

CTInstruments HPLC for Testing Cannabinoids

Supporting Document

HPLC Features

- Reciprocating Pump
- Rheodyne 7725i Injector
- CTI HPLC Software
- UV/VIS Detector
- Temperature-controlled Column Compartment

HPLC Specifications

Flow Rate	0.001 - 5mL/min
Max Pressure	6,300 psi
Flow Accuracy	≤±1%
Flow Precision	RSD <0.1%
Qualitative Repeatability	RSD ≤0.2%
Quantitative Repeatability	RSD ≤0.5%
Wavelength Range	180 – 680nm
Spectrum Bandwidth	8nm
Wavelength Accuracy	±1nm
Wavelength Precision	Below 0.1nm
Noise	≤0.25X10 ⁻⁵ AU

HPLC Column Specifications

Column Type	C18, SS body*
Dimensions	150x4.6mm
Packing	5µm particles
Guard Column	C18

Chromatographic Conditions

Mode	Isocratic
Temperature	30°C
Detection	UV at 220nm
Mobile Phase	Buffer:Acetonitrile*
Flow Rate	1.2mL/min

*Note: Specific chemistry is proprietary information



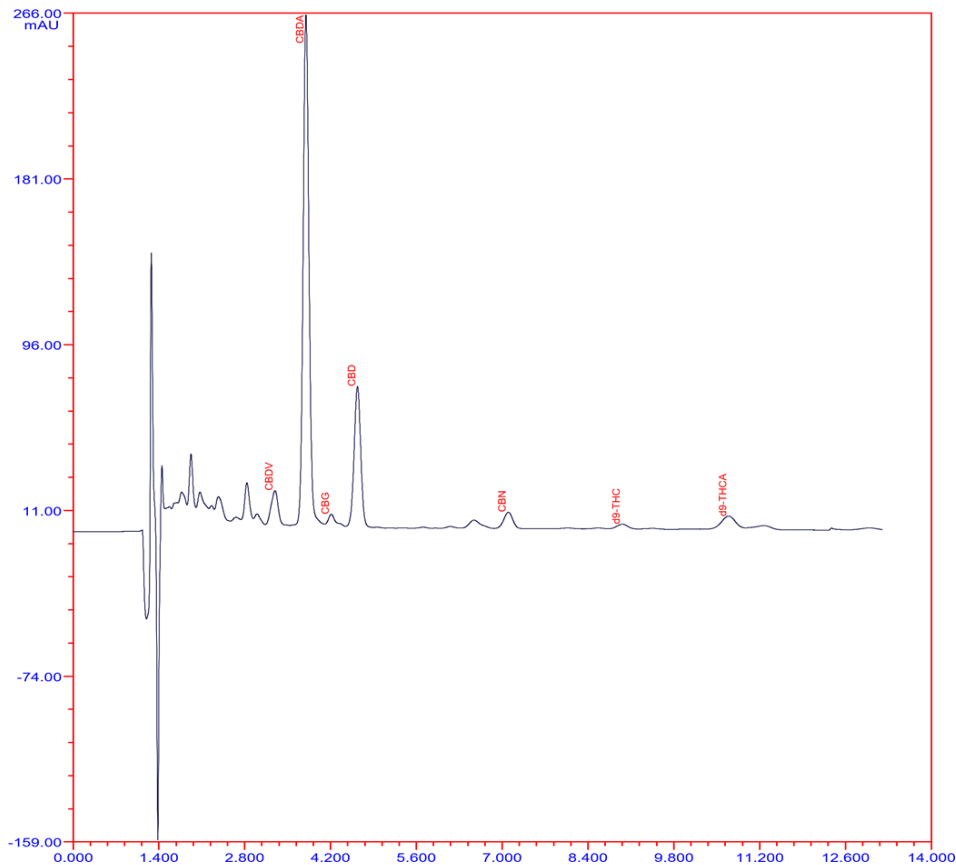
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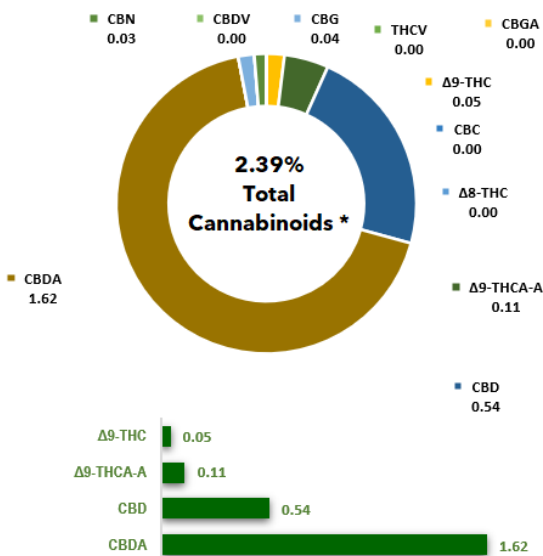
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Typical Chromatogram



Typical result reporting

CANNABINOID PROFILE



Compound	Result (% w/w)	mg/gram of sample
THCV	NR	NR
Δ8-THC	NR	NR
Δ9-THC	0.05	0.46
Δ9-THCA-A	0.11	1.14
CBD	0.54	5.38
CBDA	1.62	16.16
CBDV	<0.01	<0.05
CBG	0.04	0.41
CBGA	NR	NR
CBN	0.03	0.31
CBC	NR	NR

Compound	Result (% w/w)	mg/gram of sample
Total Cannabinoids *	2.39	23.86
Total Potential THC	0.15	1.47
Total Potential CBD	1.95	19.55
Total Potential CBG	0.04	0.41

NOTES

* Total Cannabinoids = sum of all measured cannabinoids
 Total Potential THC = Δ8-THC + Δ9-THC + Δ9-THCA-A * 0.877
 Total Potential CBD = CBD + CBDA * 0.877
 NR = None Reported, Measured amount is below detection limit for the specified method

Lower Limit of Quantification

The lower limit of quantification (LLOQ) is the lowest amount of a cannabinoid in a sample that can be quantitatively determined with suitable precision and accuracy using the corresponding method and dilution rates.

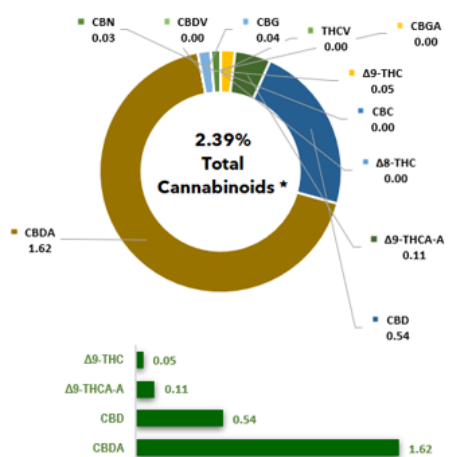
Compound	Plant LLOQ (%w/w)	High % Concentrate LLOQ (%w/w)
Δ^9 -THC	0.03	0.04
Δ^8 -THC	0.03	0.06
Δ^9 -THCA	0.03	0.06
THCV	0.03	0.04
CBD	0.03	0.03
CBDA	0.03	0.03
CBDV	0.03	0.03
CBG	0.03	0.03
CBGA	0.03	0.03
CBN	0.03	0.03
CBC	0.03	0.05

Repeatability

Below are results of measuring a typical hemp sample, followed by an image of stacked 3 consecutive measurements. There are two observations from this measurement:

1. The retention times are consistent and repeatable, giving the confidence in the ability to correctly identify the correct cannabinoids.
2. The measured concentrations are very repeatable as indicated by height/area of each peak. The calculated relative standard deviation (RSD) in this case ranges from 1.19% to 1.70%, while for cannabinoids with very low concentrations manifested as very small peaks (in this sample CBN, d9-THC, and d9-THCA) the calculated RSD ranges from 4.62% to 5.63%. In case of need to further refine the latter 3 compounds and improve both accuracy and RSD, the sample would simply be less diluted resulting in higher peaks for these 3 compounds.

CANNABINOID PROFILE

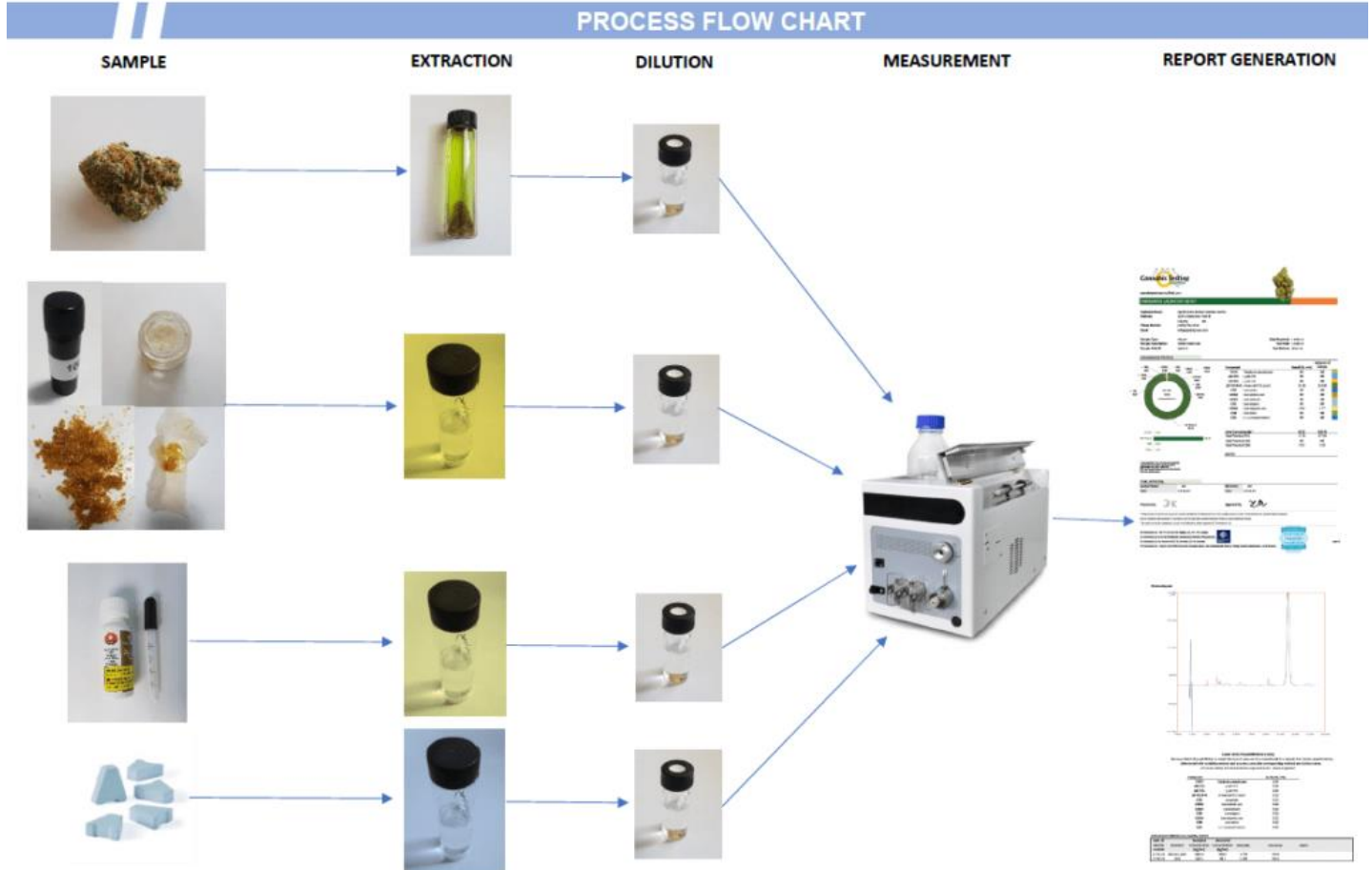


Compound	Result (% w/w)	mg/gram of sample
THCV	Tetrahydrocannabivarin	NR
Δ8-THC	(-)-Δ8-THC	NR
Δ9-THC	(-)-Δ9-THC	0.05
Δ9-THCA-A	(-)-trans-Δ9-THC acid A	0.11
CBD	Cannabidiol	0.54
CBDA	Cannabidiolic acid	1.62
CBDV	Cannabidivarin	<0.01
CBG	Cannabigerol	0.04
CBGA	Cannabigerolic acid	NR
CBN	Cannabinol	0.03
CBC	(+/-) Cannabichromene	NR
Total Cannabinoids *	2.39	23.86
Total Potential THC	0.15	1.47
Total Potential CBD	1.95	19.55
Total Potential CBG	0.04	0.41

3 consecutive measurements of hemp sample



Process Flow Chart

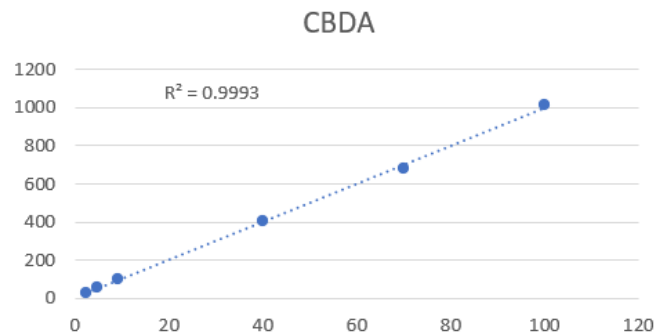
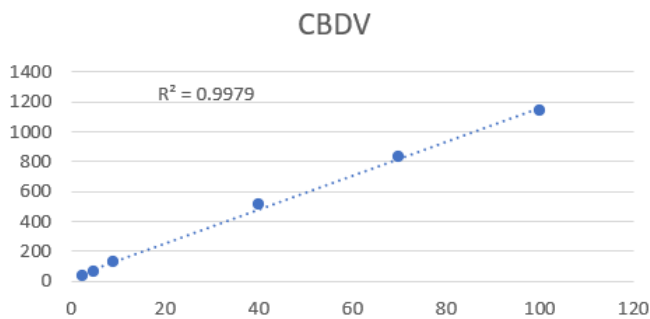


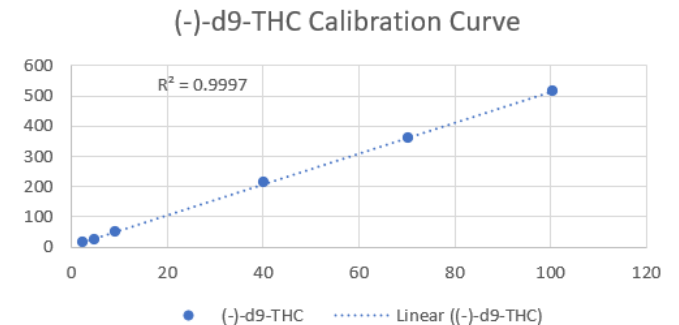
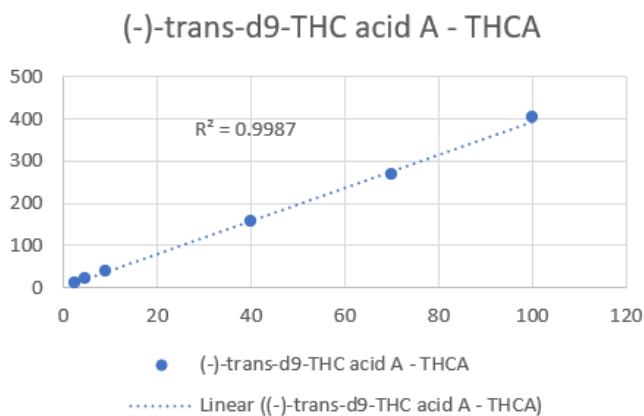
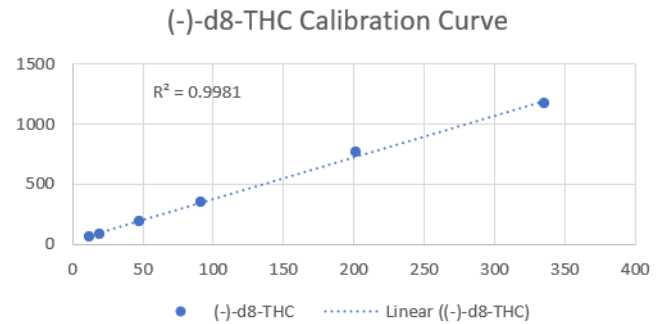
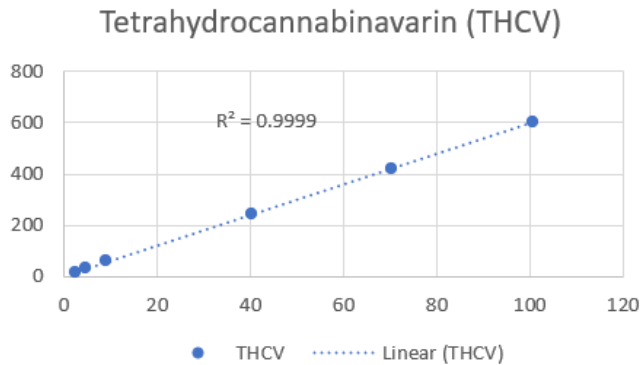
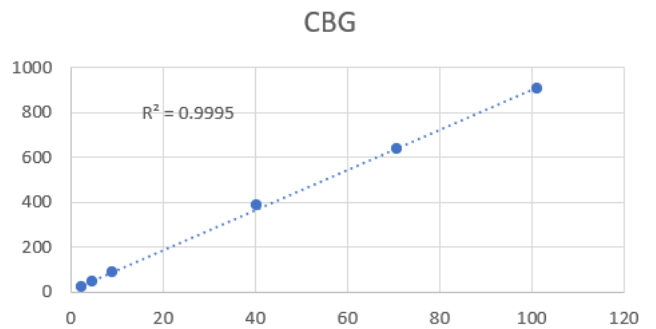
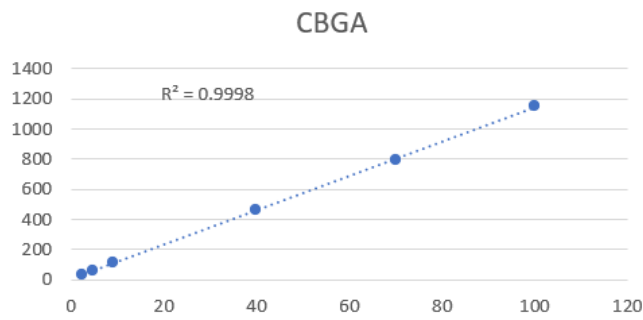
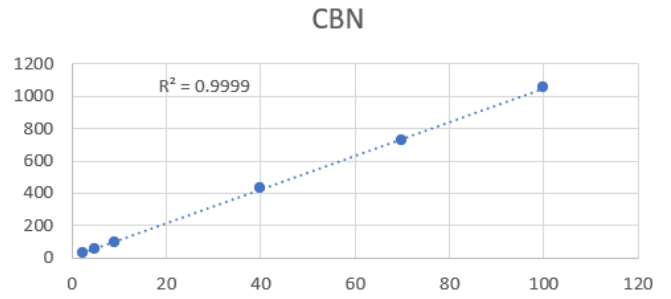
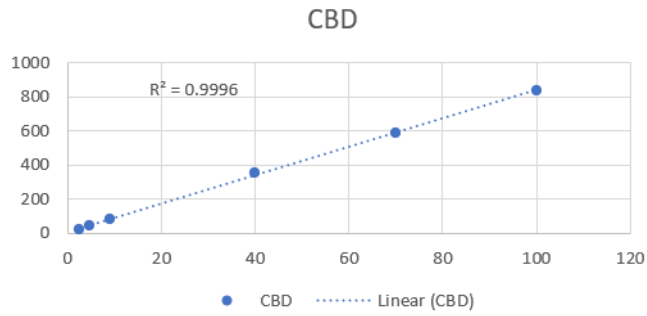
Process Flow Chart v.210228

CTInstruments Ltd.

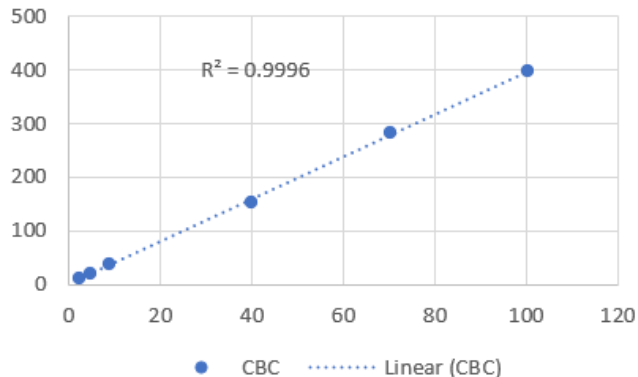
support@cannabistestingsimplified.com

Calibration Curves





(+/-) Cannabichromene (CBC)



Calibration Validation

Every day, after the HPLC is started and column is conditioned, a series of commercial standards is measured first to validate the instrument. Typically, benzoic acid (BA), CBD, and delta 9 THC (if available) are measured and results are recorded in calibration log. Pass/FAIL criteria are applied to confirm if the instrument operates as expected. RSD is calculated on the standards to trend the data and see any deterioration in condition of the instrument (UV lamp burning out, column performance). Then blanks, spikes, standards are measured as per need basis – depending on the needs of the laboratory.

Calibration log

Retention Time (min)	Standard Reported Concentration (ug/mL)	Standard Measured Concentration (ug/mL)	Date Standard Purchased	Standard Dilution Volume (uL)	Solvent Used for Dilution (uL)	Standards
Benzoic acid	1002.9	1013	12-Nov-20	20	1000	Benzoic acid
CBD	100.5	103.4	13-Jan-21	300	600	CBD
(-)-Δ9-THC	101.7	99.89	13-Jan-21	300	600	(-)-Δ9-THC

CALIBRATION LOG							
Calibration ID	Calibration Date	Standard	Standard Concentration (ug/mL)	Measured Concentration (ug/mL)	Delta (%)	Pass/Fail	Notes
1000	2020-09-20	Benzoic acid	1002.9	1021.2	1.8%	PASS	
1001	2020-09-20	(-)-Δ9-THC	101.7	99.3	-2.4%	PASS	
1002	2020-09-23	Benzoic acid	1002.9	1005.0	0.2%	PASS	
1003	2020-09-23	(-)-Δ9-THC	101.7	102.0	0.3%	PASS	
1004	2020-09-29	Benzoic acid	1002.9	1004.1	0.1%	PASS	
1005	2020-09-29	CBD	100.5	99.7	-0.8%	PASS	
1006	2020-10-01	Benzoic acid	1002.9	1007.7	0.5%	PASS	
1007	2020-10-01	CBD	100.5	103.0	2.5%	PASS	
1008							

Set PASS LIMIT
5%

Relative Standard Deviation		
Benzoic Acid	CBD	d9-THC
0.79%	2.30%	1.90%

Standards validations from the day are automatically recorded on each lab report for transparency.

Instrument Calibration & Quality Control

Date of Quality Control	Standard	Standard Concentration (ug/mL)	Measured Concentration (ug/mL)	Delta (%)	PASS/FAIL	Notes
13-May-21	Benzoic acid	1002.9	1013.0	1.0%	PASS	
13-May-21	CBD	100.5	103.4	2.9%	PASS	

3. Work order sheet generation

WORK ORDER		
SAMPLE TAG ID	100380	Date of Analysis: May 13, 2021
Customer Name:	CTInstruments Ltd.	
Date Received:	July 14, 2020	SAMPLE DENSITY
Sample Type:	Flower	1
Sample Description:	test	
Analysis Type:	cannabinoids	269

SAMPLE DENSITY (gram/mL)		
all solids	1	
MCT oil	0.95	
coconut oil	0.903	
olive oil	0.917	
hemp oil	0.922	

WORK ORDER WORKSHEET		
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	Weight of Empty Vial (g)	
	Weight of Vial with Sample (g)	Starting Weight of Material (mg)
Solids		
Plants		
Waxy Extracts		
Weighted materials		

	Cannabinoid	RT (min)	Measured Value
	CBDV		18.01
	CBDA		262.14
	CBGA		0
	CBG		5.87
	CBD		79.5
	THCV		0
	CBN		8.36
	d9-THC		3.79
	d8-THC		0
	d9-THCA		7.19
	CBC		0

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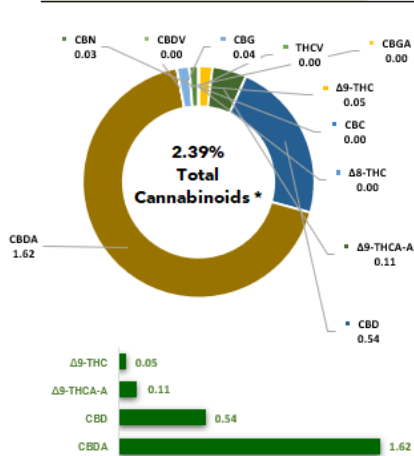
4. Lab Report Generation

Customer Name: CTInstruments Ltd.
Address: 100 111 5th Ave SW
 Calgary AB
Phone Number: 403-629-8597
Email: support@cannabistestingsimplified.com

Sample Type: Flower
Sample Description: test
Sample TAG ID: 100380

Date Received: 14-Jul-20
Test Date: 13-May-21
Test Method: HPLC-01

CANNABINOID PROFILE



Compound	Result (% w/w)	mg/gram of sample
THCV	Tetrahydrocannabivarin	NR
d8-THC	(-)-d8-THC	NR
d9-THC	(-)-d9-THC	0.05
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* Total Cannabinoids = sum of all measured cannabinoids
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 Total Potential CBD = CBD + CBDA
 NR = None Reported. Measured amount is below detection limit for the specified method.

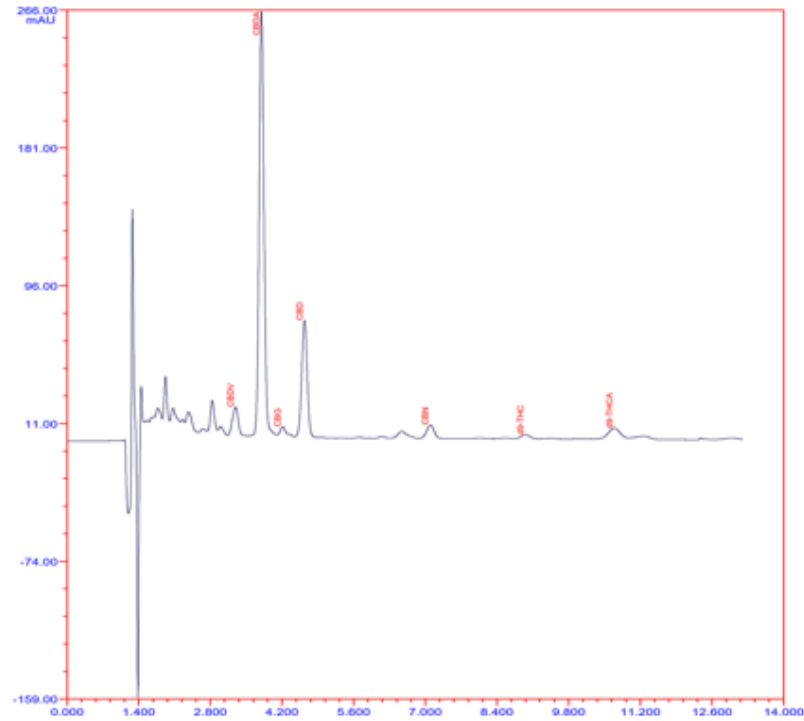
FINAL APPROVAL

Analyst Name: OVL
Date: 13-May-21

QA Name: OVL
Date: 13-May-21

Prepared By

Approved By

Chromatogram

Lower Limit of Quantification (LLOQ)

The lower limit of quantification (LLOQ) is the lowest amount of a cannabinoid in a sample that can be quantitatively determined with suitable precision and accuracy using the corresponding method and dilution rates. All values below this threshold are reported as NR - None Reported.

Compound		LLOQ (% w/w)
THCV	Tetrahydrocannabivarin	0.01
Δ8-THC	(-)-Δ8-THC	0.02
Δ9-THC	(-)-Δ9-THC	0.01
Δ9-THCA-A	(-)-trans-Δ9-THC acid A	0.02
CBD	Cannabidiol	0.01
CBDA	Cannabidiolic acid	0.01
CBDV	Cannabidivarin	0.01
CBG	Cannabigerol	0.01
CBGA	Cannabigerolic acid	0.01
CBN	Cannabinol	0.01
CBC	(+/-) Cannabichromene	0.02

Instrument Calibration & Quality Control

Date of Quality Control	Standard	Standard Concentration on (ug/mL)	Measured Concentration on (ug/mL)	Delta (%)	PASS/FAIL	Notes
13-May-21	Benzoic acid	1002.9	1013.0	1.0%	PASS	
13-May-21	CBD	100.5	103.4	2.9%	PASS	

5. Instrument Maintenance

Maintenance LOG					
Date	Analyst	Maintenance Performed/Corrective Action	Comment on Damage or Malfunction	Part replaced	Back in Service?

6. Detailed Testing Protocols included – proprietary information.

All aspects of the LIMS are customizable.