Scientific Letters

Poster Communication 51

GC-MS Terpene Profiling in Cannabis extracts: Method Optimization and Analytical Validation

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Abstract

Background: The importance of Cannabis sp. in modern medicine stems from its complex phytochemical profile, where terpenes may play a role through the entourage effect. This entourage effect proposes that Δ^9-tetrahydrocannabinol (Δ^9-THC) interacts with other compounds in the cannabis plant in ways that meaningfully modify its effects. For instance, Spindle et al. reported that D-limonene reduced the acute anxiogenic effects of Δ^9-THC [1]. To ensure the standardization of cannabis-based products, GC-MS is essential by enabling accurate quantification of these compounds in complex plant matrices [2]. **Objective:** This study aims to optimize and validate a GC-MS method for the characterization of major terpenes in cannabis leaves and flowers. **Methods:** Two cultivars of cannabis flowers were used: one rich in CBD (Blue Cheese) and the other rich in Δ^9-THC (Z-Face). In addition, leaves from a Δ^9-THC-rich cannabis cultivar provided by Avextra were also analyzed. Samples were pulverized and extracted with ethyl acetate for 10 minutes using a Retsch 400 ball mill. GC-MS analysis was performed using an SH-Rxi-5ms column under a temperature gradient. Terpenes were identified in SIM mode based on characteristic ions [3]. The method was validated according to ICH guidelines. **Results:** The method showed high precision and accuracy, demonstrating linearity ($R^2 > 0.999$) for all ten analyzed terpenes. The three most abundant terpenes identified in the leaves were β-caryophyllene (0.014%), α-bisabolol (0.011%), and α-humulene (0.09%), expressed as percentage (*w*/*w*). In the Blue Cheese cultivar, the dominant terpenes were α-bisabolol (0.174%), β-caryophyllene (0.108%), and α-pinene (0.086%). The predominant terpenes in the Z-Face cultivar included β-caryophyllene (0.445%), α-humulene (0.252%), and limonene (0.227%). Terpinolene was the only terpene not detected in all three samples. **Conclusion:** A validated GC-MS method was established for terpene characterization in cannabis extracts. Higher terpene levels were found in flowers compared to leaves, with distinct profiles observed between the CBD- and THC-rich varieties.

Keywords: Cannabis; GC-MS; Terpenes

Acknowledgments/Funding

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

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