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THE QUALITATIVE AND QUANTITATIVE ANALYSIS OF CBD IN HEMP OILS BY UHPLC WITH PDA AND APPLICATIONS

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Abstract

Cannabidiol (CBD) is the major non-psychotropic cannabinoid compound derived from the plant Cannabis sativa L. CBD was first isolated in 1940 and its structure and stereo chemistry determined in 1963. The cannabinoid CBD, a non-psychoactive isomer of the more infamous tetrahydrocannabinol (THC), is available in a growing number of administration modes, but the most commonly known is CBD oil. In just a few years, cannabidiol (CBD) has become immensely popular around the world, CBD is now sold and used to treat a wide range of medical conditions and lifestyle diseases(Hazekamp, 2018). Hemp oil from Cannabis sativa L. is a very rich natural source of important nutrients, not only polyunsaturated fatty acids and proteins, but also terpenes and cannabinoids, which contribute to the overall beneficial effects of the oil. In the European Union (EU), the cultivation of certain cannabis varieties is granted provided they are registered in the EU's Common Catalogue of Varieties of Agricultural Plant Species and the THC content does not exceed 0.2% of the dried flowers of the plant(European Commission website, 2018). There are hundreds of producers and sellers of CBD oils active in the market, and their number is increasing rapidly. Various studies done on CBD oils products around the world have come to similar conclusions about incorrect label information. For that reason, thorough analytical testing of final products by certified third-party labs is an essential tool to guarantee the safety and composition of CBD oils. Hence, it is important to have an analytical method for the determination of these components in commercial samples and their applications. The present work describes a technique for the monitoring the cannabidiol-CBD present in 3 commercial hemp oils, by UHPLC with PDA detection.

Key words: *Cannabinoids, Cannabis sativa, CBD oil, HPLC, UHPLC.*

INTRODUCTION

The present work describes a technique for the monitoring of the cannabidiol-CBD present in 3 commercial hemp oils, by UHPLC with PDA detection, for the qualitative and quantitative determination of the cannabidiol (CBD).Using chromatographic methods: HPLC and UHPLC allow the determination of the original composition of cannabinoids in oil by direct analysis.CBD oil is actively marketed for use by children (for Dravet's syndrome, ADHD, autism) (Devinsky et al., 2018), the elderly (Alzheimer's disease, dementia, Parkinson's disease, cardiovascular diseases, inflammatory diseases) (Chagas et al., 2014), patients who suffer from complications (cancer, multiple sclerosis, chronic pain,diabetic complications, arthritis, epilepsy) (Śledziński et al., 2018, Cuñetti et al., 2018, Hunter et al., 2018) and even for pets (anxiety, appetite, sleep, osteoarthritis) (Gamble et al., 2018; Scott et al., 2019); for this reason qualitative and quantitative certification is required by a selective, simple and rapid method. CBD-rich oil has become increasingly popular and is administered via sublingual drops, gel capsules or as a topical ointment (Shimadzu, 2018).

The main source of CBD-rich oil is industrial hemp. CBD oil is derived as a concentrate from CO_2 or the butane extraction of hemp, sometimes followed by steam distillation or ethanol distillation for purification (Shimadzu, 2018). Currently, the market is developing further towards more sophisticated and patentable products, including oral capsules, liposomal products, skin creams, and chewing gums containing CBD (Hazekamp, 2018).

CannaLean has developed a novel formulation of Cannabidiol (CBD), with chitosan, a biocompatible, non-toxic, and non-immunogenic compound that enhances the potential of CBD to significantly reduce cholesterol and triglycerides (Apietroaiei et al., 2016, 2018). This method is necessary to determine potency, and ensure the quality and safety of these oils.

MATERIALS AND METHODS

Chromatographic separation was achieved using a PerkinElmer Brownlee Analytical C18 column (50 mm × 2.1 mm, 1.9 µm) or equivalent, using and gradient elution with 0.1% formic acid in water as mobile phase A and 0.1% formic acid in acetonitrile as mobile phase B. The flow rate was 0.4 mL/min and the injection volume was 5µL. For quantification, the detection wavelength was set at 210 nm (Figure 1).

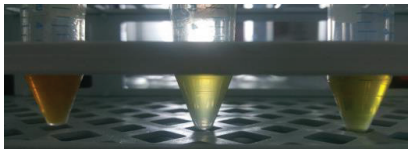

Figure 1. Appearance of samples

All solvents, reagents, and diluents used were HPLC-grade or better.

Solvents, Standards and Samples:

- Cannabidiol Solution, 1.0mg / mL SLBM6755V Analytical Batch Standard Sigma Aldrich
- Absolute methanol, 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 Holland.

The analytical platform was an PerkinElmer Flexar UHPLC System, including:

- Column Oven
- PDA Plus Detector
- Solvent Manager
- UHPLC Autosampler
- UHPLC Pump

It was used 3 variants of oils marketed on the internet, which, in order to be easier so named:
Sample 174-declared product containing 1350 mg/100 ml total cannabinoids concentration (label information),
Sample 175-product declared with a content of 2.5% CBD (label information),
Sample 181-product declared with 8% CBD content, 4 mg/drop (label information).
The UHPLC method parameters are shown in Table 1.

Table 1. The UHPLC method parameters

<table>
<tr><td>Column</td><td>Column PerkinElmer Brownlee DB , C18(50mm×2,1mm , 1,9µm) (lot# 130114Q)</td></tr>
<tr><td>Mobile Phase</td><td>Solvent A: Water with 0.1% formic acid
Solvent B: Acetonitrile with 0.1% formic acid
<table>
<tr><th colspan="4">Program</th><th colspan="3">Solvent Reservoir</th></tr>
<tr><th>Step</th><th>Type</th><th>Time (min)</th><th>Flow (mL/min)</th><th>A</th><th>B</th><th>Curve</th></tr>
<tr><td>0</td><td>Equil</td><td>1.000</td><td>0.4</td><td>50</td><td>50</td><td>0</td></tr>
<tr><td>1</td><td>Run</td><td>0.100</td><td>0.4</td><td>50</td><td>50</td><td>1</td></tr>
<tr><td>2</td><td>Run</td><td>3.000</td><td>0.4</td><td>2</td><td>98</td><td>1</td></tr>
<tr><td>3</td><td>Run</td><td>6.000</td><td>0.4</td><td>2</td><td>98</td><td>1</td></tr>
<tr><td>4</td><td>Run</td><td>1.000</td><td>0.4</td><td>50</td><td>50</td><td>1</td></tr>
<tr><td>5</td><td>Run</td><td>3.000</td><td>0.4</td><td>50</td><td>50</td><td>1</td></tr>
</table></td></tr>
<tr><td>Pressure</td><td>18000 psi/ maximum</td></tr>
<tr><td>Oven Temp</td><td>30ºC</td></tr>
<tr><td>PDA Detection Wavelength</td><td>210 nm</td></tr>
<tr><td>Injection Volume</td><td>5 µL</td></tr>
<tr><td>Sampling (Data) Rate</td><td>5 pts/sec</td></tr>
</table>

The stationary phase in the column must be kept moist before operating the UHPLC. Following an extensive literature study, it was found that the CBD had the maximum absorption in methanol at 210nm wavelength, so this wavelength was chosen for subsequent determinations. To check if the detector works, we took a sample of ultrapure water (about 60 ml). A sample of water was taken, passed through the detector, and then measured.

To save time, it was purged with a 25 ml syringe, and to speed up the process it was directly connected the detectors to the pump. We did the detection with the lamp on average detection = 0.45. So, we determined that the detector is in optimal parameters, so we can introduce the samples.

It was set the tray temperature to 5°C and column temperature to 30°C. We gave a flush to the autosampler for 4 times a with 250µL.

For quantification, the detection wavelength was set to 210nm.

Since we work V/V, 0.5 ml of water was removed from phase A and filled with 0.5 ml of formic acid, and from phase B, we removed 0.25 ml of water and filled with 0.25 ml of formic acid.

The mobile phases were placed on UHPLC. Standard CBD, 1.0 mg/ml in methanol, standard for batch drug analysis SLBMM6755V, Sigma-Aldrich, registered was used as a sample 176 from which we made a dilution in methanol of 100 ppm, 100 µg/mL (complete V/V with 900 µL absolute methanol for HPLC, LiChrosolv®).

It was homogenized everything with a Vortex Genius 3 for 30 seconds.

To check the equipment status, first it was performed an injection of absolute methanol solvent for HPLC, LiChrosolv®.Then it was diluted the samples as follows:

sample 174-0.2 ml oil + 1.8 ml isopropanol (2-propanol) - for HPLC, LiChrosolv®).

sample 175-0.2 ml oil + 1.8 ml isopropanol (2-propanol) - for HPLC, LiChrosolv®)

sample 181-0.2 ml oil + 1.8 ml isopropanol (2-propanol) - for HPLC, LiChrosolv®).

It was developed 7 variants, but we used variant no.6.A PerkinElmer Flexar™ UHPLC system was used, including a quaternary pump, and a PDA (photodiode array) detector.

RESULTS AND DISCUSSIONS

Sample analysis is significantly reduced (Table 2), while the validation of the method has confirmed that the method generates repeated and accurate results. It was developed an optimized UHPLC-PDA method with low extraction time and more environmentally friendly solvents for adoption in CBD determination laboratories. Sample preparation eliminates the use of chloroform, which has been commonly used in cannabinoid analysis, reducing materials costs, using greener solvents, and improving laboratory safety. Figure 2 shows the cromatograms of a mixture containing CBD, all separeted in a less then 4 minutes.

This method can be used in a variety of settings, from clinical trials, research, quality control, and normative assessment of this growing industry.

Table 2. The accurate quantitation of CBD for the hemp oil samples

Sample Name	Avg. Amount	Units	Avg. Plates (Foley-Dorsey)	Avg. Tailing Factor	Avg. Resolution	Avg. Area
standCBD 20ppm	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
CBD175 D100	48.3351	µg/mL	N/A	0.000	1.09	3,319,760.2
CBD181 D4500	27.3011	µg/mL	6,110	0.995	0.00	2,491,310.3

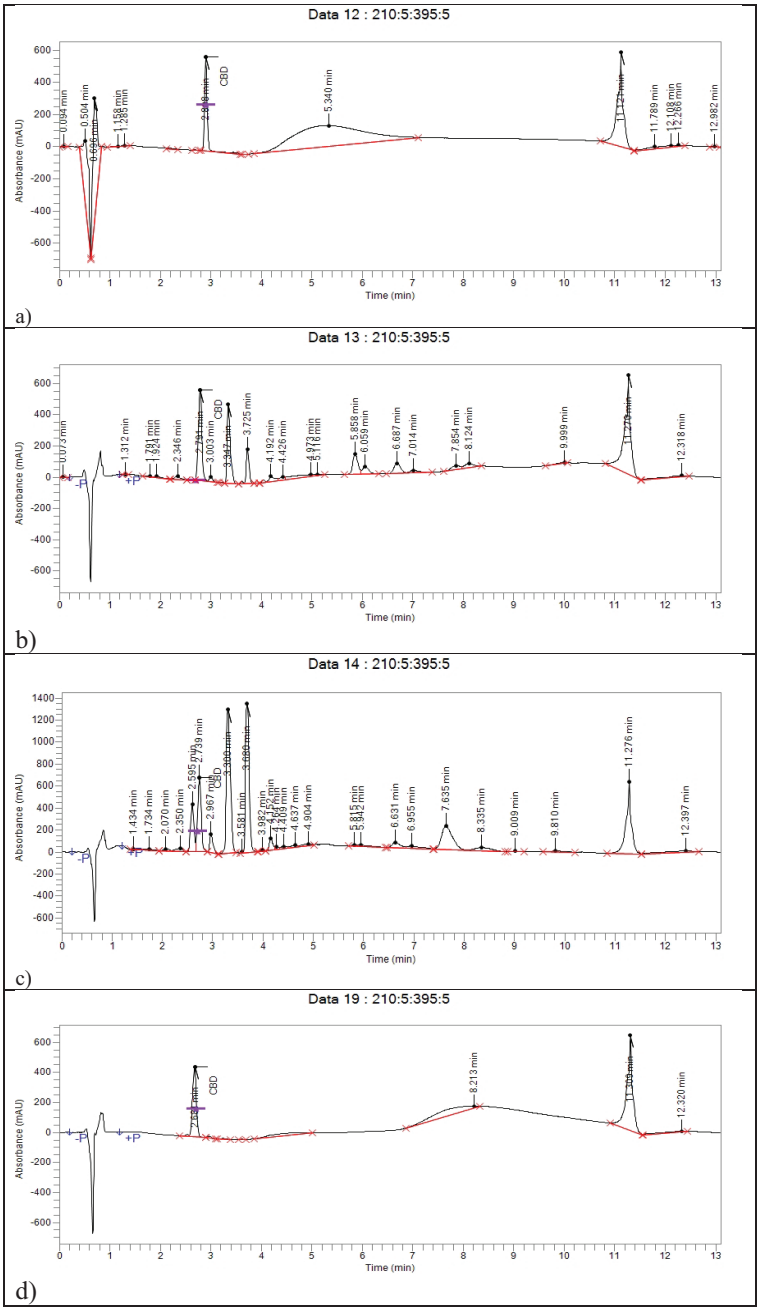

Figure 2. Chromatogram for CBD a) standard 20ppm; b) 174D100; c) 175D100; d) 181 D4500

Qualitative Analysis of Hemp Oil

Analysis of the three samples of CBD oil (Table 2) showed differences in the declared concentrations and the results of the study. Off the three analyzed samples: two samples were far below label claim and one sample was well above the label, up to 200%. To solve the problem, some manufacturers simply add to the CBD and CBD-acid content to have a higher CBD-total content on the label. When

purchasing CBD oils, one should consider: label claim, actual concentration, and the selling price.

CONCLUSIONS

The perspective of the study is to apply this method to different pharmaceutical forms but also to other types of samples (biological, soil, water etc.).It is also an interesting alternative for routine analyzes in forensic sciences, the analytical method easily characterizes and quantifies CBD in hemp oils available from commercial sources to provide a robust tool for potency, safety, and quality determinationswith uses in both human and veterinary medicine.

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