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Application News

No. HPLC-034

Supercritical Fluid Chromatography

Analysis of 9 Cannabinoids by Supercritical Fluid Chromatography

■ Introduction

HPLC has long been considered the gold standard for the quantitative analysis of cannabinoids in cannabis and hemp owing to robust methods, efficacy for both acid and neutral forms, and its simple sample preparation. A lesser known technique is supercritical fluid chromatography (SFC) which uses supercritical CO_2 as the primary mobile phase.

SFC offers advantages over HPLC. First, the use of CO_2 allows for increased flow rates due to the lower viscosity and backpressure compared to aqueous solvents. In addition, SFC is generally regarded as a "green" technique due to the minimization of organic solvents and reduced generation of hazardous waste. This has the added advantage of reducing the operating cost of the instrument as far less hazardous waste is produced.

In its chemistry, SFC is dissimilar to both reversed phase and normal phase HPLC because of the unique properties of liquid CO_2. The selectivity displayed in SFC is rather unpredictable. For this very reason, one of SFC's greatest attributes is its ability to separate peaks that have proven difficult by HPLC.

The final advantage of SFC for the analysis of cannabinoids is the complementary nature of sample preparation for RP-HPLC. A fundamental limitation of RP-HPLC is that the solvent used in the sample extraction must be water soluble. SFC has a complementary solubility where the extraction solvent can be non-polar, which is advantageous to sample preparations like liquid-liquid extractions. This eliminates steps in sample preparation like sample dry-down and reconstitution after extraction.

■ Experimental Design

Instrument Configuration (as tested)

Shimadzu Nexera UC™

- CBM-20A System Controller
- LC-30ADSF CO_2 Pump
- LC-30AD Modifier Pump
- SIL-30AC Autosampler with 5 µL Loop Injection Kit
- CTO-20AC Column Oven
- SPD-M20A Photodiode Array (PDA) Detector with SFC Flow Cell
- SFC-30A Backpressure Regulator (BPR)

Alternative Instrument Configuration

Shimadzu Nexera LC-40 UC™

- CBM-40A System Controller
- LC-30ADSF CO_2 Pump
- LC-40DXR Modifier Pump
- SIL-40CXR Autosampler with 5 µL Loop Injection Kit
- CTO-40C Column Oven
- SPD-M40 Photodiode Array (PDA) Detector with SFC Flow Cell
- SFC-30A Backpressure Regulator (BPR)

Software

LabSolutions™ LC/GC

Column

- Shim-pack™ GIS 4.6×250 mm, 5 µm C18, P/N 227-30106-08
- Shim-pack™ GIS 4.0×10 mm, 5 µm C18 Guard, P/N 227-30134-01

Reagents

- CO_2, Airgas, >99.9% purity
- Reagent Alcohol, Honeywell Chromasolv™
- Diluent: Methanol, >99.9%, Honeywell Chromasolv™

Standards

Standards were obtained from Cayman Chemical:

- Cannabindivarian (CBDV), 1 mg/mL in methanol, Part Number 20165
- Cannabidiol (CBD), 1 mg/mL in methanol, Part Number ISO60156
- Cannabigerol (CBG), 1 mg/mL in methanol, Part Number 20164
- Cannabichromene (CBC), 1 mg/mL in methanol, Part Number 26252
- Delta-9-Tetrahydrocannabinol (d9-THC), 1 mg/mL in methanol, Part Number ISO 60157
- Cannabinol (CBN), 1 mg/mL in methanol, Part Number ISO60183
- Cannabidiolic Acid (CBCA), 1 mg/mL in methanol, Part Number 18090
- Cannabigerolic Acid (CBGA), 1 mg/mL in acetonitrile, Part Number 20019
- Delta-9-Tetrahydrocannabinolic Acid (THCA), 1 mg/mL in acetonitrile, Part Number ISO+0175

Analytical Conditions

Total Flow	3.0 mL/min	
Mobile Phase A	CO_2	
Mobile Phase B	Reagent Alcohol	
Time	7 min	
Gradient	**Time** 0.00 2.50 5.00 5.01	**%B** 3 3 12 3
Column	Shim-pack™ GIS 4.6×250 mm, 5 µm C18 (227-30106-08) with Guard (227-30134-01)	
BPR Pressure	200 bar	
BPR Temperature	50 °C	
PDA	190-450 nm, monitored at 220 nm	
Column Oven	40 °C	
Injection Volume	5 µL	

■ Results

Calibration of the SFC System with a Standard Solution

A standard solution was created by combining all 9 cannabinoids and analyzed using the analytical conditions described in this application note. The resulting chromatogram (Figure 1.) was obtained.

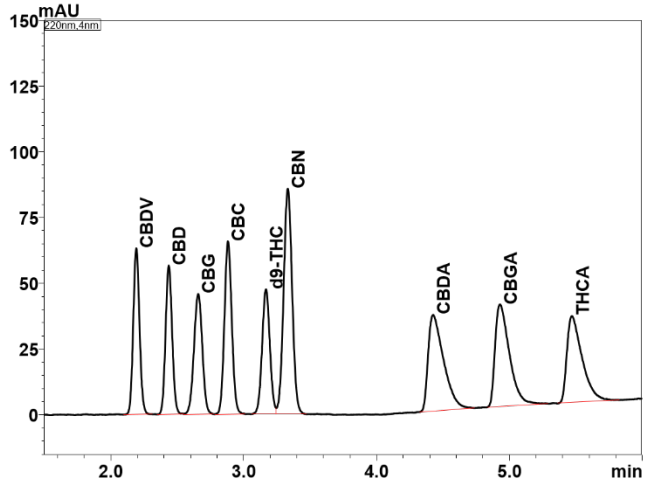
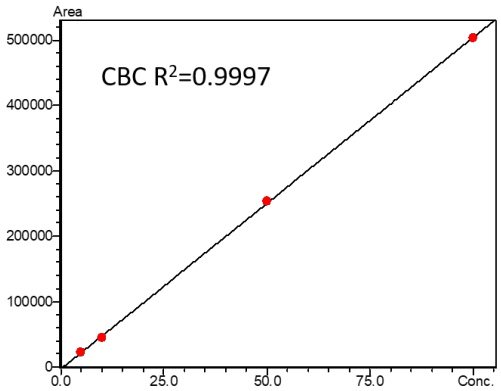


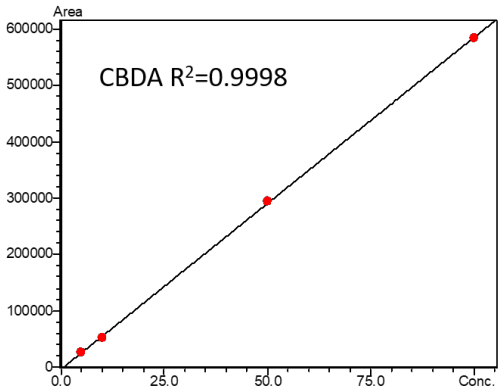
Figure 1: Chromatogram of 9 cannabinoid standard solution at 50 ppm concentration

An interesting observation is the clear differentiation of the acidic cannabinoids (CBDA, CBGA, and THCA) from the non-acidic forms. The acids are more retained on column and have a different peak shape. The clear tailing on the acidic compounds is likely attributed to secondary interactions with the column. This tailing would normally be a problem with closely eluting peaks, but the resolution exhibited by this method makes the issue irrelevant.

Standard Curves

Standard curves were prepared at 5, 10, 50 and 100 ppm by serial dilution with methanol. When the combined 9 component standard was injected it produced acceptable correlation coefficient (R^2) for all compounds.





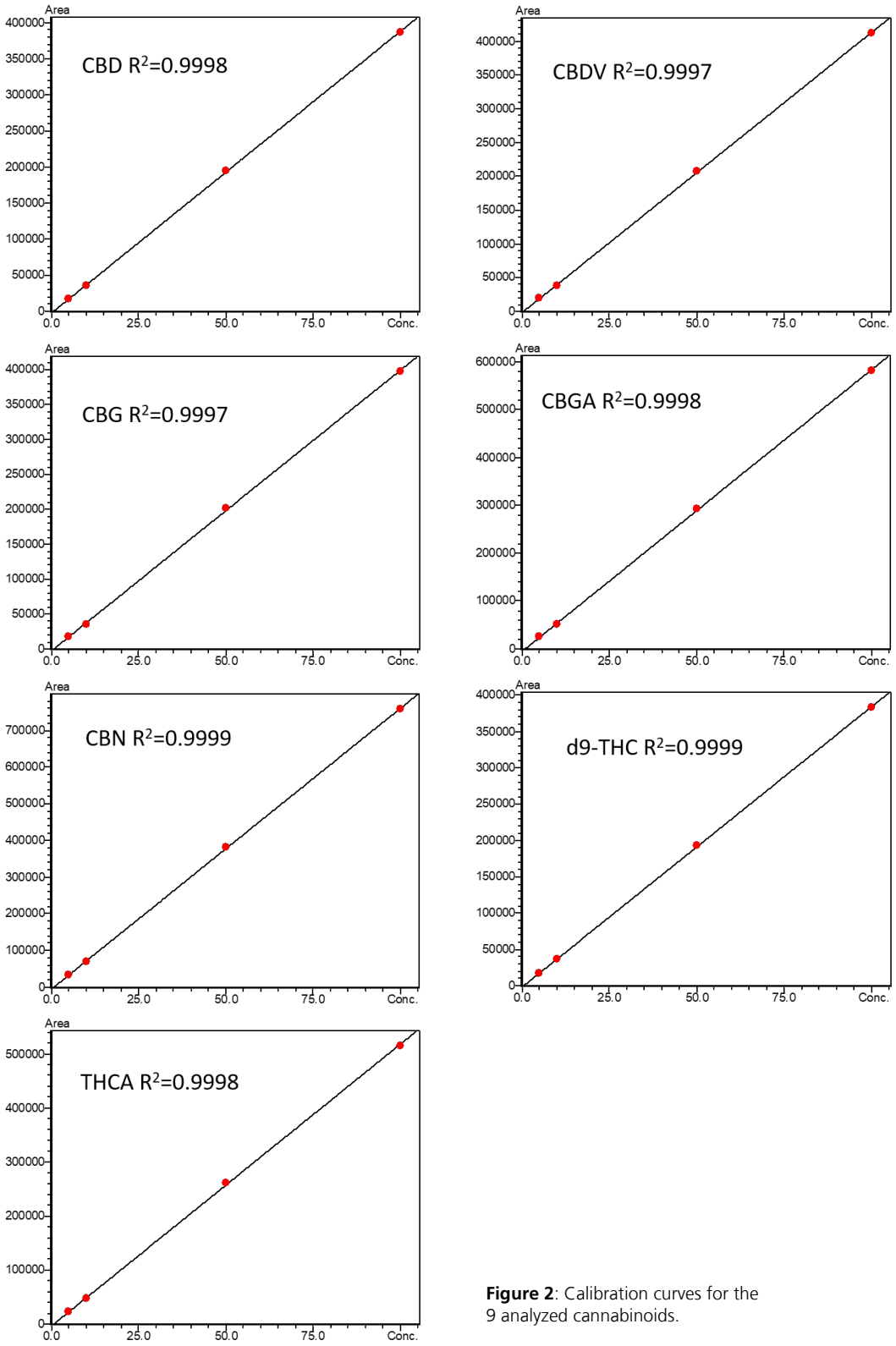


Figure 2: Calibration curves for the 9 analyzed cannabinoids.

Quantitative Analysis of Cannabinoids in Hemp Flower

A sample of dried hemp flower was prepared per the procedure outlined for use with the Shimadzu Hemp Analyzer™. When analyzed by SFC, the chromatogram in Figure 3 was obtained.

As expected, the hemp material was rich in CBD and CBDA and poor in THC and THCA. Experimental results are shown in Table 1.

Table 1: Potency of hemp material by SFC. Total THC potency is calculated by the formula (%THCA×0.877) + %D9-THC to account for decarboxylation of the acid form.

#	Compound	Conc (mg/L)	Weight %
1	CBDV	ND	ND
2	CBD	17.56	3.51
3	CBG	ND	ND
4	CBC	1.59	0.32
5	D9-THC	2.60	0.52
6	CBN	ND	ND
7	CBDA	66.59	13.32
8	CBGA	2.31	0.46
9	THCA	3.88	0.77
Total THC Potency (Weight %)			1.20

Recovery Study

To assess the recovery of the analytical method, a spike/recovery study was performed. Duplicate flower samples were prepared, and one was spiked with 5 ppm of the 9-component standard mixture and analyzed using the conditions shown above. Results are shown in Table 2. The results of the study are generally between 90-110% and considered acceptable, with CBD being slightly higher at 111.1%.

Table 2: Results of the spike/recovery study.

#	Compound	Unspiked Conc. (ppm)	Spiked Conc. (ppm)	Recovery (%)
1	CBDV	0.83	6.18	106.9
2	CBD	17.56	23.11	111.1
3	CBG	0.82	5.99	103.5
4	CBC	1.59	6.79	104.0
5	D9-THC	2.60	7.62	100.5
6	CBN	ND	5.50	110
7	CBDA	66.59	71.58	99.8
8	CBGA	2.31	7.63	106.6
9	THCA	3.88	8.76	97.7

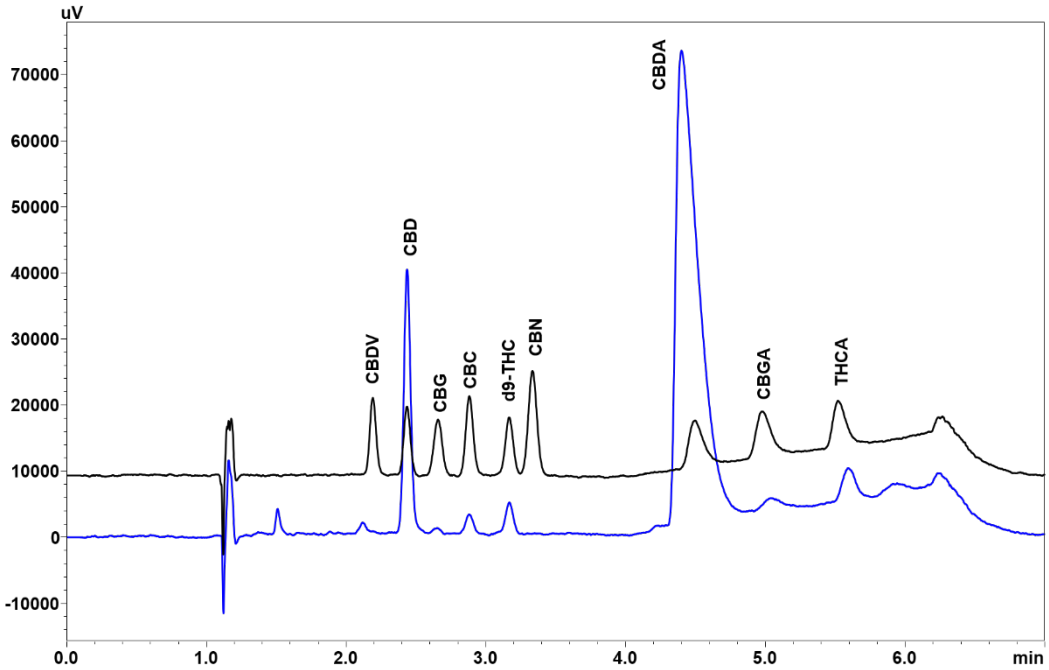


Figure 3: An overlay of the 10 ppm standard (black) and the hemp flower preparation (blue).

■ Conclusion

This application note has demonstrated the use of SFC to quantitate 9 cannabinoids in hemp flower. The method exhibits an alternative selectivity to RP-HPLC and can generally be considered a green technique. Use of this method for difficult sample matrices that do not lend themselves to RP-HPLC is a further field of exploration.

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SHIMADZU Corporation
www.shimadzu.com/an/

SHIMADZU SCIENTIFIC INSTRUMENTS
7102 Riverwood Drive, Columbia, MD 21046, USA
Phone: 800-477-1227/410-381-1227, Fax: 410-381-1222
URL: www.ssi.shimadzu.com

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