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CANNABIDIOL CONTENT EVALUATION IN COMMERCIAL DIETARY SUPPLEMENTS AND STABILITY IN OIL VEHICLE

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ABSTRACT. Cannabidiol (CBD) is one of the most studied alkaloids found in *Cannabis* species. Also, its occurrence in *Cannabis* plants is higher in most of cases compared with the addictive compound named tetrahydrocannabinol (THC). This study aimed to evaluate CBD concentrations and stability in different commercial products and reconstituted oil. An UHPLC method previously published was applied. Three CBD oils and two powders marketed online together with a reconstituted oil were analyzed. The oils presented concentrations lower than stated on the label. The content of reconstituted CBD oils prepared similarly to the commercial oils decrease linearly with small differences based on the storage conditions. Thus, the CBD oil kept at temperatures of 5±0.3°C tends to decompose slightly slower compared with the oil kept at room temperature. A stabilizer may be needed to slow the decomposition process. Future studies for reconstituted formulation may be needed to establish if the type of oil used is influencing the decomposition process.

Keywords: *CBD-oil, dietary supplements, storage, CBD powders, decomposition*

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INTRODUCTION

Cannabidiol (CBD) whose chemical structure can be found in Figure 1, belongs to the class of cannabinoids [1]. It can be found in numerous pharmaceutical formulations [2-4]. Sadly, many of them are registered as dietary supplements and this can be a huge disadvantage for patients because some studied supplements can contain lower concentrations of active substance than stated on the label [5,6]. Currently, the only pharmaceutical formulation approved worldwide that contains CBD as its active substance CBD is Epidiolex, which contains 10% CBD [7,8]

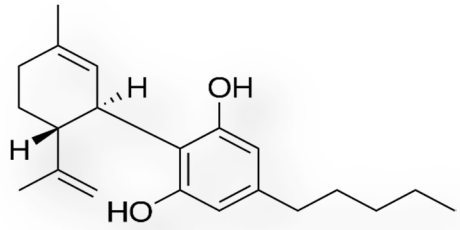

Figure 1. CBD chemical structure

CBD can be found in numerous types of pharmaceutical formulations from therapeutic oils to ointments, creams, gels and, sprays. Its external use can be attributed to the presence of hydroxyl groups which confers CBD an antioxidant property based on which it can be used in cosmetical products as an anti-aging ingredient. The internal use is sustained by a high number of studies many of them demonstrating the antiepileptic effect. Also, studies regarding the use of CBD in Alzheimer disease, cancer, or Parkinson disease were conducted [9].

Usually, the class of dietary supplements consists of vitamins and minerals, so taking into consideration that CBD is a cannabinoid that has proven therapeutic effect it could be stated that it is wrongly attributed to this class and it should only be present in the class of drugs approved by entities such as European Medicines Agency (EMA) or Food and Drug Administration (FDA) [9].

At the moment, numerous dietary supplements are marketed worldwide and many of them can be purchased online without counseling from any healthcare professional. A list of pharmaceutical formulations with CBD registered as supplements that can be purchased online in Romania can be found in Table 1.

Table 1. Pharmaceutical formulations with CBD registered as dietary supplements

Product name	Pharmaceutical form	CBD concentration (%)
CBD oil	Oily solution	5, 10, 15, 25, 30
Body cream	Ointment/cream	0.5
Hand cream	Ointment/cream	0.2
Mouthwash	Solution	0.1
Tablets for animals	Tablets	1.3, 3.2 mg/tablet
Toothpaste	Paste	0.133
Oral Spray	Spray	0.8
Soothing Gel	Gel	0.1

An important issue regarding CBD products is related to their stability and the variable degree of recovery from samples with the natural origin [10], the studies underlying in the same time the importance of non-aqueous liquid vehicles for liquid formulations or extemporaneous preparation.

This study aims to assess the concentration of CBD found in commercial oils and powders together with a stability study of CBD reconstituted oils at different temperatures and under light exposure.

RESULTS AND DISCUSSION

The results obtained show that in the case of the powders, the measured concentrations correspond to the ones mentioned on the label by the manufacturer. The same cannot be said about CBD oils.

In a study conducted by Pacifici et al. [10], it has been stated that the recovery from tea samples of CBD was variable, also mentioning the fact that different parts of the plant tend to have different concentrations of CBD. The same study concluded that the stability in an aqueous solution is very short so an extemporaneous preparation needs to be taken into consideration. In the same research, it has been shown that the CBD is more stable in oily solution compared with an aqueous solution, but a loss of 20% of the initial concentration is recommended to be taken into consideration.

It is well known that solutions represent the most unstable pharmaceutical formulations [11-13], so there is a possibility that initially, during manufacturing, the concentrations were the ones mentioned on the label by the manufacturers but keeping them in certain conditions such as high temperatures, light exposure

and the lack of a stability test might have led to a decrease in terms of concentrations. In the case of CBD oil A (5%), CBD oil B (5%) and, CBD oil C (10%), concentrations lower than 95% of the declared amount were measured (Table 2). In the case of reconstituted oils, it can be noticed that the more time passes the more the CBD concentration tends to decrease (Table 3). A slightly higher decrease can be noticed in the case of the oil kept at room temperature, which indicates the importance of the storage conditions of the oily solution. A maximum absorption at the wavelength of 208 nm (Figure 5) and a retention time of 3.2 minutes corresponding to CBD was observed (Figures 2-4). A shift might occur during the determinations between the signals belonging to the oils and external standards that could be explained by the aging of the column. The shifting process is less than 10% which is deemed acceptable by the in-force European Pharmacopoeia. In figure 3, are represented the peaks corresponding to the external standard (higher signal) and the one belonging to the evaluated CBD oils (lower signal). The concentration of CBD in the blank hemp oil used is negligible – less than 0.4%, which can be correlated with the initially higher concentration of CBD in the reconstituted CBD oil compared to the quantity of CBD powder dissolved, at about 5.38%. An accumulation of the CBD added and dissolved in the solution with the CBD present in the hemp oil caused this final higher concentration. In the Certificate of Analysis, a higher concentration is mentioned in all of the three oils studied compared to the label, which also indicates the existence of the CBD in small quantities in the hemp seed oil.

Table 2. Evaluation of CBD in the samples

Pharmaceutical formulation	Concentration on the label	Concentration at T_0	Absolute Difference (%)	Total relative loss (%)	Concentration at T_2 (%)	Absolute difference (%)	Total relative loss (%)
CBD oil (A)	5.0 %	4.240	-0.760	15.200	3.950	-1.050	21.000
Bio CBD oil (B)	5.0 %	4.580	-0.420	8.400	4.290	-0.710	14.200
CBD oil (C)	10.0 %	8.485	-1.515	30.300	8.449	-1.551	15.150
Powder A	99.8 %	99.093	-0.707	0.7080	97.090	-2.710	2.715
Powder B	99.0 %	97.530	-1.470	1.4840	96.970	-2.030	2.050

In a study conducted by Bonn-Miller et al. 84 oils from 31 companies were evaluated. It has been stated that 69% of the CBD oils were mislabelled. Regrettably, an operation such as dilution, blending and, the rectification of CBD oils is permitted legally [7]. In another study conducted by Fraguas-Sánchez CBD water and alcoholic solution were prepared to determine the t_{95} the results showed that CBD tends to be more stable in alcoholic solution than in water solution (water+0.5% Tween 80), also, the temperature influences the CBD stability, the same study showing that a solution kept at temperatures of 5°C is more stable compared with the solution kept at room temperature (25°C). The authors mentioned above linked the instability by the presence of oxygen, whilst, when the solution is saturated with another gas such as nitrogen the stability of the solution increased [14].

Table 3. Assessment of CBD concentration in the reconstituted oil

Pharmaceutical formulation	T_0 (%)	T_1 (%)	Absolute difference (%)	Total relative loss (%)	T_2 (%)	Absolute difference (%)	Total relative loss (%)
Reconstituted oil kept at (5°C)	5.380	5.170	-0.210	3.903	4.738	-0.642	11.930
Reconstituted oil kept at (25°C)		5.100	-0.280	5.204	4.730	-0.650	12.081

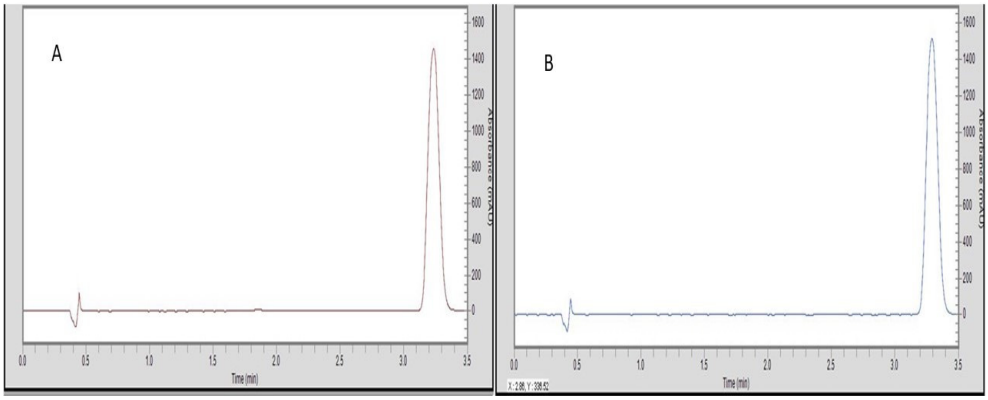

Figure 2. Chromatograms of CBD powders A and B at T_0

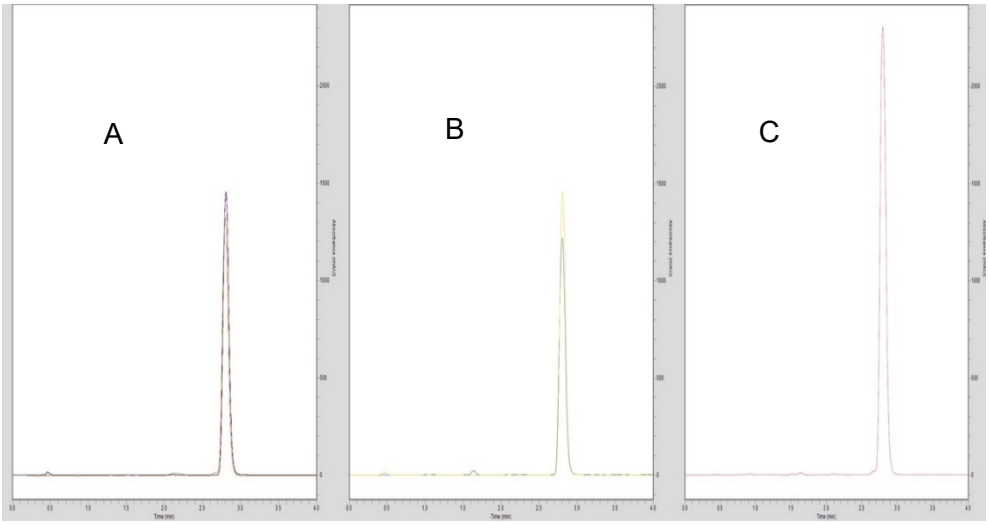

Figure 3. CBD chromatograms of oils A, B, C

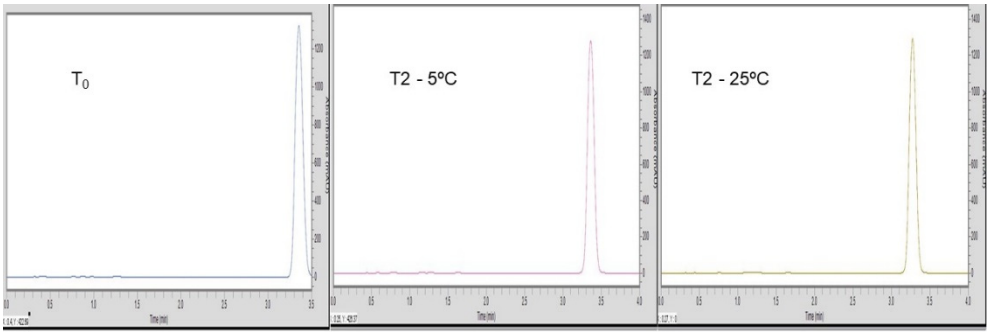

Figure 4. CBD chromatograms of reconstituted oil at T_0 and T_2.

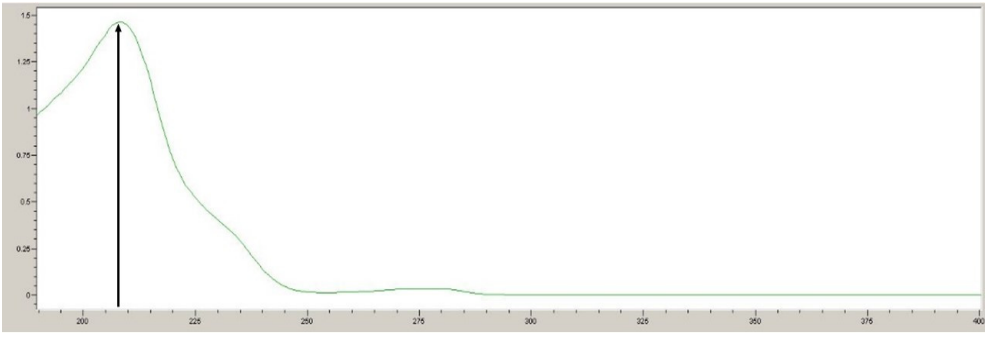

Figure 5. CBD spectra, maximum absorption at 208 nm

Shelf-life evaluation for the reconstituted oils

To determine the shelf life, the reconstituted oils were tested. The time needed for the concentration to decrease to 90% of the initial value was evaluated. After 14 days, concentrations less than 90% were assessed in both of the cases. The main difference consisted in the fact that in the case of the oil kept at room temperature 12 days were needed to reach this value, while in the case of the oil kept at 5°C the decrease in concentration to under 90% was observed after 12 and a half days.

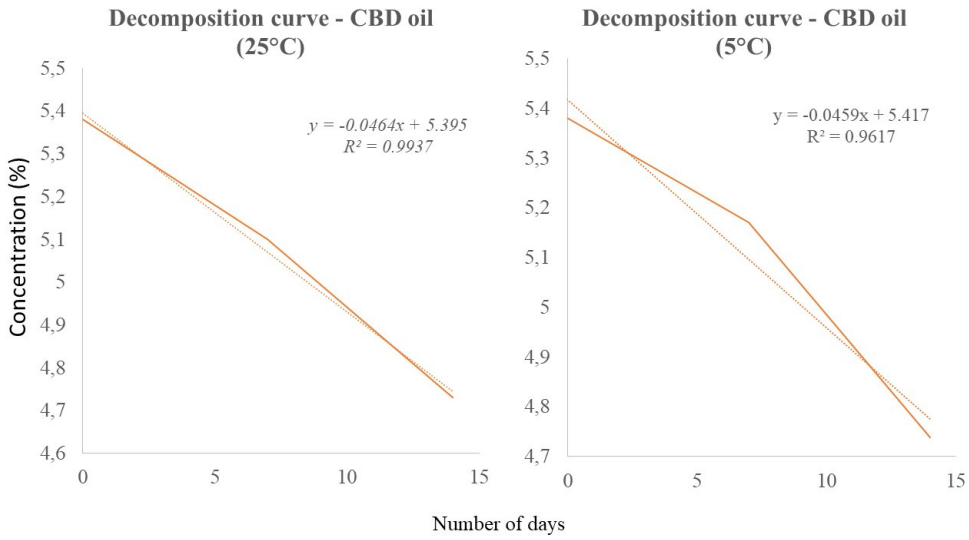

Figure 6. Decomposition curves for reconstituted CBD oil kept at different temperatures

Varying the temperature storage conditions was considered as a result of the lack of stability of CBD in ACN, while kept at room temperature, whilst when the CBD solution in ACN was kept in the refrigerator at 5°C, the stability of the solution was improved. This fact represented the starting point of the present study. It can be observed that in both of the cases the concentration tends to decrease as time passes. Small differences regarding the concentration at T_1 and T_2 were observed in the case of both CBD reconstituted oils kept in different temperature conditions.

CONCLUSIONS

Five dietary supplements found on the market were evaluated, three of them being formulated as an oily solution while two of them were powders. Besides the dietary supplements, a reconstituted CBD oil concentration was assessed. The powders concentration was within the acceptance limit whilst the oily solutions presented concentration under the lower acceptance limit. In the case of the reconstituted oil, it has been observed that as time passes the CBD concentration tends to decrease fact that can be observed in both of the reconstituted oils kept in different storage temperatures. To obtain a concentration close to the one mentioned on the label, the CBD concentration in the hemp oil has to be taken into consideration. Another possibility consists of using other oils that don't contain CBD such as sunflower oil, sesame oil where there is no risk of cumulation of CBD, and obtaining concentrations that are not in accordance with the declared ones. Also, labeling accuracy of CBD sold online might be incorrect, thus including CBD oils as a prescription or over-the-counter drug might be more suitable, due to its therapeutic properties because in the case of drugs available with prescription many analytical tests are conducted, so the concentration of the product coincides with the one written on the label.

EXPERIMENTAL SECTION

a. Reagents and reference substances

During the assessment of CBD concentrations, the following reagents and substances were used: cannabidiol (CBD) 99.5% (Trigal Pharma, Austria), acetonitrile (ACN) (Honeywell, Germany) and, hemp oil (Canah, Romania) ultrapure water obtained using a Millipore Direct Q3 purifier.

b. Chromatographic equipment and method

The chromatographic equipment consisted of a Flexar 10 (Perkin Elmer) UHPLC system with a DAD detector. Due to the aging of the initial column, we needed to replace it, and we chose a column with the same type of stationary phase and the same size but from a different manufacturer. The column used, an InfinityLab Poroshell 120 EC-C18, was manufactured by Agilent and had the following characteristics: dimensions - 3.0 x 100 mm, particle size - 2.7 µm. Other equipment used: magnetic stirrer with a heating source (VWR Hot-Plate Advanced Series) and an ultra-microcentrifuge 5430R (Eppendorf).

To establish the CBD concentration in the studied oils and powders a UHPLC method previously developed and validated was used. [15]

c. Preparing standard and test sample solutions.

The stock solution had a concentration of 1 mg/mL cannabidiol and its preparation was simple, 10 mg of CBD being dissolved in 10ml of ACN. The reference solution had a concentration of 100 µg/mL and was obtained by diluting the stock solution with the same solvent (ACN).

The evaluated samples can be found in Table 4.

Table 4. Analysed samples

Pharmaceutical formulation	Concentration
CBD oil (A)	5.00 %
Bio CBD oil (B)	5.00 %
CBD oil (C)	10.00%
Powder A	99.80 %
Powder B	99 %
CBD oil (reconstituted formulation)	5 % (CBD added)

With the scope of quantification, the CBD from the CBD oils (registered as dietary supplements) was extracted as follows: 200 µL of CBD oil were mixed with ACN in a beaker. The mixture was stirred using a magnetic stirrer at 800 rotations per minute (rpm) for one hour. The resulted solution was transferred and diluted in a 10 mL flask with ACN. The next step consisted of a new dilution of 1:10. From the obtained solution approximately 1.5 mL were centrifuged at 9000 rpm and 20°C for three minutes.

Reconstitution of the oily CBD solution was performed by using CBD – 99.5% and hemp oil, following a concentration of 5% CBD. The reconstitution consisted of the dissolution of the CBD powder (99.5%) in the hemp oil. In order to obtain a homogenous solution, we stirred the reconstituted CBD oil for 1 hour at 1200 rpm. The reconstitution was realized as a result of the variated concentration belonging to the evaluated CBD oil.

It was divided into two equal parts, each to be stored under different conditions, half of it was maintained at room temperature (23°C±2°C) without any exposure to light while the other half was maintained in the refrigerator at temperatures of 5°C±0.3°C. Different conditions of storage were chosen as a result of the instability of the CBD in ACN at room temperature.

Evaluation of the CBD concentration for the oils kept at different temperatures was realized at T_0 – when it was prepared, T_1 – at one week, T_2 – after two weeks.

The hemp oil itself which was used for preparing the reconstituted CBD oils was also tested to establish if it contains any CBD; the reconstituted formulation was prepared as similarly as possible and using the same ingredients as the purchased CBD oils.

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