Application Note

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Purification of Cannabinoids from a *Cannabis sativa* Extract

Author

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Abstract

With the growth of cannabinoid-containing products offered by the food, cosmetics, and healthcare industry, isolation of single cannabinoids to be used as ingredients or reference compounds is increasingly important. This application note demonstrates the purification of different cannabinoids on an Agilent 1290 Infinity II Autoscale Preparative LC/MSD System. Featuring dedicated analytical and preparative flow paths with a shared mass selective detector (MSD) and combined autosampler/fraction collector, this two-in-one system saves bench space and a considerable amount of investment. Analytical scouting is carried out on the analytical path, then the method is scaled-up to preparative conditions. Mass-based fraction collection enables specific isolation of the target compounds without collecting undesired fractions. For fraction reanalysis, the system is switched back to the analytical path, enabling fast and easy re-injection of the collected fractions by the combined autosampler/fraction collector.

Introduction

Over the last few years, interest in cannabinoids and their chemical and pharmaceutical properties has constantly grown. For a long time, the use and possession of cannabinoid containing goods was ill famed, although the psychoactive compound (–)-*trans*-Δ^9-tetrahydrocannabinol (THC) is only one of more than 560 cannabinoids discovered so far.[1] Only recently, some countries started recognizing the beneficial effects of cannabinoids when consumed in controlled and reasonable amounts. Today, Canada as well as 12 US states have legalized cannabis for recreational use; a total of 33 US states have medical cannabis programs in place.[2,3] In 2018, the US Food and Drug Administration (FDA) approved the first cannabis-derived drug, with cannabidiol (CBD) being the active pharmaceutical ingredient.[4]

In the light of this development, it has become ever more important to develop robust analytical methods for characterization and quality control of cannabinoid extracts, raw materials, and formulations. Due to the vastness of their chemical structures, commercial reference standards are available for only a few cannabinoids. Creating reference standards from plant extracts is therefore the only way to identify and characterize unknown compounds. For this task, preparative-scale high-performance liquid chromatography (HPLC) is usually the method of choice due to easy sample preparation and a wide choice of detection methods, such as ultraviolet (UV) or mass-selective (MS) detection.

This application note demonstrates the isolation of different cannabinoids from a cannabidiol-rich extract in hemp oil. Reversed-phase HPLC with MS detection is applied on an analytical scale to develop a separation method. After optimization, the method is scaled up to preparative conditions, applying mass-based fraction collection for selective and specific isolation of the compounds.

Experimental

Instrumentation

Analytical scouting and preparative purification were carried out on an Agilent 1290 Infinity II Autoscale Preparative LC/MSD System with the following configuration:

- Agilent 1290 Infinity II Preparative Binary Pump (G7161B) with 200 mL pump heads (option 206)
- Agilent 1290 Infinity II Preparative Open-Bed Sampler/Collector (G7158B)
- Agilent 1260 Infinity II Variable Wavelength Detector (G7114A) with 0.3 mm preparative flow cell (option 024)
- Agilent 1260 Infinity II Diode Array Detector WR (G7115A) with 10 mm analytical flow cell (option 018)
- Agilent 1290 Infinity II Preparative Column Compartment (G7163B)
- Agilent 1290 Infinity Valve Drive (G1170A) with preparative 2-position/14-port valve head (G4738A)
- Agilent 1290 Infinity II MS Flow Modulator (G7170B)
- Agilent 1260 Infinity II Quaternary Pump (G7111B)
- Agilent 1260 Infinity II Delay Coil Organizer (G9324A) with knitted delay coils for 15 to 40 mL/min (option 210)
- Agilent InfinityLab LC/MSD XT (G6135BA) with Agilent Jet Stream ion source

Columns

- Analytical column: Agilent Polaris C18-A, 4.6 × 150 mm (id × L), 5 µm (part number A2000150X046)
- Preparative column: Agilent Polaris C18-A. 21.2 × 150 mm (id × L), 5 µm (part number A2000150X212)

Software

Agilent OpenLab CDS ChemStation edition for LC and LC/MS Systems, version C.01.10 [239]

Solvents and sample

All solvents used were LC grade. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22 µm membrane point-of-use cartridge (Millipak). LC-grade formic acid was purchased from Merck (Darmstadt, Germany).

A CBD oil sample was purchased in a local drugstore. Prior to analysis, 1 mL of sample was pipetted to 4 mL methanol, mixed, and filtered through a 0.45 µm Agilent Captiva premium syringe filter with nylon membrane (part number 5190-5091). The filtrate was directly used for injection.

Method settings

The MS detector was operated in single ion monitoring (SIM) mode to increase selectivity and specificity for detection and fraction collection. Seven target masses listed in Table 1 were monitored to detect 13 different cannabinoids described in the literature.[5] The sample was separated under analytical conditions to find a suitable gradient. For preparative scale-up, a column of the same length and stationary phase was available, so the same gradient could be used for preparative purification. Chromatographic conditions and MSD parameters are given in Table 2 and Table 3, respectively.

Table 1. Target ions used for fraction collection and possible cannabinoids.[5]

m/z	Compounds
−285.2	Tetrahydrocannabivarin
−313.2	Tetrahydrocannabinol, cannabidiol, cannabichromene, cannabicyclol
+353.2	Tetrahydrocannabivarinic acid
+367.2	Tetrahydrocannabinolic acid-C4
+377.2	Cannabinolic acid
+381.2	Tetrahydrocannabinolic acid A, cannabidiolic acid, cannabichromenic acid, cannabicyclolic acid
+383.2	Cannabigerolic acid

Table 2. Chromatographic conditions of analytical and preparative runs.

Parameter	Analytical Scouting	Preparative Purification
Mobile Phase	A) 0.1 % formic acid in water B) 0.05 % formic acid in methanol	
Flow Rate	1.5 mL/min	31.86 mL/min
Gradient	0 min: 60% B 9 min: 95% B	0 min: 60% B 9 min: 95% B
Stop Time	12.0 min	13.0 min
Post-Time	4 min	3.5 min
Injection Volume	5 µL	1,000 µL
Injection Method Preset	Nonpolar sample matrix (plug setting 2)	
Post-Sample Plug	90 µL methanol	180 µL methanol
Temperature	Ambient	Ambient
UV Detection	DAD 230 nm Peak width >0.025 min (0.5 s response time) 10 Hz data rate	VWD 230 nm Peak width >0.05 min (1 s response time) 10 Hz data rate
MS Detection	Signal 1: positive SIM *m/z* 287.2 *m/z* 315.2 *m/z* 353.2 *m/z* 367.2 *m/z* 377.2 *m/z* 381.2 *m/z* 383.2 Signal 2: negative SIM *m/z* 285.2 *m/z* 313.2	
Split Ratio to MSD	Full flow	5,000:1 (mode M6) 3.50 min ON
Fraction Collection	Not applicable	3.50 min mass-based

Table 3. MSD spray chamber and fraction collection settings.

Parameter	Value
Make Up Solvent	0.1 % formic acid in methanol:water (70:30)
Make Up Flow	1.5 mL/min
Ionization Source	Agilent Jet Stream Electrospray
Drying Gas Flow	12.0 L/min
Nebulizer Pressure	35 psig
Drying Gas Temperature	300 °C
Sheath Gas Temperature	350 °C
Sheath Gas Flow	11.0 L/min
Capillary Voltage	+ 4,000 V
Nozzle Voltage	+ 600 V
Target Mass (*m/z*)	286.2; 314.2; 330.2; 344.2; 354.2; 358.2; 360.2
Ion Species	$[M+Na]^+$, $[M-H]^-$
Fraction Trigger	Slope: 10,000 counts/sec

Results and discussion

A hemp oil sample rich in cannabidiol was analyzed by HPLC. To find suitable parameters for a separation gradient and UV and MS detection, the method was developed under analytical conditions first. Figure 1 displays the optimized separation in analytical scale as an overlay of the UV with the MS detector signals. With UV detection, only the most abundant components of the extract were observed. Using an MS detector and monitoring target masses of typical cannabinoids (see Table 1) revealed that the extract contained more cannabinoids than those visible in the UV detector trace (see Figure 1). The most abundant peak in the UV trace (8.5 minutes) was found with *m/z* 313.2 in negative SIM, which is in line with the experimental mass for cannabidiol[5] (CBD, see Table 1). The second largest peak in UV (7.2 minutes) was detected with *m/z* 285.2 in negative SIM, which is an indication for tetrahydrocannabivarin (THV). Both compounds, however, could not be unambiguously identified for lack of reference standards.

After analytical scouting, the separation gradient was scaled up to preparative conditions and the injection volume was increased. The same SIM signals as under analytical conditions were applied to the preparative method, and mass-based fraction collection was used. Neutral target masses were entered as listed in Table 3. The software automatically calculated the deprotonated species and sodium adducts, which enabled a fraction trigger based on the signal most suitable for each compound.

Figure 2 depicts the separation in preparative scale. The simple gradient transfer without optimization causes a slight shift of all peaks towards earlier retention. This shift is due to the lower dwell time in preparative scale compared

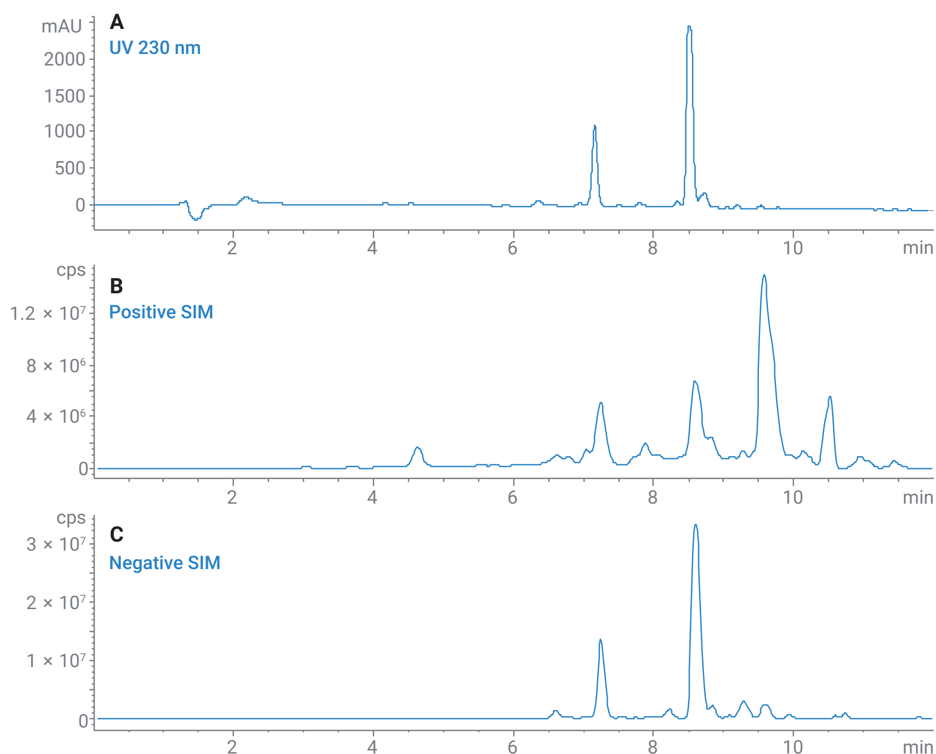

Figure 1. Separation (analytical scale) of a cannabinoid extract. The UV detector trace (A) shows only the most abundant compounds, whereas the MSD traces (B and C) reveal more compounds not visible when using UV detection.

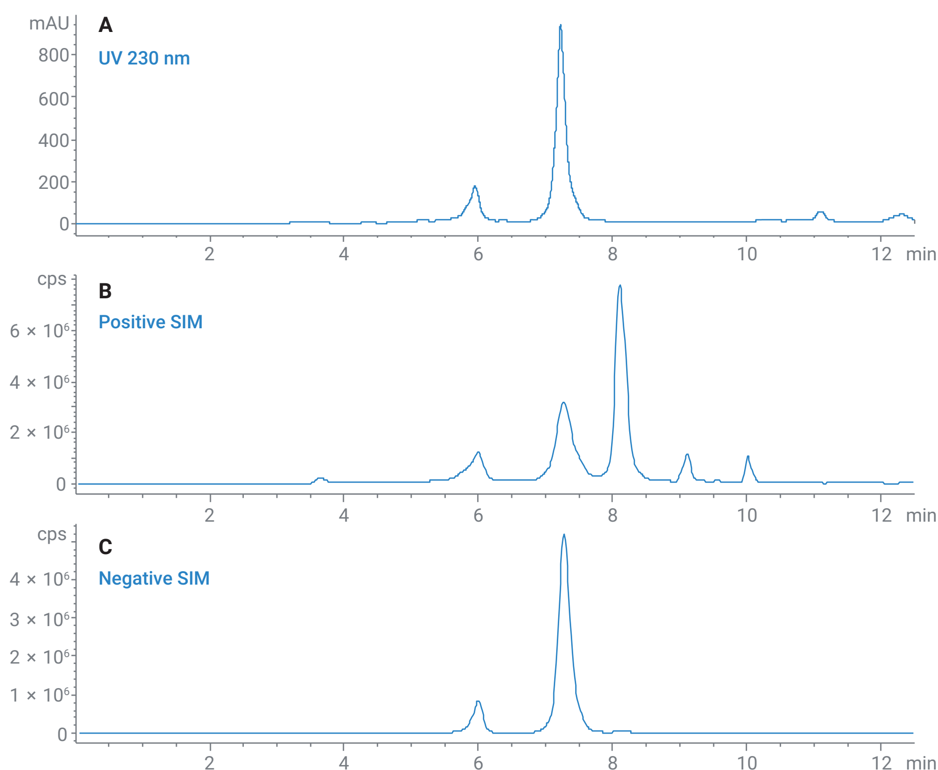

Figure 2. Separation (preparative scale) of a cannabinoid extract. The combined traces of different SIM signals in positive (B) and negative (C) mode reveal several compounds not visible in the UV trace (A).

to the analytical path of the system. Fraction collection was triggered solely by the MSD signals, which enabled highly specific and sensitive purification of the different compounds.

Figure 3 lists all MSD signals used for fraction collection, with blue bars representing collection events. Ten separate fractions were collected, of which only two (fractions 3 and 4) were visible in the UV signal. Multiple fraction collection events within a single trace indicate that the sample contained several isomeric cannabinoids. The main compound of the CBD oil sample, cannabidiol, was detected at 7.2 minutes with its molecular ion of *m/z* 313.2 in negative ionization mode. In the UV trace, this compound exhibited the largest peak area of approximately 75%. In the MSD signals, the most abundant peak was not CBD but an unknown cannabinoid detected with *m/z* 367.2 in positive ionization mode. However, due to different ionization properties of the different cannabinoids, signal heights in the MSD signal do not correlate with actual concentrations of the compounds in the sample. Nevertheless, this compound was successfully collected by the mass-based fraction trigger, which demonstrates the benefit of an MSD for purification workflows; when used in addition to a UV detector, the MSD can trigger collection of compounds otherwise missed.

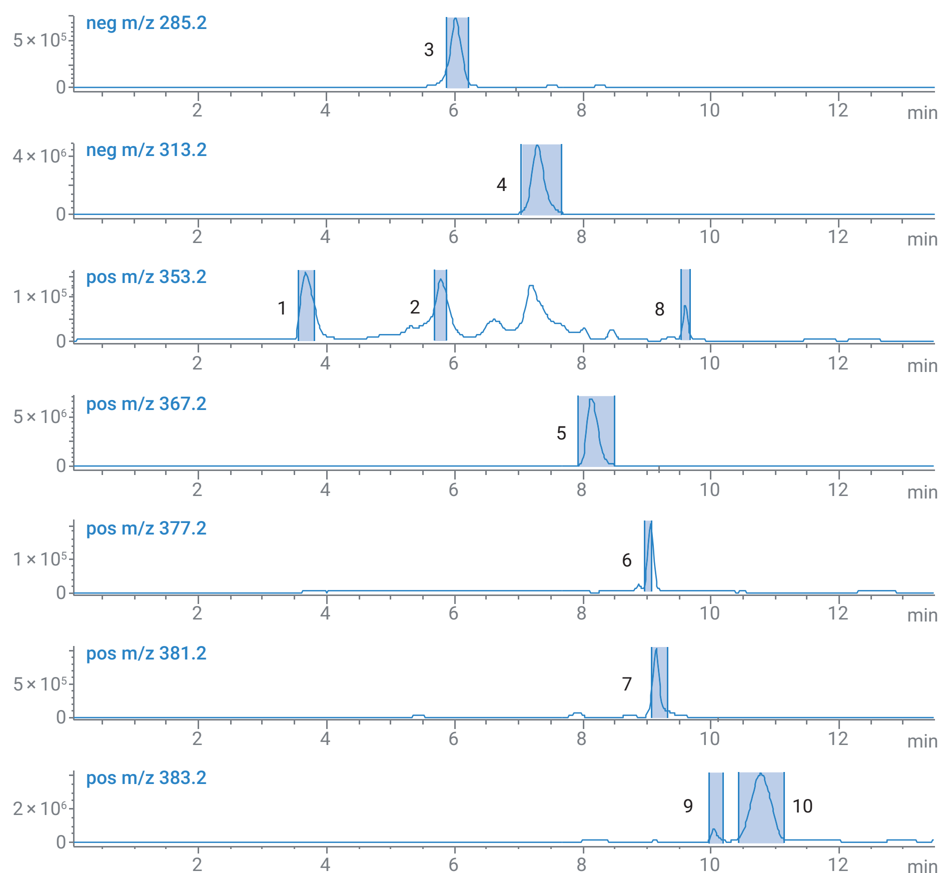

Figure 3. Extracted ion chromatograms (EICs) of target ions used for fraction collection of cannabinoids. Blue bars represent events of fraction collection.

To assess purity and recovery, fractions were reanalyzed using the analytical path of the 1290 Infinity II Autoscale Preparative LC/MSD system. Relative quantitation of the collected compounds was carried out by one-point calibrations with the diluted sample and comparison of the fraction peak area after dilution to a fixed volume. As only compounds visible by UV detection could be quantified, fraction 4 (CBD) was selected as a representative sample. Recovery of CBD ranged from 92 to 97% over a series of five injections. Purity of the fraction was calculated by the percentage of the CBD peak area in the UV signal compared to the total peak area at 230 nm, and was found to be 95%. Figure 4 compares the UV and MSD signal of the CBD fraction with MSD traces of different cannabinoids. In the MSD signals, more impurities than the one visible in the UV trace can be seen. As mentioned above, MSD signals cannot be compared on a quantitative level. However, the MSD analysis after fraction collection can help decide whether another cleanup step might be necessary to obtain pure compounds, depending on the purity requirement of the workflow. Being able to use a single MSD for analytical scouting, mass-based fraction collection, and fraction reanalysis is one of the advantages of the 1290 Infinity II Autoscale Preparative LC/MSD system.

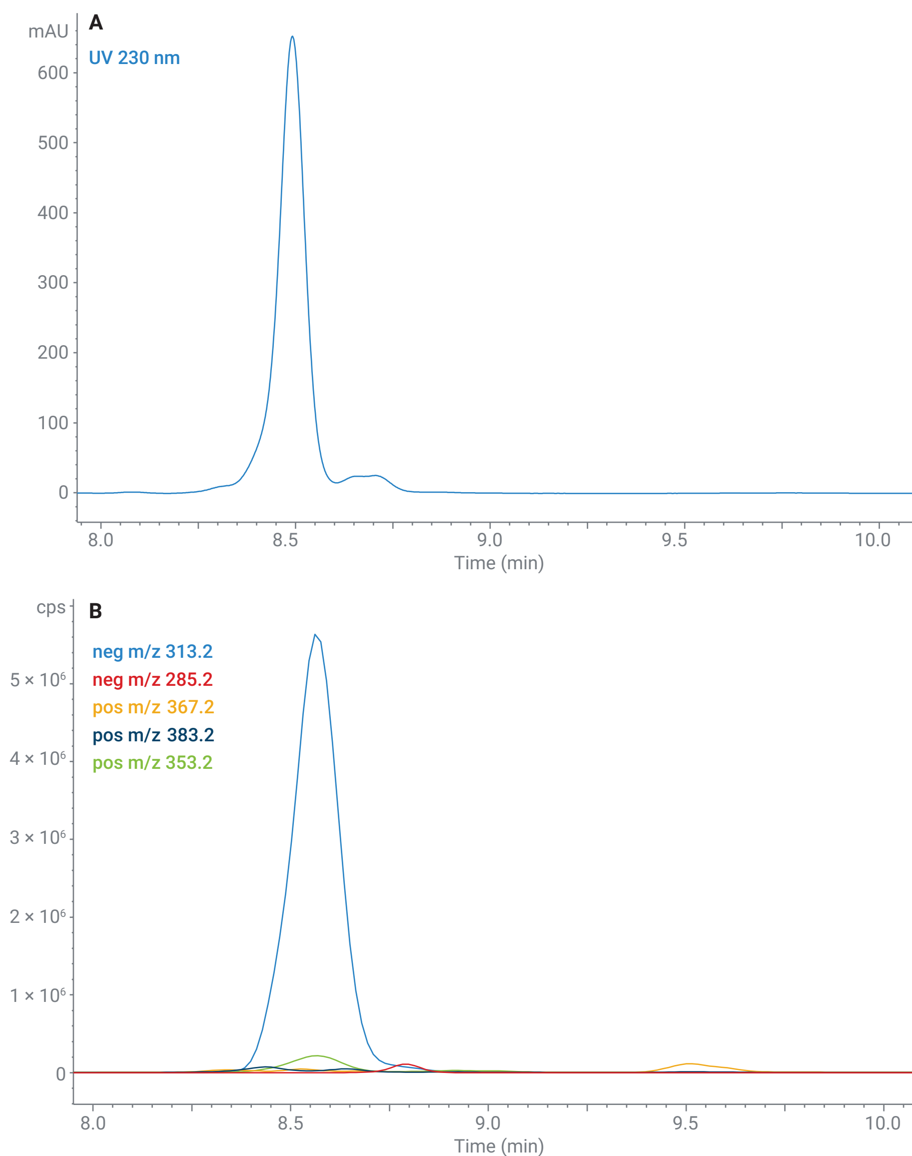

Figure 4. Reanalysis of fraction 4 by UV (A) and MS detection (B). Although not quantifiable, the MSD reveals impurities not visible in the UV signal.

Conclusion

The separation and purification of a cannabinoid extract was successfully conducted on a 1290 Infinity II Autoscale Preparative LC/MSD System. Analytical scouting and method development were carried out using the analytical path of the system. Scale-up to preparative conditions was facilitated using the same system but by switching to the preparative path. The shared MSD allowed target masses obtained during scouting to be used for mass-based fraction collection without the need to invest in a second MSD for triggering the collection. The combination of autosampler and fraction collector in a single module enabled easy fraction reanalysis on a small system footprint without manual transfer of the fractions into sample vials and to a separate analytical system. Fraction reanalysis yielded a purity and recovery of 95% on average for the main compound of the cannabinoid extract.

References

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