Application Note

Cannabis & Hemp Testing

Agilent

Trusted Answers

Dedicated Cannabinoid Potency Testing in Cannabis or Hemp Products Using the Agilent 1220 Infinity II LC System

Authors

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Abstract

Agilent offers a proven solution for rapid, robust, accurate, and precise quantification of targeted cannabinoids commonly found in cannabis flower and hemp.

- Quantify cannabinoids for quality, safety, and compliance
- Buy or lease the best analytical equipment
- Rapid method implementation with our dedicated method
- Consulting services to assist and train your staff

Introduction

The Agilent 1220 Infinity II LC system offers reliable and robust profiling and quantification for 11 of the most commonly targeted cannabinoids found in marijuana (*Cannabis Sativa*) and hemp products derived from the plant material. The method provides sample preparation guidelines, and defines the analytical column, mobile phase modifiers, and full system parameters to separate the targeted cannabinoids under 10 minutes. The method was tested and vetted on two independent 1220 Infinity II LC systems. Results of inter- and intraday accuracy, precision, and linearity are presented in Tables 5–7. A custom report providing total potency and total CBD results is also included.

The information content of this application note, along with ready to run acquisition, quantitation, etc. methods and extensive support information are available as eMethod G5277#010, Cannabis Potency test with the 1220VL LC/VWD system.

Experimental

Table 1. Target compounds.

Cannabidivarin (CBDV)
Tetrahydrocannabivarin (THCV)
(−)-*trans*-Δ9-tetrahydrocannabinol (THC)
Cannabidiol (CBD)
Cannabigerol (CBG)
Δ9-tetrahydrocannabinolic acid (THCA-A)
Cannabidiolic acid (CBDA)
Cannabinol (CBN)
Cannabigerolic acid (CBGA)
Cannabichromene (CBC)
(−)-Δ8-THC

Table 2. Hardware and consumables.

Hardware and Software	Part Number
Agilent 1220 Infinity II LC Gradient System VL Includes gradient pump (maximum pressure 400 bar) with integrated degassing unit, autosampler, column oven, and variable wavelength detector with standard flow cell.	G4290C
Agilent OpenLAB CDS VL Workstation	M8417AA

Consumables	Part Number
Agilent InfinityLab Poroshell 120 EC-C18, 3.0 × 50 mm, 2.7 µm	699975-302
0.45 µm Regenerated cellulose (RC) syringe filter	5190-5107
Formic acid	G2453-85060
LC/MS-grade water, 4 L	5190-6897
LC/MS-grade methanol. 1 L	5190-6896
Ceramic Homogenizers, 50 mL tubes, 100/pk	5982-9313

Mobile phase preparation

- **0.1 % (v/v) Formic acid aqueous phase:** In a 1-L Class A volumetric flask, add approximately 500 mL high-purity HPLC-grade water (5190-6897). Add 1.0 mL formic acid (G2452-85060). Gently swirl to mix and Q.S. to 1 L. Mix well and transfer to an HPLC mobile phase bottle.
- **0.05 % (v/v) Formic acid organic phase:** In a 1-L Class A volumetric flask, add approximately 500 mL high-purity HPLC-grade methanol (5190-6896). Add 0.5 mL formic acid (G2452-85060). Gently swirl to mix and Q.S. to 1 L. Mix well and transfer to an HPLC mobile phase bottle.

Table 3. LC conditions.

Parameter	Value
Column	Agilent InfinityLab Poroshell 120 EC-C18, 3.0 × 50 mm, 2.7 µm
Mobile phase	A) 0.1 % (V/V) Formic Acid Aqueous Phase B) 0.05 % (V/V) Formic Acid Organic Phase
Flow rate	1.0 mL/min
Stop time	9.5 minutes
Post run	1.5 minutes
Column temperature	50 °C isothermal
Injection volume	5.0 µL
Autosampler temperature	Ambient
Peak width	>0.0063 minutes (0.13 seconds response time) (80 Hz)
Variable wavelength detector	230 nm

Table 4. LC mobile phase gradient.

Time (min)	% B
0	60
1.0	60
7.0	77
8.2	95

Sample preparation for flower or hemp plant material

1. Weigh 200 mg flower/leaf cutting into a 50-mL centrifuge tube. Homogenize using ceramic homogenizers, and a commercial grinder.
2. Add 20 mL of methanol. Vortex/shake for 10 minutes (A 100-fold dilution).
3. Aliquot 1 mL into a new vial. Centrifuge at 5,000 rpm for five minutes.
4. Transfer 50 µL of supernatant to a new vial. Add 950 µL methanol. Mix briefly (20 fold dilution for a total dilution of 2,000-fold).
5. Filter with 4 mm, 0.45 µm regenerated cellulose (RC) syringe filters (p/n 5190-5107).

Sample preparation for oils and concentrates

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. Add 8 mL 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 0.45 µm regenerated cellulose (RC) syringe filter (part number 5190-5107), filter 2 mL of the solution into a clean glass vessel. Perform an additional 10-fold dilution of the filtered solution by transferring a 100 µL aliquot into an amber glass 2 mL auto-sampler vial and adding 900 µL high purity HPLC or LC-MS grade methanol. Cap and vortex briefly to mix (final dilution factor = 1,000). Please note, depending on the product higher dilution factors may be required.

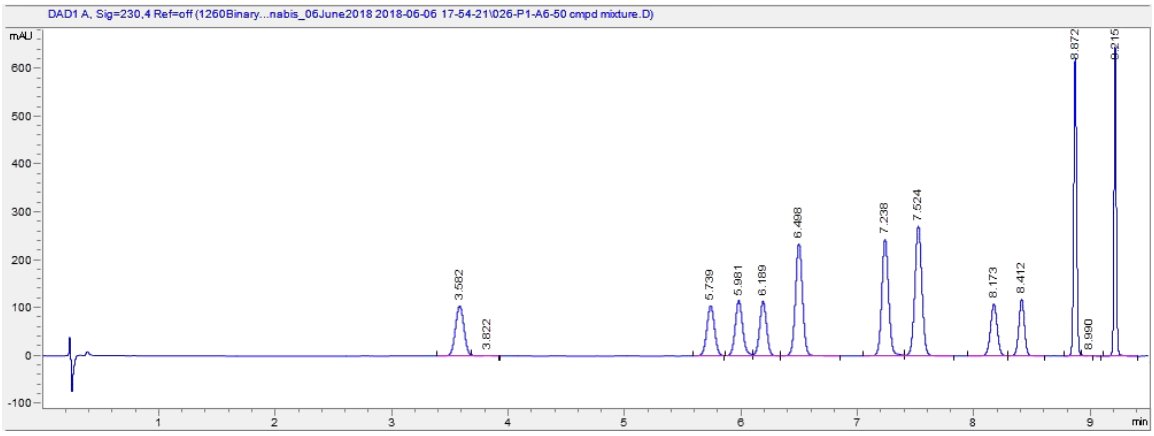
No QuEChERS sample preparation method for potency for the following reasons:

- Must add water to cannabis flower: Upon this addition, the suspension becomes very basic within two to four minutes. This converts THCA to THC and may cause additional breakdown products such as CBDA conversion to CBD.
- Addition of QuEChERS salts causes an exothermic reaction: In the presence of water, this will also cause decarboxylation of THCA to THC.

Calibration curves

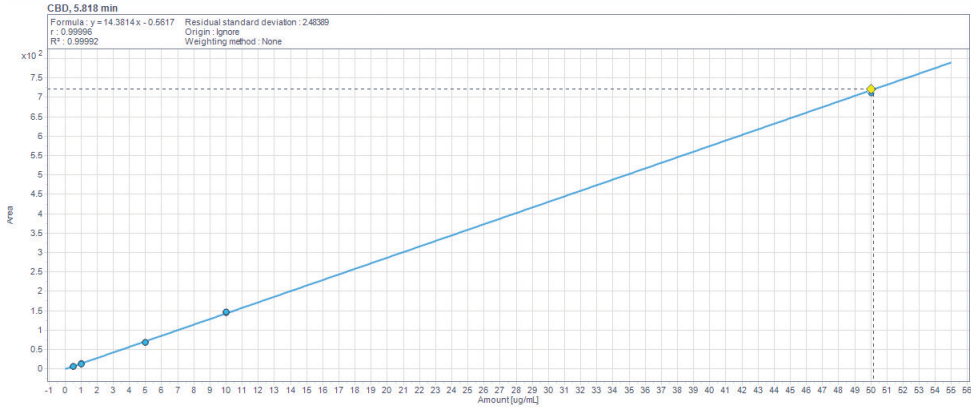
Standard calibrators were prepared from individual commercially available certified reference standards at 1.0 mg/mL each in organic solvent. Equal volumes of each reference standard were mixed and diluted with high-purity methanol to a high concentration of 50.0 µg/mL. Serial dilutions were made to 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 determinations.

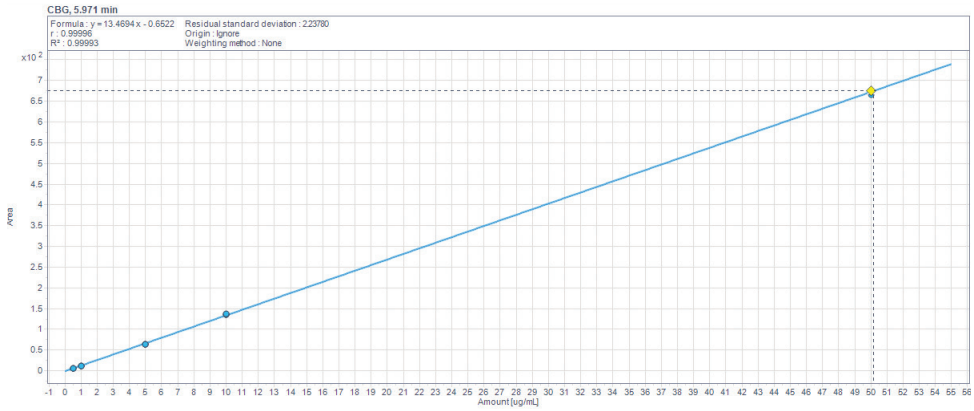
Representative chromatogram (50 µg/mL injection)

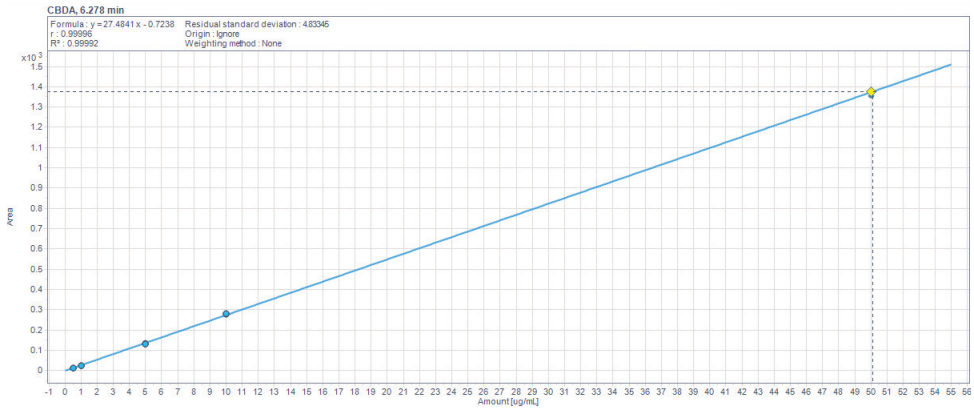


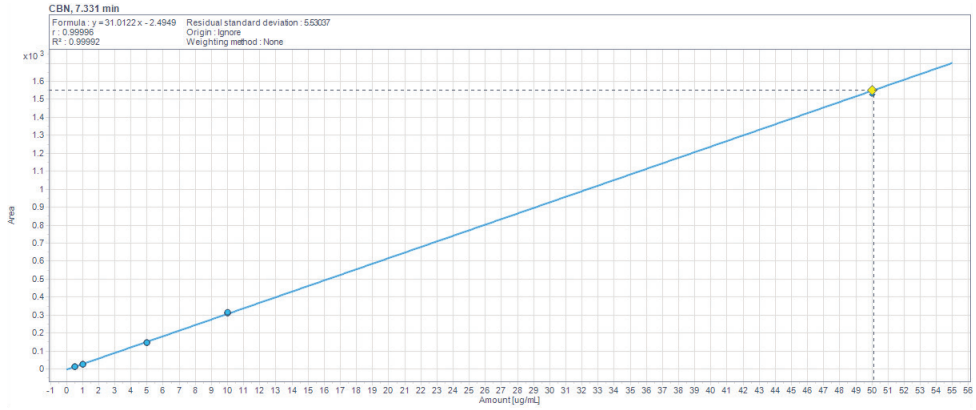
Representative calibration curves

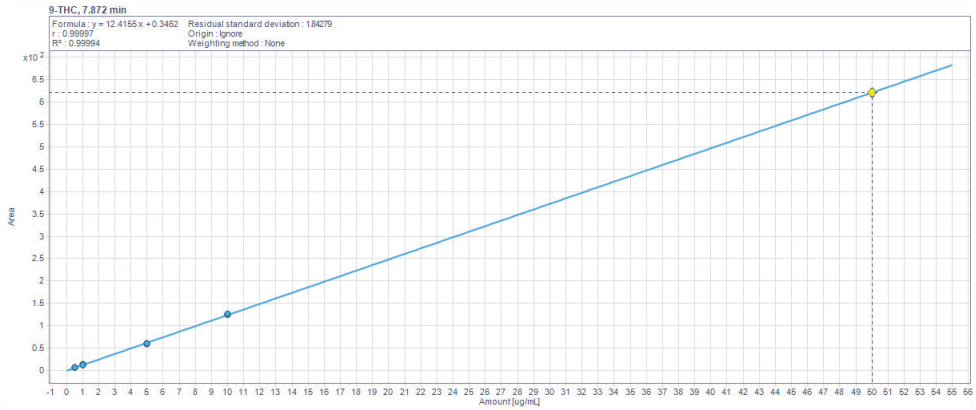
THC, THCA, CBD, CBDA, CBN, and CBG in RT order

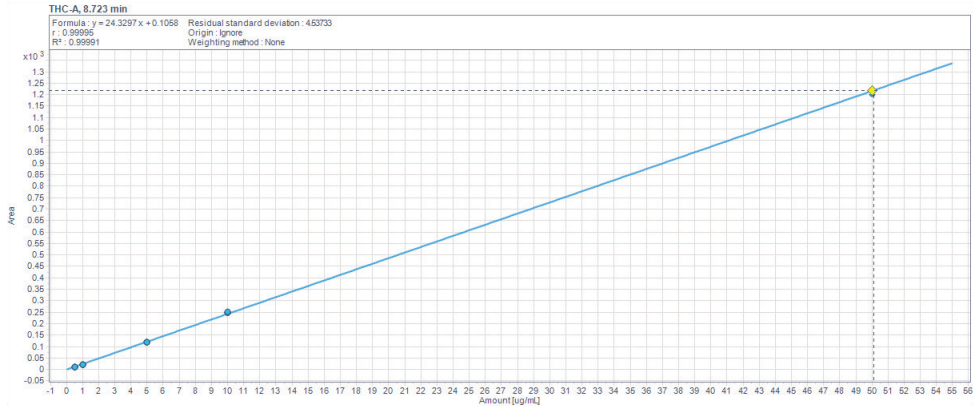







CBN, 7.331 min
Formula : y = 31.0122 x + -2.4949
r : 0.99996
R² : 0.99992
Residual standard deviation : 5.53037
Origin : Ignore
Weighting method : None
x10 3
Area
Amount [ug/mL]


9-THC, 7.872 min
Formula : y = 12.4155 x + 0.3462
r : 0.99997
R² : 0.99994
Residual standard deviation : 1.84279
Origin : Ignore
Weighting method : None
x10 2
Area
Amount [ug/mL]


THC-A, 8.723 min
Formula : y = 24.3297 x + 0.1058
r : 0.99996
R² : 0.99991
Residual standard deviation : 4.53733
Origin : Ignore
Weighting method : None
x10 3
Area
Amount [ug/mL]

Accuracy

Table 5. Inter-and intraday accuracy for the two independent Agilent 1220 LC systems.

Instrument 1	Accuracy				
	Compound (by RT)	Intraday			
Compound	Concentration (µg/mL)	Day 1	Day 2	Day 3	Average
CBDV	0.5	107.2	109.3	110.9	109.1
	1.0	100.3	99.2	100.6	100.0
	5.0	97.6	96.9	95.1	96.6
	10.0	93.4	92.9	91.3	92.5
	50.0	101.5	101.6	102.1	101.7
THCV	0.5	106.7	105.9	108.6	107.1
	1.0	99.8	101.4	102.4	101.2
	5.0	98.1	98.3	95.4	97.2
	10.0	94.0	93.0	91.6	92.9
	50.0	101.3	101.5	102.0	101.6
CBD	0.5	105.8	108.0	105.0	106.3
	1.0	101.2	100.7	97.8	99.9
	5.0	97.8	96.8	93.2	95.9
	10.0	93.7	92.9	97.8	94.8
	50.0	101.4	101.6	102.3	101.8
CBG	0.5	107.4	108.2	109.1	108.2
	1.0	99.9	100.9	101.7	100.8
	5.0	97.7	96.2	95.6	96.5
	10.0	93.5	93.0	91.6	92.7
	50.0	101.4	101.7	102.0	101.7
CBDA	0.5	106.4	108.5	108.7	107.9
	1.0	100.4	100.1	102.2	100.9
	5.0	98.0	96.8	95.5	96.8
	10.0	93.9	92.9	91.5	92.8
	50.0	101.4	101.6	102.0	101.7
CBGA	0.5	106.4	108.3	109.1	107.9
	1.0	100.2	100.4	101.9	100.8
	5.0	98.1	96.8	95.5	96.8
	10.0	94.0	92.9	91.5	92.8
	50.0	101.3	101.7	102.0	101.7

Instrument 1	Accuracy				
	Compound (by RT)	Intraday			
Compound	Concentration (µg/mL)	Day 1	Day 2	Day 3	Average
CBN	0.5	103.9	106.9	107.7	106.2
	1.0	100.0	100.9	102.4	101.1
	5.0	99.6	97.4	96.1	97.7
	10.0	95.6	93.2	92.0	93.6
	50.0	100.9	101.5	101.9	101.4
9-THC	0.5	107.4	111.5	109.3	109.4
	1.0	99.6	97.1	101.1	99.3
	5.0	97.7	96.9	95.9	96.8
	10.0	93.9	92.9	91.7	92.8
	50.0	101.4	101.7	102.0	101.7
8-THC	0.5	107.9	107.2	108.0	107.7
	1.0	99.5	101.1	102.5	101.0
	5.0	97.5	97.2	95.8	96.9
	10.0	93.6	93.0	91.8	92.8
	50.0	101.4	101.6	101.9	101.6
CBC	0.5	64.8	107.2	107.8	93.3
	1.0	95.7	100.2	101.5	99.1
	5.0	123.8	97.6	96.4	105.9
	10.0	122.1	93.6	92.5	102.7
	50.0	93.7	101.5	101.7	99.0
THC-A	0.5	78.6	106.6	107.4	97.5
	1.0	96.8	100.0	101.0	99.3
	5.0	115.5	98.0	96.9	103.5
	10.0	112.9	94.1	93.0	100.0
	50.0	96.2	101.3	101.6	99.7

Instrument 2	Accuracy				
	Compound (by RT)	Intraday			
Compound	Concentration (µg/mL)	Day 1	Day 2	Day 3	Average
CBDV	0.5	107.2	112.1	112.5	110.6
	1.0	94.8	92.2	89.1	92.1
	5.0	96.6	93.2	83.7	91.2
	10.0	101.3	102.3	116.2	106.6
	50.0	100.1	100.3	98.5	99.6
THCV	0.5	104.4	105.3	107.9	105.9
	1.0	96.7	96.7	92.8	95.4
	5.0	97.1	94.4	83.8	91.8
	10.0	101.9	103.7	117.2	107.6
	50.0	99.9	99.8	98.2	99.3
CBD	0.5	103.6	105.2	108.9	105.9
	1.0	97.5	96.7	92.3	95.5
	5.0	97.1	94.6	83.9	91.9
	10.0	101.9	103.7	116.6	107.4
	50.0	99.9	99.8	98.4	99.4
CBG	0.5	104.8	105.7	108.2	106.2
	1.0	96.8	96.6	92.8	95.4
	5.0	96.8	94.4	83.8	91.7
	10.0	101.6	103.4	116.8	107.3
	50.0	100.0	99.9	98.3	99.4
CBDA	0.5	104.8	106.6	107.9	106.4
	1.0	96.7	96.1	93.2	95.3
	5.0	96.8	94.2	84.3	91.8
	10.0	101.7	103.1	116.2	107.0
	50.0	100.0	100.0	98.4	99.5
CBGA	0.5	105.2	106.8	108.4	106.8
	1.0	96.8	96.3	93.0	95.4
	5.0	96.7	94.1	84.0	91.6
	10.0	101.2	102.7	116.0	106.6
	50.0	100.1	100.1	98.4	99.5

Instrument 2	Accuracy				
	Compound (by RT)	Intraday			
Compound	Concentration (µg/mL)	Day 1	Day 2	Day 3	Average
CBN	0.5	104.5	105.5	107.1	105.7
	1.0	96.8	96.6	92.7	95.4
	5.0	97.0	94.3	84.2	91.9
	10.0	101.8	103.6	117.9	107.8
	50.0	99.9	99.9	98.1	99.3
9-THC	0.5	104.2	105.7	107.8	105.9
	1.0	96.9	96.4	92.8	95.4
	5.0	97.2	94.4	84.0	91.8
	10.0	101.8	103.7	117.3	107.6
	50.0	99.9	99.8	98.2	99.3
8-THC	0.5	104.1	105.5	108.1	105.9
	1.0	97.0	96.2	92.0	95.1
	5.0	97.0	94.6	84.2	91.9
	10.0	101.9	103.8	117.5	107.7
	50.0	99.9	99.8	98.2	99.3
CBC	0.5	102.2	103.4	105.7	103.8
	1.0	96.8	96.3	92.2	95.1
	5.0	98.3	95.7	85.3	93.1
	10.0	103.1	105.1	119.0	109.1
	50.0	99.6	99.4	97.8	98.9
THC-A	0.5	102.2	103.8	106.2	104.1
	1.0	96.7	96.1	91.9	94.9
	5.0	98.4	95.8	85.7	93.3
	10.0	103.2	104.8	118.4	108.8
	50.0	99.6	99.5	97.9	99.0

Precision

Table 6. Inter-and intraday precision for the two independent Agilent 1220 HPLC systems.

Instrument 1 precision (%RSD) – 5 µg/mL				
Compound	Interday			Intraday
CBDV	3.39	0.59	0.47	3.44
THCV	0.13	0.56	0.59	3.71
CBD	0.30	0.36	0.15	3.80
CBG	0.39	0.24	0.19	4.00
CBDA	0.23	0.66	0.59	3.25
CBGA	0.19	0.28	0.62	3.39
CBN	0.27	0.36	0.31	3.80
9-THC	1.30	1.08	0.69	1.59
8-THC	1.31	0.88	0.48	1.82
CBC	0.32	0.19	0.13	3.06
THC-A	0.12	0.15	0.30	3.07

Instrument 2 precision (%RSD) – 5 µg/mL				
Compound	Interday			Intraday
CBDV	1.25	0.80	0.55	6.62
THCV	0.33	0.65	0.30	5.81
CBD	0.08	0.34	0.56	6.67
CBG	0.32	0.28	0.32	6.80
CBDA	0.34	0.51	0.16	6.06
CBGA	0.23	0.43	0.32	6.38
CBN	0.22	0.13	0.13	6.69
9-THC	0.33	0.68	0.43	6.91
8-THC	0.65	0.47	0.13	5.64
CBC	0.20	0.20	0.05	4.75
THC-A	0.19	0.22	0.14	6.26

Linearity

Table 7. Ignore origin, no weighting.

Instrument 1 linearity (R^2)				
Compound	Day 1	Day 2	Day 3	Average
CBDV	0.9997	0.9995	0.9995	0.9996
THCV	0.9997	0.9995	0.9995	0.9996
CBD	0.9997	0.9995	0.9995	0.9996
CBG	0.9997	0.9995	0.9995	0.9996
CBDA	0.9998	0.9995	0.9995	0.9996
CBGA	0.9997	0.9995	0.9995	0.9996
CBN	0.9997	0.9995	0.9996	0.9996
9-THC	0.9997	0.9991	0.9995	0.9994
8-THC	0.9997	0.9971	0.9984	0.9984
CBC	0.9998	0.9996	0.9996	0.9997
THC-A	0.9998	0.9996	0.9996	0.9997

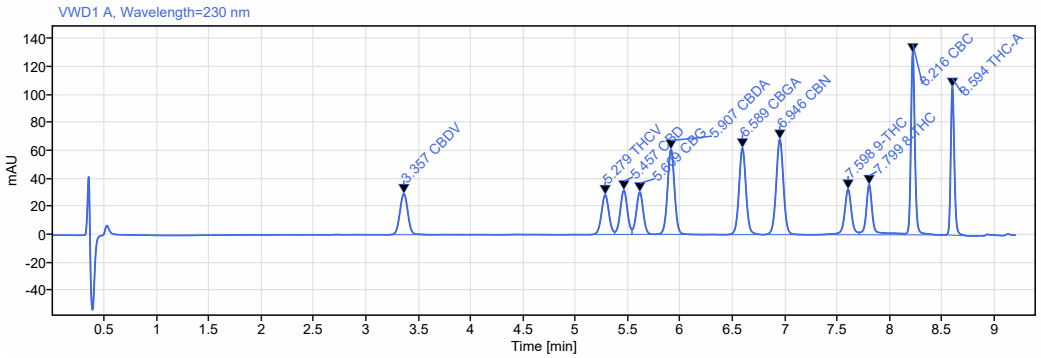
Instrument 2 linearity (R^2)				
Compound	Day 1	Day 2	Day 3	Average
CBDV	0.9999	0.9998	0.9977	0.9991
THCV	0.9999	0.9998	0.9975	0.9991
CBD	0.9999	0.9998	0.9977	0.9991
CBG	0.9999	0.9998	0.9977	0.9991
CBDA	0.9999	0.9999	0.9977	0.9992
CBGA	0.9999	0.9999	0.9978	0.9992
CBN	0.9999	0.9998	0.9975	0.9991
9-THC	0.9999	0.9999	0.9978	0.9992
8-THC	0.9999	0.9998	0.9975	0.9991
CBC	0.9999	0.9998	0.9972	0.9990
THC-A	0.9999	0.9998	0.9974	0.9990

Appendix: Example sample report

Single Injection Report

Agilent Technologies

Sample name:	10 11 cmpd mix		
Data file:	022-5-25 11 cmpd mix.D	**Operator:**	SYSTEM
Instrument:	1220 Right	**Injection date:**	2018-02-01 09:44:39-07:00
Inj. volume:	5.0	**Location:**	5
Acq. method:	Agilent_Cannabis_EC.M	**Type:**	Sample
Processing method:	*GC_LC Quantitative_DefaultMethod.pmx	**Calib Level:**	
		Sample amount:	0.00
Manually modified:	None		



Signal: VWD1 A, Wavelength=230 nm

Name	RT [min]	RF	Area	Amount [ug/mL]	Concentration [ug/mL]
CBDV	3.36	16.762	155.076	9.252	9.252
THCV	5.28	15.188	140.236	9.234	9.234
CBD	5.46	16.167	149.623	9.255	9.255
CBG	5.61	14.960	138.518	9.259	9.259
CBDA	5.91	30.625	282.626	9.229	9.229
CBGA	6.59	30.972	286.065	9.236	9.236
CBN	6.95	35.386	327.881	9.266	9.266
9-THC	7.60	15.223	150.199	9.866	9.866
8-THC	7.80	14.998	154.177	10.280	10.280
CBC	8.22	36.808	346.170	9.405	9.405
THC-A	8.59	27.174	252.937	9.308	9.308

Total Potency [ug/mL]	Total CBD [ug/mL]
18.03	17.35

Sample Potency Report.rdl [Rev. 7] Printed: 2018-03-16 08:45:10-06:00 Page 1 of 1

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.

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DE.6467939815

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Printed in the USA, May 22, 2020
5991-9285EN

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