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RESEARCH ARTICLE

New UHPLC Method for Cannabidiol Determination in Hard Capsules

Robert-Alexandru Vlad*, Lenard Farczadi, Silvia Imre, Adriana Daniela Ciurba, Nicoleta Todoran, Emoke Redai, Paula Antonoaea, Daniela Lucia Muntean

University of Medicine, Pharmacy, Sciences and Technology of Târgu Mureș, Romania

Objectives: The aim of the study was to propose a new UHPLC method for the determination of cannabidiol (CBD) from supplements and drugs available on the Romanian market. **Materials and methods**: The HPLC assay of CBD was achieved by using a Phenomenex Gemini NX-C18 column. The mobile phase consisted of 70% acetonitrile and 30% water. Elution was performed in isocratic mode and the detection was done at 208 nm. The method was tested on hard capsules containing 150 mg of CBD. **Results and discussions**: The retention time of CBD was 2.8 minutes. Regression analysis showed good linearity over the 1-100 ug/ml concentration range. The lowest limit of quantification was established at 1 µg/ml. The method was developed by using reconstituted capsules. The substance proved low stability in solution at room temperature and stability at temperatures between 2-8ºC. The recovery of reconstituted samples was 96.77%. The commercial capsules had a very low content of 15-20% from declared content. **Conclusions**: The proposed method can be used for CBD determination in different pharmaceutical formulations – hard and soft capsules with coconut oil as excipient.

Keywords: cannabidiol, UHPLC, assay, hard capsules

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Introduction:

Cannabidiol (CBD), with the IUPAC naming: 2-[(1R,6R)-3-methyl-6-prop-1-en-2-ylcyclohex-2-en-1-yl]-5-pentylbenzene-1,3-diol is an alkaloid found in the species of Cannabis sativa and Cannabis ruderalis; usually it occurs in higher concentrations in Cannabis sativa [1, 2, 3].

CBD is a white crystalline powder with a low melting point of 63º C, practically insoluble in water 11.6 mg/L but soluble in ethanol, methanol, acetonitrile, dimethylsulfoxide (DMSO) [3].

From the pharmacological point of view CBD has a low affinity for CB1 and CB2 receptors. Many studies demonstrated that the substance is an antagonist on the CB1 and CB2 receptors. The alkaloid proved also an antagonist effect of the GPR 55 receptor, inverse agonist on GPR 3, 6 and 12 receptor and a partially agonist on 5 HT_{1A} receptor, having as a consequence antidepressant, anxiolytic and neuroprotective effects [4-6]. The oral route bioavailability is between 20-33% due to its lipophilic character (log P 6.33) and due to the first hepatic passage; as a result of these characteristics CBD is classified in the second class in Biopharmaceutic Classification System (BCS) because it has a low solubility but a high permeability [7]. Other effects are anti-dystonic [8], antiemetic [9,10] and anti-inflammatory [11-13].

As a result of the low solubility in water we can found on the pharmaceutical market formulas that contain CBD dissolved in different types of oils due to the much higher solubility. THC can be found besides CBD, a substance needing a prescription in some pharmaceutical formulations which is prohibited in many countries or it can be prescribed under various restrictions due to the fact that cannabis extract is included in the oily phase. In Romania CBD could be find as supplements, soft capsules that contain cannabis oil, and formulations, hard capsules with 150 mg CBD. The number of HPLC published methods for CBD determination is low, therefore the aim of this study was to propose a new UHPLC method for CBD determination in hard capsules.

Material and methods

Chemicals and reagents

The reference substances and reagents used were: CBD – 99.5 % purity obtained from Trigal Pharma, ACN - Acetonitrile supplied by SLW Chemicals.

Instrumentation and chromatographic equipment

Chromatographic equipment consisted of UHPLC Flexar 10 system (Perkin Elmer). The analysis was performed on a Gemini NX-C18 column, 3.0 x 100 mm, 3 µm. Other equipments: a water purification system - Direct Q3 System (Millipore); magnetic stirrer with heater– VWR Hotplate Advanced Series; refrigerated ultramicrocentrifuge 5430 R (Eppendorf).

Stock and quality control samples

The stock solution of 2 mg/mL was obtained by dissolving 20 mg of CBD in 10 mL of acetonitrile. A number of six concentrations were further obtained: 1 µg/ml, 5 µg/ml, 10 µg/ml, 25 µg/ml, 50 µg/ml and 100 µg/ml. The quality control samples were stored in the freezer at 5ºC.

* Correspondence to: Robert-Alexandru Vlad
E-mail: alexiandru@gmail.com

Extraction procedure

Ten capsules were weighted empty and with the mixture of CBD and coconut oil in order to determine the average mass of the content. A mass of 427 mg of was transfered in a 10 ml volumetric flask and extracted with ACN. The resulted composition was stirred at 1200 rpm for 60 minutes, without heating; the next step was to centrifugate at 12000 rpm for 5 minutes and in the end from the solution obtained a volume of 0.1 ml has been taken diluted 50 times with ACN.

A quantity of a reconstitued samples equivalent to 15 mg of CBD and 25 mg of coconut oil was extracted by applying the same procedure as for the hard capsules.

Calibration curve and linearity

Calibration curve consisted of a blank sample, a placebo sample (sample with coconut oil and ACN) and 6 non-zero samples, ranging from 1-100 µg/mL. The calibration curve plotted the peak areas of the 6 non-zero samples.

Results

HPLC method development

During the method development, several columns have been tested: Zorbax SB-Solvent Saver plus, Supelcosil LC-18, Luna C18 (2) and Gemini NX-C18. Due to the relatively high tendency of decomposition of CBD under temperature effect, the column temperature was set at an optimum temperature of 13 ºC and the samples were kept at 10 ºC.

The chromatography analysis was finally conducted on a Gemini NX-C18, 3.0 x 100 mm column, 3 µm, at 13 °C. The analysis time was set for 3.5 minutes. The elution was isocratic and a mixture of 70% ACN and 30% water as a mobile phase was used, whereas the flow rate was set for 1 mL/min and the injection volume at 10 µl. The chromatograms were obtained at 208 nm, however spectra were recorded between 200-400 nm.

At the begining of the chromatographic method development the purpose was to find a suitable mobile phase which leads to adequate peak's shape and short analysis time. Initially CBD was eluted with a mixture of 40% water and 60% acetonitrile gradient composition, resulting in broad peak with a retention time of 4 minutes. The chromatographic behaviour of the analyte allowed the reducing the percentage of water to 30% which determined the CBD retention time of 2.8 minutes and a total run-time of 3.5 min, providing symmetric and narrow peak. Based on the UV spectra, the optimal wavelength was set at 208 nm.

Analytical performances of the method

Specificity

The retention time of the CBD in the optimised chromatographic conditions was 2.8 min. No interferences were detected at the retention time of the CBD on the chromatogram of a blank sample (Figure 1).

Selectivity

Good selectivity was observed as no peak from the coconut oil interfered with CBD. The peaks showed small variability, RSD% being less than 5%.

Calibration curve and carry-over

The calibration curve was obtained as area versus concentration by using 6 calibration points. The concentrations ranged between 1-100 µg/ml and each calibration point was determined by replicate analysis (n=5). The value of the correlation coefficient was higher than 0.99. No carry-over was observed by injecting high-concentration standard before blank sample analysis.

Accuracy and precision

Accuracy and precision were calculated at three levels of concentration: low L (10 µg/ml), intermmediate I (25 µg/ml) and high H (100 µg/ml). The intra-day and inter-day variability were less than 2% at all the 3 concentrations tak-

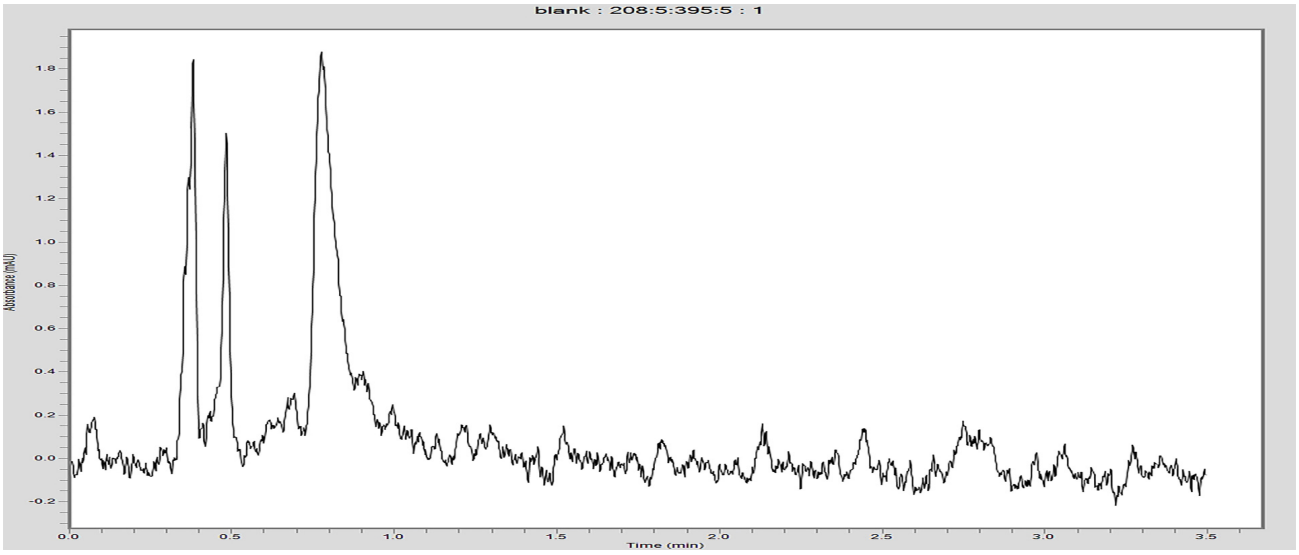

Fig. 1. Chromatogram of a blank sample

en into consideration, showing that the method was precise and accurate. The CV for intra-day assays ranged from 0.11% to 1.84% while the inter-day values of CV were between 0.31-0.51%. The mean accuracy for the intra-day assay ranged between 97.83%-102.99% while the inter-day accuracy was between 98.34%-102.81% (Table I).

Stability

It has been observed that if the CBD capsules are storaged at room temperature the concentration of CBD is 5 to 6 time smaller compared to when it is kept at temperatures between 2-8 ºC, also the CBD solution kept at room temperature gets colored into yellow. No specific storage conditions were labelled on the studied commercial samples. The indication for storage at low temperature is not a common label for this substance and commercial products. Our findings confirm the Sigma Aldrich storage indication at 2-8ºC.

Limits of quantification

These two parameters were analyzed on acetonitrile solutions, diluted from the stock solution. The lowest limit of quatification was 1 µg/ml (Figure 2) and the highest limit of quantification was 100 µg/ml (Figure 3).

Table I. Accuracy and precision for within batch and between-batch assays (n=5)

Level	Nominal concentration, µg/mL	Intra-day		Inter day	
		RSD %	Mean accuracy	RSD %	Mean accuracy
L level	10	1.84	99.19	0.50	102.5
I level	25	0.29	97.83	0.31	98.34
H level	100	0.11	102.99	0.51	102.81

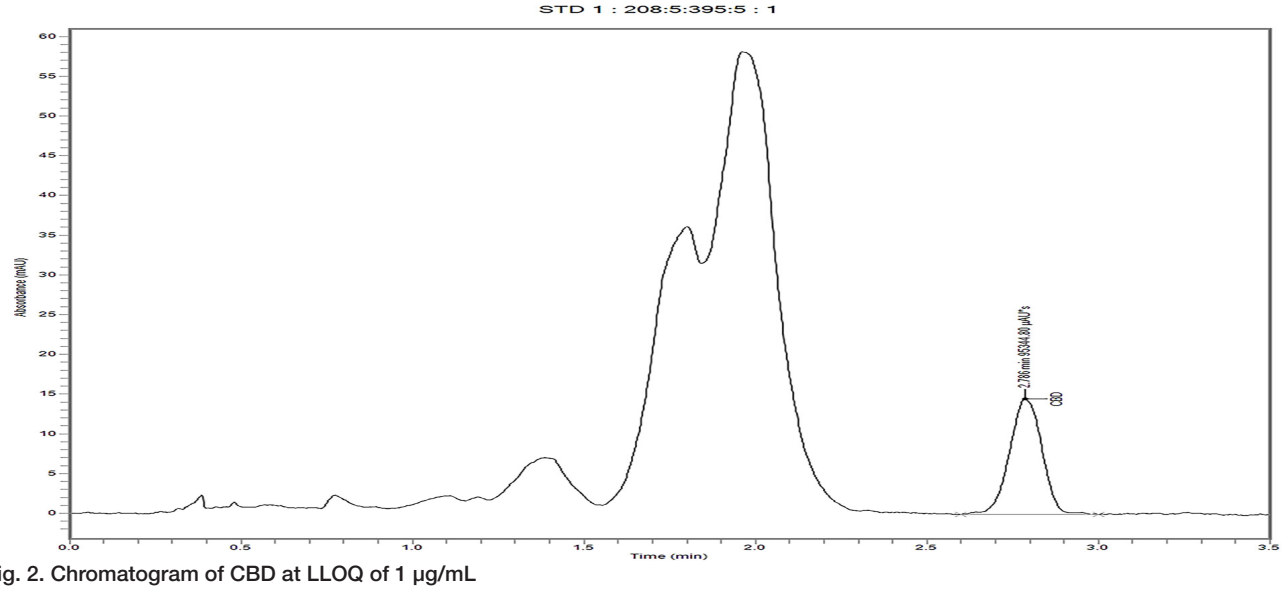

Fig. 2. Chromatogram of CBD at LLOQ of 1 µg/mL

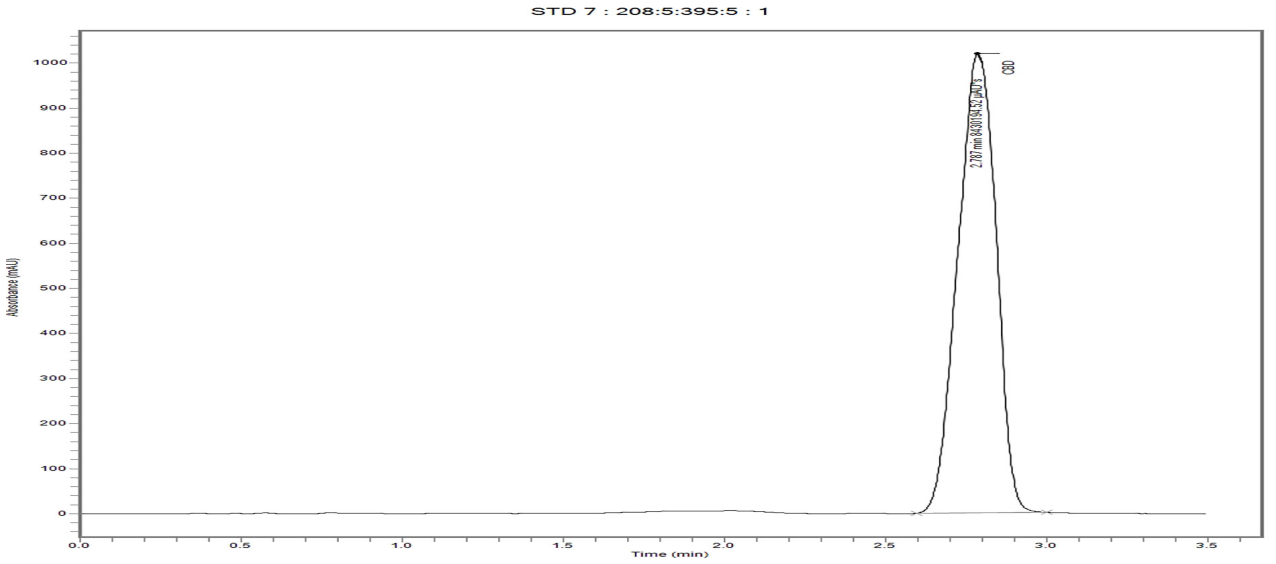

Fig. 3. Chromatogram of CBD at ULOQ of 100 µg/ml

Method application for CBD determination in the pharmaceutical product

The HPLC UV method was applied for CBD determination in capsules. The CBD content of the capsules kept at room temperature was about 15-20% of the declared concentration, while on the reconstituted capsules which were kept at temperatures between 2-8ºC for 7 days, the CBD content was 96.77%.

Discussions

In this study, a new HPLC-UV method was developed for the screening of CBD in hard capsules. A simple mobile phase composition allowed a rapid analysis of CBD (retention time of 2.8 min) on a middle-bore HPLC column, providing symmetric and efficient peak.

The CBD content of capsules was 96.77% on the reconstituted samples while on the ones found on the market it was ranged within 15-20%. Beneath the storage mentions an important factor which could conduct to low CBD concentratios may have been the fact that the capsules were close to the expiry date. However a decrease of concentration of 80% before expiration date indicates that in this case the storage below 8 °C is necessary.

There are few literature data regarding CBD determination in formulations. *Ravula A* et al. proposed a similar method in terms of efficiency and analytical performances [14], but in comparison with the method published by *Zgair A* et al. with a retention time about 8.3 minutes, the current method is closed to a high-throughput one [15].

Conclusions

The method obtained is simple, accurate, precise and specific and it can be used for CBD determination in formulations, hard capsules with cannabidiol and coconut oil.

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Authors' contribution

Alexandru Robert Vlad (Data curation; Formal analysis; Resources; Software; Validation; Writing – original draft; Writing – review & editing)
Lenard Farczadi (Investigation; Resources; Software; Supervision; Validation; Writing – review & editing)
Silvia Imre (Conceptualization; Data curation; Formal analysis; Methodology; Project administration; Writing – review & editing)
Adriana Daniela Ciurba (Conceptualization; Funding acquisition; Methodology; Project administration; Writing – review & editing)
Todoran Nicoleta (Conceptualization; Formal analysis; Funding acquisition; Writing – review & editing)
Redai Emoke (Visualization; Writing – review & editing)
Antonoaea Paula, Assist prof. (Conceptualization; Validation; Writing – review & editing)
Muntean Daniela Lucia (Funding acquisition; Project administration; Software; Writing – review & editing)

Conflict of interest

None to declare

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