Macedonian Pharmaceutical Bulletin, 72 (Suppl 1) 235 - 236 (2026)
Online ISSN: 1857-8969
DOI: 10.33320/maced.pharm.bull.2026.72.03.112
Short communication

An RP–HPLC method for determination of cannabidiol in topical dosage forms

Zhaklina Poposka Svirkova[1,2*], Dragica Doneva[1], Marija Andonovska[1,3], Zharko Tanturovski[1,2], Zana Mustafa[1], Sanija Zulfikjari[1], Aleksandra Dimitrovska[1], Zorica Arsova Sarafinovska[1,2], Sandra Zinoski[1]

[1]*Institute for Public Health of the Republic of Macedonia, 50 Divizija No 6, 1000 Skopje, North Macedonia*
[2]*Faculty of Medical Sciences, Goce Delcev University, Krste Misirkov 10A, 2000 Stip, North Macedonia*
[3]*Faculty of Dentistry – Skopje, Mother Teresa No. 43, Ss. Cyril and Methodius University in Skopje, 1000 Skopje, North Macedonia*

Introduction

The increasing incorporation of cannabidiol (CBD) into topical products has created a need for reliable analytical methods to ensure product quality and safety. CBD, a non-psychoactive cannabinoid from cannabis, possesses anti-inflammatory, antioxidant, and antimicrobial properties, making it a popular ingredient in creams, lotions, serums, and balms (Baker et al., 2003; Schettino et al., 2021).

However, CBD concentration in commercial topicals varies widely, typically ranging from 0.1% to 15% w/w. This variability, combined with the complexity of topical matrices containing oils, surfactants, emulsifiers, and preservatives, poses significant challenges for accurate quantification.

High-performance liquid chromatography (HPLC) has emerged as one of the most widely used and reliable techniques for the quantification of CBD in cosmetic products (Analakkattillam et al., 2022; Schettino et al., 2021). HPLC offers several key advantages, including high sensitivity, selectivity, and the ability to analyze both neutral and acidic cannabinoids without thermal degradation or derivatization (Analakkattillam et al., 2022). Coupled with ultraviolet (UV) or diode array detection (DAD), HPLC provides robust and reproducible results suitable for routine quality control analysis (Shishovska et al., 2016).

Sample preparation remains a critical step prior to HPLC analysis, as cannabinoids are highly hydrophobic and may be embedded in complex emulsion systems. Common techniques include ultrasound-assisted extraction using solvents such as methanol, ethanol, or acetonitrile, often followed by filtration or centrifugation (Schettino et al., 2021).

Therefore, the aim of this work is to develop and validate a simple, fast, and sensitive reversed-phase (RP)–HPLC method for CBD determination in topical products.

Materials and methods

The method was applied to topical preparations containing varying concentrations of CBD, available on the Macedonian market, such as: Rheuma & Pain Relieve Ointment (500 mg CBD/ 50 g), Ointment (125 mg CBD/ 50 g) and Hemorrhoid Ointment (300 mg CBD/ 30 g). The standard substances were: CBD((-)-trans-2-p-mentha-1,8-dien-3-yl-5-pentyl-resorcinol), CBN (6,6,9-trimethyl-3-pentyl-6H-benzo[c]chromen-1-ol) and Δ9-THC (3-pentyl-

*z.poposka.svirkova@iph.mk

6,6,9-trimethyl-6a,7,8,10a-tetrahydro-6H-dibenzo (b, d)pyran-1-ol), delivered as 1 mL ampoule solution (1 mg/ mL prepared in methanol, with purity 99.52%, m/m, 99.05%, m/m and 98.40%, m/m, respectively). The standard substances were obtained from Supelco (Merck KGaA, Germany). All solvents used were of HPLC grade.

Chromatographic analysis was performed on an Agilent Technologies 1200 series HPLC system (Germany), using a Purospher® Star RP18e column (150 mm × 4 mm ID, 5 μm) from Merck KGaA (Germany). The mobile phase consisted of acetonitrile and water acidified with o-phosphoric acid at pH 2.6, in gradient mode from 50% to 80% acetonitrile (*V/V*). The flow rate was 1.5 mL/min, column temperature 30°C, detection wavelength 220 nm, injection volume 10 μL, and the total run time was 31 min (Shishovska et al., 2016).

The working solutions of standard substance CBD were prepared in absolute ethanol in concentration range: 0.021 mg/mL – 0.380 mg/mL. The working concentration was 0.1 mg/ml.

The sample solutions were prepared by liquid/liquid extraction using absolute ethanol. The extraction was carried out in an ultrasonic bath. When the layers were clearly separated, the ethanolic layer was filtered and injected into the HPLC/DAD system.

Results and discussion

Three different Cannabis sativa L. topical oil preparations, purchased from the Macedonian market, were analyzed. The declared CBD content in the preparations was 0.25% and 1%, as stated on the product label.

In the samples analyzed, CBD was identified and quantified. Identifications were done by comparing the retention times and UV spectra of standard compounds with retention times of peaks at the chromatograms of samples and their UV spectra. The retention times of CBD peaks were around 9 min.

For the quantification, data obtained from the chromatograms were calculated using the method of the calibration curve (constructed as concentration, c (mg/ml), versus peak area, A (mAU)). The analyzed working solutions of the active substance CBD showed high linearity in the working ranges. The estimated coefficient of the linearity for CBD working solutions was 0.9997 (A = 26.03 c + 0.2973). The symmetry factor for CBD peak was found to be 0.950, while the resolution between CBD and CBN was found to be 4.7. The limit of detection was 0.37 ng CBD, while the limit of quantification was 1.11 ng (corresponding to 0.037 mcg/ml and 0.11 mcg/ml, respectively). The content of cannabidiol in analyzed samples was found to be from 93.2% to 98.1% of the declared values.

Conclusion

The proposed RP-HPLC method is simple, accurate, and precise for CBD determination in topical preparations, making it suitable for routine quality control and stability studies. Accurate analytical methods for CBD quantification are essential for ensuring product quality and patient safety of cannabis-based products.

References

Analakkattillam, S. et al., 2022. Analytical method validation for assay determination of cannabidiol and tetrahydrocannabinol in hemp oil infused products by RP-HPLC. Sci. Rep. 12, 12453. https://doi.org/10.1038/s41598-022-13737-6

Baker, D. et al., 2003. The therapeutic potential of cannabis. Lancet Neurol. 2(5), 291-298. https://doi.org/10.1016/S1474-4422(03)00381-8

Schettino, L. et al., 2021. A Rapid and Sensitive Method for the Determination of Cannabidiol in Cosmetic Products by Liquid Chromatography-Tandem Mass Spectrometry. Cosmet. 8(2), 30. https://doi.org/10.3390/cosmetics8020030

Shishovska, M. et al., 2016. Determination of cannabidiol and Δ9-tetrahydrocannabinol in Cannabis sativa L. preparations present in the European market by HPLC/DAD. Maced. Pharm. Bull. 62(Supplement), 239. https://bulletin.mfd.org.mk/volume-62-supp/