Application Note

Agilent
Trusted Answers

Separation of 16 Cannabinoids Using the Agilent 1260 Infinity II LC System

Authors

Brian Whiteley and
William Long
Agilent Technologies, Inc.

Abstract

This application note uses the Agilent 1260 Infinity II LC system to successfully separate and quantify 16 of the prevalent cannabinoid compounds contained in cannabis plants. A total of 16 cannabinoids were chromatographically resolved in under 12 minutes, and baseline resolution was achieved for all 16 compounds.

Introduction

The continued growth of cannabis-infused products will lead to more companies needing to identify and quantify not only the delta-9-THC and CBD content of their products, but to identify all cannabinoids in their products. A search of the literature indicates that as many as 104 cannabinoids have been isolated from *Cannabis sativa* L.[1] Studies have shown that several of these cannabinoids are precursors in the biosynthetic pathway to the formation of CBD and THC.[2] Identifying and calculating all cannabinoid content contained in plant material could be critical for cultivators and processors in identifying material that may fall out of specification based on legal requirements of the 2018 U.S. Farm Bill.

Agilent continues to build on their expertise within the cannabinoid testing market by offering methodology to separate and quantify 16 cannabinoids. This includes:

- Quick, efficient, and reliable separation of 16 cannabinoids
- Integrated hardware and software with intelligent reporting
- Applicability to testing labs, cultivators, processors, and product developers for determining cannabinoid content
- Consulting services to assist and train staff

The methodology outlined in this application note uses gradient conditions to separate and quantify 16 of the prevalent cannabinoids contained in cannabis.

Experimental

Table 1 outlines the target cannabinoids; 11 of the 16 target cannabinoids were provided by Agilent and the rest were provided by Cerilliant Corporation.

Table 2 outlines the LC conditions, and Table 3 outlines the mobile phase gradient. The methodology was performed on an Agilent 1260 Infinity II LC system (Table 4) in low delay volume configuration.

Table 1. Target compounds.

Compound	Agilent Part Number
Cannabidivarinic acid (CBDVA)	
Cannabidivarin (CBDV)	5191-3920
Cannabidiolic acid (CBDA)	5191-3930
Cannabigerolic acid (CBGA)	5191-3927
Cannabigerol (CBG)	5191-3923
Cannabidiol (CBD)	5191-3924
Tetrahydrocannabivarin (THCV)	5191-3921
Tetrahydrocannabivarinic acid (THCVA)	
Cannabinol (CBN)	5191-3926
Cannabinolic acid (CBNA)	
(-)-Δ9-THC (d9-THC)	5191-3929
(-)-Δ8-THC (d8-THC)	5191-3922
(±)-Cannabicyclol (CBL)	
Cannabichromene (CBC)	5191-3928
Δ9-Tetrahydrocannabinolic acid (THCA-A)	5191-3925
Cannabichromenic acid (CBCA)	

Table 2. LC conditions.

Parameter	Value
Column	Agilent InfinityLab Poroshell 120 EC-C18, 3.0 × 150 mm, 2.7 µm (p/n 693575-302)
Guard Column	Agilent InfinityLab Poroshell 120 EC-C18, 3.0 × 5 mm, 2.7 µm (p/n 823750-911)
Mobile Phase	A) 5 mM ammonium formate, 0.1% (v/v) formic acid aqueous phase B) 0.1% (v/v) formic acid in acetonitrile
Flow Rate	0.8 mL/min
Stop Time	11 min
Postrun	3.5
Column Temperature	30 °C
Injection Volume	5 µL
Autosampler Temperature	Ambient
Peak Width	>0.0063 min (0.13 s response time) (40 Hz)
Diode Array Detector	230 nm

Table 3. LC mobile phase gradient.

Time (min)	%B
0	75
10	90
11	90

Table 4. Instrument configuration

Hardware and Software	Product Number
Agilent 1260 Infinity II binary pump	G7112B
Agilent 1260 Infinity II vialsampler	G7129C
Agilent 1260 Infinity II multicolumn thermostat	G7116A
Agilent 1260 Infinity II diode array detector HS	G7117C
Agilent OpenLab CDS Workstation software	Version 2.7

When samples are analyzed, it is suggested that following the procedure in this application note will lead to reliable results and minimum fowling of equipment and columns. The equipment listed can be found in the Agilent brochure entitled "Consumables Kits for Cannabis Analysis" (publication number 5994-1607EN). The following Agilent consumables are used in routine work (described in the following procedure):

- Vial, screw top, amber, write-on spot, deactivated (salinized), certified, 2 mL, 100/pk (part number 5183-2072)
- Cap, screw, green, PTFE/red silicone septa, 100/pk (part number 5182-0718)
- 50 mL centrifuge tubes, 25/pk (part number 5610-2049)
- 15 mL centrifuge tubes, 50/pk (part number 5610-2039)
- 0.45 µm regenerated cellulose (RC) syringe filter, 100/pk (part number 5190-5107)
- Syringe, 5 mL, 100/pk (part number 9301-6476)
- Ceramic homogenizers, for 50 mL tubes, 100/pk (part number 5982-9313)

Sample preparation for plant material

1. Weigh 200 mg of leaf cutting into a 50 mL centrifuge tube. Homogenize using ceramic homogenizers and a commercial grinder.
2. Add 20 mL of methanol. Vortex or shake for 10 minutes (100-fold dilution).
3. Aliquot 1 mL into a new vial. Centrifuge at 5,000 rpm for 5 minutes.
4. Transfer 50 µL of supernatant to a new vial. Add 950 µL of methanol. Mix briefly (20-fold dilution for a total of 2,000-fold).
5. Filter with Agilent Captiva premium syringe filters, regenerated cellulose (RC) membrane, 4 mm, 0.45 µm into an analytical vial.

Sample preparation for oils and concentrates

1. Pipette a 100 µL aliquot of homogenized hemp oil, CBD oil, concentrate, tincture, or resin into a tared 10 mL volumetric flask. Accurately determine and record the weight of the collected product.
2. Add 8 mL of high-purity HPLC- or LC/MS-grade ethanol. Cap and mix well. Bring the volume to 10 mL with ethanol (100-fold dilution). Using a glass syringe fitted with a Captiva premium syringe filter such as 0.45 µm regenerated cellulose (RC) syringe filter, 100/pk, filter 2 mL of the solution into a clean analytical vial.
3. Perform an additional 10-fold dilution of the filtered solution by transferring a 100 µL aliquot into an amber glass 2 mL autosampler vial and adding 900 µL of high-purity HPLC- or LC/MS-grade methanol. Cap and vortex briefly to mix (final dilution factor = 1,000). **Note:** higher dilution factors may be required depending on the product.

Results and discussion

A total of 16 cannabinoids were chromatically resolved in under 12 minutes (Figures 1 and 2). Baseline resolution (>1.5) was achieved for all 16 compounds with emphasis on Δ^8-THC, Δ^9-THC, CBD, CBDA, and THCA, which were deemed critical for regulatory requirements. While 16 compounds are included in this analysis, many additional compounds could potentially be analyzed using this method as there is additional chromatographic space between several compound groups. It could easily expand to 22 or more compounds if the analytes are chosen based on spacing. As can be seen in Figures 1 and 2, the baselines are smooth and slope slightly, allowing easy integration of peaks. In addition, the gradient shown in Table 3 follows a gentle rise in acetonitrile to 90%, allowing removal of all the anticipated impurities.

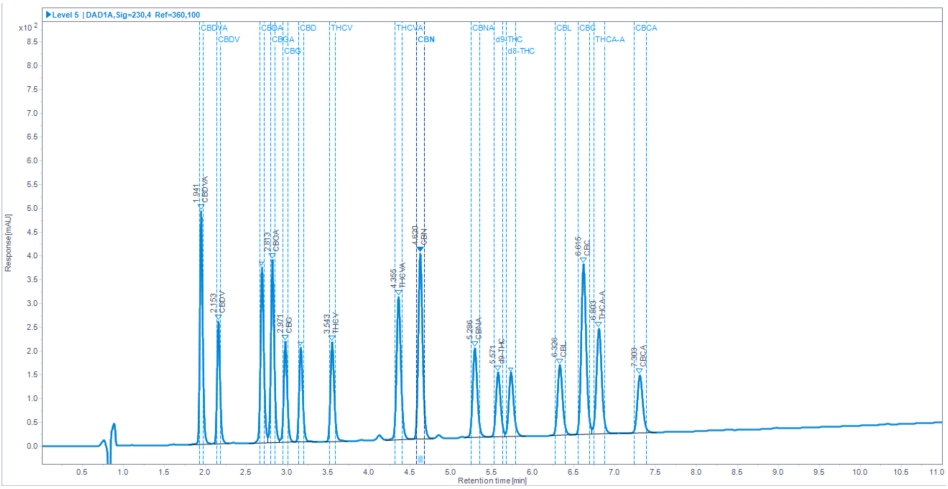


Figure 1. Representative chromatogram: 50 µg/mL injection.

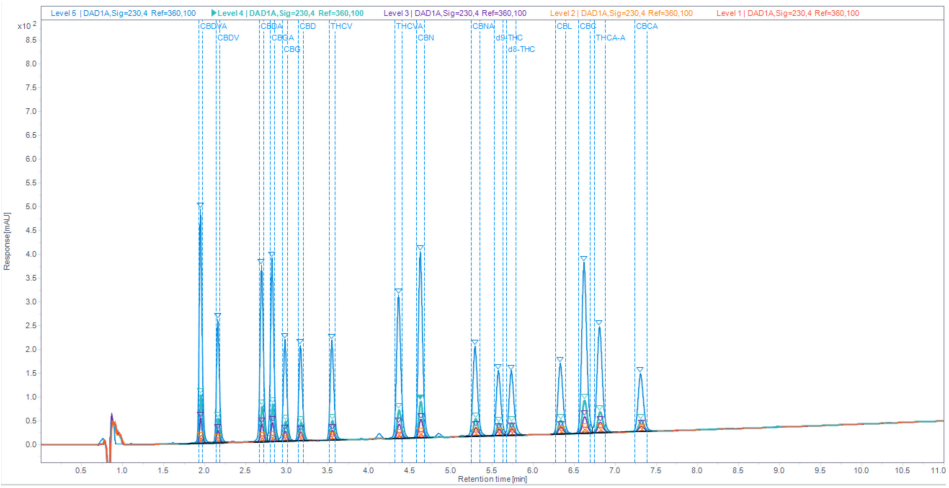
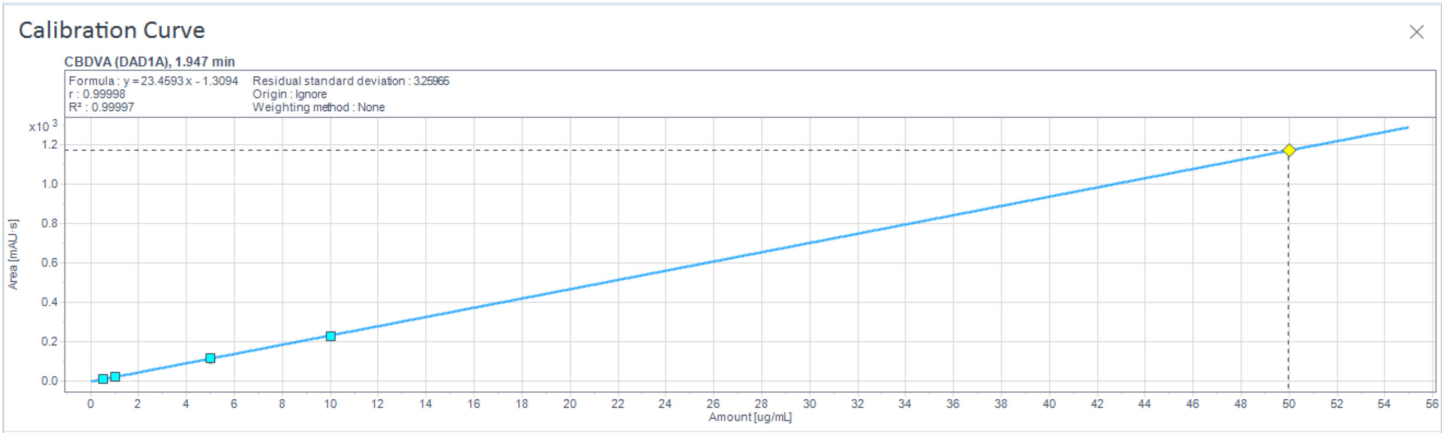
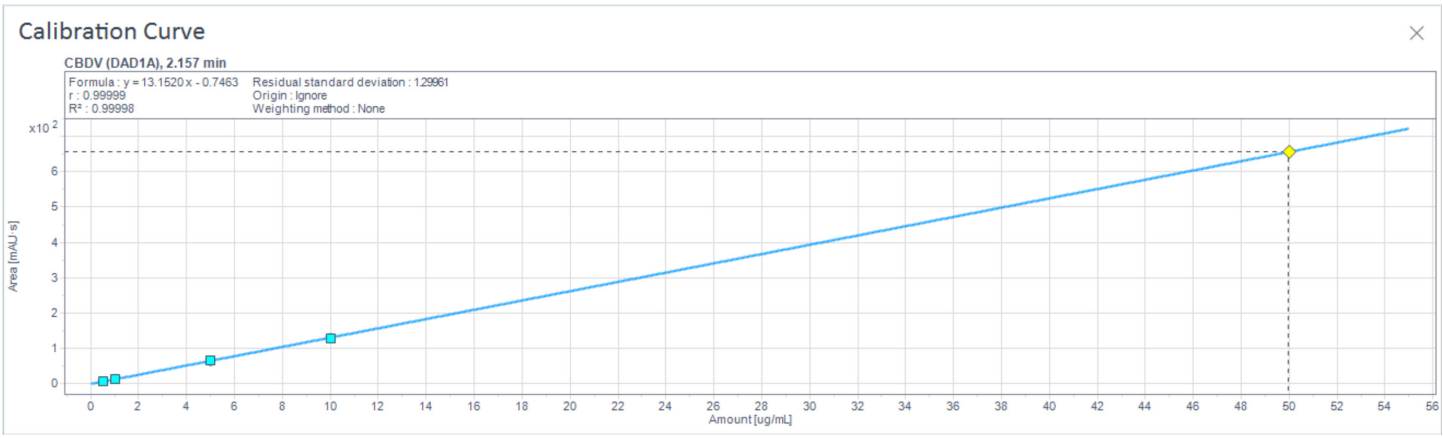
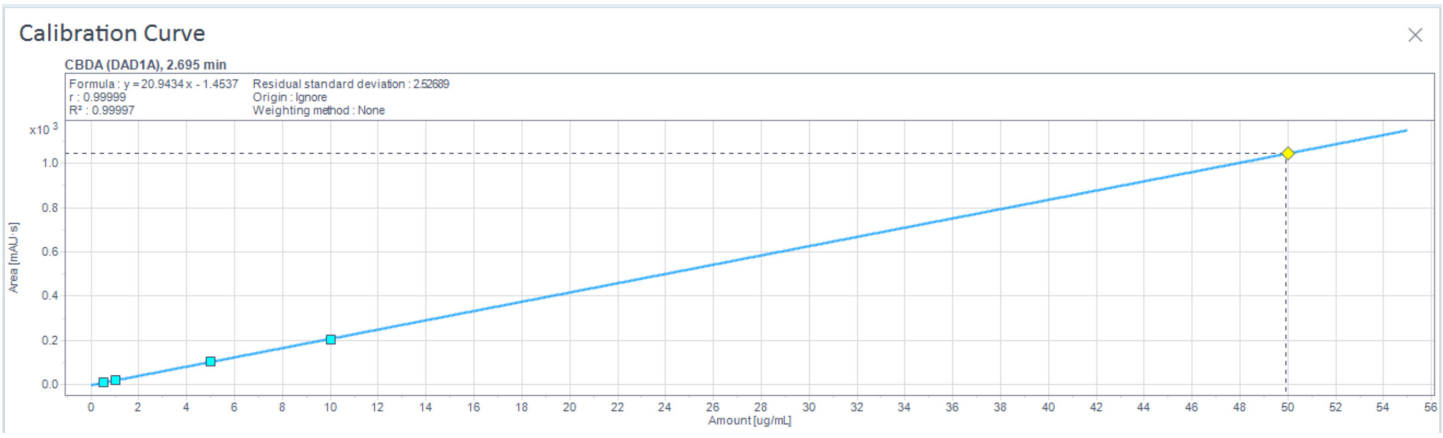


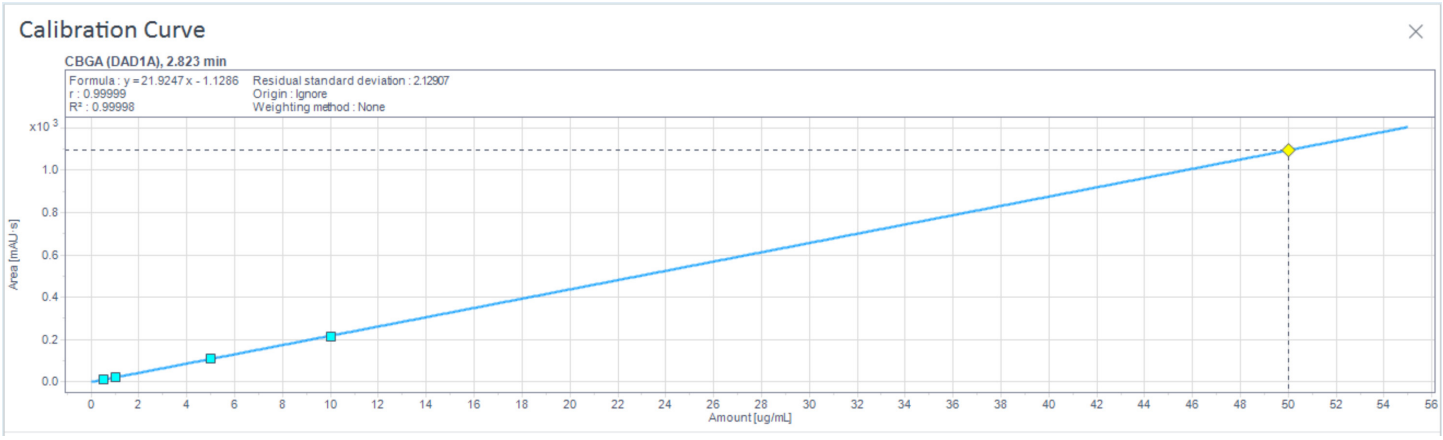
Figure 2. Representative chromatogram: overlay of five injections (0.5 to 50 µg/mL).

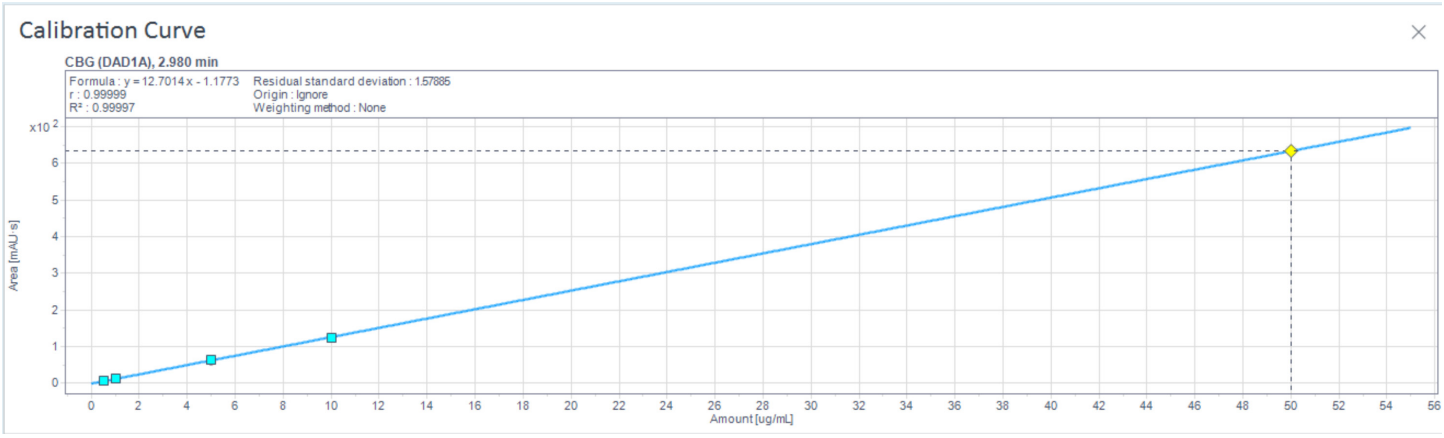
Standard calibrators were prepared from individual, commercially available, certified reference standards (Table 1) at 1.0 mg/mL each in organic solvent. Equal volumes of each reference standard were mixed and diluted with high purity methanol to a high concentration of 50.0 µg/mL. Serial dilutions were made to create a five-point calibration curve at concentrations of 50.0, 10.0, 5.0, 1.0, and 0.5 µg/mL. Externally standardized calibration curves were constructed as response against concentration and used for accuracy, precision, and linearity. Limit of detection (LOD) and limit of quantitation (LOQ) were determined by collecting eight replicate injections of three lowest calibrators. The resulting standard deviation calculation (s) determined via OpenLab CDS was used to calculate LOD as 3.3(s) and LOQ 10(s). Calibration curves are presented in order of retention time (Figure 3). Excellent linearity is demonstrated for all compounds in Figure 3 (calibration curves) and Figure 4 (summary of linearity data). This linearity is shown to hold true across multiple days (three), with correlation coefficients of 0.999 or better. Accuracy of measurements is demonstrated by comparing multiple injections to the calibration curve. This data shows that nearly perfect correlation at 5 µg/mL to 50 µg/mL. Data at 1 µg/mL is generally found to be within 10%.

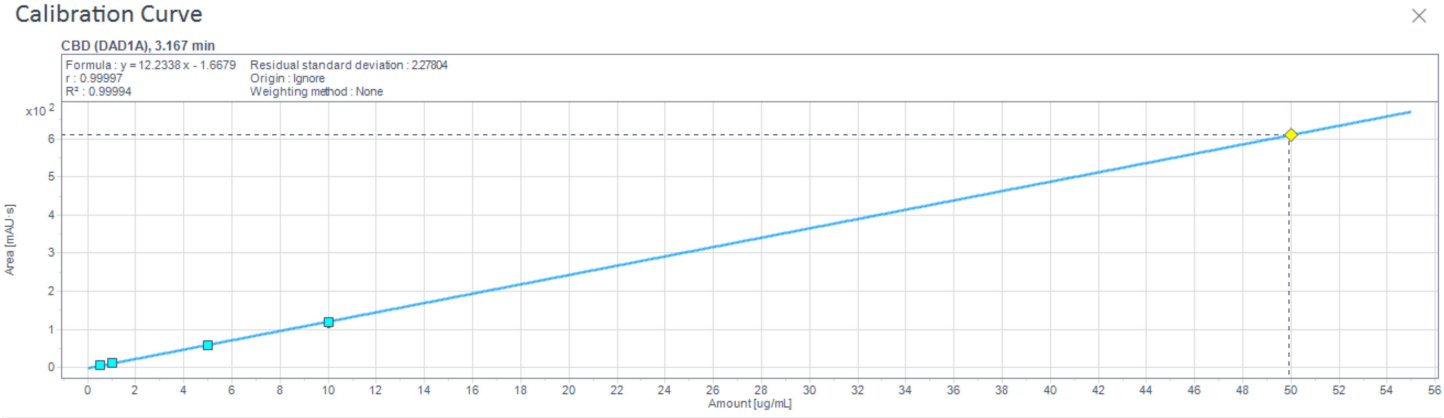

Calibration Curve
CBDVA (DAD1A), 1.947 min
Formula : y = 23.4593 x - 1.3094
r : 0.99998
R² : 0.99997
Residual standard deviation : 3.25965
Origin : Ignore
Weighting method : None
x10 3
Area [mAU*s]
Amount [ug/mL]

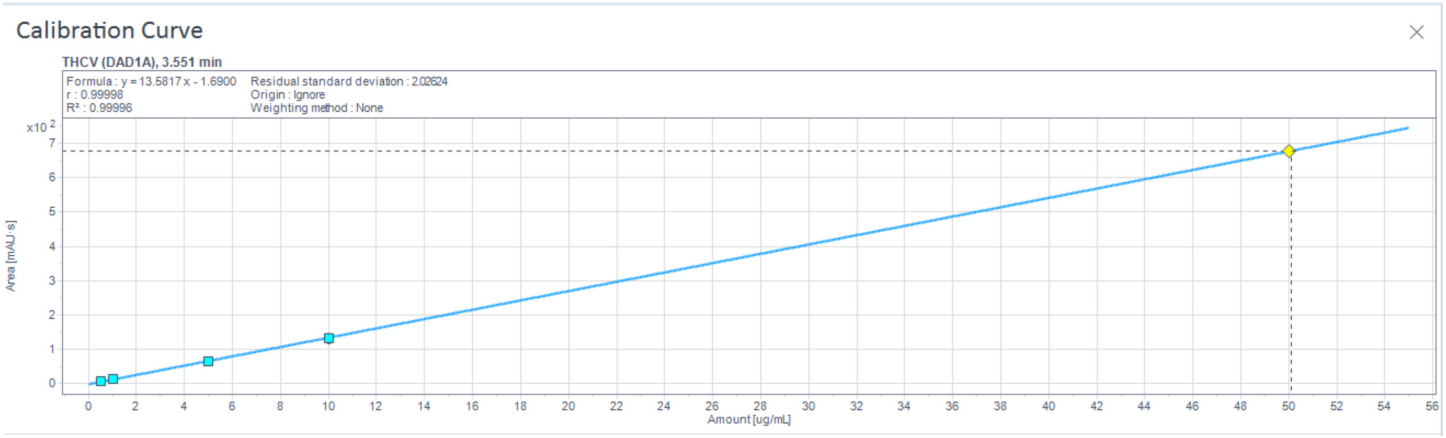

Calibration Curve
CBDV (DAD1A), 2.157 min
Formula : y = 13.1520 x - 0.7463
r : 0.99999
R² : 0.99998
Residual standard deviation : 1.29961
Origin : Ignore
Weighting method : None
x10 2
Area [mAU*s]
Amount [ug/mL]

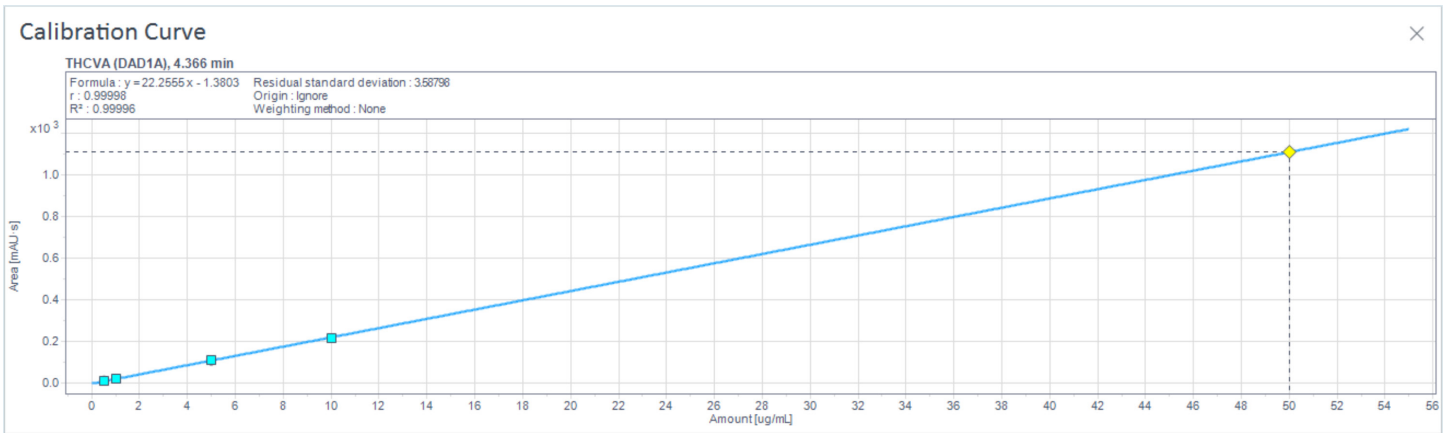

Calibration Curve
CBDA (DAD1A), 2.695 min
Formula : y = 20.9434 x - 1.4537
r : 0.99999
R² : 0.99997
Residual standard deviation : 2.52689
Origin : Ignore
Weighting method : None
x10 3
Area [mAU*s]
Amount [ug/mL]

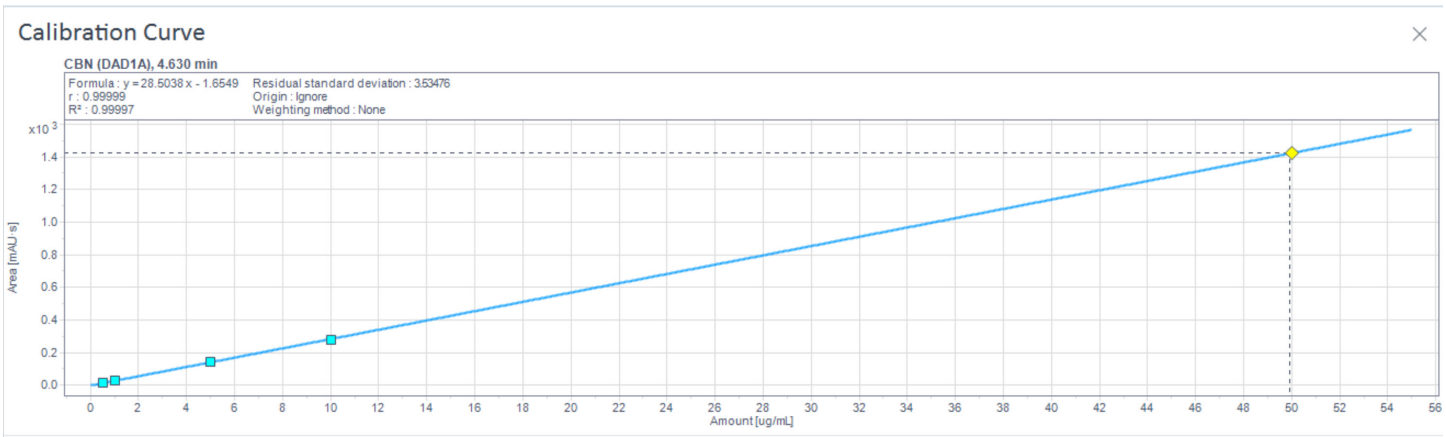

Calibration Curve
CBGA (DAD1A), 2.823 min
Formula : y = 21.9247 x - 1.1286
r : 0.99999
R² : 0.99998
Residual standard deviation : 2.12907
Origin : Ignore
Weighting method : None
x10 3
Area [mAU s]
Amount [ug/mL]


Calibration Curve
CBG (DAD1A), 2.980 min
Formula : y = 12.7014 x - 1.1773
r : 0.99999
R² : 0.99997
Residual standard deviation : 1.57885
Origin : Ignore
Weighting method : None
x10 2
Area [mAU s]
Amount [ug/mL]


Calibration Curve
CBD (DAD1A), 3.167 min
Formula : y = 12.2338 x - 1.6679
r : 0.99997
R² : 0.99994
Residual standard deviation : 2.27804
Origin : Ignore
Weighting method : None
x10 2
Area [mAU s]
Amount [ug/mL]


Calibration Curve
THCV (DAD1A), 3.551 min
Formula : y = 13.5817 x - 1.6900
r : 0.99998
R² : 0.99996
Residual standard deviation : 2.02624
Origin : Ignore
Weighting method : None
x10 2
Area [mAU*s]
Amount [ug/mL]

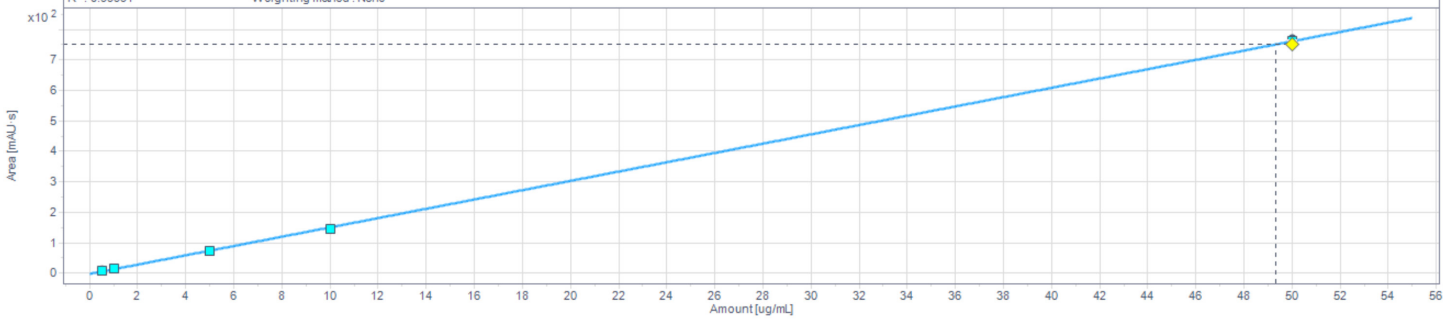

Calibration Curve
THCVA (DAD1A), 4.366 min
Formula : y = 22.2555 x - 1.3803
r : 0.99998
R² : 0.99996
Residual standard deviation : 3.58798
Origin : Ignore
Weighting method : None
x10 3
Area [mAU*s]
Amount [ug/mL]


Calibration Curve
CBN (DAD1A), 4.630 min
Formula : y = 28.5038 x - 1.6549
r : 0.99999
R² : 0.99997
Residual standard deviation : 3.53476
Origin : Ignore
Weighting method : None
x10 3
Area [mAU*s]
Amount [ug/mL]

Calibration Curve

CBNA (DAD1A), 5.302 min

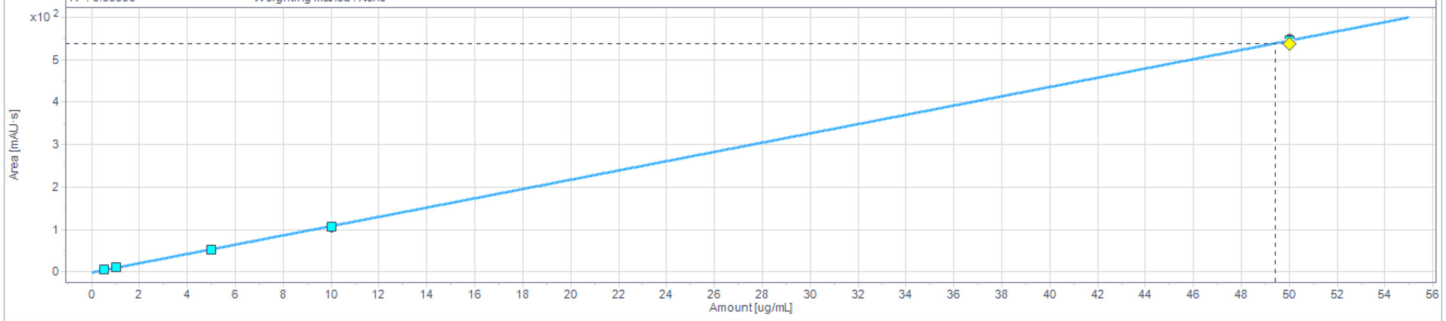
Formula : y = 15.2824 x - 2.2715
r : 0.99995
R² : 0.99991
Residual standard deviation : 3.56544
Origin : Ignore
Weighting method : None



Calibration Curve

d9-THC (DAD1A), 5.586 min

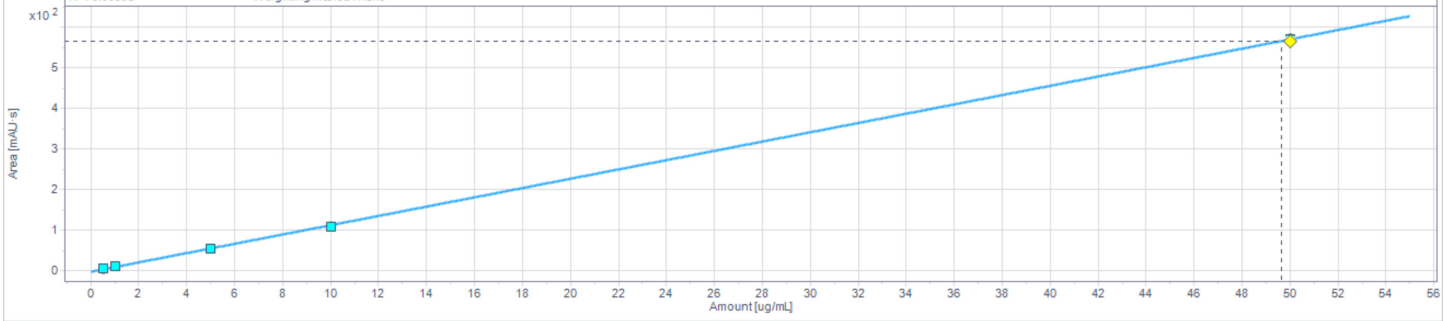
Formula : y = 10.9369 x - 1.2993
r : 0.99998
R² : 0.99996
Residual standard deviation : 1.57469
Origin : Ignore
Weighting method : None

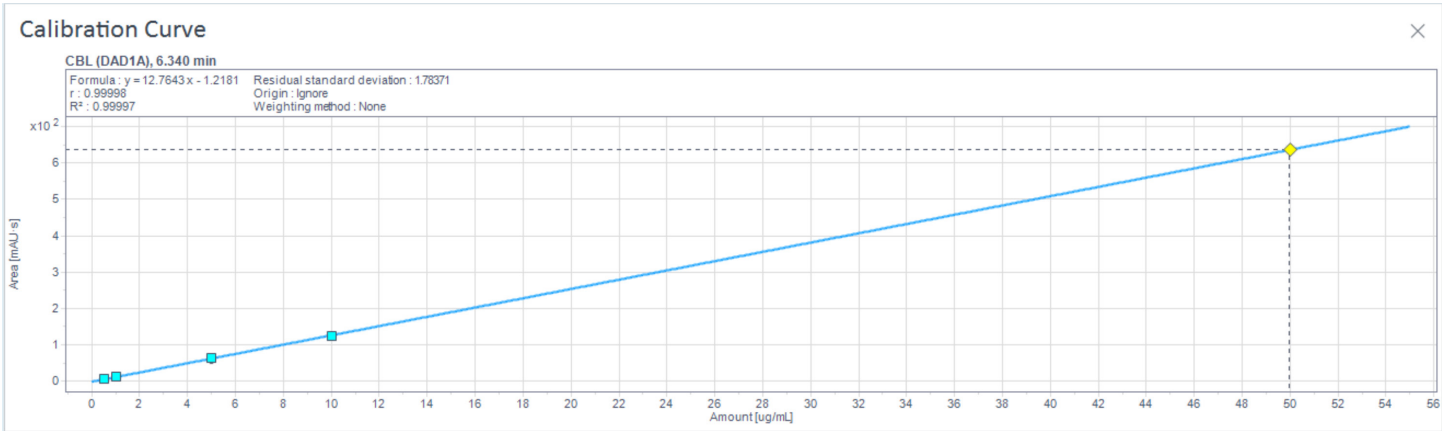


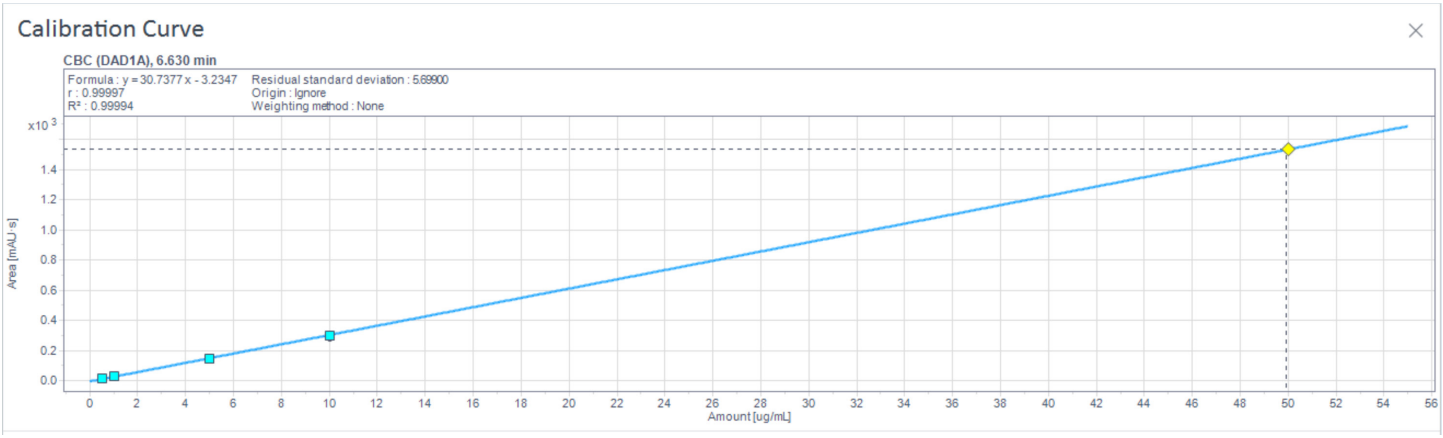
Calibration Curve

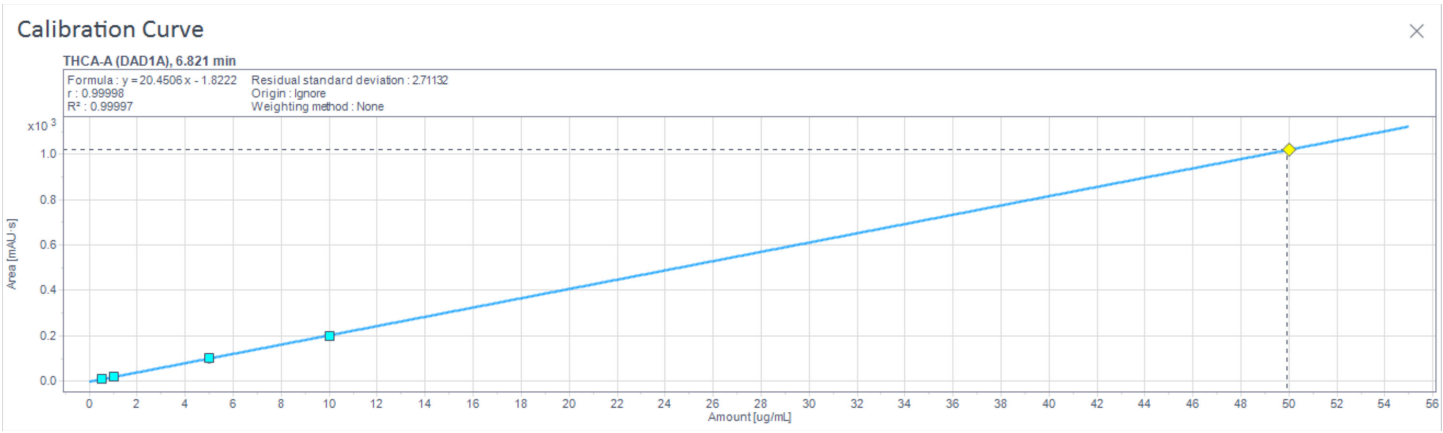
d8-THC (DAD1A), 5.743 min

Formula : y = 11.4414 x - 1.8716
r : 0.99998
R² : 0.99995
Residual standard deviation : 1.91044
Origin : Ignore
Weighting method : None




Calibration Curve
CBL (DAD1A), 6.340 min
Formula : y = 12.7643 x - 1.2181
r : 0.99998
R² : 0.99997
Residual standard deviation : 1.78371
Origin : Ignore
Weighting method : None
x10 2
Area [mAU s]
Amount [ug/mL]


Calibration Curve
CBC (DAD1A), 6.630 min
Formula : y = 30.7377 x - 3.2347
r : 0.99997
R² : 0.99994
Residual standard deviation : 5.69900
Origin : Ignore
Weighting method : None
x10 3
Area [mAU s]
Amount [ug/mL]


Calibration Curve
THCA-A (DAD1A), 6.821 min
Formula : y = 20.4506 x - 1.8222
r : 0.99998
R² : 0.99997
Residual standard deviation : 2.71132
Origin : Ignore
Weighting method : None
x10 3
Area [mAU s]
Amount [ug/mL]

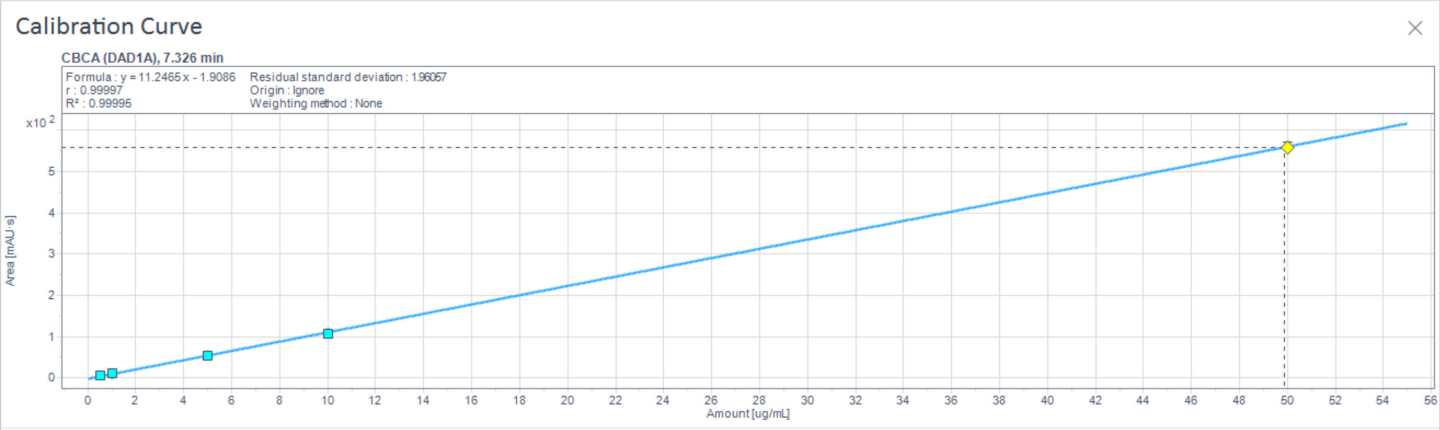


Figure 3. Calibration curves in order of retention time.

Table 5. Instrument linearity.

Compound	Instrument Linearity (R^2): Ignore Origin, No Weighting			
	Day 1	Day 2	Day 3	Average
CBDVA	0.9999	0.9999	0.9998	0.9999
CBDV	0.9999	0.9999	0.9996	0.9998
CBDA	0.9999	0.9999	0.9999	0.9999
CBGA	0.9999	0.9999	0.9998	0.9999
CBG	0.9999	0.9999	0.9995	0.9998
CBD	0.9999	0.9999	0.9995	0.9998
THCV	0.9999	0.9999	0.9998	0.9999
THCVA	0.9999	0.9999	0.9997	0.9998
CBN	0.9999	0.9999	0.9998	0.9999
CBNA	0.9999	0.9999	0.9996	0.9998
d9-THC	0.9999	0.9999	0.9996	0.9998
d8-THC	0.9999	0.9999	0.9997	0.9998
CBL	0.9999	0.9999	0.9998	0.9999
CBC	0.9999	0.9999	0.9997	0.9998
THCA-A	0.9999	0.9999	0.9998	0.9999
CBCA	0.9999	0.9999	0.9997	0.9998

Table 6. Accuracy of analysis.

Compound by RT	Concentration (g/mL)	Accuracy (%)			
		Day 1	Day 2	Day 3	Average
CBDVA	50	100.1	100.1	100.1	100.1
	10	98	98.2	97	97.7
	5	99.9	99.9	97.4	99.1
	1	109.2	109.3	123.9	114.1
	0.5	116	111.5	125	117.5
CBDV	50	100.1	100.1	100.2	100.1
	10	98.5	97.9	94.5	97.0
	5	100.3	99.4	96.4	98.7
	1	106.2	111.5	134.9	117.5
	0.5	109.1	116.7	152.2	126.0
CBDA	50	100.1	100.1	100.1	100.1
	10	98.5	98.6	96.6	97.9
	5	100.3	100.1	97.4	99.3
	1	105.7	105.6	124.3	111.9
	0.5	109.6	111.3	130	117.0
CBGA	50	100.1	100.1	100.1	100.1
	10	98.5	98.6	96.6	97.9
	5	100.3	100.1	97.4	99.3
	1	105.7	105.6	124.3	111.9
	0.5	109.6	111.3	130	117.0
CBG	50	100.1	100.1	100.3	100.2
	10	98.2	97.2	93.4	96.3
	5	100	99.6	95.4	98.3
	1	106.2	111.8	140.3	119.4
	0.5	117.6	126.8	169.2	137.9
CBD	50	100.1	100.1	100.3	100.2
	10	97.6	98	93.8	96.5
	5	98.5	98.1	94.9	97.2
	1	112.46	111.9	139.8	121.4
	0.5	128.6	125	168.8	140.8
THCV	50	100.1	100.1	100.2	100.1
	10	98.1	98.8	96.4	97.8
	5	98.7	98.3	96	97.7
	1	110.8	107	128.4	115.4
	0.5	121.8	121.2	137.7	126.9
THCVA	50	100.1	100.1	100.2	100.1
	10	97.7	97.7	95.3	96.9
	5	99.5	98.7	95.7	98.0
	1	109.9	113.5	133.3	118.9
	0.5	122	123.4	149.8	131.7

Compound by RT	Concentration (g/mL)	Accuracy (%)			
		Day 1	Day 2	Day 3	Average
CBN	50	100.1	100.1	100.2	100.1
	10	98.2	98.5	96.1	97.6
	5	100	99.5	96.7	98.7
	1	108.5	108	128.7	115.1
	0.5	113.1	112.1	136.4	120.5
CBNA	50	100.1	100.1	100.3	100.2
	10	96.8	96.9	94.2	96.0
	5	98.8	98.4	95.4	97.5
	1	111.3	115.1	138.3	121.6
	0.5	140	134.3	159.9	144.7
d9-THC	50	100.1	100.1	100.2	100.1
	10	98	97.8	94.6	96.8
	5	99.5	98.4	96.2	98.0
	1	109.3	112.7	136.7	119.6
	0.5	119.3	125	149.1	131.1
d8-THC	50	100.1	100.1	100.2	100.1
	10	97.5	97.9	95.6	97.0
	5	100.1	98.8	96	98.3
	1	109.8	111.8	133.5	118.4
	0.5	120.2	121.2	140.8	127.4
CBL	50	100.1	100.1	100.2	100.1
	10	98	98.7	96.1	97.6
	5	99.9	99.1	96.9	98.6
	1	107.3	108.3	127.6	114.4
	0.5	120	113.2	135.8	123.0
CBC	50	100.1	100.1	100.2	100.1
	10	97.2	97.9	95.4	96.8
	5	99.5	99.1	96.4	98.3
	1	111.6	110.6	130.8	117.7
	0.5	124.5	120.5	146.6	130.5
THCA-A	50	100.1	100.1	100.2	100.1
	10	90.9	98.5	96.5	95.3
	5	100.1	99.8	97.1	99.0
	1	104.4	107.3	124.5	112.1
	0.5	121.7	111.4	133.8	122.3
CBCA	50	100.1	100.1	100.2	100.1
	10	97.5	98.2	95.3	97.0
	5	99.7	98.6	96.4	98.2
	1	107.6	108.2	130.8	115.5
	0.5	128.3	125.3	148.2	133.9

Table 7. Precision of analysis.

Compound by RT	Concentration (µg/mL)	Precision (%RSD)		
		Day 1	Day 2	Day 3
CBDVA	50	0.12	0.41	0.19
	10	0.22	0.33	0.50
	5	0.56	1.06	0.98
	1	2.34	2.69	1.29
	0.5	6.79	1.61	2.67
CBDV	50	0.12	0.82	0.21
	10	0.81	0.36	0.82
	5	1.10	1.57	1.62
	1	1.05	1.84	1.33
	0.5	0.31	1.94	3.25
CBDA	50	0.17	0.28	0.16
	10	0.16	1.05	1.62
	5	0.26	0.85	0.50
	1	1.65	3.52	0.70
	0.5	0.76	4.18	4.98
CBGA	50	0.11	0.32	0.29
	10	0.13	1.87	1.78
	5	0.36	1.33	0.79
	1	1.63	0.90	0.46
	0.5	1.35	2.70	1.43
CBG	50	0.11	0.27	0.20
	10	0.31	2.58	2.19
	5	0.51	1.92	1.03
	1	1.69	0.80	1.76
	0.5	4.03	2.51	5.01
CBD	50	0.16	0.30	0.20
	10	0.66	0.36	1.66
	5	0.16	0.28	0.42
	1	1.19	0.57	0.63
	0.5	2.99	1.62	1.62
THCV	50	0.13	0.20	0.29
	10	1.83	0.68	1.51
	5	1.08	0.64	1.44
	1	0.60	0.65	0.41
	0.5	4.19	10.12	1.39
THCVA	50	0.11	0.32	0.16
	10	0.56	0.44	1.42
	5	0.20	0.70	1.17
	1	2.97	1.41	1.27
	0.5	17.61	1.80	6.66

Compound by RT	Concentration (µg/mL)	Precision (%RSD)		
		Day 1	Day 2	Day 3
CBN	50	0.18	0.27	0.12
	10	0.21	0.44	1.72
	5	0.21	0.55	0.42
	1	1.05	0.54	0.52
	0.5	2.62	0.57	1.28
CBNA	50	1.22	0.28	0.22
	10	0.68	1.51	2.75
	5	1.16	0.35	0.36
	1	0.63	1.33	0.47
	0.5	17.85	2.55	1.14
d9-THC	50	1.14	0.18	0.24
	10	1.11	1.06	1.94
	5	0.13	0.41	1.76
	1	2.13	2.55	4.14
	0.5	8.10	6.99	2.60
d8-THC	50	0.62	0.35	0.31
	10	0.27	0.97	1.64
	5	0.69	0.43	1.62
	1	2.82	4.06	6.22
	0.5	7.22	4.25	2.09
CBL	50	0.16	0.35	0.22
	10	0.15	0.15	0.15
	5	1.35	0.47	1.77
	1	1.62	1.32	5.92
	0.5	12.77	3.78	11.13
CBC	50	0.17	0.27	0.15
	10	0.13	0.16	1.73
	5	0.21	0.49	0.34
	1	1.17	1.67	0.97
	0.5	3.12	3.59	43.67
THCA-A	50	0.25	0.20	0.23
	10	0.35	0.27	1.72
	5	1.11	0.67	0.93
	1	2.67	3.56	3.62
	0.5	5.77	6.23	2.82
CBCA	50	0.25	0.32	0.15
	10	0.96	0.77	1.69
	5	1.70	15.21	0.60
	1	3.81	3.62	2.59
	0.5	6.49	1.45	1.59

Table 8. LOD/LOQ calculation at 0.5 µg/mL.

Compound	LOD	LOQ
CBDVA	0.02	0.19
CBDV	0.05	0.15
CBDA	0.06	0.19
CBGA	0.03	0.08
CBG	0.03	0.10
CBD	0.02	0.07
THCV	0.07	0.20
THCVA	0.07	0.22
CBN	0.04	0.12
CBNA	0.04	0.13
d9-THC	0.06	0.19
d9-THC	0.07	0.21
CBL	0.04	0.12
CBC	0.08	0.24
THCA-A	0.08	0.25
CBCA	0.14	0.43

Conclusion

This application note outlines a method to separate and quantify 16 cannabinoids in under 12 minutes. The Agilent 1260 Infinity II LC system coupled with the Agilent OpenLab CDS Workstation software provides a convenient platform for testing labs, cultivators, processors, and product developers in the cannabis industry to evaluate cannabinoid content in various products. Agilent also offers consulting services to assist customers in implementing this methodology in their laboratory.

References

1. ElSohly, M.; Gul, W. Constituents of *Cannabis Sativa*. *In* Handbook of Cannabis, Pertwee, R., Oxford University Press, **2014**.
2. Hillig, K. W.; Mahlberg, P. G. A Chemotaxonomic Analysis of Cannabinoid Variation in Cannabis (*Cannabaceae*). *Am. J. Bot.* **2004**, *91*, 966–975. DOI: 10.3732/ajb.91.6.966

Note: Agilent products and solutions are intended to be used for cannabis quality control and safety testing in laboratories where such use is permitted under state/country law.

www.agilent.com

DE26918354

Printed in the USA, November 21, 2022
5994-5519EN

Agilent
Trusted Answers