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Application News

No. HPLC-045

High Performance Liquid Chromatography

Natural Cannabinoid and Cannflavin Profiling by HPLC-PDA

This application was developed by our partner Front Range Biosciences with generous support from MilliporeSigma.

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■ Introduction

Cannabinoids are a diverse group diterpenoid compounds primarily observed in Cannabis and Rhododendron species. To date, over 120 phytocannabinoids have been identified and quantified in Cannabis extracts using analytical techniques such as High Performance Liquid Chromatography (HPLC). With the federal legalization of hemp, a type of Cannabis, and state-supported legalization measures for high-THC Cannabis, HPLC testing of dried plant material for psychotropic potency and therapeutic dosing has become part of nearly every piece of legislation. While numerous chromatographic methods have been developed for the detection and quantification of THCA, CBDA, CBGA, CBNA, and their decarboxylated forms, many do not account for the possibility of coelutions with other secondary metabolites in plant samples such as cannabinoids, flavonoids, and terpenes. To complicate analyses further, the metabolomes of different Cannabis varieties can vary greatly, resulting in chromatographic coelutions that are present in some extracts but not in others.

The method presented in this application note attempts to resolve most of the significant coelutions common to different types of Cannabis and was designed for laboratories interested in the quantification of minor cannabinoid and cannflavin constituents. Using this method, a total of 34 unique Cannabis analytes were quantified in less than 32 minutes. The method described has been successfully applied to not only leaf and flower Cannabis tissue, but cannabis/hemp products such as concentrates, oils, and cosmetic products.

■ Sample Preparation

Air-dried samples were milled to a powder using stainless steel ball-bearings with stems and seeds mechanically removed after pulverization. Between 0.2 and 0.5 grams of powder aliquots were solvent extracted in 10mL of HPLC-grade acetone using ultrasonication for a total of 30 minutes at a water temperature no greater than 35°C. Sample extracts were syringe-filtered with 0.22 µm PTFE filters, followed by either a 2-fold dilution for leaf extracts or a 5-fold dilution for floral extracts.

■ Experimental

A Shimadzu Prominence-i LC-2030 C 3D Plus system, equipped with an MilliporeSigma Ascentis-C18 Express column and a photodiode array detector (PDA) was utilized to quantitate cannabinoid and cannflavin analytes in dried hemp tissues.

Table 1: Instrument and Mobile Phase Conditions

Column	Ascentis® Express C18, 2.7 µm x 150mm x 3mm
Mobile Phase A	HPLC Water, 8% (v/v) Methanol, 0.035% (v/v) Formic Acid, 1.8mM Ammonium Formate
Mobile Phase B	HPLC Acetonitrile
Column Temperature	24°C
Autosampler Temperature	15°C
Injection Volume	2 µL
Flow Rate	0.45 mL/min

Table 2: Gradient Conditions

Time (min)	% Mobile Phase A	% Mobile Phase B
0	59	41
1	58	42
10	37	63
16	32	68
26	19	81
28	13	87
29.5	0	100
30.5	0	100
31	59	41

Recommended Equilibration Time: 4 Minutes

Table 3: Photodiode Array Detector Conditions

Analyte	Quantitative Wavelength	Analyte	Quantitative Wavelength	Analyte	Quantitative Wavelength
Cannflavin B	340nm	CBG	230nm	CBL	230nm
CBDO	230nm	CBD	230nm	Δ9-THCA	270nm
CBDVA	270nm	THCV	230nm	CBC	280nm
CBDV	230nm	Δ9-THCVA	270nm	CBCA	258nm
CBGV	230nm	CBCV	280nm	CBLA	270nm
CBGVA	270nm	CBDPA	270nm	CBDM	230nm
CBE	230nm	CBCVA	258nm	CBGM	230nm
CBDB	230nm	CBN	280nm	Δ9-THCP	230nm
CBCO	280nm	CBNA	258nm	CBT	230nm
CBDA	270nm	CBDP	230nm	Δ9-THCPA	270nm
Cannflavin A	340nm	Δ9-THC	230nm	**PDA Conditions** Lamp: D2 Cell Temperature: 40°C Polarity: + Slit Width: 8nm	
CBGA	270nm	Δ8-THC	230nm		

Calibrations

Calibration standards were prepared gravimetrically for 34 unique cannabinoids and cannflavins at concentrations ranging from 0.1 ug/mL to 800 ug/mL. Certified Reference Materials (CRM) standards or research grade isolates were obtained from MilliporeSigma, Restek, Caymen Chemical, Purisys, and Toronto Research Chemicals.

The linearity for all compounds was ≥ 0.99 R^2 using linear correlations and a best-fit weighting of 1/Concentration. The UV spectra of each analyte was recorded in a spectral library to assist in positive identification of cannabinoids and cannflavins in plant tissue extracts.

Figure 1: Calibration Curves and UV Spectra with Lambda Max Values

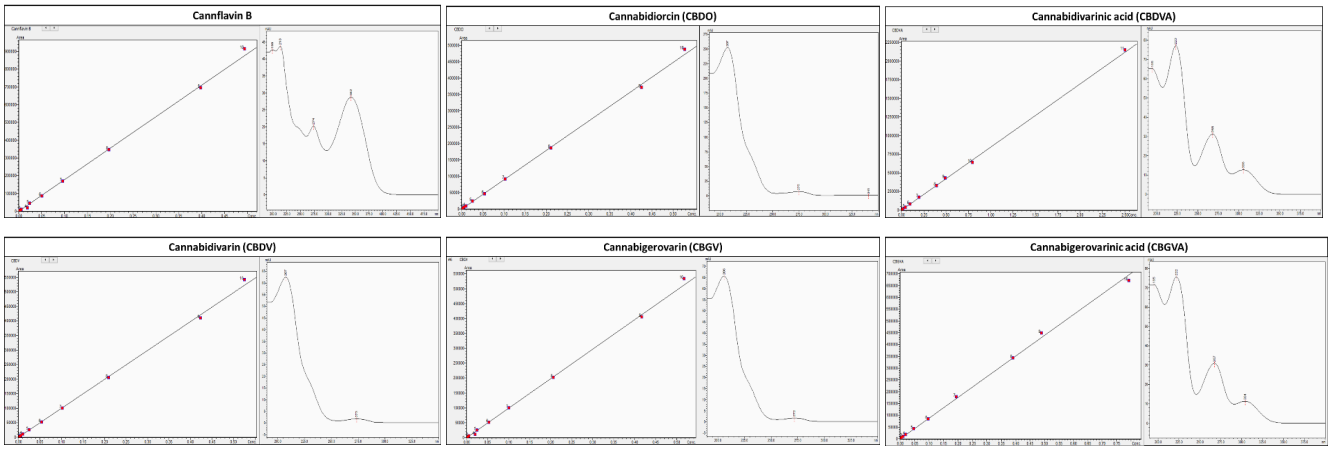


Figure 1 *(Continued)*: Calibration Curves and UV Spectra with Lambda Max Values

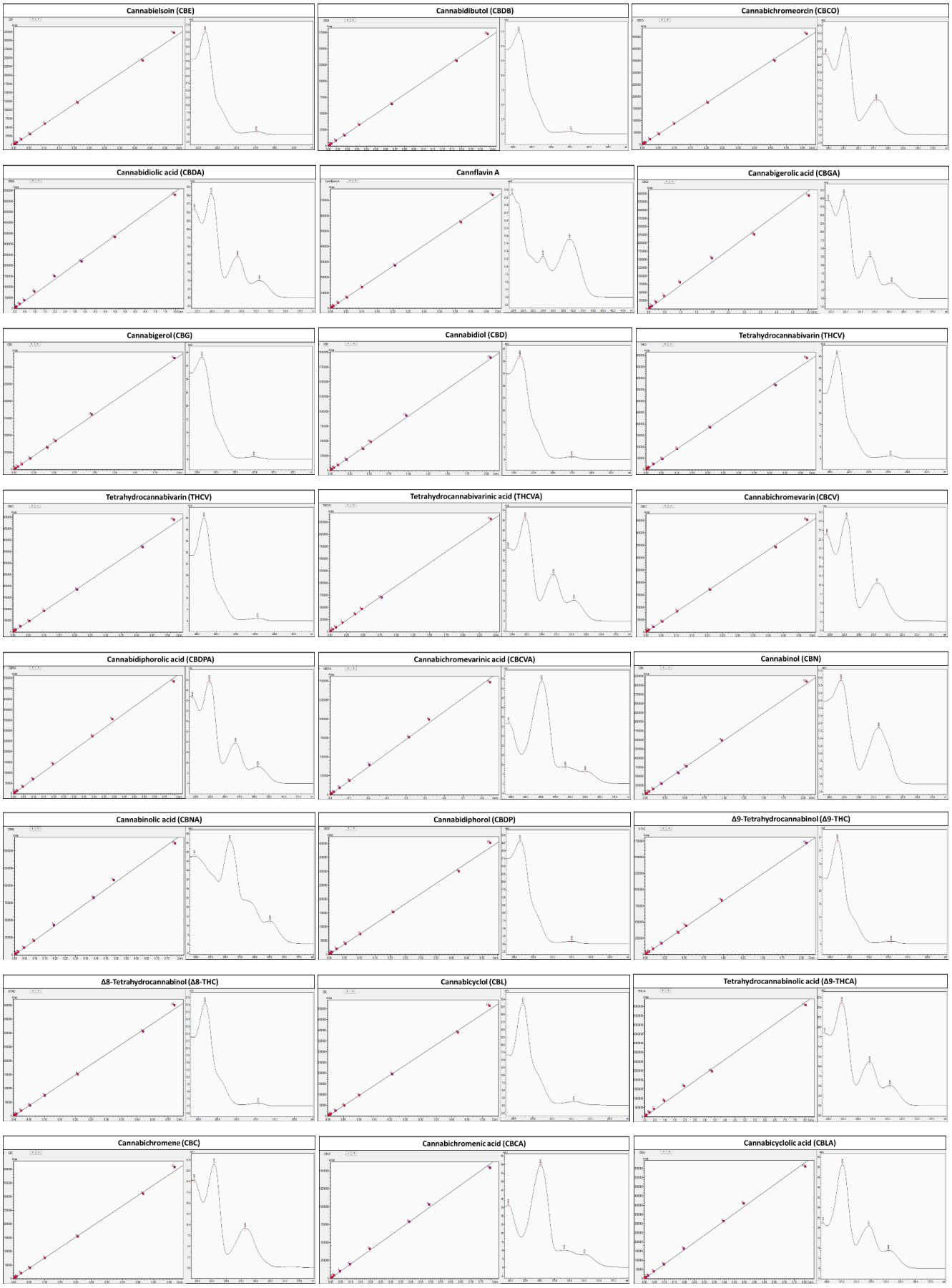
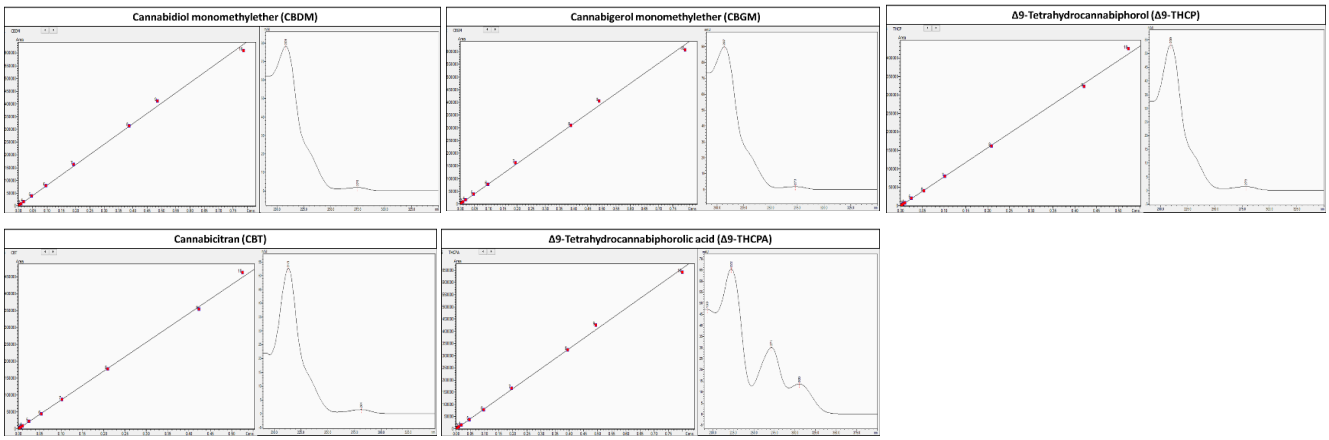


Figure 1 *(Continued)*: Calibration Curves and UV Spectra with Lambda Max Values

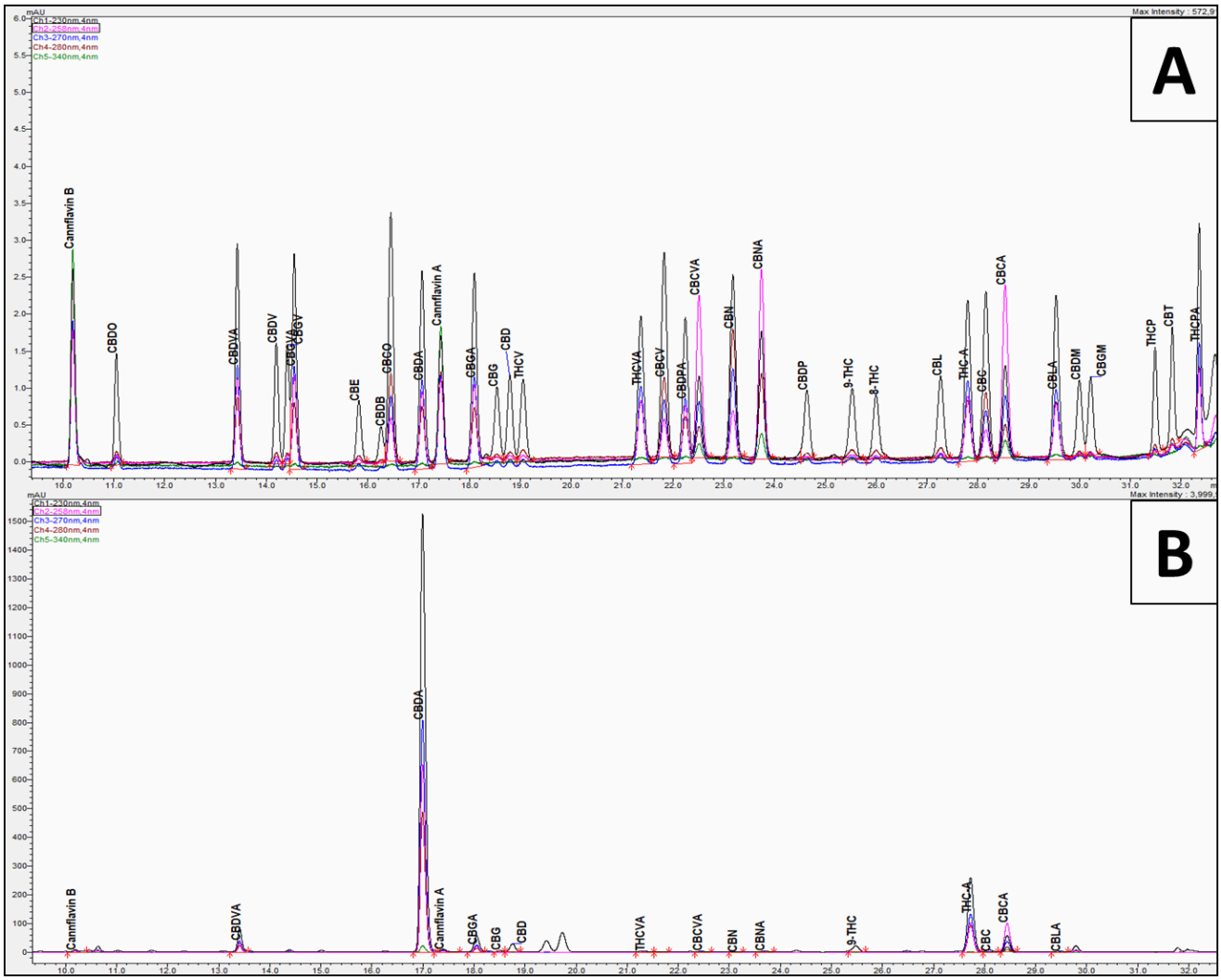


■ Results

Calibrations

USP resolution greater than 1.0 was observed for all analytes. A solvent containing no analytes was applied to all standards and samples for consistent baseline identification.

Figure 2A: Chromatogram of 34 cannabinoids/cannflavins at approximately 1 ug/mL. Single injection with quantitation wavelengths overlaid.
Figure 2B: Chromatogram of mix of hemp acetone extracts at a total dilution of 50X. Single injection with quantitation wavelengths overlaid.



Accuracy and Precision

Accuracy and precision were evaluated by spiking all 34 analytes on to homogenized low-cannabinoid producing Cannabis plant material (Table 4). The concentration of cannabinoids and cannaflavins present in non-spiked Cannabis plant material was subtracted from the observed concentrations in the spiked samples.

To further evaluate the method's accuracy and precision, performance tests were provided by MilliporeSigma and diluted by 5X (Table 5).

Table 4: Average Percent Recoveries and Percent Relative Standard Deviations at Approximately 5 ug/mL On-Column or Approximately 0.05 Weight %. N=3 Replicates.

Analyte	Average Recovery (%)	Relative Standard Deviation (%)	Analyte	Average Recovery (%)	Relative Standard Deviation (%)
Cannflavin B	101	3.5	CBDPA	102	2.4
CBDO	120	7.5	CBCVA	97.4	2.3
CBDVA	97.6	2.5	CBN	95.3	2.8
CBDV	112	2.8	CBNA	97	2.4
CBGV	108	2.0	CBDP	114	3.3
CBGVA	108	3.2	Δ9-THC	99.8	1.9
CBE	111	2.9	Δ8-THC	103	1.3
CBDB	99.5	1.8	CBL	105	5.0
CBCO	101	2.8	Δ9-THCA	99.7	2.1
CBDA	103	2.3	CBC	85.6	4.9
Cannflavin A	111	7.5	CBCA	98.4	2.4
CBGA	100	1.5	CBLA	98.7	2.6
CBG	89.7	3.7	CBDM	101	1.4
CBD	103	3.7	CBGM	98.9	2.1
Δ9-THCV	94.2	3.3	Δ9-THCP	103	2.6
Δ9-THCVA	91.5	0.63	CBT	101	2.1
CBCV	102	2.0	Δ9-THCPA	96.0	0.70

Table 5: Average Percent Recoveries and Percent Relative Standard Deviations of MilliporeSigma PEC6001 (8 Part Neutral Potency) and PEC6002 (6 Part Acid Potency). N=3 Replicates Per Performance Test

Analyte	Average Recovery (%)	Relative Standard Deviation (%)	Analyte	Average Recovery (%)	Relative Standard Deviation (%)
CBDVA	106	2.0	Δ9-THCVA	98.8	1.9
CBDV	103	1.3	CBN	101	1.3
CBDA	123	1.8	Δ9-THC	111	1.2
CBGA	113	2.0	Δ8-THC	107	1.4
CBG	113	1.1	Δ9-THCA	108	1.9
CBD	102	1.5	CBC	106	1.3
Δ9-THCV	104	1.4	CBCA	105	2.1

Limits of Detection

Table 6: Calculated Method Limits of Detection at 50X Total Dilution Factor and 0.2 Grams of Sample.

Analyte	LOD (S/N > 3:1) Wt %	Analyte	LOD (S/N > 3:1) Wt %
Cannflavin B	0.002	CBDPA	0.006
CBDO	0.003	CBCVA	0.006
CBDVA	0.006	CBN	0.003
CBDV	0.003	CBNA	0.006
CBGV	0.003	CBDP	0.003
CBGVA	0.006	Δ9-THC	0.003
CBE	0.003	Δ8-THC	0.003
CBDB	0.001	CBL	0.003
CBCO	0.003	Δ9-THCA	0.005
CBDA	0.005	CBC	0.003
Cannflavin A	0.003	CBCA	0.006
CBGA	0.005	CBLA	0.006
CBG	0.003	CBDM	0.006
CBD	0.003	CBGM	0.006
Δ9-THCV	0.003	Δ9-THCP	0.003
Δ9-THCVA	0.006	CBT	0.003
CBCV	0.003	Δ9-THCPA	0.006

■ Conclusions

A gradient HPLC method was developed for the quantification of 34 unique compounds in Cannabis within a single injection. Solvent consumption per injection was less than 16mL with an injection-to-injection runtime of 35 minutes. The method described allows for the quantitation of major and minor phytocannabinoids in Cannabis with minimal coelutions from flavonoids or terpenes; thus, reducing limits of detection while maintaining accuracy at ≤ ±20% and precision at ≤ ±10%.

First Edition: January 2023

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