OPEN ACCESS **Scientific** Letters

IV 1H-TOXRUN INTERNATIONAL CONGRESS 2025 | 08-09 MAY, 2025 Porto, Portugal

Poster 47

GC-MS-Based Study of 14 Cannabinoids Separation in Cannabis sativa L. Extracts using a Derivatization Approach

Bruna A. S. Nunes[1,2], Albertina M. Capita[1,2], Ana F. Morais[1,2,3], Ricardo J. Dinis-Oliveira[1,2,3,4], Carlos J. A. Ribeiro[1,2,5] and Eduarda M. P. Silva[1,2] *

[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, 4200-319 Porto, Portugal.
[4] FOREN—Forensic Science Experts, 1400-136 Lisbon, Portugal.
[5] Avextra Portugal SA, 7570-003 Grândola, Portugal.

* Correspondence: eduarda.silva@iucs.cespu.pt

Abstract

Background: Cannabinoids from *Cannabis sativa* L. are increasingly studied due to their potential as medicinal drugs, offering therapeutic benefits such as pain relief and anti-inflammatory effects, while also raising concerns as substances of abuse due to their psychoactive properties [1,2]. Gas chromatography-mass spectrometry (GC-MS) is a powerful tool for identifying and quantifying cannabinoids, offering high sensitivity and specificity. However, due to the thermal instability of cannabinoids, derivatization is a crucial step to improve their detectability and chromatographic behavior in GC-MS analysis. **Objective:** This study aims to develop a derivatization protocol and a GC-MS-based analytical method for cannabinoid detection in extracts of the *Cannabis* sp cultivar ZF plant. **Methods:** Extraction of cannabinoids from dried cannabis flowers was achieved following the European Pharmacopoeia protocol [3]. The standards and extracts were derivatized with 120 µL of *N*,*O*-bis(trimethylsilyl)trifluoroacetamide with 1% of trimethylchlorosilane (BSTFA + 1% TMCS), 80 µL of pyridine in 200 µL of anhydrous ethyl acetate. The mixture reacted for 30 min at 60 ºC, then cooled to room temperature and injected directly into the GC-MS for analysis. The chromatographic conditions were established using a capillary column containing 5% diphenyl 95% dimethylpolysiloxane (30 m x 0.25 mm x 0.25 µm), injector temperature set to 280 ºC followed by a temperature ramp from 180 up to 280 ºC at a helium flow rate of 1 mL/min to a total run of 25 min. **Results:** Several derivatization conditions were tested to allow high yields of the derivatized cannabinoids while preventing decarboxylation of the acidic forms. Hence, reaction time and temperature, the quantity of derivatizing agent, the use or not of pyridine, and the use of solvents like ethyl acetate, dichloromethane, and acetonitrile were studied. Chromatographic conditions were also optimized to allow the simultaneous separation and detection of 14 compounds in the same run. **Conclusions:** The optimized derivatization conditions ensured the stability of the different cannabinoids avoiding decarboxylation of the acidic forms and formation of byproducts. The established chromatographic conditions provided an adequate separation and peak resolution of a total of 14 cannabinoids. The GC-MS-based analytical method was successfully applied to the identification and detection of these cannabinoids in cannabis extracts.

Keywords: medicinal cannabis; phytochemical analysis; gas chromatography; mass spectrometry

Acknowledgments/Funding

This work received financial support through the annual funding of 1H-TOXRUN of the University Institute of Health Sciences (IUCS-CESPU). The authors also acknowledge the funding provided by Avextra Portugal SA.

References

1. Pellati, F.B. et al. Cannabis sativa L. and Nonpsychoactive Cannabinoids: Their Chemistry and Role against Oxidative Stress, Inflammation, and Cancer. *BioMed Res. Int*, **2018**, doi: 10.1155/2018/1691428.
2. Baron, E.P. Comprehensive Review of Medicinal Marijuana, Cannabinoids, and Therapeutic Implications in Medicine and Headache: What a Long Strange Trip It's Been. *Headache* **2015**, 55, 885-916, doi: 10.1111/head.12570.
3. Ph. Eur. 11.5. Cannabis Flower Monograph, 07/**2024**:3028.