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A sustainable approach to analyzing neutral cannabinoids using HPLC-DAD

Carlos J. A. Ribeiro [1,2,3,*]

[1] Avextra Portugal SA, 1070-060 Lisboa, Portugal
[2] Associate Laboratory i4HB - Institute for Health and Bioeconomy, University Institute of Health Sciences - CESPU, 4585-116 Gandra, Portugal
[3] UCIBIO - Applied Molecular Biosciences Unit, Translational Toxicology Research Laboratory, University Institute of Health Sciences (1H-TOXRUN, IUCS-CESPU), 4585-116 Gandra, Portugal

* Correspondence: carlos.ribeiro@avextra.com

Abstract

Background: Gałuszka *et al.* [1] proposed 12 principles of green analytical chemistry that underscore the importance of minimizing environmental impact and enhancing analytical efficiency in scientific practices, all the while safeguarding operator safety. Researchers are encouraged to utilize HPLC techniques that facilitate quicker and more efficient separation of cannabinoids. In the literature, this separation is primarily accomplished using reversed-phase HPLC methods, with acetonitrile (ACN) and methanol (MeOH) consistently identified as the preferred organic modifiers [2]. However, their toxic and flammable characteristics present significant risks. ACN can be metabolized in the liver into harmful substances, and chronic exposure can lead to negative health effects. Furthermore, even slight exposure to MeOH, whether through inhalation or skin contact, can negatively impact the nervous system, liver, and kidneys [3]. Therefore, it is crucial to explore alternative organic solvents for the mobile phase to achieve a more environmentally friendly LC separation of cannabinoids [4]. **Objective:** This study aims to develop an HPLC-DAD analytical method for the separation of six neutral cannabinoids, using ethanol (EtOH) as the organic mobile phase as an alternative to MeOH and ACN. **Methods:** The chromatographic separation of cannabinoids was performed on an Agilent 1260 Infinity II HPLC-DAD system, utilizing an InfinityLab Poroshell 120 EC-C18 column (3.0 x 150 mm, 2.7 µm) that was protected by a Poroshell 120 EC-C18 3.0 mm guard column. A gradient elution was carried out using a mixture of ethanol and deionized water, both containing formic acid, at a flow rate of 0.5 mL/min over a duration of 18 minutes. The method developed demonstrated great selectivity, linearity, precision, and accuracy. **Results:** The optimized method was achieved by modifying chromatographic conditions, including gradient, flow rate, run time, and column temperature. Diode array analysis was conducted to evaluate specificity, while UV quantification was carried out at 230 nm. To ensure that the analytical method meets its intended purpose, parameters such as linearity. accuracy, precision, and lower range limits were established in accordance with regulatory guidelines, including ICH Q2 and M10, and AOAC appendix F. **Conclusions:** A greener analytical method using EtOH as organic mobile phase was successfully developed for the quantification of six neutral cannabinoids.

Keywords: cannabis; CBD; Δ^9-THC; HPLC-DAD; green analytical chemistry

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