining plant chemotypes by determination of (THCA/CBDA) ratio (Aizpurua-Olaizola et al., 2016a) ; Ultrasonication was used for cannabinoid extraction from *C. sativa* L. followed by HPLC analysis (Agarwal et al., 2018); (Calvi et al., 2018a; Ciolino et al., 2018; Sexton et al., 2018).

Labs-Mart recently developed and validated analytical methods using high-pressure liquid chromatography (HPLC-DAD) to quantify cannabinoids and gas chromatography with mass spectroscopy (GC-MS) to quantify terpenes in cannabis raw material (Jin et al., 2018); A generic HPLC workflow was developed, validated, and successfully applied to the analysis of phytocannabinoids in *C. sativa* extracts (Fekete et al., 2018); THC and other acid and neutral cannabinoids were quantified in Cannabis plants by fast-HPLC-DAD (Burnier et al., 2019)

By the year 2017-

Evaluation of cannabinoids concentration and stability in standardized preparations of cannabis tea and cannabis oil by ultra-high performance liquid chromatography tandem mass spectrometry (Pacifici et al., 2017); An HPLC method was used for the analysis of non-psychoactive cannabinoids in fibre-type *Cannabis sativa* L. (hemp) using a new extraction method (Brighenti et al., 2017); While Patel et al. made a quantitative and qualitative analysis of cannabinoids in cannabis using a modified HPLC/DAD method (Patel et al., 2017); By HPLC-MS/MS coupled with chemometric analysis: quality evaluation and a pilot human study in Taiwan for determination of cannabinoids in hemp nut products (Chang et al., 2017); Methods Unheated and heated extracts of cannabis seizures were prepared from the dried flowering tops and leaves (marijuana) or from the resin (hashish) and subjected to analysis using high performance liquid chromatography (HPLC) (Souleman et al., 2017); *Cannabis sativa* (Hemp) Seeds, Δ^9-

Tetrahydrocannabinol, and Potential Overdose (Yang et al., 2017).

By the year 2016-

Simultaneous determination of three kinds of effective constituents in Cannabis plants by reversed-phase HPLC (Fu et al., 2016); a non-targeted approach to compare the variance in the major cannabinoids to the entire HPLC-UV chemical profiles at 220 nm (Mudge et al., 2016). High-performance liquid chromatography with diode-array detection (HPLC/DAD) was used to determine THC, Δ9-tetrahydrocannabinolic acid A (THCA), cannabinol (CBN), cannabidiol (CBD), cannabidiolic acid (CBDA), cannabigerol (CBG), cannabigerolic acid (CBGA), cannflavin A/B, and total phenolics (Peschel, 2016). The qualitative and quantitative of polyphenolic compounds and antioxidant activity of cold-pressed seed oil from *Finola cultivar* of industrial hemp (*Cannabis sativa* L.) were performed by HPLC analyses (Smeriglio et al., 2016). In vitro validation of 5 commercial vaporizers was performed with THC-type and CBD-Type Cannabis using gas chromatography/mass spectrometry was used to determine recoveries of total THC and total CBD in the vapor using HPLC-PDA (Lanz et al., 2016); by using (HPLC/DAD) to determine some cannabinoids in tinctures from a Δ9-tetrahydrocannabinol (THC)-type chemovar (Peschel, 2016).

By the year 2015-

Quantification of Δ^9-THC and THCA was carried out to check the stability of cannabis samples. The determination of the degradation of THCA to Δ9-THC in 29 cannabis products seized in Austria was monitored by HPLC-UV (Taschwer and Schmid, 2015); Determination of 11 cannabinoids in biomass and extracts of different varieties of cannabis using high-performance liquid chromatography (Gul et al., 2015)**;** using HPLC-PDA for analysis of cannabinoids in hemp seed oils (Layton et al., 2015). Growth in the application of SFC for cannabis analysis is expected to increase, because it is considered greener than HPLC. The amounts of solvents used are greatly reduced, and as such, the amount of waste that needs to be

disposed is minimal. Obtainable efficiency and specificity by SFC are comparable to that of HPLC (Geryk et al., 2015); using HPLC/DAD (two unique analytical methods for cannabinoids and other compounds fingerprinting in Cannabis (Peschel and Politi, 2015); using HPLC, for cannabinoids analysis in "marijuana nourishments" (Vandrey et al., 2015); using HPLC, for doing a comparative study between THCA and THC as percentage in C. sativa, in addition to, the storage temperature effect on cannabinoids stability (Taschwer and Schmid, 2015)

UHPLC

By the year 2018-

A liquid chromatography coupled with tandem mass spectrometry (UPLC-MS) was used for quantifying cannabinoids (THC, CBD, THCA, CBDA and CBN) concentrations in mixed olive oil cannabis preparations (Carcieri et al., 2018); Pacifici et al. used UHPLC for evaluation of long-term stability of cannabinoids in standardized preparations of cannabis flowering tops and cannabis oil (Carcieri et al., 2018; Noestheden et al., 2018a; Pacifici et al., 2018); UPLC method was used for simultaneous analysis of cannabinoid and synthetic cannabinoids in dietary supplements (Heo et al., 2016); Wang et al. developed and validated an UHPLC-UV-MS method for determination of 11 acid and neutral cannabinoids in cannabis samples (Wang et al., 2018). Eleven cannabinoids were characterized in Cannabis matrix using quantitative and fit-to-purpose performance as a function of extra-column variance and UPLC technique. (Noestheden et al., 2018a); the bulk diffusion coefficients and van Deemter curves using UPLC were used for the development of a rapid quantitative method for determination of 11 cannabinoids (Noestheden et al., 2018b). Using a liquid chromatography coupled to tandem mass spectrometry on a QTRAP 4000; Di Marco Pisciottano et al. developed a fast method for determination of nine phytocannabinoids in beverages and food derived from *Cannabis sativa* (Di Marco Pisciottano et al., 2018).

By the year 2017-

By UPLC combined with ionic mobility mass spectrometry (TWIM-MS) cannabinoids were successfully separated (Tose et al., 2017). Determination of three kinds of cannabinoids in cannabis using ultra-high performance liquid chromatography-tandem mass spectrometry and analysis of phenotype of cannabis (Sun et al., 2017). An ultra-high liquid chromatography coupled to traveling wave ion mobility mass spectrometry (UPLC-TWIM-MS) were applied to both electrospray ionization modes (ESI($\pm$)) and used to analyze hashish, marijuana, and parts of the *Cannabis Sativa* L. plant (flower and leaf). (Tose et al., 2017). Supercritical fluid chromatography (SFC) analysis of cannabinoids was reported by Backstrom et al. and by Wang et al. (Wang et al., 2017).

By the year 2016-
Development and validation of an UHPSFC-DAD/MS method for the qualitative and quantitative determination of major cannabinoids in cannabis plant extracts and products   (Wang et al., 2016). While Wei et al. developed UPLC/MS/MS method for the sensitive quantification of cannabinoids in milk by alkaline saponification–solid phase extraction (Wei et al., 2016). Decarboxylation study of acidic cannabinoids: a novel approach using ultra-high-performance supercritical fluid chromatography/photodiode array-mass Spectrometry. (Mei Wang et al., 2016); Simultaneous Analysis of Cannabinoid and synthetic cannabinoids in dietary supplements using UPLC with UV and UPLC–MS-MS (Heo et al., 2016). Principal cannabinoids concentration and their stability evaluated by a high performance liquid chromatography coupled to diode array and quadrupole time of flight mass spectrometry method (Citti et al., 2016); by UPC2, for the determination of the cannabinoids in cannabis (Thomas and ElSohly, 2016).

By the year 2015-
By UHPLC-UV/MS fingerprint method for quantitative determination of THC, CBD, CBG, and other eight cannabinoids from *Cannabis sativa* L. varieties. (Wang et al., 2015); By a validated UPLC-MS method

cannabinoid content was determined in dietary supplements (Lindberg and Johnson, 2015); Quantification of THC, CBD, cannabigerol (CBG), $\Delta 9$ tetrahydrocannabinolic acid (THCA), cannabidiolic acid (CBDA) and cannabigerolic acid (CBGA) was performed using a Shimadzu prominence UFLC system Cannabinoids and terpenes as chemotaxonomic markers in cannabis (Elzinga et al., 2015). Cannabinoids production by hairy root cultures of Cannabis and their content were determined using LC/MS (Farag and Kayser, 2015). By a validated method for determination of cannabinoid content in dietary supplements by UPLC-MS, 81(Lindberg and Johnson, 2015); using LC/MS, to study Cannabis DNA genotypes (Welling et al., 2016); using compact mass spectrometry for detection and quantification of cannabis-related compounds (Eike et al., 2015).

Gas chromatography techniques (GC)
Gas chromatography /flame ionization detector (GC/FID) Techniques
By the year 2018-
 by GC/FID, is commonly used technique in determination of cannabinoids and studying their stability. However, this technique limitation is the decarboxylation of acid cannabinoids into neutral ones; due to heat of the GC port; silylation technique was possible as reported by Elsayed et al. Who succeeded to determine acid and neutral cannabinoids in one run using trimethylsilane as derivatizing reagent (Ibrahim et al., 2018)
It was reported many GC/FID methods for determination of phytocannabinoids and for evaluation of stage growth of the Cannabis plant. The evaluation of the inflorescence yields and the content of $\Delta 9$-tetrahydrocannabinol ($\Delta 9$-THC) and cannabidiol (CBD) of seven traditional genotypes of cannabis (Janatová et al., 2018)
Gas chromatography /Mass (GC/MS) techniques
By the year 2018-
Using a GC/MS validated method for determination of cannabinoids in hemp

inflorescence by comparing two derivaatization techniques.(Cardenia et al., 2018); Commercial cannabis consumer products were tested using GC–MS qualitative analysis of cannabis cannabinoids (Ciolino et al., 2018); Comprehensive quality evaluation of medical *Cannabis sativa* L. inflorescence and macerated oils based on HS-SPME coupled to GC–MS and LC-HRMS (q-exactive orbitrap®) approach. (Calvi et al., 2018b); Headspace solid-phase microextraction/gas chromatography–mass spectrometry HS-SPME/GC–MS technique followed by multivariate statistical tools were used for determination of the complete volatile organic compound (VOC) emission profiles of 48 seized samples. (Calvi et al., 2018a)

By the year 2017-
By using open Probe fast GC-MS - combining ambient sampling ultra-fast separation and in-vacuum ionization for real-time analysis of cannabis samples (Keshet et al., 2017).

By the year 2016-
 Using GC-FID for quantification of 28 terpenes and verified via GC-MS (Aizpurua-Olaizola et al., 2016b).

Advanced Techniques

By the year 2018-
The potential of near infrared spectroscopy to estimate the content of cannabinoids in *Cannabis sativa* L.(Sánchez-Carnerero Callado et al., 2018); validated high resolution mass-spectrometry LC-HRMS (Orbitrap®) method was used for "the in-depth profiling and fingerprinting of cannabinoids" and in two authorized medical grade varieties of *Cannabis sativa* L. flowers (Calvi et al., 2018a); Thermal desorption-ion mobility spectrometry: A rapid sensor for the detection of cannabinoids and discrimination of *Cannabis sativa* L. chemotypes (Contreras et al., 2018); high-resolution ion mobility spectrometry for rapid cannabis potency testing (Hädener et al., 2018); extraction and isolation of cannabinoids from marijuana seizures and characterization by H NMR allied to chemometric tools (Leite et al., 2018)

By the year 2017-

Chemical profiling and classification of cannabis through electrospray ionization coupled to Fourier transform ion cyclotron resonance mass spectrometry and chemometrics (Borille et al., 2017)

By the year 2016-
Tsujikawa et al. developed of a novel immunoassay for herbal cannabis using a new fluorescent antibody probe, "Ultra Quenchbody" (Tsujikawa et al., 2016); Beasley et al. used matrix-assisted laser desorption/ionization-MS (MALDI-MS) to detect and image cannabinoids in hair samples. MALDI-MS is another technique capable of detecting low-level cannabinoids, though in this case, also requiring derivatization (Beasley et al., 2016); 200 samples were analyzed using Infrared and Raman screening of seized novel psychoactive substances (Jones et al., 2016); Evaluating the selectivity of colorimetric test (Fast Blue BB salt) for the cannabinoids identification in marijuana street samples by UV–Vis, TLC, ESI (+) FT-ICR MS and ESI (+) MS/MS (dos Santos et al., 2016).

By the year 2015-
Compact Mass Spectrometry was used for Detection and Quantitation of Cannabis-Related Compounds (Jack and Henion, 2015); By HILIC, as part of an RP-HPLC study for the determination of the cannabinoids in cannabis and a marijuana concentrate (Hung et al., 2015); By 2D-LC with chemiluminescence detection, for screening of cannabinoids in industrial-grade hemp (Pandohee et al., 2015); By EI-LC/MS with supersonic molecular beams (an introductory technique study, including cannabis) (Seemann et al., 2015).

Conclusions

Cannabis testing and quality control of the plant extracts are growing due to the legalization all over the world as it is used for recreational or medicinal purposes. Therefore, there is a need to develop and validate different analytical techniques for cannabis testing. Here we just reported a scientific survey for analysis of cannabis extracts, matrices, or preparations.

Acknowledgment

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