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Short communication

Development of powerful chromatographic methods for better separation of tetrahydrocannabinol isomers during HPLC analysis of cannabis flowers and extracts

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Introduction

THC (tetrahydrocannabinol) usually refers to the naturally existing isomer of Δ^9-THC, but also may include Δ^8-THC (Grotenhermen & Russo, 2002). Out of some five hundred components of Cannabis, Δ^9-THC is the primary and perhaps the only compound (Δ^8-THC is also active but its concentration is very low) responsible for its psychoactive effects. Most of the pharmacological effects of this natural product are due to the activation of two types of G-protein-coupled receptors, the cannabinoid CB_1 (distributed in the brain) and CB_2 receptors (present almost uniquely in the immune system). This well explains the actions of Δ^9-THC on cognitive and motor functions, as well as its immune-modulatory effect (Di Marzo, 2004).

In the scientific papers and brochures of vendors of chromatographic equipment and columns, Shimadzu, Waters (Aubin, Layton & Helmueller, 2018), Agilent (Storm, Zumwalt & Macherone, 2019), Knauer (Loxterkamp, Stephan & Monks, 2020) etc., many different methods are published for separation of cannabinoids. These methods achieve separation of eight, up to seventeen different cannabinoids, most commonly and dominantly present in cannabis flowers and extracts. All of them focus their attention on achieving a maximal resolution between the peaks of cannabinoids, using gradient and/or isocratic elution, achieving a maximal resolution of about 1.2 between the critical separation pairs, CBGA/CBDA, CBG/CBD, and most important Δ^9-THC/Δ^8-THC.

Our experience in analysis of different cannabis flowers and extracts showed a necessity for much higher resolution between the psychoactive cannabinoid Δ^9-THC and its positional isomer Δ^8-THC, as well as other peaks of cannabinoids that might eventually co-elute with them, since as psychoactive they are very strictly limited in the cannabis extracts and products**.** Thus, our research was aimed at this objective.

Materials and methods

The following reagents were used: methanol, acetonitrile, isopropanol, 85% *o*-phosphoric acid, 70-72% perchloric acid, 99 % formic acid and 99 % trifluoroacetic acid, purchased from Merck, Darmstdat, Germany and Sigma-Aldrich, USA. The deionized water was an "in-house" product prepared with a conductivity of 0.05 µS/cm.

The following instruments were used: analytical balance Mettler Toledo AG285, pH–meter Metrohm 827 pH Lab, US bath Branson 3510 and IKA orbital shaker KS 260 basic. The RC (regenerated cellulose) 0.45 µm syringe filters were purchased from Agilent Technologies (USA).

The following HPLC systems were used: Shimadzu Prominence LC2030-i Cannabis Potency Analyzer, Shimadzu Prominence LC2040-i 3D, Dionex Ultimate 3000 UHPLC system and Agilent HPLC 1260 system.

The following columns were used: Zorbax ODS 250 mm × 4.6 mm, 3.5 µm and Shimazdu Nex-Leaf SH-SPP

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ODS and Poroshell ODS HPLC, both with dimensions 150 mm × 4.6 mm, 2.7 µm.
The test samples were prepared from cannabis flowers and extracts obtained from Replek Farm Ltd., and the certified reference materials of the cannabinoids of interest were purchased from Cerilliant, Sigma-Aldrich, USA and Cayman Chemical, USA.

Results and discussion

In our routine analytical experience, we clashed with cannabis extracts yielding unknown peaks overlapping with the peaks of THC isomers, which we found very hard to be resolved by use of the existing, published methods. This was the main reason for the development of the simple and fast RP-HPLC-UV/DAD method, for better separation of THC positional isomers in order to additionally separate them from the other co-eluting peaks.

We developed simple methods with the increased resolution of critical elution pair, Δ^9-THC and Δ^8-THC, up to 3.2, by use of C18 column with core shell-based particles with 25000 NTP (number of theoretical plates), and up to 4.3 by use of C18 column with standard fully porous bead particles with 35700 NTP, and mobile phase composed only of methanol, acetonitrile and acidified water.

It showed that the polar ODS (C18) matrixes, as expected, have better separation power because of the higher number of heterogenic interactions between the analyte and the stationary phase. The columns used, Poroshell and Nex-Leaf columns were of superficially porous particles (SPP) type, thus expected to yield the highest resolution per unit length, but their separation power is compromised by the highly hydrophobic biological nature of the samples making them prone to clogging and less lasting.

The acid used for the preparation of the acidified water did not significantly influence the separation of the peaks of the nonionic compounds of interest. By use of all four tested acids: *o*-phosphoric acid, perchloric acid, formic acid and trifluoroacetic acid, suitably diluted in water, satisfying resolution between Δ^9-THC and Δ^8-THC was obtained.

The mobile phase composition and flow rate, column temperature and characteristics, injection volume, and finally the equipment characteristics and quality, contribute to separation quality and method selectivity

Conclusion

Achieving the best possible separation between the psychoactive Δ^9-THC and its positional isomer Δ^8-THC is crucial in order to avoid co-elution of other possibly interfering peaks that might contribute to false higher content of strictly limited Δ^9-THC in the tested sample.

This was achieved by the use of a proper combination of simple chromatographic conditions: L1 (ODS) HPLC column and simple ternary mobile phase composed only of acidified water, methanol and acetonitrile. Various combinations of chromatographic conditions were tested and optimized to be used for this purpose.

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