Optimizing for High Throughput Analysis of Cannabinoids in Cannabis Products

Success with the Ascentis Express C18 Column

Seamus Riordan-Short, Senior Chemist; **Yevgen Kovalenko,** Technician; **Matthew Noestheden,** Director of Operations, **Supra Research and Development**

Katherine K. Stenerson, Analytical Sciences Liaison, **Jennifer King,** Program Marketing Manager, **MilliporeSigma**

With increasing cannabis and hemp legislation, there has been increased demand for development and validation of accurate and precise testing methods for potency quantitation. Cannabinoids present a number of challenges, and there is also the additional burden of dealing with a variety of matrix types. HPLC/UV is the technique most commonly used, and the HPLC parameters must be optimized to maintain good separation and stable retention over many injections and with the various sample types.

Scientists at Supra Research and Development ("SupraRnD") located in Kelowna, British Columbia, Canada (www.suprarnd.ca) have developed a high throughput and reliable method for cannabinoids that is applicable to a variety of matrices. SupraRnd's involvement in cannabis testing began in 2015 when they obtained a license from Health Canada for testing cannabis products. In 2018 they were one of the first laboratories in Canada to obtain their ISO 17025 accreditation for cannabis testing. Their potency method has evolved over time to meet the changing needs of their customers, and is now validated for several different matrices.

Experimental Conditions

Whole flower samples were frozen in hermetically sealed bags at -80°C for a minimum of 30 minutes and then homogenized immediately. It is critical that a representative sample be homogenized and subsampled when analyzing cannabis flower, as there can be considerable variance in phytocannabinoid concentrations between and within a given plant. The subsequent workflow involved a simple extraction of a 0.2 g sample size with methanol, followed by sonication and stabilization of the extract at -20°C for 1 hour. The sample was then centrifuged, and the supernatant diluted 100:1 for HPLC analysis. The small sample size in combination with the pre-analysis dilution minimizes the potential for matrix-related issues (e.g., interferences, column longevity, etc.). The HPLC portion of the analysis has a cycle time of 8 minutes injection to injection. This allows 60 injections per 8-hour interval, which enables more customer samples to be run in a work shift. The cannabinoids analyzed by the method are listed in **Table 1.**

Table 1. 17 Phytocannabinoids separated by HPLC method

1.	CBDVA	10.	CBNA
2.	CBDV	11.	Δ9-THC
3.	CBDA	12.	Δ8-THC
4.	CBGA	13.	CBL
5.	CBG	14.	CBC
6.	CBD	15.	THCA
7.	THCV	16.	CBCA
8.	THCVA	17.	CBLA
9.	CBN		

The final, optimized HPLC parameters are summarized in **Table 2.** When developing this method, the following were considerations:

- Chromatographic resolution of all 17 compounds.
- Cycle time (i.e. run time plus equilibration) of less than 10 minutes total.
- A rugged method with consistent performance for >1000 injections with stable retention times, while maintaining good peak shape and response.
- Suitable for use with different matrices such as flower, chocolate, ointment, oil, concentrate, etc.

Table 2. Optimized method HPLC parameters

column:	Ascentis® Express C18, 15 cm x 2.1 mm I.D., 2 µm
mobile phase:	(A) 5 mM ammonium formate in water + 0.1% formic acid; (B) 0.1% formic acid in acetonitrile
gradient:	70 to 90% B in 3 min; held at 90% B for 2 min; to 98% B in 0.1 min; held at 98% B for 0.9 min; to 70% B in 0.1 min; held at 70% B for 0.9 min
flow rate:	0.4 mL/min
pressure:	533 bar
column temp.:	30°C
detector:	UV, 228 nm
injection:	25 µL
sample:	methanolic extract of cannabis derived samples (oil, concentrate, ointment, etc.)

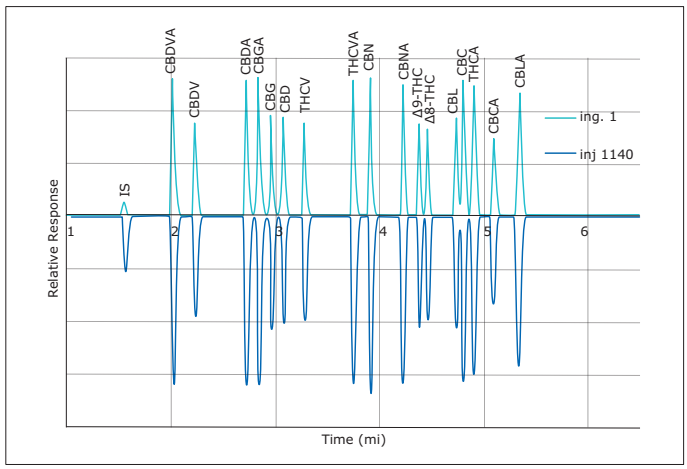
Calibration for the method was from 0.01 µg/mL to 40 ug/mL. This required a high dilution for some samples in order to bring them within this analytical range. For calibration and spiking, Cerilliant® certified reference materials (CRMs) were used. Individual cannabinoid CRMs at 1 mg/mL (with the exception of CBLA at 0.5 mg/mL) were diluted, along with the internal standard solution, directly into HPLC mobile phase component A, to prepare a 17-component stock solution at 40 µg/mL. This stock was then diluted further into a 30:70 mixture of HPLC mobile phases A:B, for the lower concentration calibration standards.

The HPLC column used for the analysis was an Ascentis® Express C18 column, 15 cm x 2.1 mm I.D., 2 µm. Ascentis® Express columns contain Fused-Core® particles with a solid core and porous shell architecture, also referred to as superficially porous. This particle structure provides higher separation efficiency than fully porous particles of the same size, and allows for faster analysis times with lower backpressure than approaches using smaller (< 2 µm) fully porous particles. The particle architecture of Ascentis® Express columns allows for the use of larger particles, making them suitable for both conventional and UHPLC systems. For this method, SupraRnD used a UHPLC system, although with the proper instrument optimization, successful separation can be obtained on conventional systems as well. Specifically, this would involve minimization of system dispersion. This can be done by reducing tubing length and ID of the column inlet and outlet; and for UV detectors, using a flow cell with a volume of < 5 µL.

Method Validation and Performance

Prior to choosing the Ascentis® Express C18 for method validation, SupraRnD screened six other columns of similar chemistry from various manufacturers. They were able to achieve chromatographic resolution and a short run time with several columns but found that the Ascentis® Express C18 was the only column that provided retention time stability – especially for the acidic cannabinoids. This is illustrated in **Figure 1** which shows chromatograms of a check standard at injection #1 and injection #1140, in between which numerous sample extracts were run.

Figure 1. Cannabinoid standard on Ascentis® Express C18 column; comparison of injection #1 and injection #1140.



The method using the Ascentis® Express C18 was validated in several different matrices including hop flowers (as a surrogate matrix to cannabis), hemp seed oil, CBD concentrate, and topical ointments. Recoveries from hops ranged from 85-115% over a spiking range of 0.05 to 20% by weight. A summary of this validation, as well as the other matrices, is summarized in **Table 3.** The method reporting limits (MRLs) achieved for the cannabinoids (except for CBDV, CBG, CBD and CBC in the concentrate) were all 0.05 wt.%. Repeatability, as %RSDr, was < 4% for all matrices. Further evaluation was done using proficiency testing in which the method successfully passed for samples of cannabis flower and hemp oil.

Table 3. Summary of method validation data for cannabinoid method in several matrices

	% Recovery range of all 17 cannabinoids spiked into matrix			RSD	MRL (wt%)
Spiking level (wt%)	**.05%**	**1%**	**20%**		
Hops (surrogate matrix)	86-106	96-115	100.5-116	< 1.5%	0.05
Hemp seed oil	92-118	104-116	101.5-113	< 4%	0.05
ointment 1					
(CBD isolate)	83-120*	80-122*	--	< 3%	0.05
ointment 2	79-129**	86-117**	--	< 2.5%	0.05
CBD concentrate	71-123.5*	92-118*	--	< 3.5%	0.05Ŧ

*CBD recovery not quantitated due to high incurred levels

**Δ9-THC recovery not quantitated due to high incurred levels

ŦCBDV, CBG, CBD, CBC incurred in matrix led to issues preventing calculation of MRL for these compounds

Figure 2 shows example chromatograms of hop flowers spiked at 1% w/w and at the MRL concentration of 0.05% w/w. At the much lower spiking level, where matrix interference was more apparent, all 17 cannabinoids were discernable from background peaks and could be analyzed. The specific interferences eluting next to THCV and CBL were probably due to specific terpenes present in the hop sample. These peaks were not observed in cannabis flower. In a spiked ointment sample **(Figure 3)**, all cannabinoids were clearly detected at the MRL.

To date, > 1,550 injections have been made on a single Ascentis® Express C18 column. SupraRnD has noted that thus far there has been no significant increase in column backpressure, or degradation in performance. Data collected on backpressure over the course of this use, showed a net increase of 2%. They also noted that retention times were stable, allowing them to identify cannabinoid peaks in samples with more confidence. An example is illustrated in **Figure 4** in which two different matrices, dark chocolate and cannabis flower, are compared. Both samples contained measurable amounts of Δ9-THC, and the difference in the retention time between the two matrices were minimal.

Conclusion

After evaluating several HPLC columns, SupraRnD has successfully developed a robust and rugged method using the Ascentis® Express C18 column for the analysis of 17 cannabinoids in a variety of matrices. Thus far, the method has been successfully applied to five different sample types including flower, ointments, chocolate, concentrates and gummies. The Ascentis® Express C18 column was chosen for the final method based on retention time stability over repeated use, and ability to maintain chromatographic performance for the cannabinoids. In addition, the column currently in use has shown minimal increase in backpressure over the course of > 1500 injections.

Figure 2. Hop flowers, spiked at 1% and .05% with cannabinoids.

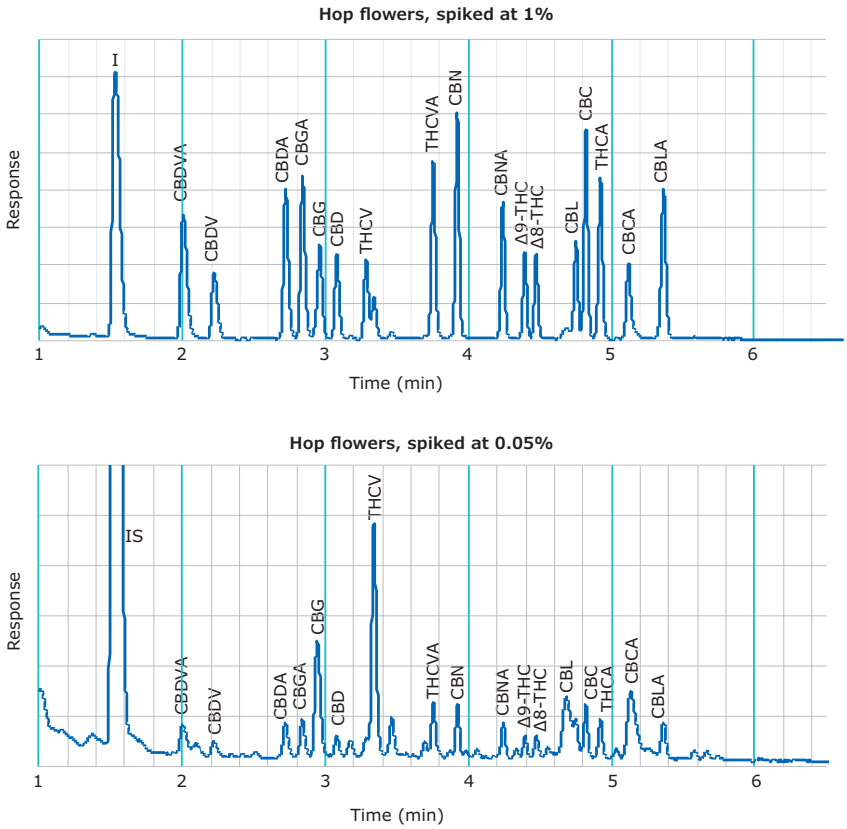


Figure 3. Ointment made from cannabis extract, spiked at 0.05% by weight.

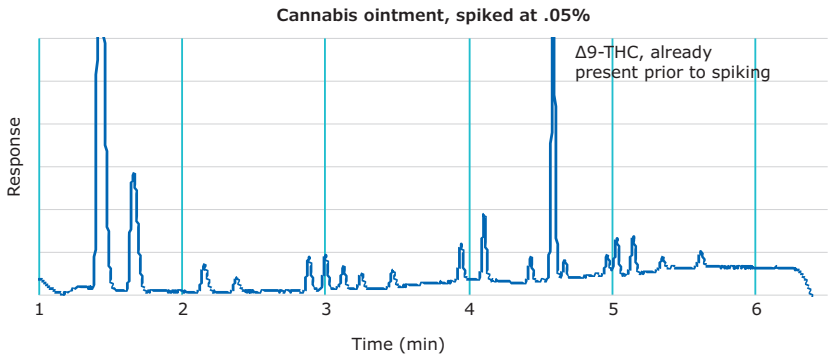
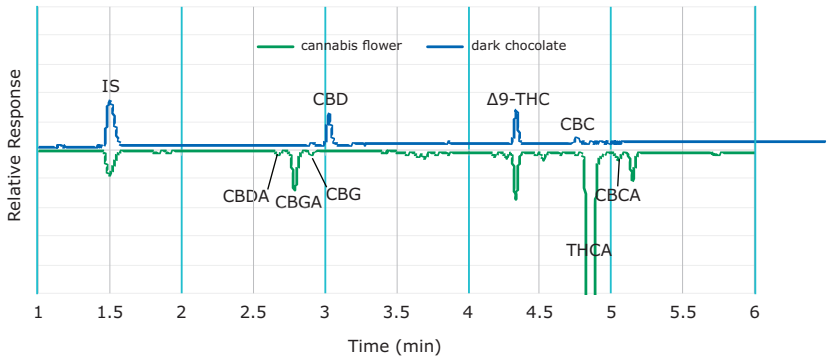


Figure 4. Comparison of elution pattern between dark chocolate and cannabis flower samples (unspiked).



Featured and Related Products

	Product Description	Brand	Mfr. No.	Thomas No.
	HPLC Columns			
	Ascentis® Express C18 column, 15 cm x 2.1 mm I.D., 2 µm	Supelco	**50814-U**	**1145P16**
	Ascentis® Express C18, 2 µm guard cartridge 5 mm × 2.1 mm, pkg of 3 ea	Supelco	**50822-U**	**1145P17**
	Ascentis® Express Guard Cartridge Holder	Supelco	**53500-U**	**1179R66**
	Accessories			
	Certified Vial Kit, Low Adsorption (LA), 2 mL, pk of 100 volume 2 mL, clear glass vial (with marking spot), natural PTFE/ silicone septa (with slit), thread for 9 mm	Supelco	**29652-U**	**21A00M257**
	Certified Vial Kit, Low Adsorption (LA), 2 mL, pk of 100 volume 2 mL, amber glass vial (with marking spot), natural PTFE/ silicone septa, thread 9 mm	Supelco	**29653-U**	**21A00M258**
	Certified Reference Materials			
	Cannabidivarinic Acid (CBDVA) solution 1.0 mg/mL in acetonitrile, certified reference material, ampule of 1 mL, Cerilliant®	Supelco	**C-152**	**C753Q16**
	1.0 mg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	Supelco	**C-140**	**C753Q11**
	Cannabidiolic acid (CBDA), 1.0 mg/mL in acetonitrile, ampule of 1 mL, certified reference material, Cerilliant®	Supelco	**C-144**	**C753Q15**
	Cannabigerolic acid (CBGA), 1.0 mg/mL in acetonitrile, ampule of 1 mL, certified reference material, Cerilliant®	Supelco	**C-142**	**C753Q13**
	Cannabigerol (CBG), 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	Supelco	**C-141**	**C753Q12**
	Cannabidiol solution, 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	Supelco	**C-045**	**C915P98**
	Tetrahydrocannabivarin (THCV), 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	Supelco	**T-094**	**C753Q39**
	Tetrahydrocannabivarinic acid (THCVA), 1.0 mg/mL in acetonitrile, certified reference material, ampule of 1 mL, Cerilliant®	Supelco	**T-111**	---
	Cannabinol (CBN), 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	Supelco	**C-046**	**C915Q01**
	Cannabinolic acid (CBNA), 1.0 mg/mL in acetonitrile, certified reference material, ampule of 1 mL, Cerilliant®	Supelco	**C-153**	---
	Δ9-tetrahydrocannabinol (Δ9-THC), 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	Supelco	**T-005**	**C940Y88**
	Δ8-tetrahydrocannabinol (Δ8-THC), 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	Supelco	**T-032**	**C940Z47**
	Cannabicyclol (CBL), 1.0 mg/mL in acetonitrile, certified reference material, ampule of 1 mL, Cerilliant®	Supelco	**C-154**	**C745K49**
	Cannabichromene (CBC), 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	Supelco	**C-143**	**C753Q14**
	Δ9-tetrahydrocannabinolic acid (THCA), 1.0 mg/mL in acetonitrile, ampule of 1 mL, certified reference material, Cerilliant®	Supelco	**T-093**	**C753Q38**
	Cannabichromenic acid (CBCA), 1.0 mg/mL in acetonitrile, certified reference material, ampule of 1 mL, Cerilliant®	Supelco	**C-150**	---
	Cannabicyclolic acid (CBLA), 0.5 mg/mL in acetonitrile, certified reference material, ampule of 1 mL, Cerilliant®	Supelco	**C-171**	**C745K50**
	Water, Solvents and Chemicals			
	Methanol, UHPLC grade	Sigma-Aldrich	**900688**	**C761T93**
	Formic acid 98% - 100%, for LC-MS, LiChropur™	Supelco	**5330020050**	**C820P83**
	Acetonitrile with 0.1 % (v/v) formic acid for UHPLC	Sigma-Aldrich	**900686**	**C761T99**
	Ammonium formate ≥99.0%, for LC-MS, LiChropur™	Supelco	**70221**	**C989U89**
	Ultrapure water from Milli-Q® system or bottled water	Milli-Q	**Milli-Q® IQ 7005 or 101262**	--- --- or ---

MS_AN6577EN Ver. 1.0
32848
10/2020

REV 8/5/22