Application Note

# THC PLANT MATERIAL ANALYSIS

## Using Compact Mass Spectrometry for product control and law enforcement of cannabis related plant products

Advion

### INTRODUCTION

The expression$^S$ compact mass spectrometer (CMS) is a novel high performance, easy-to-use single quadrupole mass spectrometer with enhanced scan speed and the ability for on-line polarity switching and in-source fragmentation. Priced substantially lower than other MS systems and having a small footprint to fit in space restricted laboratories, the CMS brings the analytical benefits of mass spectrometry to more laboratories than ever before.

Analysis of the active components of the *cannabis sativa* plant is required both for law enforcement where these compounds are illegal and also as a tool for product control and optimization in the increasing number of legal markets for cannabis plant products. Whenever simple, unequivocal and legally defensible analysis methods for the detection and quantification of plant metabolites are required, mass spectrometry is the detector of choice.

Here, we present two simple workflows for the analysis of cannabinoids such as the naturally occuring cannabinol (CBN), the psychoactive ingredient of *cannabis sativa*, tetrahydrocannabinol (THC) and the degradation product cannabidiol (CBD). Both TLC/FIA/CMS for qualitative detection of cannabinoids, as well as HPLC/CMS for the quantitative determination of THC demonstrate the added benefit of compact mass spectrometry in the analysis of natural products.


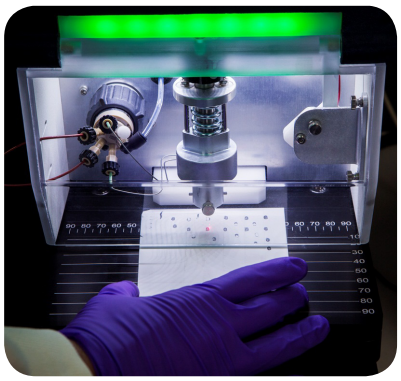

**Figure 1:** A Compact Mass Spectrometer (expression, Advion, NY, 1a) connected to the Plate Express (1b and 1c), a device for the extraction of samples from a TLC plate. CMS provides valuable additional information in natural product analysis workflows.

### METHOD

*Thin layer chromatography (TLC)*: Cannabinoids were separated on TLC silica gel 60 F254 (Merck, NJ) with a run solvent of 80/20 Petrolether (60-80 bp) / Dioxane (Sigma Aldrich, MO)[1]. *TLC/FIA/MS analysis:* Utilizing the Plate Express™ (Advion, NY) with a solvent flow rate of 200 µL/min methanol 0.1 vol% formic acid. *HPLC analysis:* Samples were analyzed with a 1220 HPLC system including a UV detector (Agilent, CA) on a Supelco Titan 2.1 mm column (Sigma-Aldrich, MO) at a flow rate of 350 µL/min and a 5 min gradient from 50 % to 90 % Acetonitrile 0.1 vol% formic acid. *MS analysis:* A mass range of m/z 100 to m/z 1000 was scanned using both polarity switching and in-source CID. SIM scanning in negative ion mode MS was used at *m/z* 313.2 (THC and CBD) and *m/z* 309.2 (CBN) for the quantitative analysis approach. Both MS methods used the expression$^S$ CMS mass spectrometer (Advion, NY).

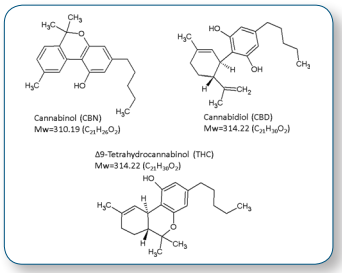


**Figure 2:** Chemical structures of the three compounds studied.

## RESULTS

The Plate Express sample extraction device can singly or sequentially analyze a lane on a TLC plate, extract the compounds present and transport to the CMS for further analysis (Fig. 3). A typically qualitative TLC/FIA/MS analysis of THC is shown in 3a with the resulting negative ion mode in-source CID MS (3b) to unequivocally determine the presence of THC in this sample (simultaneously acquired positive ion mode MS and in-source CID MS data not shown). Despite a different molecular structure, both THC and CBD have not only the same isotopic mass, but also fragment identically in positive ion mode ESI/MS (data not shown, compare[2]). However, in negative ion mode, in-source CID results in the same *m/z* fragments, but at significantly different relative intensities which allows a distinction between THC and CBD in negative ion mode MS.

To demonstrate a quantitative workflow an HPLC/CMS method was set-up to detect CBN, CBD and THC based on SIM scanning within a 10 min separation time and detection in negative ion mode (Fig. 4a). Triplicate analysis of cannabinoid standards show calibration functions with good linearity in a range of 2.5 to 250 ng on column, which is sufficient to quantify from cannabis plant material with only 0.1 % w/w content such as roots, stems and leaves of the plant.

The expression$^S$ CMS greatly enhances the information content gained from natural products and can help in the quality control of *cannabis sativa*-based products as well as in law enforcement.

## CONCLUSIONS

- The expression$^S$ –a CMS with faster scan speed, on-line polarity switching and in-source CID generates valuable information in the analysis of natural products.
- The new Plate Express, a sample extraction decive generates targeted mass spectra from thin layer chromatography plates and analyte spots of interest.
- HPLC/CMS provides a robust and reliable quantification method for THC, CBN and CBD.

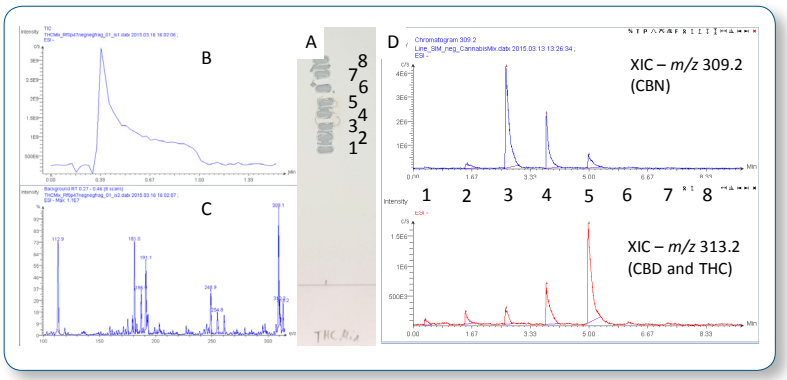


**Figure 3:** TLC/FIA/MS analysis of cannabinoids. 3a shows a typical TLC separation of an analytical mixture of CBD, CBN and THC at 1 µg material on the lane. TLC/FIA/MS analysis of the Rf region of THC (Rf=0.47) shows a strong MS TIC signal (3b) with a prominent negative ion signal at *m/z* 313.2 (data not shown) and the characteristic in-source CID fragments of THC (3c). An alternative approach analyzing the whole TLC lane shows that CBN, CBD and THC are not baseline separated during TLC analysis (3d), so quantitative analysis should use HPLC/MS.

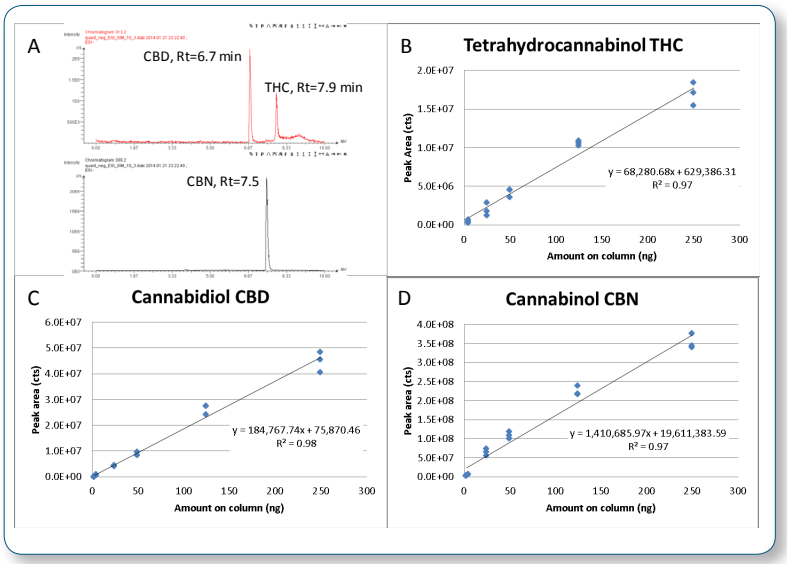


**Figure 4:** 4a shows a typical HPLC/CMS chromatogram for all three analytes in negative ion mode SRM with the upper trace being the XIC of *m/z* 313.2 and the lower trace XIC of *m/z* 309.2. Good linearity calibration functions can be obtained for all three compounds (4b: THC, 4c: CBD and 4d: CBN) covering a range from 250 to 2.5 ng analyte on the 2.1 mm ID column used – sufficient to analyze plant material with as little as 0.1 % w/w THC content.


LITERATURE

[1] Recommended Methods for the Identification and Analysis of Cannabis and Cannabis Products' United Nations Office on Drugs and Crime 2009; ISBN 978-92-1-148242-3

[2] Broecker S, Pragst F. Isomerization of cannabidiol and Δ9-tetrahydrocannabinol during positive electrospray ionization. In-source hydrogen/deuterium exchange experiments by flow injection hybrid quadrupole-time-of-flight mass spectrometry. Rapid Communication in Mass Spectrometry 2012; 26(12):1407-1414.



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