METHODS FOR QUANTIFICATION OF THE MAIN CANNABINOIDS IN CBD OIL

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ABSTRACT

Cannabidiol (CBD) is an alkaloid present in *Cannabis sativa*, together with tetrahydrocannabinol (THC) and more than 120 other substances belonging to a group of compounds named cannabinoids. Due to the continuous increased usage of CBD oils, it became necessary to be developed efficient methods for the identification of its compounds and especially for the characterization of the cannabinoids from the commercial specimens. Cannabinoids may be detected by many and different analytical methods, including immunoassays (EMIT®, Elisa, fluorescent polarization, radioimmunotest), techniques of flat chromatography: classic thin layer chromatography (TLC), optimum performance laminar chromatography (OPLC) and multiple development automatization (AMD), gas chromatography-mass spectrometry (GC-MS), high-performance liquid chromatography-mass spectrometry (HPLC-MS). Ultraviolet signal (UV) is used for the quantification of major cannabinoids and the mass spectrometer is used for the quantification of minor cannabinoids. The purpose of this study was to compare the performances of TLC, Ultra High-Performance Liquid chromatography with Photodiode Array Detection (UHPLC with PDA) and LC-MS/ MS technique for the qualitative and quantitative determination of cannabinoids in 3 commercial oils with CBD. Having in view that CBD may be found in many forms of oils, on the legal market of the internet, we believe that the development of a method for the qualitative and quantitative determination may be an interesting subject for the pharmaceutical professional persons.

Keywords: *CBD oils, cannabinoids, analytical, THC, quantification.*

INTRODUCTION

The Cannabis family includes *Cannabis sativa* and *Cannabis indica s*pecies. *Cannabis sativa* contains over 500 unique compounds, including over 120 natural cannabinoids. There were reported many therapeutical properties attributed to their pharmacological characteristics, which leads to a significant interest in their use in nutraceuticals and other consumption products [1]. The *Cannabis* plant was used from the oldest times for producing hemp fibers (for clothes, rope and paper), seeds that may be used as food for animals and also as a medicinal plant. CBD is one of the main alkaloids from the composition of cannabis plants together with other identified alkaloids [2]. The two major neuroactive compounds from the cannabis

plants are: the main psychoactive alkaloid, tetrahydrocannabinol (THC) and non-psychoactive alkaloid CBD [3].

Taking into account the present legislation, there are small differences regarding the quantities of THC admitted in the hemp products, the concentrations varying between 0,05 and 0,6% [4]. In the plant, the cannabinoids are mostly present under the form of acids, which are decompounded by decarboxylation. CBD is widely used although its benefic effects are reported especially based on the casuistical observations [5],[6]. In some countries, the products with CBD are legal, while in other countries they are forbidden, thus aggravating the confusion. At present, CBD is used as an active ingredient in the following products: Epidiolex® - oral solution (contains only CBD), approved in 2018 by Food and Drug Administration (FDA) as a medical product used for the treatment of seizures associated with Dravet and Lennox-Gastaut syndromes and Sativex® - oral-mucosal spray (contains both CBD and THC, in the percentage of 1:1) [7], [8], [9]. More clinical studies are in progress for the potential treatment of neurological and behavioral disorders. As CBD has a complex mechanism of action, there is a high potential of its use in the treatment of different pathologies [4].

The quantification of cannabinoids is essential for the proper labeling of cannabis products, for quality control, as well as for establishing the legality regarding the content of THC. The oils with CBD contain potentially useful nontoxic phyto cannabinoid substances. Together with the increase of the interest of the patients for the oils with CBD, there are indicated more researches for a better understanding of their therapeutic potential and of the safety profile [1], [10], [11], [14].

The present paper describes a series of analytical methods used for the separation of cannabinoids necessary for the analysis of the oils with CBD from the market. Using chromatographic methods we can determine the original composition of cannabinoids in oils by direct analysis [11]. CBD oil is traded for being used by children (for Dravet syndrome, ADHD, autism) [12], old persons (Alzheimer disease, dementia, Parkinson disease, cardiovascular diseases, inflammatory diseases) [13], patients that suffer from complications (cancer, multiple sclerosis, chronic pain, diabetic complications, arthritis, epilepsy) [11] and even for pets (anxiety, appetite, sleep, osteoarthritis) [15]. From this reason, the qualitative and quantitative certification is necessary through a selective, simple and fast method. The oil-rich in CBD became more and more popular and it is administrated under the form of sublingual drops, gelatinous capsules or as unguent local ointment [11]. At present, the market is in the progress of developing towards more sophisticated products, including oral capsules, liposomal products, skin lotions and chewing gums that contain CBD [6], [7].

MATERIALS AND METHODS

TLC

With the help of this technique and of the kit from alpha-CAT ® the main ingredients from cannabis may be visualized due to a reaction of a specific coloration, which represents the cannabinoids present in the oils with CBD or from

the cannabis product. Up to 4 specimens may be tested for each chromatographical plate.

TLC is the first method used for the chemical analysis of cannabis. It has the advantage of parallel analyses, although it has limits in resolution and sensitivity. The advantages are the reduced costs, the simple preparation of the specimens and the nondestructive method. Moreover, it allows the use of a wide range of chemical reactive substances for detection.

I used 3 modalities of oils traded on the internet, which I named as follows for easier identification: specimen 174 – product declared with a content of 1350 mg/100 ml total concentration of cannabinoids, specimen 175 - product declared with a content of 2, 5% CBD, specimen 181 - product declared with a content of 8% CBD, 4mg/drop.

Whether our specimen has a decreased or increased rate of cannabinoids, we can calculate a factor of multiplication for testing of the rate of THC is decreased or increased in the oils with CBD analyzed. We apply this simple rule based on the weight of the specimen: Factor of multiplication = (100 mg x 2µl) / weight of the specimens (mg) X extraction fluid (µl).

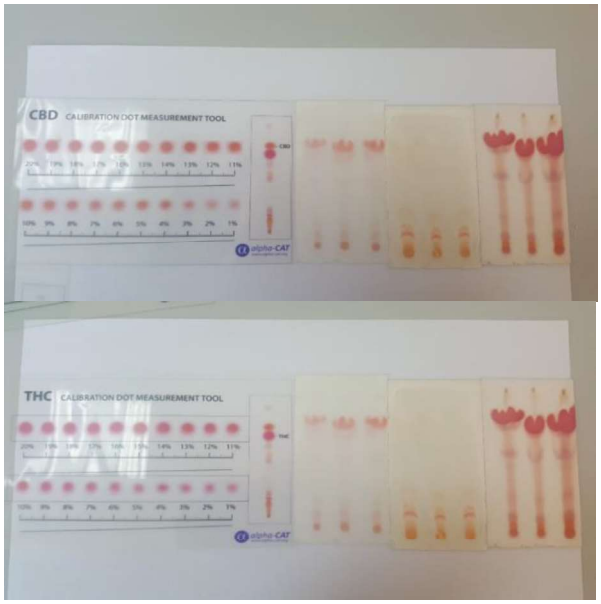

Fig.1. *Comparing with the area cu rigla graphical ruler of cannabinoids alpha-CAT® for CBD and THC, specimens 174, 175, 181.*

At present, TLC is a cheap method for the analysis of cannabinoids approved by the Office of the United Nations for Drugs and Crimes for the routine control of the content of cannabinoids. For the applications that require a high sensitivity for instance in pharmacology, it is not an indicated method.

UHPLC-PDA

Through UHPLC technique with PDA detection it was followed the separation and quantitative determination of CBD present in 3 oils. The chromatographical separation was made using a PerkinElmer Brownlee Analytical C18 column (50mm × 4,6mm id, 5µm) or the equivalent, using the evaluation in gradient with 0,1% acid formic in water as mobile phase A and 0,1% acid formic in acetonitrile as mobile phase B. For quantification, the length of detection wave was set at 210 nm.

The method was established and optimized in the following chromatographical conditions: debit-1mL/min, temperature of the column -30°C, injecting volume-5µL, mobile phases - formic acid and formic acid with acetonitrile(V/V).

Solvents, standards and specimens: Solution of cannabidiol, 1,0 mg/ml SLBM6755V; Standard Analytic Standard Sigma Aldrich; Methanol absolute, for HPLC, LiChrosolv®; Acetonitrile, for HPLC, LiChrosolv®; Formic Acid for LC / MS, Fischer Chemical®; 2-propanol, for HPLC, LiChrosolv®; 3 variants of hemp oils from Romania and Netherlands.

Table 1. *Exact Quantification of CBD for the 3 oil specimens*

Sample Name	Avg. Amount	Units	Avg. Plates (Foley-Dorsey)	Avg. Tailing Factor	Avg. Resolution	Avg. Area
Stand CBD 20 ppm	0.0000	µg/mL	11,217	1.407	2.27	2,203,748.7
CBD 174 D100	35.2245	µg/mL	7,385	1.083	1.25	2,803,382.6
CBD 175 D100	48.3351	µg/mL	N/A	0.000	1.09	3,319,760.2
CBD 181 D4500	27.3011	µg/mL	6,110	0.995	0.00	2,491,310.3

The optimized method is a simple, fast, selective, sensitive and useful method for the verification of the stability of CBD in the pharmaceutical forms, may be useful for quality control of the medicine products, both under the form of active substance and pharmaceutical formulations. The perspective of the study consists in applying this method on different pharmaceutical forms but also on different types of specimens (biological, soil, water, etc.)

LC-MS

I researched a technique of qualitative and quantitative analysis of CBD and CBG from three commercial hemp oils with the help of a UHPLC device with MS detector. The fluid chromatography system PerkinElmer® Flexar UHPLC connected to a LC/MS PerkinElmer® 5500 QTRAP model detector allows the analysis of different compounds at low concentrations (e.g. pesticides, contaminant, mycotoxin, dopant substances, drugs). The technique combines the separation power of UHPLC and the MS capacity of obtaining information about the mass and structure of the analytes. The mass spectrometer separates ions in gas phase based on m/z (load/mass). The chromatographical separation was performed using a

Perkin Elmer Brownlee Analytic DB AQ C18 (1,9 µm 100x2,1 mm) column or an equivalent with elution in gradient, debit 400 µL/min, temperature of the column 40°C, injecting volume 5µL.

The method developed detected the corresponding drops for the two compounds CBD and CBG. The calibration curve was outlined in the interval 10 - 100 ng / mL. The detection limit at the level of 3, 12 ng / mL was validated. The preparation of the specimens eliminates the use of chloroform, which was used regularly in the analysis of cannabinoids, decreasing the costs of materials, using more ecological solvents and improving the safety of the laboratory.

Table 2. *Exact quantification of CBD for the specimen of hemp oil*

Product statement	**CBD content mg/mL**
CBD oil 13.5 mg/mL	3.194
Ozonated hemp oil with 2.5% CBD and terpene oil	2.250
CBD oil 4 mg/drop	123.525

In the research literature it is widely reported that CBD coelutions with a CBG related cannabinoid but their molecular weights are different [1], [10]. For this reason, the analytic result UHPLC-PDA was confirmed with a MS complementary technique. This analytical method may be used for different applications, for instance for the quantitative and qualitative control of the CBD oil by a selective, simple and fast method.

The content of CBD of the commercial specimens analyzed in this study is not clearly specified by the manufacturer. The analysis of the three specimens of hemp oil revealed the real concentration of CBD from the specimens, highlighting the necessity of this type of analytical method.

CONCLUSION

The hemp oil from *Cannabis sativa* L. is a natural source rich in important nutritive substances, not only polyunsaturated fat acids and proteins, but also terpenes and cannabinoids, which contribute to the therapeutical benefits of the oil with CBD. Therefore, it is important to exist an analytical method for the determination and quantification of cannabinoids for establishing the exact concentration from the commercial specimens, an interesting subject for the professionals from the pharmaceutical domain.

HPLC-PDA is widely applied in the quantification of cannabinoids, as the approach is a facile one, robust and cheap. Nevertheless, LC-MS / MS is the most versatile among the methods, both regarding the dynamic range, and related to the offer of a real image of the content of CBD of the oils analyzed. The most frequently are used columns with reverse phase (mainly C18 variant or biphenyl) with a solvent of methanol-water containing 0,1% formic acid. The analytical benefits of the triple-quadrupole LC-MS / MS system are sensitivity, selectivity and

identification of the mass. As for the analysis of terpenes, UHPLC-PDA is not able to detect most of the terpenes, the connection to MS being compulsory. Flavonoids may be very well analyzed with all the detection methods connected to LC.

Based on the information presented in this study, ideal quantification method of cannabinoids is LC-MS / MS for the cannabinoids using the PerkinElmer system, especially in clinical research. This analytical method may be used for different applications, quantitative and qualitative control of CBD oil by a selective, simple and fast method.

The perspective of the study is to apply these methods to the different pharmaceutical forms, but also to other types of specimens (biological, soil, water, etc.). It is also an interesting alternative for the routine analyses in the criminalistic sciences. The analytical methods easily characterize and quantify CBD in the oils available from the commercial sources for offering a robust instrument for the determination of the potency, safety and quality, with usages both in human medicine and in the veterinary one.

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