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Optimization and validation of an HPLC-DAD method for the identification of 14 cannabinoids: application in *Cannabis sativa* L extracts

Ana F. Morais [1,2,3], **Robin Fertig** [4], **Ricardo Jorge Dinis-Oliveira** [1,2,3,5] **and Carlos J. A. Ribeiro** [2,3,6,*]

[1] Associate Laboratory i4HB - Institute for Health and Bioeconomy, University Institute of Health Sciences - CESPU, 4585-116 Gandra, Portugal
[2] UCIBIO - Applied Molecular Biosciences Unit, Translational Toxicology Research Laboratory, University Institute of Health Sciences (1H-TOXRUN, IUCS-CESPU), 4585-116 Gandra, Portugal
[3] Department of Public Health and Forensic Sciences, and Medical Education, Faculty of Medicine, University of Porto, Porto, Portugal
[4] Avextra Innovations GmbH, 64625 Bensheim, Germany
[5] FOREN—Forensic Science Experts, 1400-136 Lisbon, Portugal
[6] Avextra Portugal SA, 7570-003 Grândola, Portugal

* Correspondence: carlos.ribeiro@avextra.com

Abstract

Background: *Cannabis* sp. plant has its origins in East Asia, where it was initially used for recreational and religious purposes. This plant encompasses hundreds of chemical compounds, including phytocannabinoids, terpenoids, and flavonoids. The cannabinoids of greatest medicinal interest are Δ^9-tetrahydrocannabinol (Δ^9-THC) and cannabidiol (CBD), which are present in the plant in their acid form [1]. Sativex® is an oromucosal spray containing standardized extract with Δ^9-THC and CBD already approved as a treatment option for neuropathic pain associated with multiple sclerosis [2]. With cannabis increasing recognition as a medicinal option, there are growing concerns over how to extract, detect and quantify cannabinoids properly and efficiently. High-performance liquid chromatography (HPLC) coupled with ultraviolet (UV) detection is considered the gold standard for cannabinoid analytical assessment included in cannabis monographs present in several pharmacopeias [3,4]. **Objective:** The main goal of this project was to optimize and validate an HPLC-DAD analytical method for the quantification of 14 cannabinoids in cannabis extracts. **Methods:** Flower pulverization was accomplished with a Retsch MM 400 ball mill. Extraction was performed according to the European Pharmacopoeia [4]. Chromatographic separation of cannabinoids was achieved on an Agilent 1260 Infinity II HPLC-DAD system, using an InfinityLab Poroshell 120 EC-C18 (3.0 x 150 mm, 2.7 µm) column protected with a Poroshell 120 EC-C18 3.0 mm, 2.7 µm guard column. The gradient elution was performed using methanol with 0,05% formic acid and deionized water with 0,1% formic acid mixtures, with a flow rate of 0,5 mL/min, run time of 30 min, and injection volume of 5 µL. **Results:** The optimized method resulted from adjusting chromatographic conditions: mobile phases (solvents, gradient, pH and flow rate: 0,5 – 1 mL/min), column length (50 – 150 mm) and temperature (30 – 50 ºC). Diode array analysis was performed for specificity assessment and UV quantification was performed at 224, 230, 260, 272 and 280 nm. To demonstrate that the analytical method fits its purpose, accuracy, precision, linearity, and range were established based on regulatory guidelines – ICH Q2. **Conclusions:** The developed and validated method successfully separates 14 cannabinoids, as well as other compounds present in the cannabis extracts tested.

Keywords: cannabis; Δ^9-THC; HPLC-DAD

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