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Short communication

Development of powerful chromatographic methods for improved 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 may also include Δ^8-THC (Grotenhermen & Russo, 2002). Out of approximately 500 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 activation of two types of G-protein-coupled receptors, the cannabinoid CB_1 receptors (distributed in the brain) and CB_2 receptors (present almost uniquely in the immune system). This fact explains the actions of Δ^9-THC on cognitive and motor functions, as well as its immune-modulatory effect (Di Marzo, 2004).

The review of scientific literature and application notes of chromatographic equipment and columns, Shimadzu, Waters (Aubin et al., 2018), Agilent (Storm et al., 2019), Knauer (Loxterkamp et al., 2020) etc., reveals different methods for separation of cannabinoids. These methods achieve separation of eight, up to seventeen different cannabinoids, most commonly and dominantly present in the cannabis flowers and extracts. They focus on achieving maximal resolution between peaks of cannabinoids, using gradient and/or isocratic elution, obtaining 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 the 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 between other peaks of cannabinoids that might eventually co-elute with them, since they are psychoactive and their content in extracts and products is strictly limited.

The aim of this work was to develop powerful RP-HPLC methods for optimal separation of tetrahydrocannabinol isomers during chromatographic analysis of cannabis flowers and extracts, in order to achieve accurate determination of strictly regulated psychoactive cannabinoid Δ^9-THC.

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 "in house" product prepared with conductivity of 0.05 µS/cm.

The following instruments were used: analytical balance Mettler Toledo AG285, pH–metter Metrohm 827 pH Lab, US bath Branson 3510 and IKA orbital shaker KS 260 basic. The regenerated cellulose (RC) 0.45 µm syringe filters for sample filtration 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 chromatographic columns were used: Zorbax ODS (250 mm × 4.6 mm, 3.5 µm), Shimadzu

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Nex-Leaf SH-SPP ODS (150 mm × 4.6 mm, 2.7 µm) and Poroshell ODS HPLC (150 mm × 4.6 mm, 2.7 µm).

Certified reference materials containing cannabinoids of interest were purchased from Cerilliant, Sigma-Aldrich, USA and Cayman Chemical, USA.

The test samples were prepared from cannabis flowers and extracts obtained from Replek Farm Ltd.

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 resolve using existing, published methods. This was the main reason for development of 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 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 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.

The results from our experiments showed that the polar C18 (ODS) 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 contained superficially porous particles (SPP) type, thus expected to yield 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 having shorter column life.

The acid used for 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.

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

Various combinations of chromatographic conditions were tested and optimized to be used for this purpose.

Obtaining 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 regulated Δ^9-THC in the tested samples.

This was achieved by use of proper combination of simple chromatographic conditions: L1 (ODS) HPLC column and simple ternary mobile phase composed only of acidified water, methanol and acetonitrile.

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