APPLICATION NOTE

Confirming the presence of cannabinoids in *Cannabis sativa* by HPTLC-MS

A-98.1

Keywords

THC, cannabinol, cannabidiol, Marihuana, mass detection

Introduction

In recent years a lot of research has been done on the medicinal use of *Cannabis sativa*. Cannabinol (CBN), cannabidiol (CBD) and ∆9-tetrahydrocannabinol (∆9-THC) are the most studied active ingredients for medicinal use treating several health disorders, *e.g.* migraine, epilepsy, appetite loss. In addition to its medicinal use cannabis has been legalized by several countries as marihuana.

Scope

This method is suitable for confirming the presence of the cannabinoids CBN, CBD and THC in *Cannabis sativa*. The TLC-MS Interface 2 (CAMAG) is used to directly elute target zones from the HPTLC plate into the Waters ACQUITY QDa® for mass detection. This HPTLC-MS method offers a rapid way for substance confirmation, which can be used for screening and for quality control. Several samples can be separated in parallel and only the zones of interest are analyzed, generating less data.

Required or recommended devices

Automatic TLC Sampler 4 or Linomat 5, Automatic Developing Chamber ADC 2, Immersion Device 3, TLC Visualizer, visionCATS software, TLC-MS Interface 2, Waters ACQUITY QDa Detector (Performance), Empower® or MassLynx® software, optional: smartCut, UV Cabinet, TLC Scanner

Derivatization reagent

Reagent name: Fast Blue salt B
Reagent preparation: Weigh 1 g of fast blue salt B (o-dianisidine bis(diazotized) zinc double salt) into a glass bottle and dissolve it in 200 mL of water.

Sample

500 mg of each loosely ground sample is mixed with 10 mL of methanol, sonicated for 10 minutes, and then centrifuged for 5 min (2750 RCF). The supernatant is collected and used as test solution. Cannabis oil and cannabis tincture are diluted 1:10 with methanol.

Standards

Standards were provided by Lipomed AG (Arlesheim, Switzerland), CBN and CBD with a concentration of 1 mg/mL in methanol and ∆9-THC with a concentration of 1 mg/mL in ethanol.
Standards are diluted 1:10 with methanol.

Chromatography

Stationary phase	HPTLC Si 60 F254, 20 x 10 cm (Merck).
Sample application	Bandwise application with ATS 4 or Linomat 5, 15 tracks, band length 8 mm, track distance 11.4 mm, distance from left edge 20 mm, distance from lower edge 8 mm, application volume of 5 µL of each standard, 2 µL of the Cannabis tincture (1:10), 5 µL of Cannabis oil (1:10), and 8 µL of each Cannabis sample, same application scheme on the left and right half of the plate
Developing solvent	Cyclohexane – di-isopropyl ether – diethyl amine 52:40:8 (according to [1], System B)
Development	In the ADC 2 with chamber saturation (with filter paper) 20 min and after conditioning at 33% relative humidity for 10 min using a saturated solution of magnesium chloride
Developing distance	70 mm (from the lower edge)
Plate drying	Drying 5 min in the ADC 2
(1) Derivatization	Reagent name: Fast Blue salt B Reagent use: The plate is immersed into Fast Blue salt B reagent using the Chromatogram Immersion Device, immersion time 5 s and immersion speed 3 cm/s and then dried in flow of cold air in the fume hood for 5 minutes.
Documentation	With the TLC Visualizer under white light (after derivatization)
(2) MS confirmation	There are three options for localizing the cannabinoids on the HPTLC plate: Marking zones to be eluted under UV 254 nm using the UV cabinet or TLC Visualizer (+ quick, - less sensitive) Derivatization of part of the plate (+ sensitive, - samples have to be applied twice). Marking positions on not derivatized tracks based on RF values of reference substances Position taken from TLC Scanner measured in absorbance mode at 210 nm (+ very sensitive, + allows also quantitative evaluation, -higher investment costs) Target zones are directly eluted using the TLC-MS Interface 2 with oval elution head into the ACQUITY QDa Detector at a flow rate of 0.5 mL/min with methanol (with 0.1% ammonium hydroxide). For a full scan spectrum it is recommended to first elute a blank, which can be subtracted from the spectra of the target zones. There is no need for Single Ion Recording (SIR). For confirmation of substances between 50 and 500 ng per zone are required.
MS parameter	The ACQUITY QDa Detector is operated in ESI- mode. The ESI capillary is set to 0.8 kV, cone voltage to 15 V, and desolvation temperature at 600 °C. A full scan mass spectrum between m/z 50 and 650 is acquired at a sampling rate of 10.0 points/sec (continuum). Data processing and evaluation of mass spectra are performed with Empower. For routine use in quality control Single Ion Recording (SIR) can be performed.

Results

System Suitability Test (SST) under white light (remission):
CBD shows an orange-brownish zone at R_F ~ 0.45
THC shows a red-pinkish zone at R_F ~ 0.41
CBN shows a purple zone at R_F ~ 0.29

NOTE: The presented results are to be regarded as examples only!

Please contact CAMAG for more application notes and products!

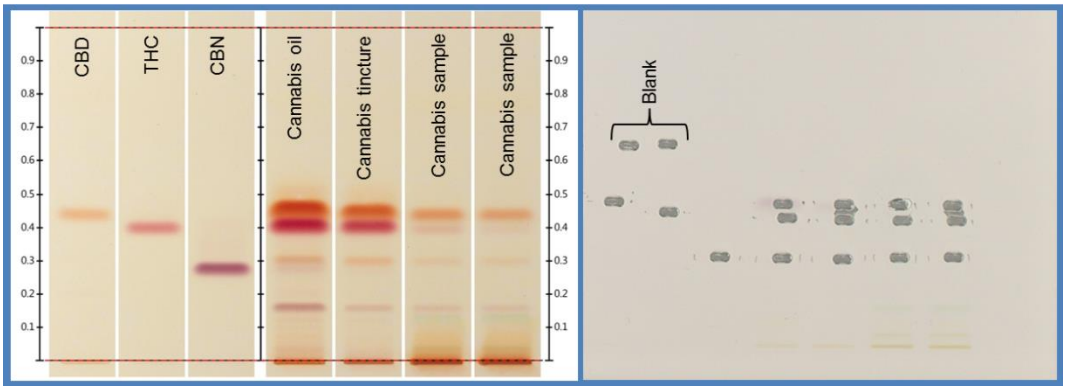

Fig. 1 Plate under white light (remission); left: plate derivatized with Fast Blue salt B reagent, right: not derivatized part of the plate after elution of zones of interest

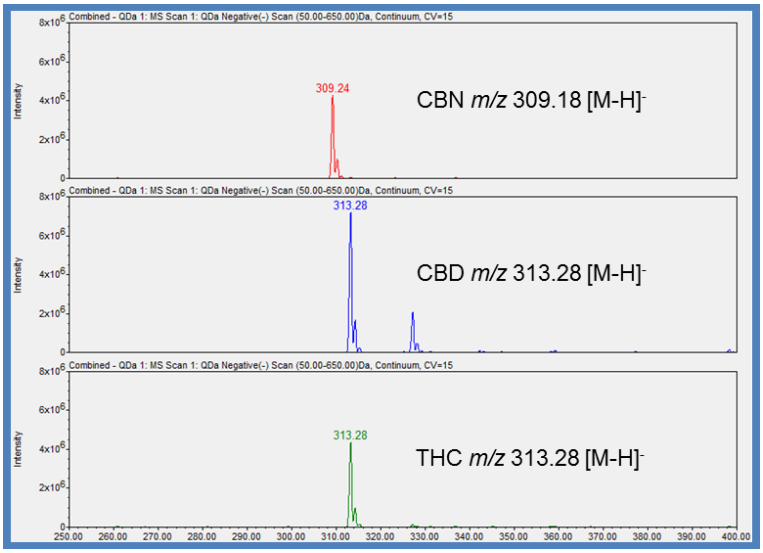

Fig. 2 HPTLC-MS spectra of CBN (standard), CBD and THC (Cannabis oil sample), displayed range m/z 250 to 400

Literature

[1] Recommended methods for the identification and analysis of cannabis and cannabis products, United Nations (2009), https://www.unodc.org/documents/scientific/ST-NAR-40-Ebook.pdf