PURIFICATION & TESTING FOR CANNABIS AND HEMP

Advion Interchim scientific

PURIFICATION & TESTING FOR CANNABIS AND HEMP

A complete range of instruments & consumables for analysis and purification of cannabinoids

Quality control of raw materials & collected fractions

Purification systems designed to match your production scale

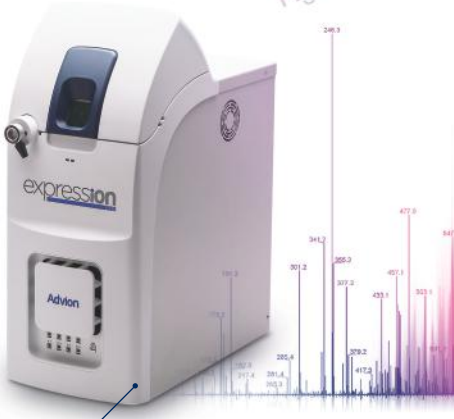
PURIFLASH® L-CANNABIS
/128 mm id column
CBD output/day 4.3 kg

PLATE EXPRESS
TLC Plate Reader

Due to variability in the cultivation and manufacturing processes the THC-Free output values can not constitute a guarantee.

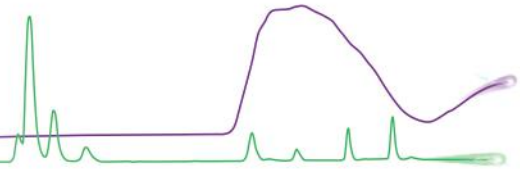
AVANT (U)HPLC

PURIFLASH® XL-CANNABIS
/200 mm id column
CBD output/day 12.2 Kg



expression COMPACT
MASS SPECTROMETER

HPC PRODUCTION COLUMN



Minor Cannabinoid Purification,
THC & Pesticide Remediation,
Rapid Winterization

Advion Interchim
scientific

HIGH THROUGHPUT PURIFICATION SOLUTIONS

Advion Interchim Scientific instruments relieve the purification bottleneck

Minor Cannabinoid Purification

- Purification of cannabinoids at low concentration is accomplished using smaller particles in the column
- Various column sizes provide linear scale up after method optimization

Turnkey THC and Pesticide Remediation systems

- Detailed SOP's and training provided
- Simple software for ease of use

Rapid Winterization

- Room temperature methodology
- 30 minute method excluding solvent recovery

PURIFICATION AND CONFIRMATION

AVANT (U)HPLC

Save time and money with in-house analytical

Testing your product throughout your process allows you to monitor the efficiency of your workflow. Obtain analytical data in minutes.

PILOT AND PROCESS SCALE

puriFlash® L-Cannabis

7 - 30 kg per day*
P/N: BU14K0 (220v)
P/N: BU14K1 (110v)

- High pressure pump for minor cannabinoid purification
- Columns up to 15 cm ID

puriFlash® XL-Cannabis

20 - 70 kg per day*
P/N: BU14L0 (220v)
P/N: BU14L1 (110v)

- Higher throughput for manufacturing
- Columns up to 20 cm ID

HPC Production Column

- Easy to pack and repack: <60 minutes to unpack and pack the column
- 15 cm and 20 cm ID

**Throughput values based on THC and pesticide remediation standard operating procedures.*

APPLICATION NOTE

A COMPLETE SOLUTION FOR THC REMEDIATION AND QUANTITATION OF CANNABINOIDS FROM DISTILLED HEMP EXTRACTS

Instrumentations

HPLC-UV: AVANT
Process Purification: puriFlash® XL-Cannabis

Authors

Changtong Hao
Lee Collier
Advion Interchim Scientific

In this application note, both the purification and analytical processes for THC remediation using the Interchim puriFlash® XL-Cannabis and Advion AVANT™ HPLC-UV systems are shown to form a complete solution for THC remediation in the hemp industry.

Introduction

Hemp contains hundreds of cannabinoids with Cannabidiol (CBD) being the most prevalent in the plant and Δ9-Tetrahydrocannabinol (THC) being the active ingredient causing psychotropic effects. However, many more compounds are formed by the hemp plant and have been investigated for their medical effects.

This limit often requires THC remediation of the distilled hemp extract (starting material) and can be achieved using preparative scale chromatography such as the puriFlash® XL-Cannabis system (Figure 1).

HPLC analysis of the starting material (third pass distillate), fractions collected during the remediation process, and the finished product can be performed using the Advion AVANT HPLC-UV analytical system (Figure 2). Both the purification and analytical processes are shown in this application note to form a complete solution for THC remediation in the hemp industry.

1

Figure 1: puriFlash® XL-Cannabis process purification system.

2

Figure 2: Advion AVANT HPLC-UV system.

HPLC-UV Method

The HPLC baseline separation of 11 cannabinoids is presented in Figure 3. All 11 cannabinoids elute within 10 minutes whilst achieving baseline resolution. Note that Δ^9-THC (peak 7) and Δ^8-THC (peak 8) separation is also possible with the current method.

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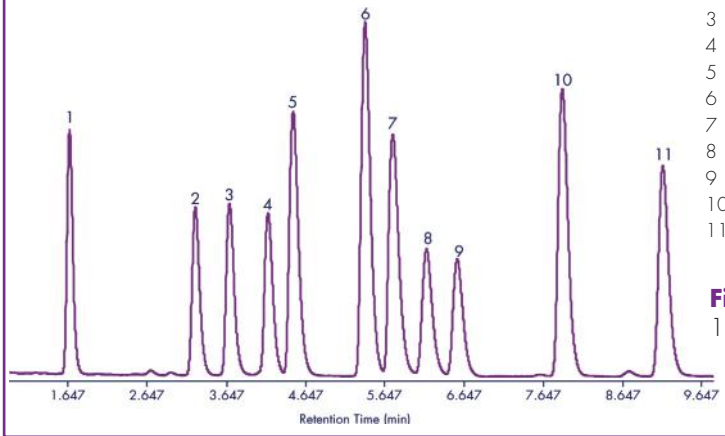

1 Cannabidivarin (CBDV)
2 Tetrahydrocannabivarin (THCV)
3 Cannabidiol (CBD)
4 Cannabigerol (CBG)
5 Cannabidiolic Acid (CBDA)
6 Cannabinol (CBN)
7 Cannabigerolic Acid (CBGA)
8 Δ^9-Tetrahydrocannabinol (Δ^9-THC)
9 Δ^8-Tetrahydrocannabinol (Δ^8-THC)
10 (±)-Cannabichromene (CBC)
11 Tetrahydrocannabinolic Acid A, (THCA-A)

Figure 3: HPLC-UV chromatogram of an 11 Phytocannabinoid standard.

To simplify CBD and Δ9-THC analysis during the purification process, a 5 phytocannabinoid standard was used to generate calibration curves for CBD and Δ^9-THC. The HPLC-UV chromatogram with baseline separation of the 5 phytocannabinoids is shown in Figure 4.

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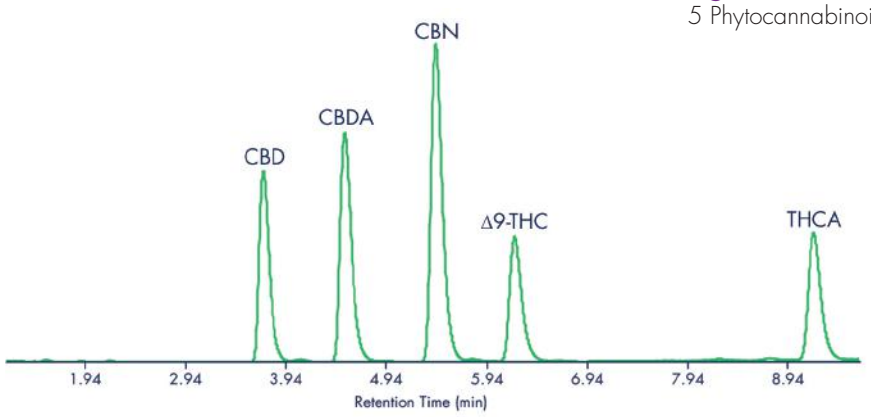

Figure 4: HPLC-UV chromatogram of a 5 Phytocannabinoid standard.

In Figure 5, the calibration curves of the five phytocannabinoids are shown from 1 to 100 ug/mL with linear regression analysis showing an R2 factor of greater than 0.9995 (details listed in Table 1).

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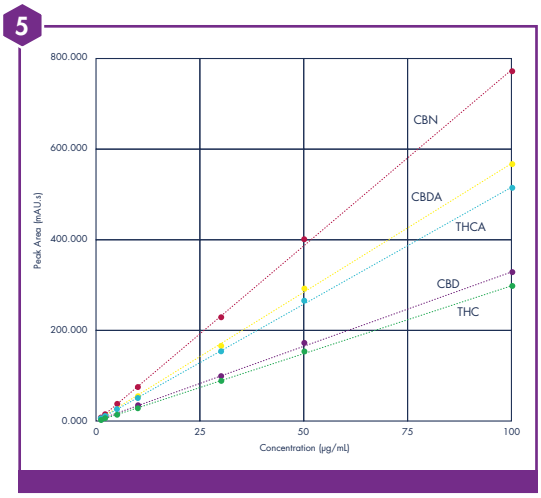

Figure 5: HPLC-UV calibration curves of 5 phytocannabinoids.

Table 1:
Linear Regression of 5 Compounds

	CBD	CBDA	CBN	THC	THCA
Slope	3.291	5.725	7.797	3.009	5.189
Intercept	0.765	-2.730	-2.183	-2.117	-1.325
Error Slope	2.712	3.610	5.876	1.856	3.336
LOQ			≈2 µg/mL		
LOD			≈1 µg/mL		

Hemp Distillate Analysis

Before running the third pass distillate (starting material), a small portion of the material was diluted 500 times in methanol and then analyzed by HPLC to determine the amount of CBD and Δ⁹-THC. A typical chromatogram example of hemp distillate analysis is shown in Figure 6.

The CBD concentration in the distillate was calculated to be 238.8 mg/mL and Δ⁹-THC was calculated to be 9.22 mg/mL (3.86% Δ⁹-THC (calculated as a % of CBD in the distillate solution)) - requiring remediation.

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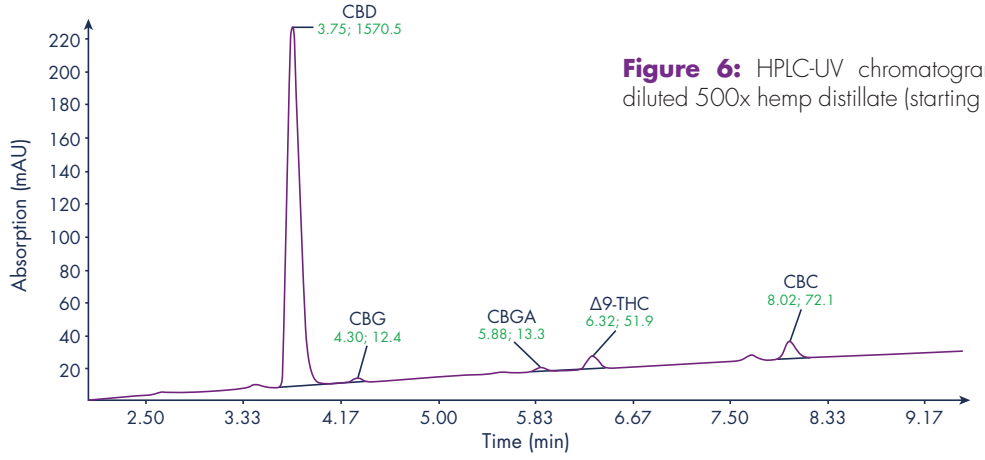


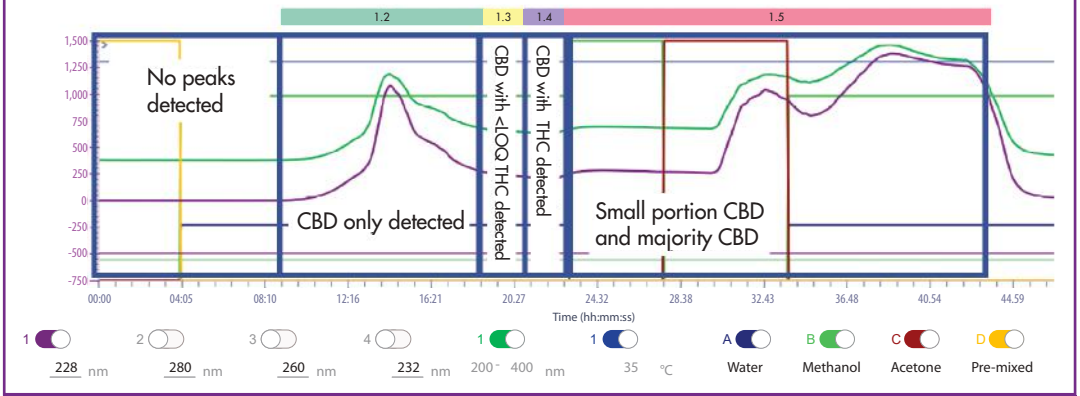
Figure 6: HPLC-UV chromatogram of the diluted 500x hemp distillate (starting material).

THC Remediation

The THC remediation is performed on a puriFlash® XL-Cannabis system with a C18 column ID of 20 cm (MJRP 40x20 cm). An example of a typical UV trace during the purification run is shown in Figure 7.

7

Figure 7: The chromatogram of THC Remediation by puriFlash® XL-Cannabis system.



To monitor the purification process, fractions were taken at multiple time points during the run and analyzed using the Advion AVANT HPLC method described above. In the fractions collected in the first 9 minutes, no CBD or Δ^9-THC is detected. These fractions correspond to the void volume of the system and the start of the sample purification without analyte present (see Figure 8A).

CBD is detected starting at the 9 min fraction. Until the 19 min fraction, the CBD collected shows no Δ^9-THC content (see Figure 8B). From 19 to 23 min, large amounts of CBD and only small amounts of Δ^9-THC are detected (see Figure 8C). These safety fractions 1.3 and 1.4 indicate the start of the elution of THC from the column. The fractions collected up to 21 min, including safety fraction 1.3 can be used for legal product, whereas the later fraction 1.4 is discarded. Fractions collected beyond 23 minutes contain large amounts of THC and are directed to waste.

8

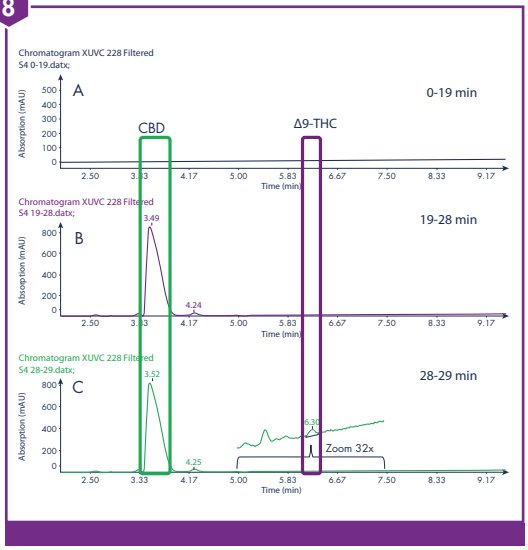
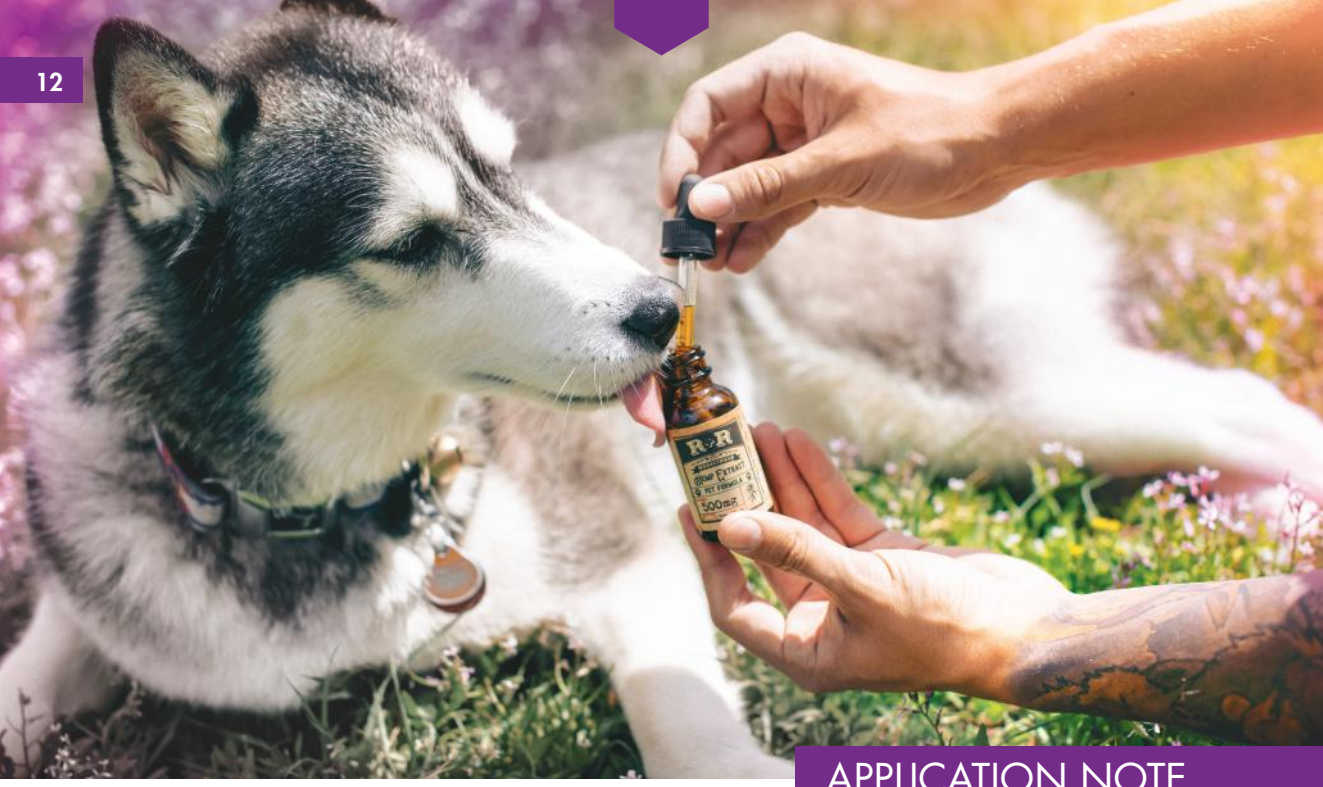


Figure 8: Chromatograms of CBD collection in three time segments. THC is not detected in the fraction from 0-28 minutes. THC is detected in the fraction from 28-29 minutes.

Summary

A combination of Interchim puriFlash® XL-Cannabis system large scale THC remediation and Advion AVANT HPLC-UV systems provides a total solution for THC remediation /cannabinoid purification and analytical quantitation quality control of cannabinoids in hemp products from starting material to finished product.

With the demonstrated methods, distilled hemp extract can be purified to provide broad spectrum oil with complete confidence of meeting allowable requirements for Δ^9-THC concentrations of less than 0.3%.



APPLICATION NOTE

MEDICINAL CANNABIS PRODUCTS FOR VETERINARY MEDICINE

Application of LC/CMS for the analysis of commercial hemp products

Instrumentations

Mass Spec: expression CMS
LC: AVANT UHPLC

Authors

Ben Nie
Jack D. Henion
Advion, Inc.

Joe Wakshlag
University of Florida,
College of Veterinary Medicine

In this application note, the Advion expression Compact Mass Spectrometer coupled with the Advion AVANT UHPLC (LC/CMS) was used to measure the concentration of cannabinoids from commercially available CBD oils for a comparative test against the product labels.

The research in this application note was published in Cannabis Science & Technology Magazine May/June 2019 and presented at the 2019 Cannabis Science Conference East in Baltimore, MD.

Introduction

Using separation from the Advion AVANT UHPLC coupled to the Advion expression single quadrupole Compact Mass Spectrometer (CMS), cannabinoids are measured for concentration against product label claims for a comparative test. Selected ion monitoring (SIM) LC/CMS analysis of multiple CBD oil samples from three different sources was used to compare concentrations with other 3rd party analytical laboratory claims:

- 13 commercial veterinary medicinal CBD oils from Cornell University College of Veterinary Medicine
- 10 CBD oils from a commercial hemp and cannabis grower
- 1 commercially available CBD oil for human consumption for which we had its certificate of analysis

Confusing Labeling of Veterinary CBD

Name	CBD Labeled
1	25 mg hemp extract/mL
2	N/A
3	200 mg/oz
4	10 mg/mL
5	4.2 mg/mL
6	56 mg hemp leaf extract/mL
7	250 mg cannabinoids
8	5 mg of hemp oil/0.5 mL
9	350 mg/30 mL
10	225 mg total in 15 mL
11	150 mg/18mL
12	website 3000 mg/60 mL
13	8 mg/mL

Method

1

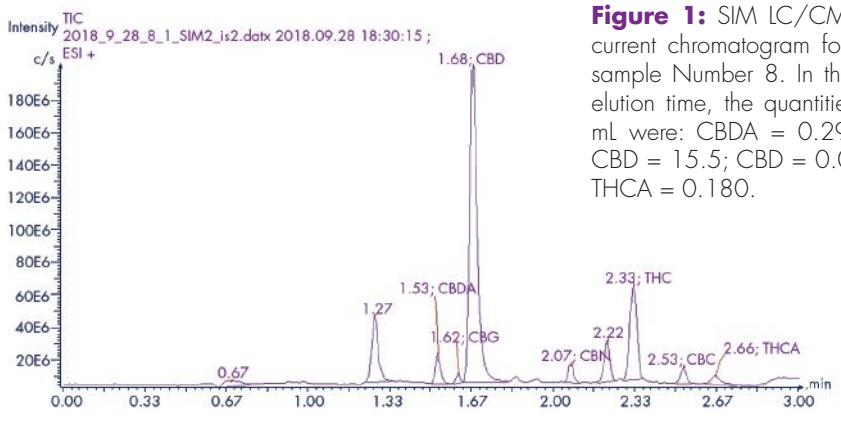


Figure 1: SIM LC/CMS total selected ion current chromatogram for veterinary CBD oil sample Number 8. In the order of LC/CMS elution time, the quantities measured in mg/mL were: CBDA = 0.291; CBG = 0.274; CBD = 15.5; CBD = 0.062; THC = 0.348; THCA = 0.180.

Results

Table 1: Summary of veterinary CBD oil concentrations for the six cannabinoids measured in Samples 1-13.

Concentrations are mg/mL of indicated cannabinoid. It should be noted that Sample 6 was a crude hemp leaf extract without subsequent sample cleanup or concentration.

1

Vet CBD OilTabl	CBDA	CBG	CBD	CBN	THC	THCA
1	0.005	0.070	13.4	0.078	0.347	0.107
2	0.007	0.051	10.6	0.005	0.194	0.026
3	0.001	N/D	1.65	0.030	0.023	0.075
4	N/D	N/D	6.51	0.003	0.001	0.088
5	0.042	0.026	4.34	0.018	0.103	0.076
6	0.153	N/D	0.88	0.002	0.014	0.244
7	1.42	0.061	5.76	N/D	0.205	0.049
8	0.291	0.274	15.5	0.062	0.348	0.180
9	0.087	0.147	8.82	0.009	0.278	N/D
10	N/D	0.102	11.6	0.017	0.352	N/D
11	N/D	0.018	7.86	N/D	0.049	0.079
12	11.8	0.372	27.5	0.005	1.29	0.693
13	0.123	0.116	13.9	N/D	0.374	0.032

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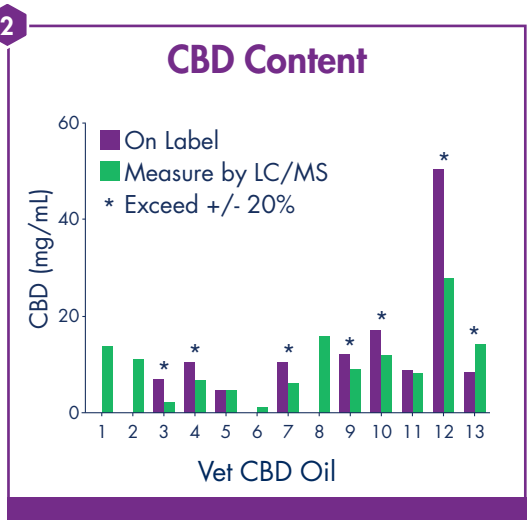

Figure 2: A comparison of the CBD concentration in all 13 medicinal oil samples determined by SIM LC/CMS against the indicated levels on the respective product label. It was not possible to know the labeled CBD concentrations for Samples 1, 2, 6, and 8 due to the units used, which was indicated to be mg of hemp extract/mL.

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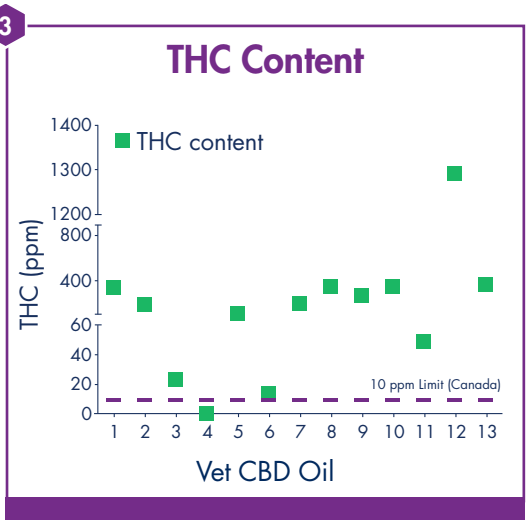

Figure 3: A comparison plot of the THC concentration in parts per million (ppm) for each of the 13 medicinal veterinary CBD oils relative to the Canadian acceptance level, which limits the THC concentration to 10 ppm.

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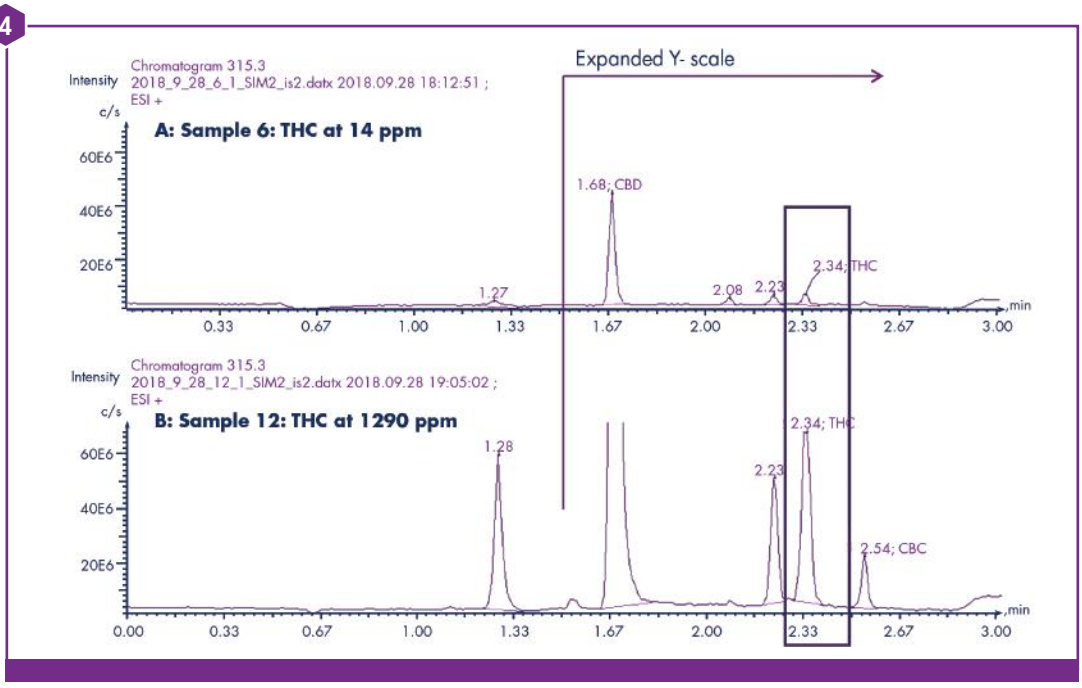

Figure 4: (A) SIM LC/CMS chromatogram for medicinal oil Sample 6, which as shown in Figure 3 had a THC concentration just above the 10 ppm allowed level. (B) THC peak in Sample 12 is a much more abundant component relative to the other cannabinoids, again with the exception being the CBD peak at 1.68 minute retention time. Note that the y-scale in both Figure 4A and 4B is the same, so the reader can directly compare the relative quantities of THC in these two samples.

2

Sample	CBDA (mg/mL)	CBD (mg/mL)	CBN (mg/mL)	THC (mg/mL)	THCA (mg/mL)
1	1.06	0.44	N/A	0.013	0.045
2	2.35	1.15	N/A	0.038	0.043
3	1.21	0.30	N/A	0.009	0.018
4	2.30	1.38	N/A	0.065	0.051
5	0.41	0.51	N/A	0.013	0.003
6	1.41	0.11	N/A	0.00069	0.059
7	0.51	0.50	N/A	0.018	0.006
8	1.26	0.34	N/A	0.010	0.091
9	1.37	0.50	N/A	0.021	0.009
10	0.020	0.002	N/A	1.906	1.955

Table 2: Analytical results of a blind test for the concentrations of five indicated cannabinoids from an anonymous grower. The grower used heated extraction to increase the concentrations of CBD and THC. Sample 10 is a cannabis extract.

3

CBD Oil	This work	Lab1	Lab2
CBDA (mg/mL)	0.442	0.440	0.53
CBG (mg/mL)	0.083	0.0948	
CBD (mg/mL)	7.78	7.64	7.89
CBN (mg/mL)	0.058	0.0568	N/D
THC (mg/mL)	0.233	0.361	0.28
THCA (mg/mL)	N/D	<0.046	N/D

Summary

It is noteworthy to see that the chemical analysis of the commercial oils suggests that the labeled contents on the bottle may differ significantly from the actual contents determined by chemical analysis.

These results suggest how perhaps in the future, commercial vendors should include independent chemical analyses of their products so that customers may have confidence in the composition and therapeutic benefits of the products.

Acknowledgements

Analysis of veterinary hemp-based oils for product integrity by LC/MS, Cannabis Science & Technology, May/June 2019, 36-45. In Press.
Table 3: Analytical results for the concentrations of six indicated cannabinoids from a different medicinal product by three independent labs. The second column summaries the results obtained by this laboratory while Columns 3 and 4 are the analytical results from two other independent laboratories labeled here as Lab 1 and 2.

APPLICATION NOTE

RAPID CANNABINOID TESTING METHOD FOR CANNABIS QUALITY CONTROL

Instrumentations

Mass Spec: expression CMS
Sampling: ASAP

Authors

Ben Nie
Jack Henion
Advion, Inc.

Seth Richardson
Kingsland Partners, Inc.

In this application note, a simple, sensitive and selective sample introduction approach with the expression Compact Mass Spectrometer (CMS) and Atmospheric Solids Analysis Probe (ASAP) was used to measure the presence of two isobaric compounds, CBDA and THCA, contained in a complex sample such as hemp or cannabis plants or their corresponding extraction products.

The data in this application note was presented at the 2018 Cannabis Science Conference in Portland, OR

Introduction

A simple, sensitive and selective ASAP sample introduction approach to measuring the presence of two isobaric compounds, CBDA and THCA, contained in a complex sample such as hemp or cannabis plants or their corresponding extraction products. Measurements are made of differences in the relative composition of CBDA and THCA fragment ions originating from the same precursor ion. Applicability to screening plants and plant product materials such as hemp or marijuana to monitor out-of-specification composition is demonstrated.

In the absence of on-line sample preparation or separation sciences such as HPLC, it is shown that a single quadrupole mass spectrometer operated at unit mass resolution in the selected ion monitoring (SIM) mode can produce ion current ratios from CBDA and THCA (isobaric compounds) contained in the gaseous plume of a sample which reflect their relative quantities in the sample. This analytical capability may be used for screening a variety of samples and providing information that may be useful for indicating cannabinoid composition in plants or extraction products and differentiating cannabis strains/cultivars (i.e. marijuana vs. hemp).

Method

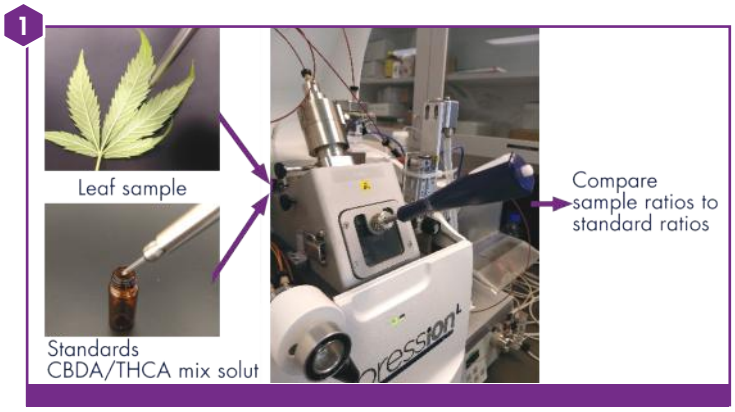

Figure 1: Workflow for measurement of CBDA/THCA relative quantities in cannabis leaf using ASAP/CMS.

2

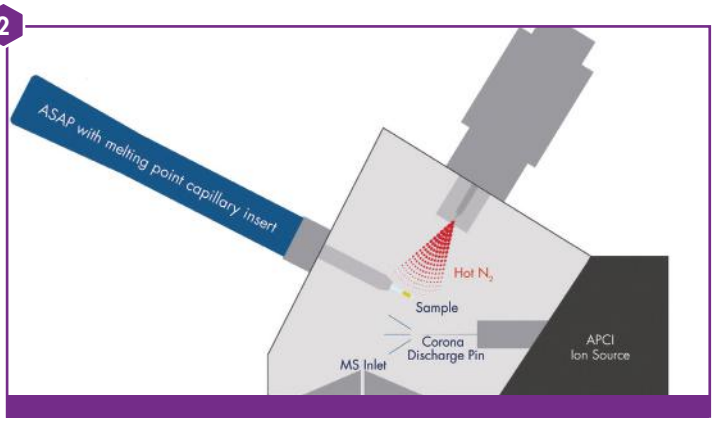

Figure 2: Schematic of ASAP with sample inserted directly into the APCI source of the CMS.

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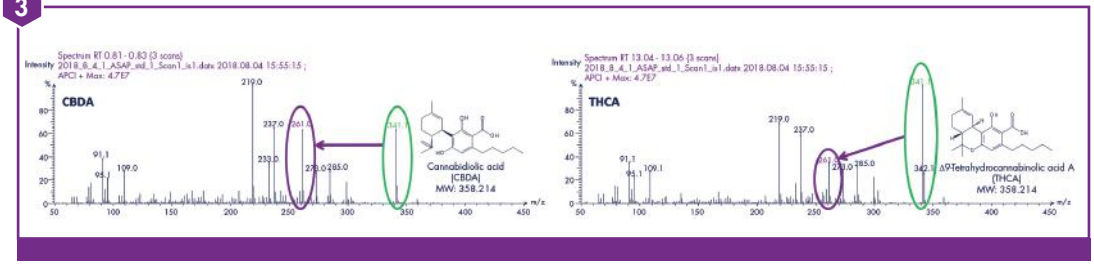

Figure 3: Mass spectra of CBDA and THCA. Under the same experimental conditions, the ion current abundance of m/z 261 for CBDA is 95-100% compared to the relative abundance of m/z 341, while the abundance of m/z 261 for THCA is much reduced at 25-30% compared to the relative abundance of m/z 341.

Results

4

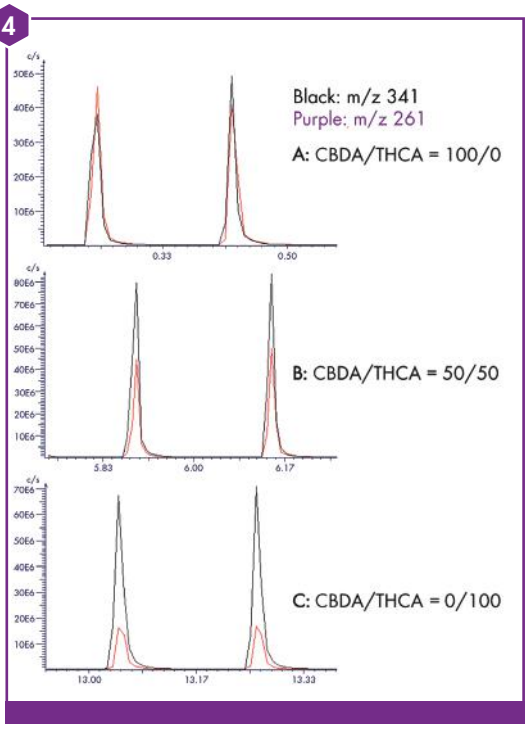

Figure 4: SIM ion current of CBDA/THCA mixture at different ratios (CBDA/THCA at 100/0, 50/50, 0/100, respectively) from Table 1. CBDA/THCA Standard Mixture Reference.

Table 1: CBDA/THCA Standard Mixture Reference

Concentration Ratio CBDA/THCA	Ion Ratio 261/341
100/0	1.043
90/10	0.920
80/20	0.841
70/30	0.756
60/40	0.598
50/50	0.515
40/60	0.465
30/70	0.437
20/80	0.346
10/90	0.309

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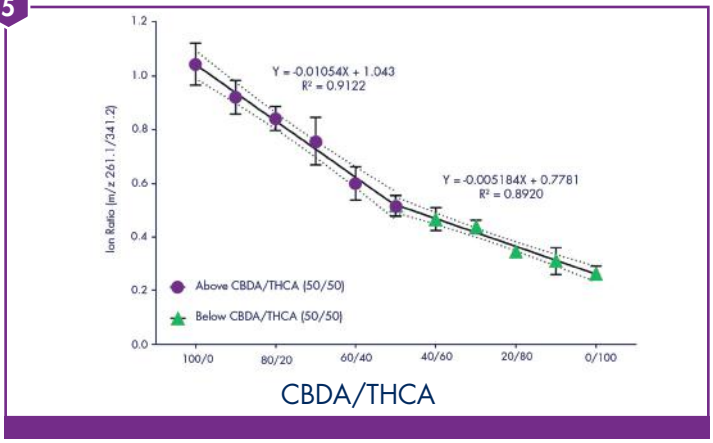

Figure 5: ASAP Calibration Curves (Hops as Matrix). Due to the ion abundance difference of m/z 261 for CBDA and THCA, the calibration curve is split into two sections at CBDA/THCA 50/50 relative concentrations.

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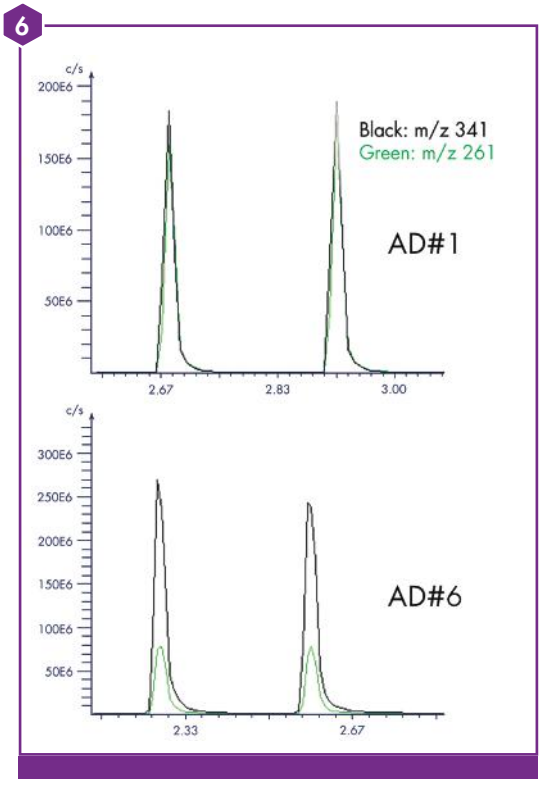
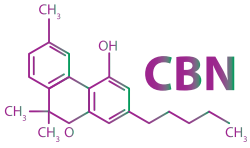
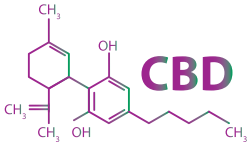


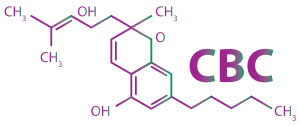
Figure 6: SIM ion current of CBDA/THCA detected in Cannabis samples (AD#1 and AD#6, respectively) from Table 2. Cannabis samples from an anonymous source by ASAP/CMS, cross-validated with LC/MS (blind test).

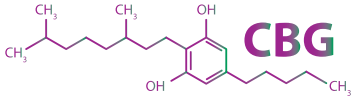
Table 2: Cannabis samples from an anonymous source by ASAP/CMS, cross-validated with LC/MS (blind test)

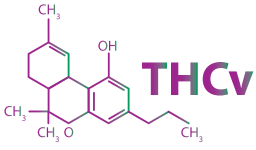
	ASAP/CMS		LC/CMS
Unknown Cannabis Samples	Ion Ratio (*m/z* 261/341)	Conc. Ratio (CBDA/ THCA)	Conc. Ratio (CBDA/ THCA)
AD#1	0.999	96/4	96/4
AD#2	1.028	99/1	97/3
AD#3	0.390	25/75	12/88
AD#4	1.039	99/1	98/2
AD#5	1.050	99/1	98/2
AD#6	0.311	10/99	1/99
MRS*	0.287	6/94	1/99

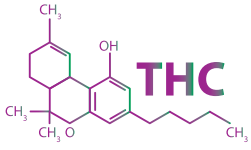












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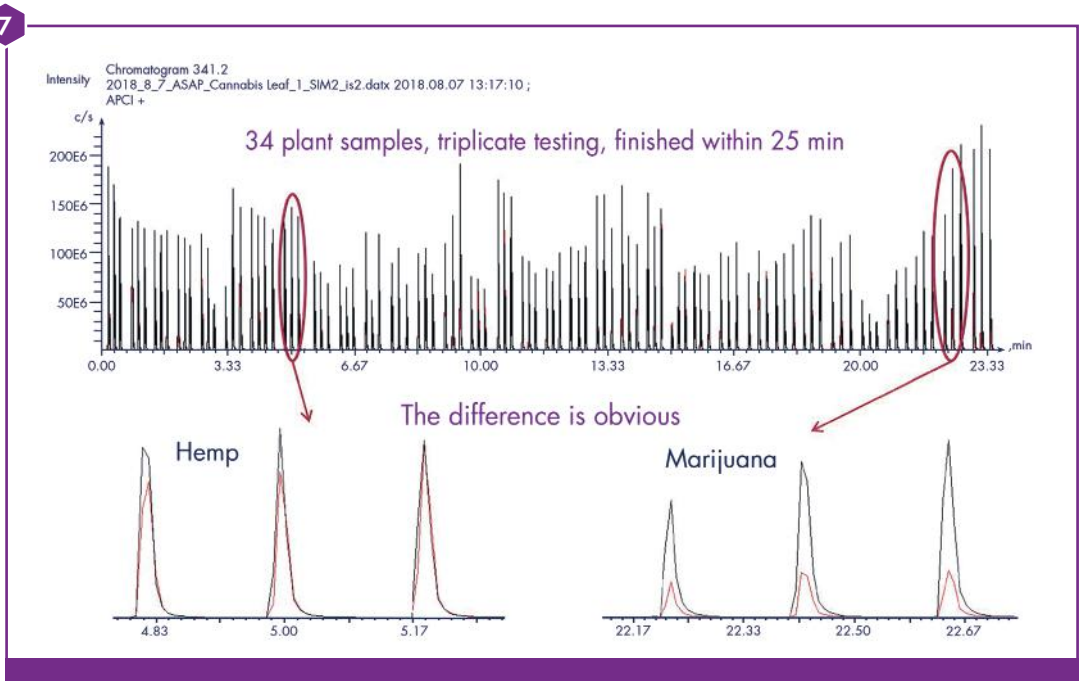

Figure 7: High-throughput screening (blind) for 34 different cannabis plants. These results show the use of ASAP-CMS to determine the relative composition of CBDA and THCA in the plants.

Summary

- A novel approach is reported to determine the relative composition of CBDA and THCA in hemp and marijuana plants using the ion current abundance differences in their respective.
- precursor/fragment ion transitions (m/z 341 to m/z 261).
- The method is simple, sensitive and relatively selective.
- The described method provides a simple, relatively high-throughput screening approach to potentially differentiate hemp and marijuana or possibly other cannabis strains.

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