

Dedicated and Factory Verified Cannabinoid Potency Testing Using the Agilent 1220 Infinity II LC System

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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.

A method for potency testing in cannabis flower and related products

- Rapid, robust, accurate, and precise
- Quantify cannabinoids for quality, safety, and compliance
- Rapid method implementation
- Consulting services to assist and train your staff

Target Cannabinoids in retention time order

1. Cannabidivarin (CBDV)
2. Tetrahydrocannabivarin (THCV)
3. (-)- Δ 9-tetrahydrocannabinol (THC)
4. Cannabidiol (CBD)
5. Cannabigerol (CBG)
6. Δ 9-tetrahydrocannabinolic acid (THCA-A)
7. Cannabidiolic acid (CBDA)
8. Cannabinol (CBN)
9. Cannabigerolic acid (CBGA)
10. Cannabichromene (CBC)
11. (-)- Δ 8-THC

Potency Testing Goals

Solution must:

- Be competitively priced
- Be < 10 minutes in total run time
- Chromatographically resolve:
 - Required cannabinoids: Δ 9-THC, THCA, CBD, CBDA, CBG, and CBN
 - Desired cannabinoids: CBDV, THCV, CBGA, CBC, Δ 8-THC
- Demonstrate:
 - LOQ's of ≤ 0.5 ng on column
 - Inter-day and intra-day quantitative precision of < 5% RSD
 - Cross-instrument reproducibility
- Calculate total potency & total CBD
- Provide a basic reporting template
- Be field deliverable via 1-day onsite training

Hardware, software, and consumables

Hardware and Software	Agilent 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 2.2 data acquisition/analysis software	M8413AA
Consumables	Agilent Part Number
Agilent Poroshell 120 EC-C18, 3.0 × 50 mm, 2.7 µm	699975-302
Formic Acid	G2453-85060
LC/MS Grade Water	5190-6897
LC/MS Grade Methanol	5190-6896

Method Conditions

Parameter	Value
Column	Agilent Poroshell 120 EC-C18, 3.0 × 50mm, 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	0.75 mL/min
Run time	9.2 minutes
Post run	2.5 minutes
Column temperature	50 °C isothermal
Injection volume	5.0 μL
Autosampler temperature	Ambient
Peak width	> 0.0063 min (0.13 s response time) (80 Hz)
Variable wavelength detector	230 nm

Time (min)	% B
0	60
6.0	77
7.2	95

Sample Preparation

- The 11 standards were obtained individually at 1 mg/mL concentrations each
- They were then mixed to create a 50 $\mu\text{g/mL}$ concentration which was then diluted in methanol to create the following dilution series:

<u>Level</u>	<u>Concentration ($\mu\text{g/mL}$)</u>
1	0.5
2	1.0
3	5.0
4	10.0
5	50.0

Representative Chromatogram

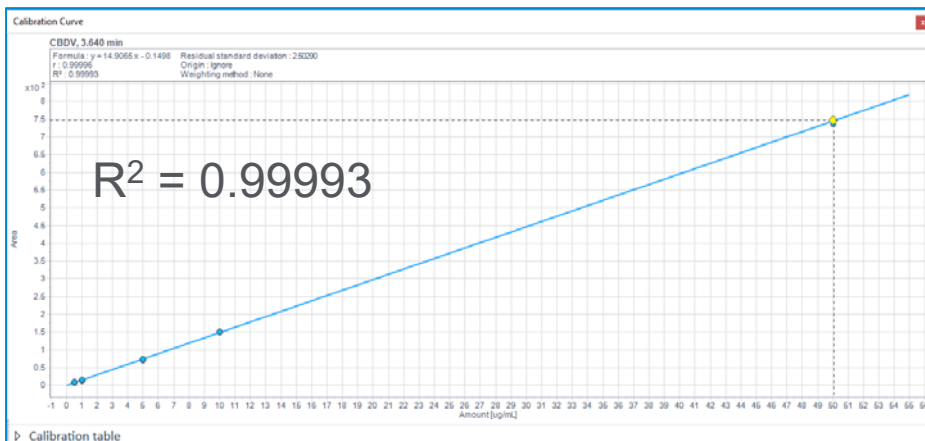


USP Resolution of critical pairs - cannabinoids at 50 µg/mL level

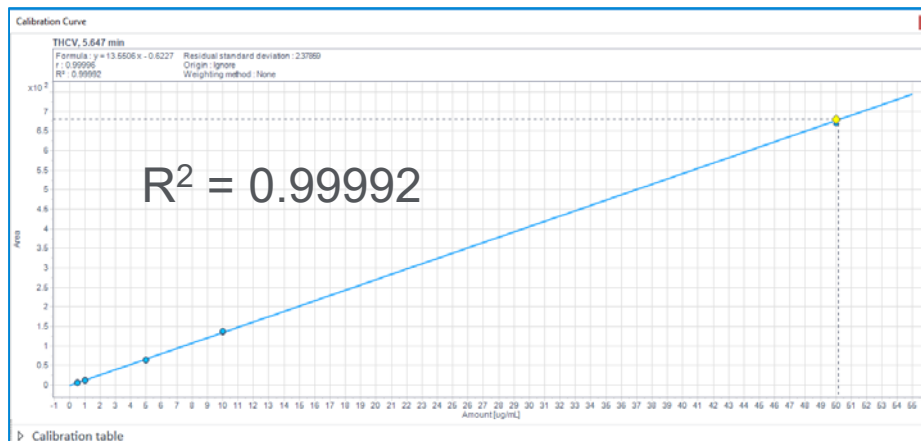
Compounds (1 and 2)	(Inj 1)	(Inj 2)	(Inj 3)	(Inj 4)	(Inj 5)
THCV/CBD	1.386	1.385	1.387	1.382	1.383
CBD/CBG	1.226	1.223	1.225	1.223	1.225
9-THC/8-THC	1.989	1.991	1.995	1.99	1.994

Calibration curves (0.5 – 50 µg/mL), no weighting, origin not included, externally standardized

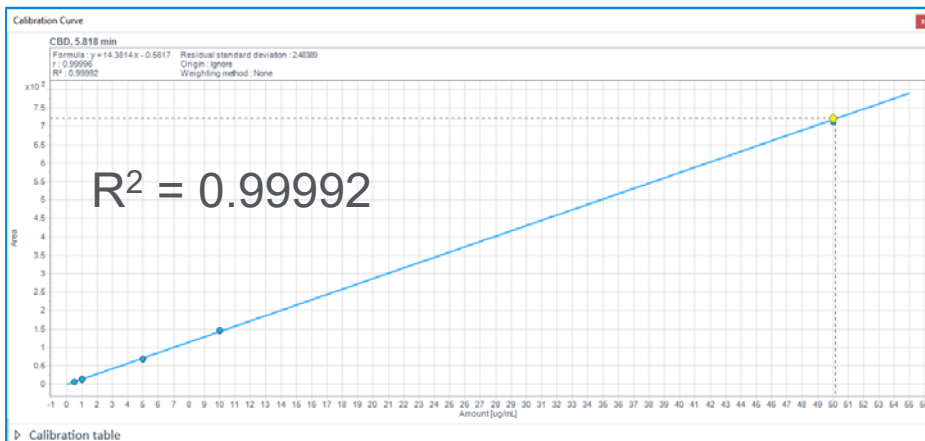
CBDV



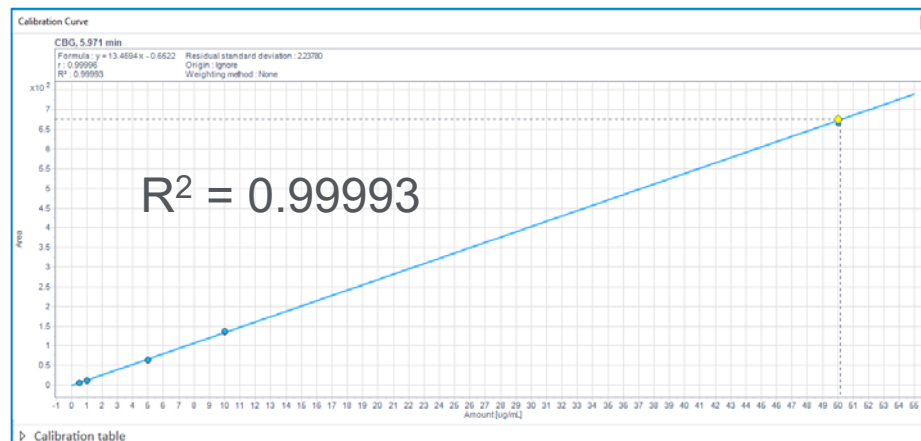
THCV



CBD

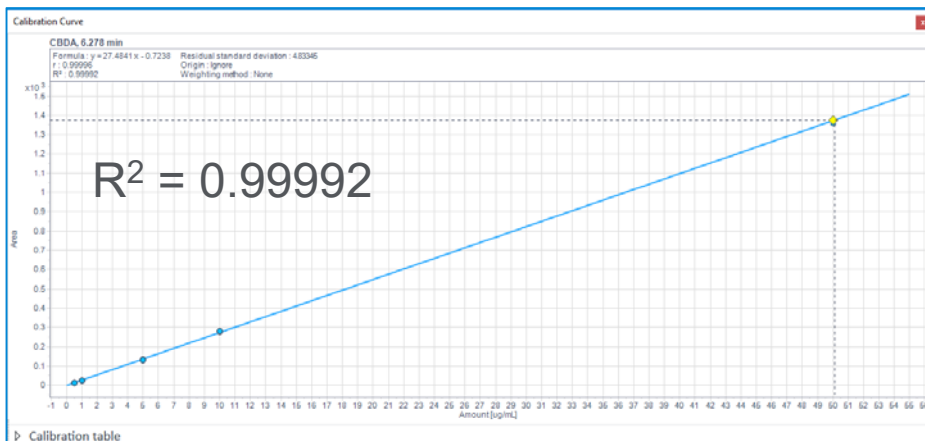


CBG

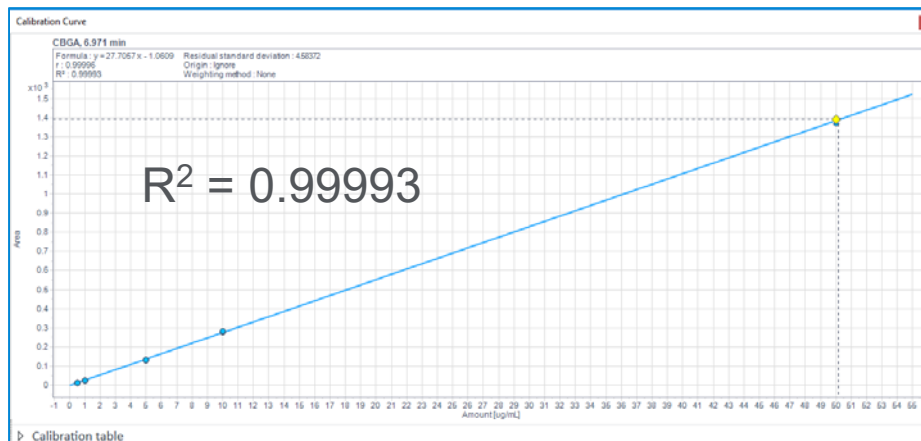


Calibration curves (0.5 – 50 µg/mL), no weighting, origin not included, externally standardized

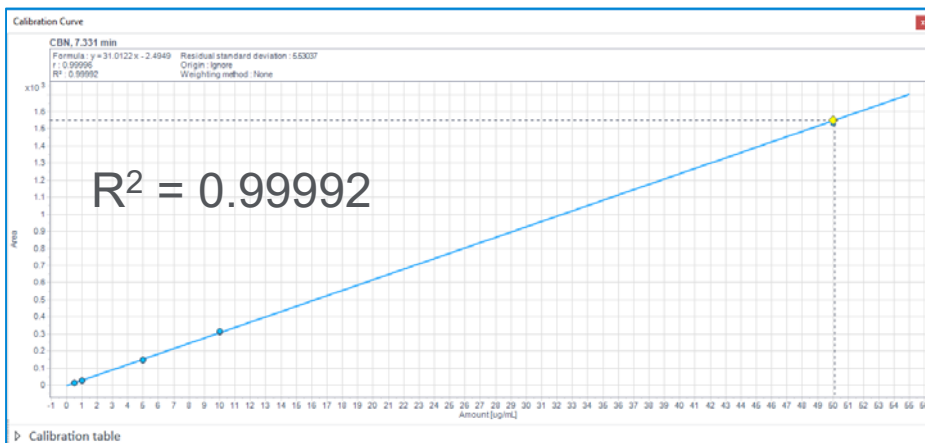
CBDA



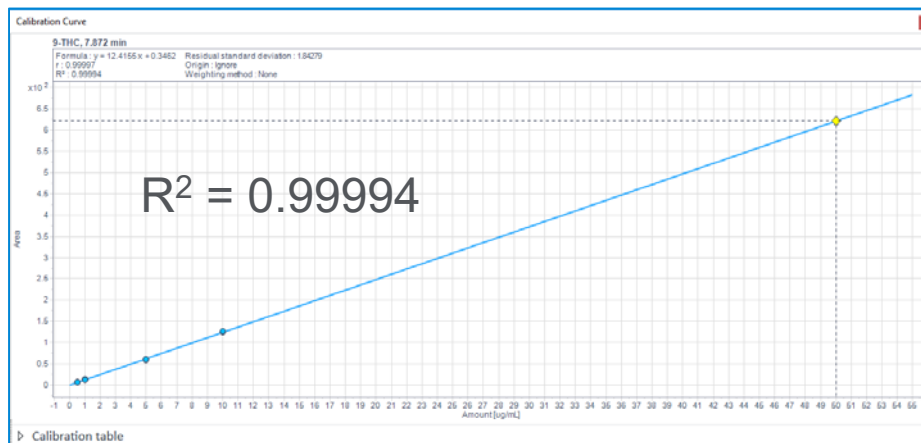
CBGA



CBN

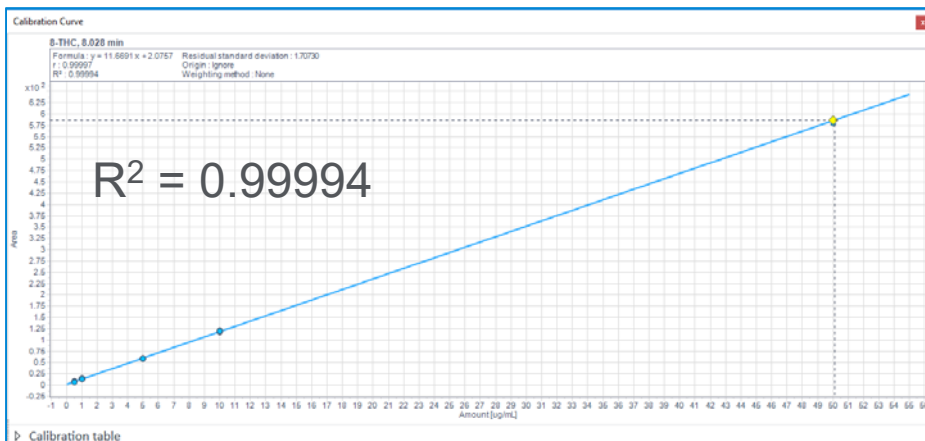


Δ⁹ - THC

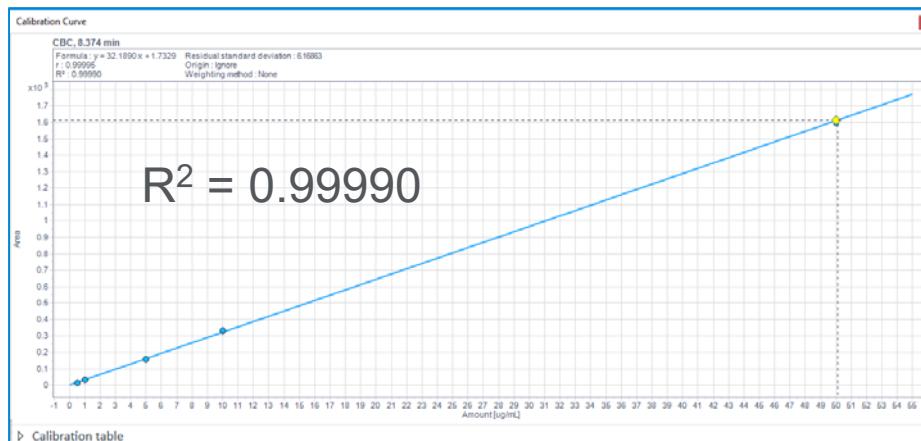


Calibration curves (0.5 – 50 µg/mL), no weighting, origin not included, externally standardized

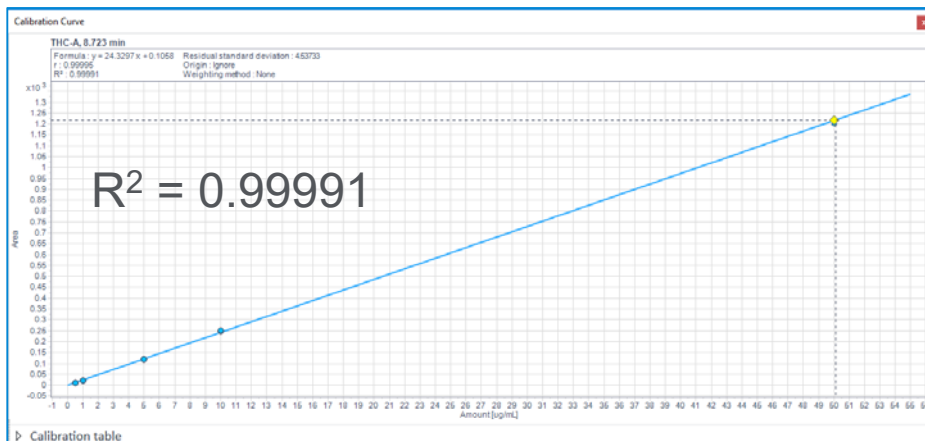
Δ^8 - THC



CBC



THCA



OpenLab CDS 2.2 Data Analysis

Cannabis - Data Analysis

File Home Processing Audit/E-Sign Injection Tree

Open Data Close Data Open Method Save Method Close Method Reprocess All Save All Results Print All View PDF Results Chromatograms Compounds Copy Delete Reset

Acquisition Setpoints Injection List Peak Details Calibration Curve Injection Results Sample Information Chromatograms Processing Method Peak Explorer

Data Processing

by Sequence

- 0.5 11 cmpd mix - 007-2-0.5 11 cmpd mix.D ✓
- 1 11 cmpd mix - 008-3-1 11 cmpd mix.D ✓
- 1 11 cmpd mix - 009-3-1 11 cmpd mix.D ✓
- 1 11 cmpd mix - 010-3-1 11 cmpd mix.D ✓
- 1 11 cmpd mix - 011-3-1 11 cmpd mix.D ✓
- 1 11 cmpd mix - 012-3-1 11 cmpd mix.D ✓
- 5 11 cmpd mix - 013-4-5 11 cmpd mix.D ✓
- 5 11 cmpd mix - 014-4-5 11 cmpd mix.D ✓
- 5 11 cmpd mix - 015-4-5 11 cmpd mix.D ✓
- 5 11 cmpd mix - 016-4-5 11 cmpd mix.D ✓
- 5 11 cmpd mix - 017-4-5 11 cmpd mix.D ✓
- 10 11 cmpd mix - 018-5-25 11 cmpd mix.D ✓
- 10 11 cmpd mix - 019-5-25 11 cmpd mix.D ✓
- 10 11 cmpd mix - 020-5-25 11 cmpd mix.D ✓
- 10 11 cmpd mix - 021-5-25 11 cmpd mix.D ✓
- 10 11 cmpd mix - 022-5-25 11 cmpd mix.D ✓
- 050 11 cmpd mix - 023-6-050 11 cmpd mix.D ✓
- 050 11 cmpd mix - 024-6-050 11 cmpd mix.D ✓
- 050 11 cmpd mix - 025-6-050 11 cmpd mix.D ✓

Signals

- VWD1 A, Wavelength=230 nm

Instrument Traces

Methods

- *GC_LC Quantitative_DefaultMethod

Data Selection

Data Processing

Reporting

Current user: SYSTEM

Chromatograms

050 11 cmpd mix | 023-6-050 11 cmpd mix.D (of)

VWD1 A, Wavelength=230 nm

Response [mAU] x10²

Retention time [min]

3.354 CBDV
5.454 CBD
5.902 CBDA
6.582 CBGA
6.942 CBN
7.788 8-THC
8.218 CBC
8.596 THC-A
18.927

Linked views for easy navigation

Calibration Curve

THC-A, 8.593 min

Formula: $y = 27.6615x - 6.7049$ Residual standard deviation: 10.30143
 $r = 0.99945$ Origin: Ignore
 $R^2 = 0.99891$ Weighting method: None

Area x10³

Amount [ug/mL]

Calibration table

Injection Results

Peaks Summary

#	Name	Signal description	RT (min)
2	THCV	VWD1 A, Wavelength=230 nm	
3	CBD	VWD1 A, Wavelength=230 nm	
4	CBG	VWD1 A, Wavelength=230 nm	
5	CBDA	VWD1 A, Wavelength=230 nm	
6	CBGA	VWD1 A, Wavelength=230 nm	
7	CBN	VWD1 A, Wavelength=230 nm	
8	9-THC	VWD1 A, Wavelength=230 nm	
9	8-THC	VWD1 A, Wavelength=230 nm	
10	CBC	VWD1 A, Wavelength=230 nm	
11	THC-A	VWD1 A, Wavelength=230 nm	
12		VWD1 A, Wavelength=230 nm	

Connected

Precision (Inter-day and Intra-day)

Instrument 1 Precision (%RSD) – 5 µg/mL					Instrument 2 Precision (%RSD) – 5 µg/mL				
Compound Name	Inter-Day			Intra-Day		Inter-Day			Intra-Day
	CBDV	3.39	0.59			0.47	3.44		
THCV	0.13	0.56	0.59	3.71		0.33	0.65	0.30	5.81
CBD	0.30	0.36	0.15	3.80		0.08	0.34	0.56	6.67
CBG	0.39	0.24	0.19	4.00		0.32	0.28	0.32	6.80
CBDA	0.23	0.66	0.59	3.25		0.34	0.51	0.16	6.06
CBGA	0.19	0.28	0.62	3.39		0.23	0.43	0.32	6.38
CBN	0.27	0.36	0.31	3.80		0.22	0.13	0.13	6.69
9-THC	1.30	1.08	0.69	1.59		0.33	0.68	0.43	6.91
8-THC	1.31	0.88	0.48	1.82		0.65	0.47	0.13	5.64
CBC	0.32	0.19	0.13	3.06		0.20	0.20	0.05	4.75
THC-A	0.12	0.15	0.30	3.07		0.19	0.22	0.14	6.26

Accuracy

Instrument 1	Accuracy					Instrument 2	Accuracy				
	Compound (by RT)	Intraday					Compound (by RT)	Intraday			
Compound Name	Concentration (µg/mL)	Day 1	Day 2	Day 3	Average	Compound Name	Concentration (µg/mL)	Day 1	Day 2	Day 3	Average
CBD	0.5	105.8	108.0	105.0	106.3	CBD	0.5	103.6	105.2	108.9	105.9
	1.0	101.2	100.7	97.8	99.9		1.0	97.5	96.7	92.3	95.5
	5.0	97.8	96.8	93.2	95.9		5.0	97.1	94.6	83.9	91.9
	10.0	93.7	92.9	97.8	94.8		10.0	101.9	103.7	116.6	107.4
	50.0	101.4	101.6	102.3	101.8		50.0	99.9	99.8	98.4	99.4
CBDA	0.5	106.4	108.5	108.7	107.9	CBDA	0.5	104.8	106.6	107.9	106.4
	1.0	100.4	100.1	102.2	100.9		1.0	96.7	96.1	93.2	95.3
	5.0	98.0	96.8	95.5	96.8		5.0	96.8	94.2	84.3	91.8
	10.0	93.9	92.9	91.5	92.8		10.0	101.7	103.1	116.2	107.0
	50.0	101.4	101.6	102.0	101.7		50.0	100.0	100.0	98.4	99.5
9-THC	0.5	107.4	111.5	109.3	109.4	9-THC	0.5	104.2	105.7	107.8	105.9
	1.0	99.6	97.1	101.1	99.3		1.0	96.9	96.4	92.8	95.4
	5.0	97.7	96.9	95.9	96.8		5.0	97.2	94.4	84.0	91.8
	10.0	93.9	92.9	91.7	92.8		10.0	101.8	103.7	117.3	107.6
	50.0	101.4	101.7	102.0	101.7		50.0	99.9	99.8	98.2	99.3
THC-A	0.5	78.6	106.6	107.4	97.5	THC-A	0.5	102.2	103.8	106.2	104.1
	1.0	96.8	100.0	101.0	99.3		1.0	96.7	96.1	91.9	94.9
	5.0	115.5	98.0	96.9	103.5		5.0	98.4	95.8	85.7	93.3
	10.0	112.9	94.1	93.0	100.0		10.0	103.2	104.8	118.4	108.8
	50.0	96.2	101.3	101.6	99.7		50.0	99.6	99.5	97.9	99.0

Linearity

Instrument 1 Linearity (R ²)					Instrument 2 Linearity (R ²)				
Compound Name	Day 1	Day 2	Day 3	Average		Day 1	Day 2	Day 3	Average
CBDV	0.9997	0.9995	0.9995	0.9996		0.9999	0.9998	0.9977	0.9991
THCV	0.9997	0.9995	0.9995	0.9996		0.9999	0.9998	0.9975	0.9991
CBD	0.9997	0.9995	0.9995	0.9996		0.9999	0.9998	0.9977	0.9991
CBG	0.9997	0.9995	0.9995	0.9996		0.9999	0.9998	0.9977	0.9991
CBDA	0.9998	0.9995	0.9995	0.9996		0.9999	0.9999	0.9977	0.9992
CBGA	0.9997	0.9995	0.9995	0.9996		0.9999	0.9999	0.9978	0.9992
CBN	0.9997	0.9995	0.9996	0.9996		0.9999	0.9998	0.9975	0.9991
9-THC	0.9997	0.9991	0.9995	0.9994		0.9999	0.9999	0.9978	0.9992
8-THC	0.9997	0.9971	0.9984	0.9984		0.9999	0.9998	0.9975	0.9991
CBC	0.9998	0.9996	0.9996	0.9997		0.9999	0.9998	0.9972	0.9990
THC-A	0.9998	0.9996	0.9996	0.9997		0.9999	0.9998	0.9974	0.9990

Instrument Detection Limit (IDL)

Averaged over 2 instruments over three days (n=6)

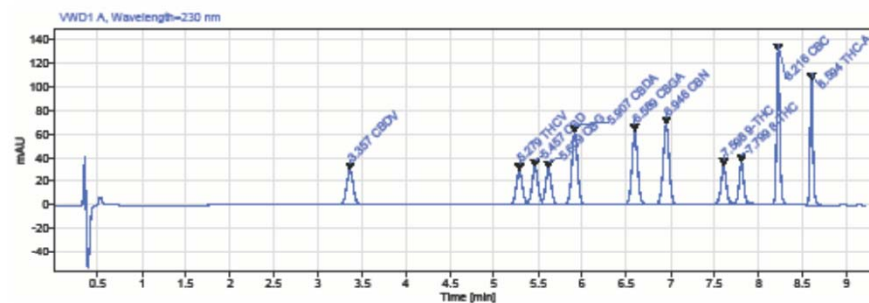
Compound by RT	IDL (ng on column)
CBDV	0.14
THCV	0.12
CBD	0.12
CBG	0.11
CBDA	0.11
CBGA	0.10
CBN	0.10
9-THC	0.10
8-THC	0.08
CBC	0.07
THC-A	0.08

Cannabis Potency Report using OpenLab CDS 2.2

Single Injection Report



Sample name: 10 11 cmpd mix
Data file: 022-5-25 11 cmpd mix.D
Instrument: 1220 Right
Inj. volume: 5.0
Acq. method: Agilent_Cannabis_EC.M
Processing method: *GC_LC
 Quantitative_DefaultMethod.pmx
Operator: SYSTEM
Injection date: 2018-02-01 09:44:39-07:00
Location: 5
Type: Sample
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	10.762	155.076	0.252	0.252
THCV	5.28	15.188	140.236	0.234	0.234
CBD	5.46	16.167	149.623	0.255	0.255
CBG	5.61	14.960	138.518	0.259	0.259
CBDA	5.91	30.625	282.626	0.229	0.229
CBGA	6.50	30.972	286.065	0.236	0.236
CBN	6.95	35.386	327.881	0.266	0.266
9-THC	7.80	15.223	150.199	0.866	0.866
8-THC	7.80	14.998	154.177	10.280	10.280
CBC	8.22	36.808	346.170	0.405	0.405
THC-A	8.59	27.174	252.937	0.308	0.308

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

OpenLab CDS 2.2 – Intelligent Reporting

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

Custom Calculation

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

$$\text{Total Potency} = \text{THC} + 0.877 * \text{THCA}$$

$$\text{Total CBD} = \text{CBD} + 0.877 * \text{CBDA}$$

On-Site Consulting Service



Sold with Agilent 1220 Infinity II LC System

Includes:

- Full day of method setup, implementation and training by Field Service Engineer
- Instruction Manual (lab exercises and example data)
- Agilent Poroshell 120 EC-C18, 3.0 × 50 mm, 2.7 μm LC column
- LC solvents (water and MeOH)
- Formic acid

Acknowledgements

Christy Storm

LC Regional Support Specialist

Agilent Technologies

Sue D'Antonio

Applications Chemist

Agilent Technologies

Anthony Macherone

Senior Scientist

Agilent Technologies

Thank you

Questions?



Appendix

Resolution (USP, ASTM) R

Tangent method (pertaining to peaks 1 and 2, t_R of peak 1 < t_R of peak 2; t_R in min)

$$R = 2 \cdot \frac{t_{R2} - t_{R1}}{W_t(2) + W_t(1)}$$

where

t_R Retention time

W_t Tangent width [min]

Sample preparation recommendations for flower

1. Weigh 200 mg sample 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.
3. Aliquot 1 mL into a new vial. Centrifuge at 5,000 rpm for five minutes.
4. Transfer 50 μ L of supernatant to a new vial. Add 950 μ L methanol. Mix briefly.
5. Filter with 4 mm, 0.45 μ m regenerated cellulose (RC) syringe filters

No QuEChERS 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.

- Converts THCA to THC
- May cause additional breakdown products
 - 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

Substantial dilution is required

- May be as much as 1,000-fold to 10,000-fold

Minimize sample manipulation to avoid the pitfalls noted above