

Analytical Testing Instruments - Cannabis and Hemp



Rod Finney Technical Sales and Support Product Manager for SFC/SFE





Discussion Points

- Prominence-I Plus potency HPLC
- Service Plans for HPLC
- Terpene Profiles
- Nexera UC SFE/SFC
- Residual Solvents



Cannabis – Active Ingredient Definition

- An active ingredient is a therapeutically active component in the medicines final formulation that is responsible for it's physiological or pharmacological action (Section 4 of TGO 93).
- In addition, the following ingredients are considered to be active ingredients in section 4(2) of TGO 93 for the purposes of TGO 93:

Cannabis - Active Ingredient Definition

- Any tetrahydrocannabinol (Including any corresponding acid) greater than or equal to 1.0% w/w or w/v of the product
- Any other cannabinoids (Including any corresponding acid) greater than or equal to 2.0% w/w or w/v of the product

• Ref: TGA Standard for Medicinal Cannabis (TGO 93) Order 2017, Version 1.2, May 2019

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Right Tool for the Job? Wood Screw?









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One Tool or Multiple Tools in the Tool Box





"Comprehensive" Cannabis Analysis by LC-MS/MS

- Merriam-Webster
 Dictionary
 - covering completely or broadly
- Cambridge Dictionary
 - Complete and including everything that is necessary
- Dictionary.com
 - broad, wide, extensive, full
- Oxford Dictionary
 - Including or dealing with all or nearly all elements or aspects of something





Cannabis Analytical Testing

- **Potency profiling**
- **Terpene Profiling**
- **Extraction with SFE**
- **Pesticide screening**
- **Residual solvents**
- Heavy metals
- Mycotoxin/aflatoxin
- **Moisture content**



Cannabinoids

In cannabis, cannabinoids are the target compounds of potency testing.

There are at least 113 different cannabinoids isolated from cannabis, exhibiting varied effects.

THC and CBD are known as key compounds.



Certificate of Analysis for CBD Oil



Certificate of Analysis November 10, 2017

FP-17-0981 Sample ID:

Product name Plus cbd oil ps80 free spray peppermint

Batch/Lot#:	7305319

2oz 500mg Strength:

Cannabinoid:	mg:
CBD	497.69
CBDV	10.40
CBDA	15.56
CBG	5.35
CBGA	0.00
CBC	20.13
CBN	3.64
∆ ⁹ -THC	8.96
THCA	0.00
Total cannabinoids:	561.7 mg
Sample size:	54 g
THC by mass:	0.0166 %



Analyst	Date

Vandana Kothari, M.S., B.Pharm Analytical Chemist

	0
IENCES	
A LIFE CONTRICT CONTRACTOR	Sample Name

sclabs

Sample Name:	(FP-17-0981) Plus CBD Oil 2oz Extra Strengt h Peppermint Spray PS80 Free Lot: 7305319
Tested for:	CV Sciences
Sample ID:	171109Q093
Date Submitted:	11/09/2017
Sample Type:	Infused

Total Sample Weight: 54.0000 Grams

Cannabinoid Test Results Cannabinoid analysis utilizing High Performance Liquid

concentration and a but not	asi saray
Cannabinoid Summary	
Total THC	∆9THC+THCa
Total CBD	CBD+CBDa

Full Canabinoid Profile

	THC		0.21 mg N
	CBD		8.85 mg
	CBDa		0.04 mg
			0.05 mg
	CBN		
	CBDV		N
	CBDVa		N
	CBG		N
	CBGa		N
	THCV		N
	A8 - THC		N
	CBC		0.41 mg
	CBC		0.41 mg
	Total Active	Cannabinoids:	9.56 mg
	Pesticide Te	est Results	
	Pesticide, Fun HPLC-Mass Sp	gicide and plant growth regulat sectrometry	or analysis utilizing
		Reporting	
		Limit	
	Acequinocyl	Not Detected 1	
	Abamectin	Not Detected 0.25	
	Bifenezate	Not Detected 0.1	
	Daminozide	Not Detected 0.5	
	Fenoxycarb	Not Detected 0.1	
	Imidacloprid	Not Detected 0.2	
	Myclobutanil	Not Detected 0.1	
17	Paclobutrazol	Not Detected 0.2	
(3)	Pyrethrins	Not Detected 0.5	
	Spinosad	Not Detected 0.1	
NOV 10	Spiromesiten	Not Detected 0.1	
1101 10	Spirotetramat	Not Detected 0.1	

Reviewed By

Method:

HPLC

HPLC

HPLC

HPLC

HPLC

HPLC

HPLC

HPLC

HPLC

Dalia Chandler, M.S., B. QA/QC Manager

Certificate of Analysis

*Amendment to CoA 171109Q093-001

Microbiological Test Results

Terpene Test Results

a Rissholol

a Pipene

3 Carene

Borneol

Geranio

a Humulen

Terpinolene

Valencene

Menthol

Nerolido

Camphene

Eucalyptol

α Cedrene

(-)-Isopulego

Camphor

Sahinene

y Terpineni

(GC - FID)

Propane

Methanol

Isobutane

Isopentane

n Butane

n Hexane

Total Terpene Concentrat

2,2-Dimethylbutane N/A

3-Methylpentane

Residual Solvent Test Result

N/A

N/A

N/A

N/A

N/A

N/A

N/A

β Caryophyller

11.34 mg

480.06 ma

0.21 mg/g ND

8.85 mg/g 0.04 mg/g 0.05 mg/g ND

0.41 mg/g

9.56 mg/g

ND ND

ND ND

3M Petrifilm and plate counts for microbiological contamination Total Yeast and Mold <1,000 cfu/g E. coli ND Pseudomonas ND Coliforms <100 cfu/a Total Aerobic Plate <1,000 cfu/a Salmonella ND Count

Tenpene analysis utilizing Gas Chromatography - Flame Ionization Detection (GC - FID)

mg/g / %

N/A

Residual Solvent analysis utilizing Gas Chromatography - Flame Ionization De

a Teminene

Linalool

Limonene

Myrcene

Terpineol

8 Pinene

Citronellol

p-Cymene

Ocimene

Isoborneo

Guaiol

Phytol

Ethanol

Isopropanol

Mercaptan

Neopentane

n Heptane

n Pentane

2-Methylpentane

Cyclohexane + Benzen

α Phellandrene

R-(+)-Pulegone

Geranyl Acetate

Caryophyllene Oxide

Fencho



CV Sciences

Sum HIT

Clert

Cless #

Collector

Sample Type:

Parameter

Amenic

Logt

N/A

N/A N/A

N/A

N/A

N/A

N/A

N/A

Cubrings

Mesney

Analytical Report Nevember 14, 2017 mg Vanduna Kothari Page Lift1 W17K0336 WO Number 3030 Battlet Hill St. San Diego, CA 97310 CV Sciences C2662 Castoner PO 13/9/2017 122662 Date Recents Liquid Product Mass Date Completes 11/14/2011 Discard Date 11/28/2017 Client CSL#: 17K1053 Plus CBD Oil Extra Strength Peppermint Spray Tox P598 Free (FP-17-0981) - Lot#: 7305319 Result PQL 0.50 Method Bate 11/13/17 Analyst -0.50 ppm ICF MS liń 0.25 11/13/17 <0.25 ppm ICTF MS ЛG <0.050 ppm 0.150 109 MS 11/09/17 A/III «0.10 ppm 0.10 1CP MS 11/13/17 HO Respectfully Submitted Chemical Solutions, Lad. Nervedro Naugle Reviewed and Appreved Nov 14 2017 12:09 PM

Murdes 2. No QA Representative

None CONFEDENTIAL REPORT. This report is confidential and is for the sole use of the addresses This report can only be reproduced in full.

The units for the PQL are the same as these shown for the result.

Sample Certification



zer. President

931 north seventh at harrisburg, pi 17102 phone 712/697-7536 fax 712/697-4400 seww.chemicalsolutionaltd.com

SC Laboratories - 100 Pioneer Street, Santa Cruz, CA 95060

CoA ID: 171109Q093-002 - Page 1 of 1

These results relate only to the items tested. This test report shall not be reproduced except in full, without written approval of the laboratory is uncertainty of measurement associated with the measurement result reported in this certificate is available from SC Laboratories upon reque

10 Potency, Pesticides, Micro, Terpenes, Residual Solvents, Heavy Metals

Cannabinoids for Medical Treatment



- CBD Treatment
- Mood/Behavior
 - Anxiety
 - ADD/ADHD
 - PTSD
 - Depression
- Neurological
 - Epilepsy
 - Seizures
 - Alzheimer's
 - Parkinson's
- Gastrointestinal
 - Nausea
 - Crohn's
 - Diabetes
- Pain/Sleep
 - Pain/Arthritis

https://www.leafly.com/news/cannabis-101/cannabis-entourage-effect-why-thc-and-cbd-only-medicines-arent-g

Potency by LC-MS/MS or HPLC/UV

- LC-MS/MS
- Cannabinoids: 1-30
- Cost: \$350,000
 - 7-9x more cost
- Level of Expertise: Experience MS (Higher)
 - Higher Salary
- Linear Dynamic Range
 - Less then HPLC/UV
- Calibration Frequency
 - 2-3 Days maximum
- Detector Stability: Lower
- Source Switching (3 types)
 - ESI to APCI time consuming
- Research Labs

- HPLC/UV
- Cannabinoids: 1-17
- Cost: \$40,000-\$50,000
 - 7-9x less cost
- Level of Expertise: Technician Level (Lower)
 - Lower Salary
- Linear Dynamic Range
 - 0.1% -100% Flower or Concentrates
- Calibration Frequency
 - QC Check in the morning
- Detector Stability: Higher
- Source Switching
 - Not Required
- QA/QC Labs

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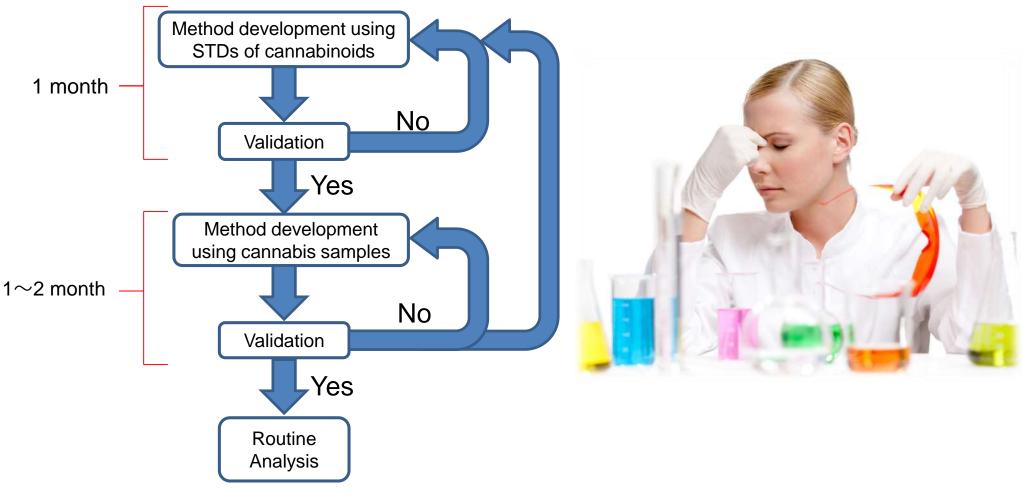
Cannabis Analyzer for Potency





Method Development and Validation

Method Development and validation are tedious and challenging...



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Introducing Cannabis Analyzer for Potency

- 1. Easy to Use
- 2. Low Cost

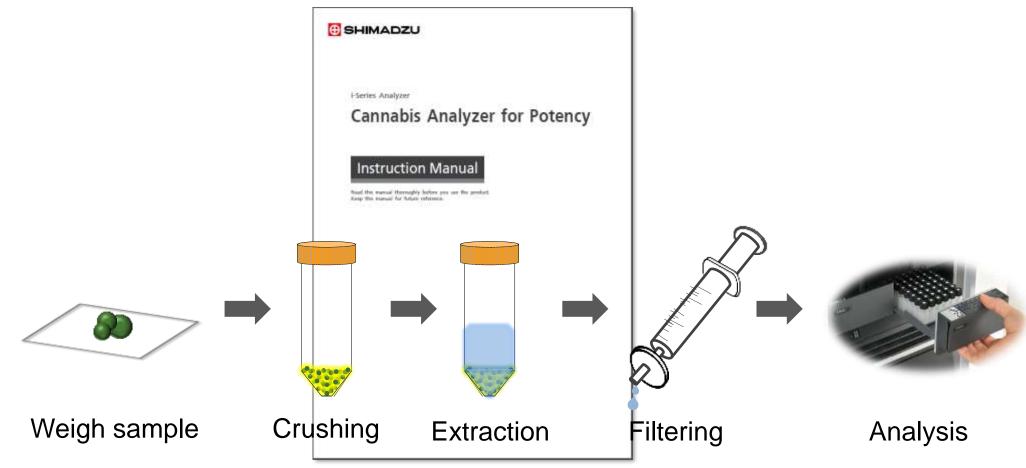


- 3. USP Guideline Methods
- 4. Service Plans
- 5. Turn-Key, All-In-One

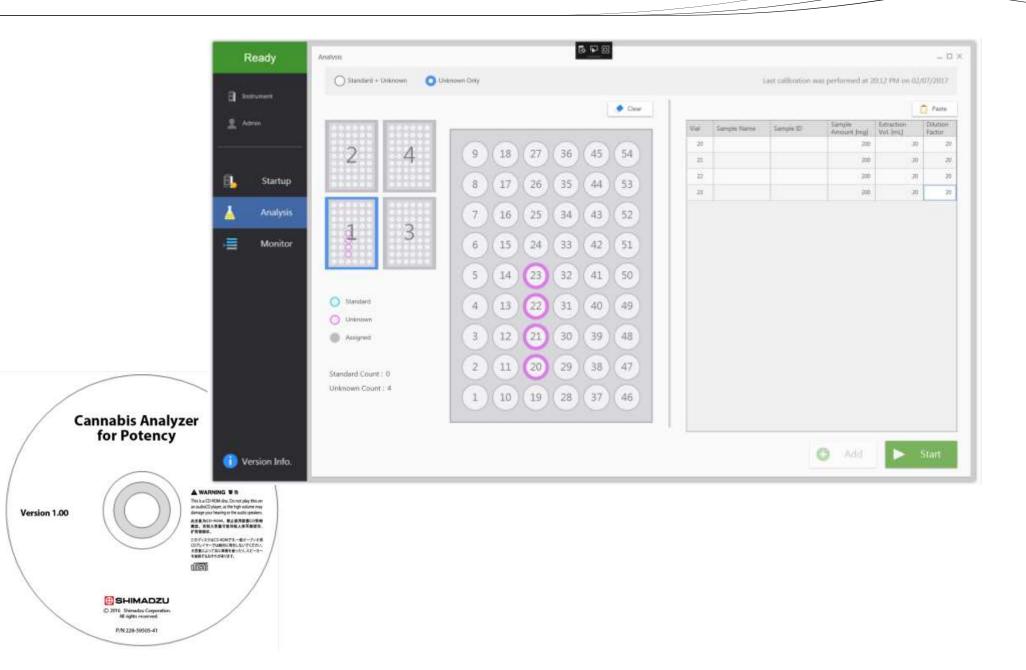


1. Easy to Use (Sample Prep)

Pretreatment Procedure



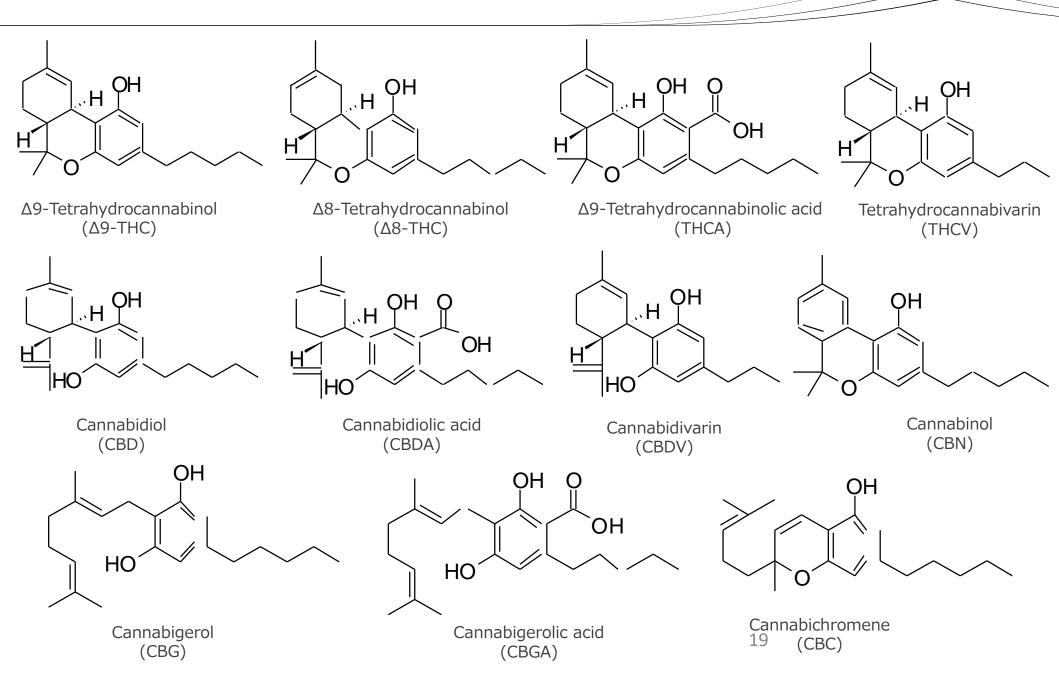
1. Easy to Use (Software)



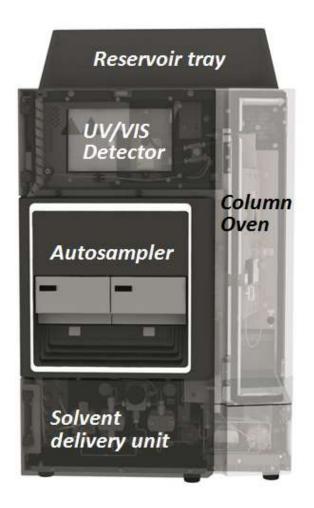
2. Low Cost



3. Validated Methods: 10 or 11 Cannabinoids



Service Plans

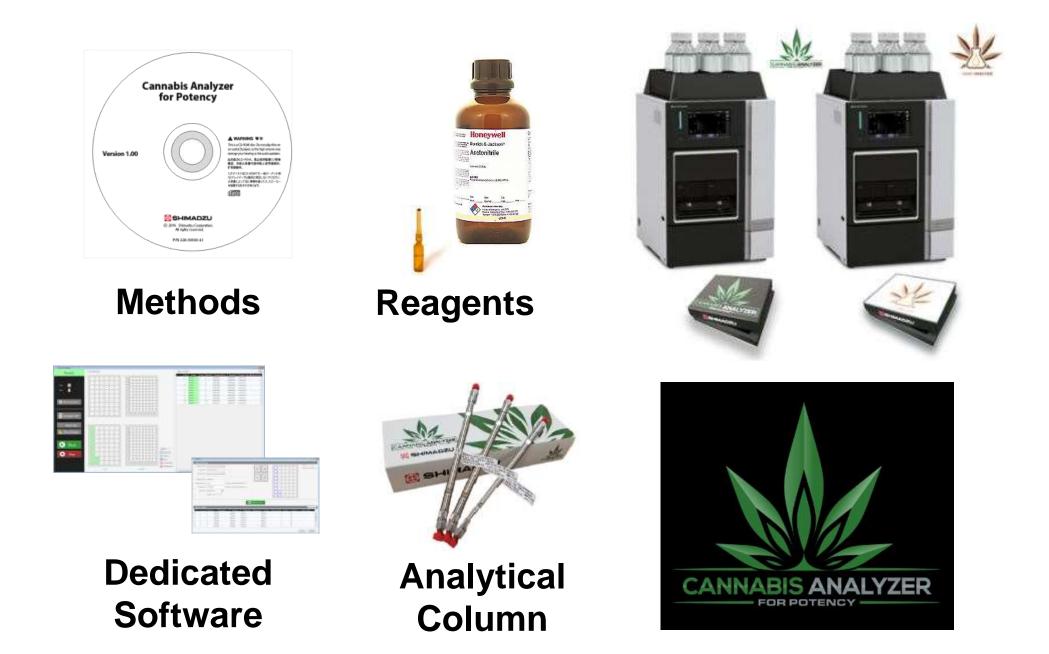


- Three types of plans offered
- Platinum Plus, Platinum and Gold. PM only also available
- Warranty
- Phone Technical Support



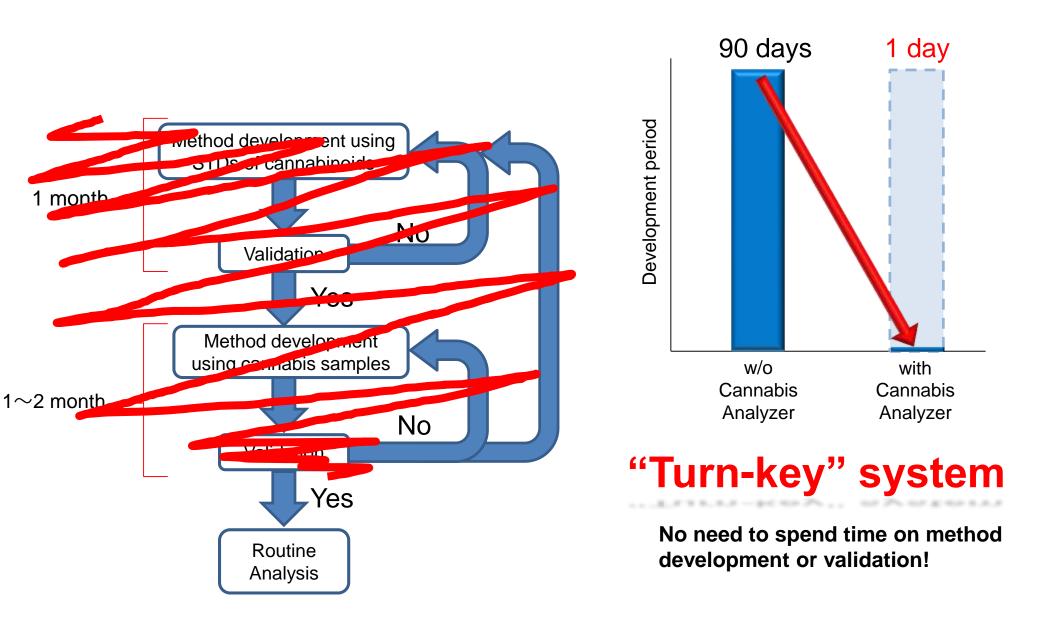
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5. "Turn-Key, All-in-One" Package

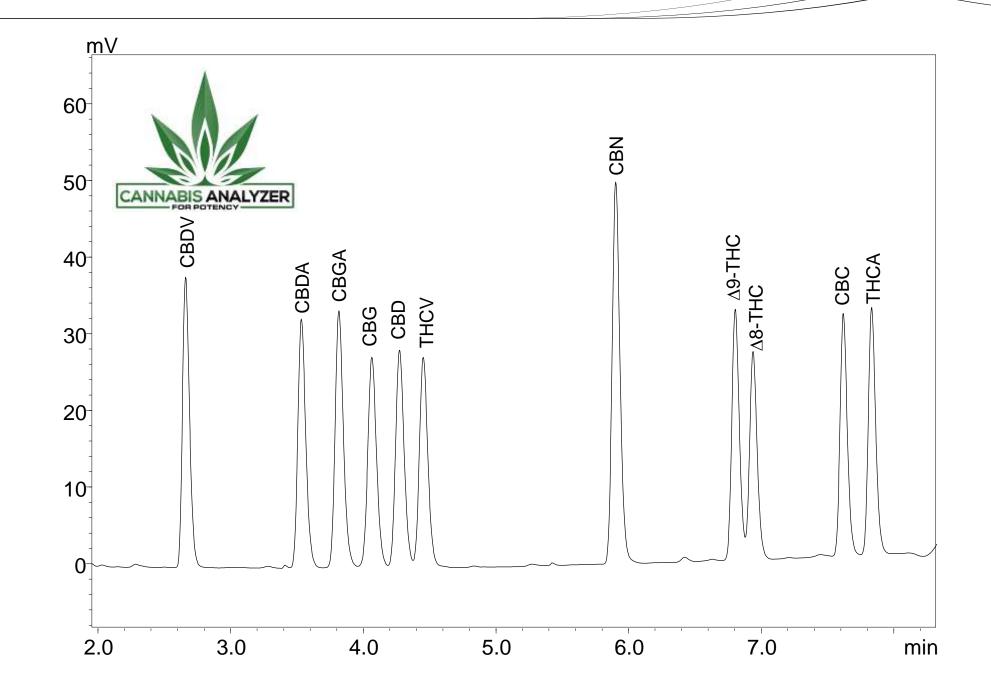




Cannabis Analyzers



High-Sensitivity method



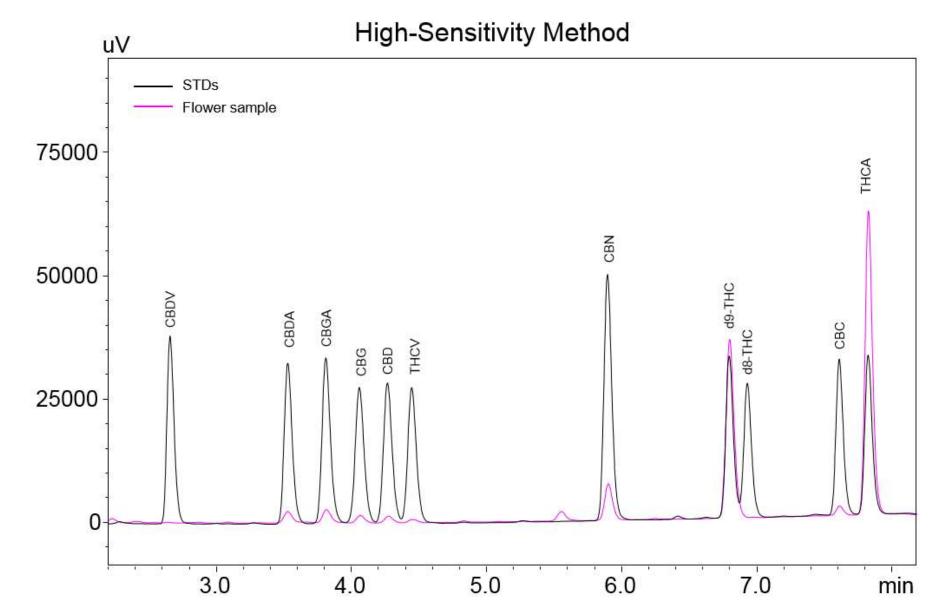
() SHIMADZU

Farm Bill – Passed December 2018

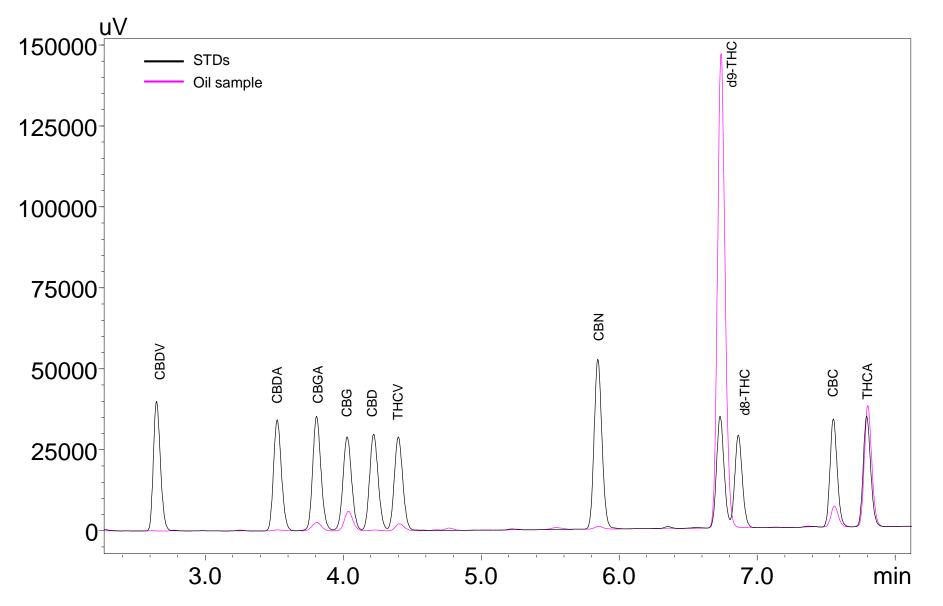
- Cannabis Sativa L. is removed from the Controlled substances act if:
 - Plant contains no more than (NMT) **<u>0.3% THC</u>** on a dry-weight basis



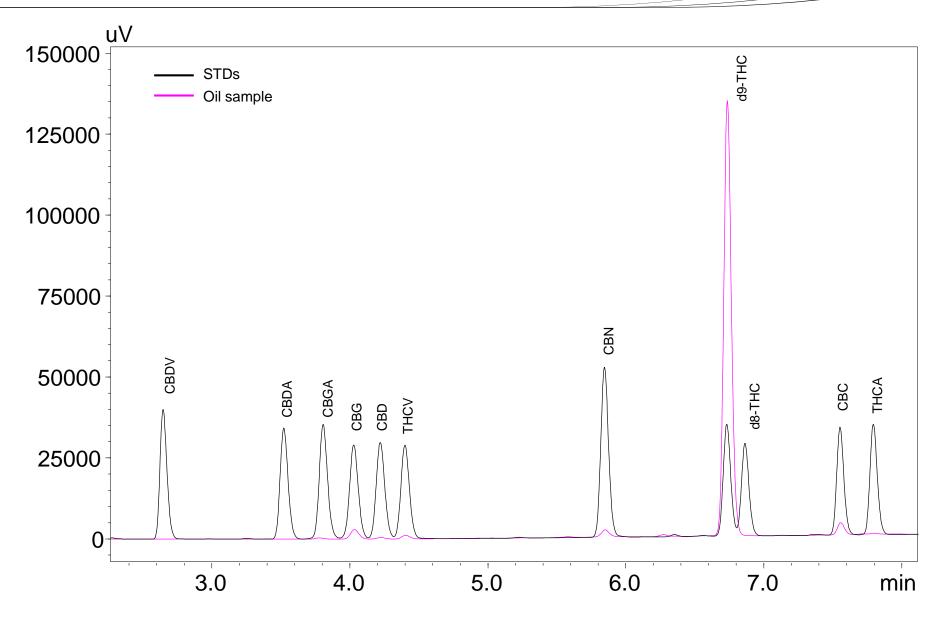
Flower Samples



Oil sample 1 (Raw)



Oil Sample 3 (Refine 2)



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CBD in Hemp Oils



Application News

High Performance Liquid Chromatography

OMPLE

The Determination of CBD and General Cannabinoid Content in Hemp Oils Using **HPLC with UV Detection**









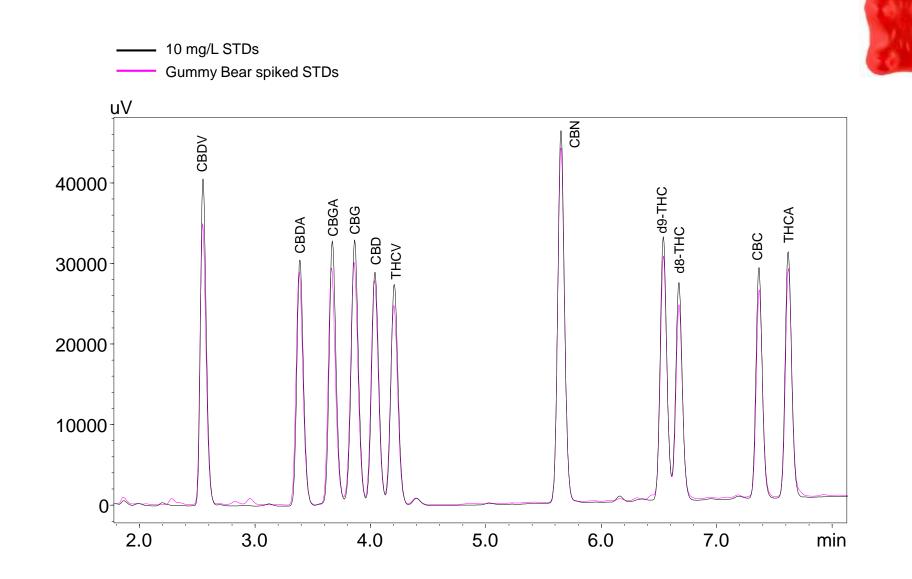




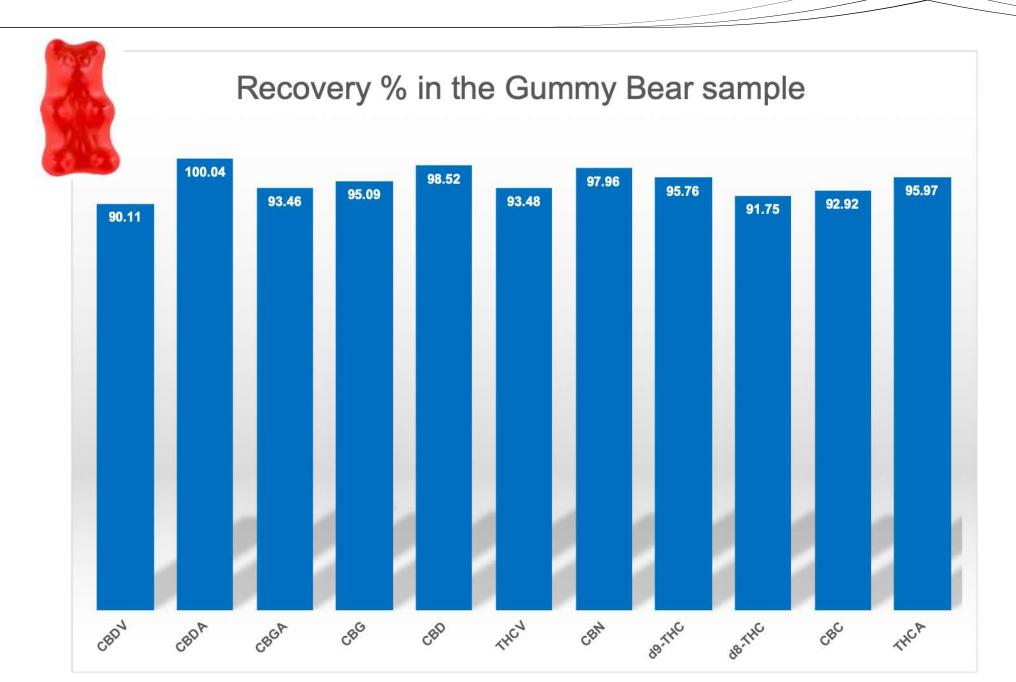


Recovery Results, 100 ug/g spike

Using Q110 (EN)



Cannabinoid Recovery Results





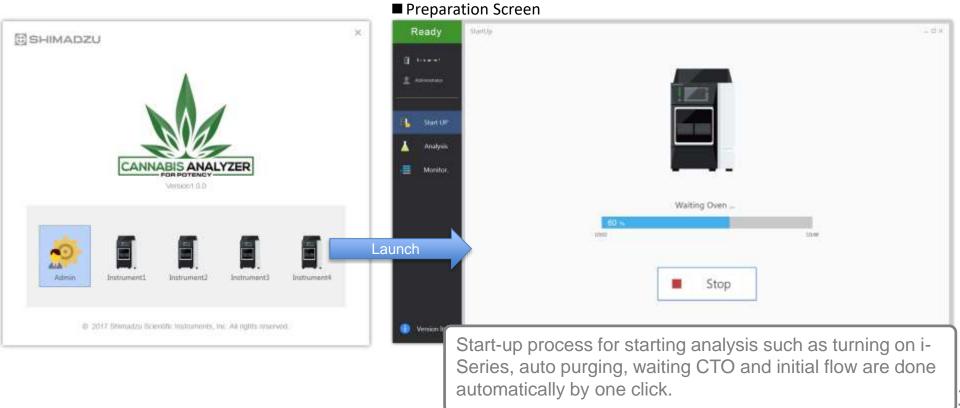
1. Ease of use _ Cannabis Analyzer Software

Outline

- Dedicated software for Cannabis Analyzer with the simple user interface
- Overlay software working on LabSolutions

Features

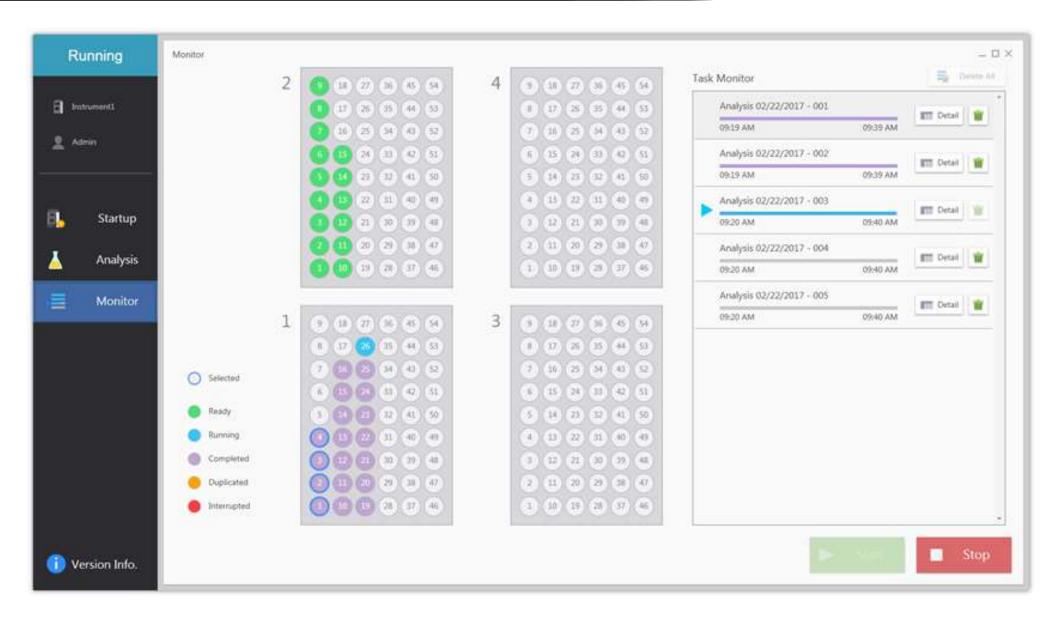
1. One Click Start-up



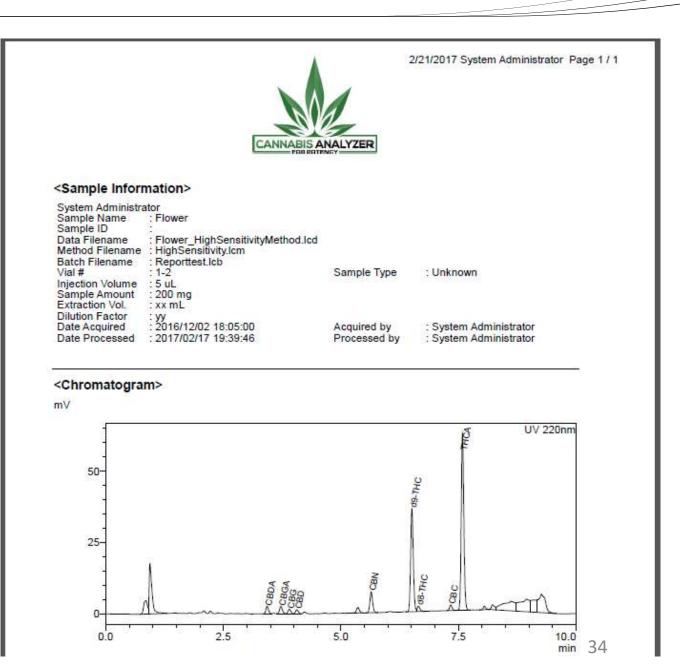
Step 2. Analysis

	Standard + Unknown	nknown Only			Last calibration w	as performed at 2	0:12 PM on 02	2/07/2017
Instrument	•	Clear						🗂 Paste
Admin		Crear	Vial	Sample Name	Sample ID	Sample Amount [mg]	Extraction Vol. (mL)	Dilution
	2 4	9 18 27 36 45 54	20			200	20) 2(
<i></i>	4		21			200	20	
Startup		8 17 26 35 44 53	23			200	20	
Analysis		7 16 25 34 43 52						
Monitor	1 3	6 15 24 33 42 51						
		5 14 23 32 41 50						
	O Standard	4 13 22 31 40 49						
	O Unknown							
	Assigned	3 12 21 30 39 48						
	Assigned Standard Count : 0	3 12 21 30 39 48 2 11 20 29 38 47						
	Standard Count : 0	2 11 20 29 38 47						

Step 3. Monitor



Sample Info & Chromatogram



Quantitative Results

<Quantitative Results>

UV			
ID#	Name	Ret. Time	Dry weight %
1	CBDV		0.00
2	CBDA	3.430	0.13
3	CBGA	3.723	0.13
4	CBG	3.904	0.07
5	CBD	4.063	0.06
6	THCV		0.00
7	CBN	5.643	0.27
8	d9-THC	6.509	2.00
9	d8-THC	6.648	0.11
10	CBC	7.335	0.11
11	THCA	7.584	3.74

Total THC	5.27	%
Total THC	52.74	mg/g
Total CBD	0.17	%
Total CBD	1.75	mg/g

Smart Phones

System Status



Chromatogram Progress



Terpene Profiling

- Greater than 200 Terpenes found in Cannabis
- Synergistic effect with cannabinoids
- "Entourage Effect"
- Pinene Linalool

Limonene



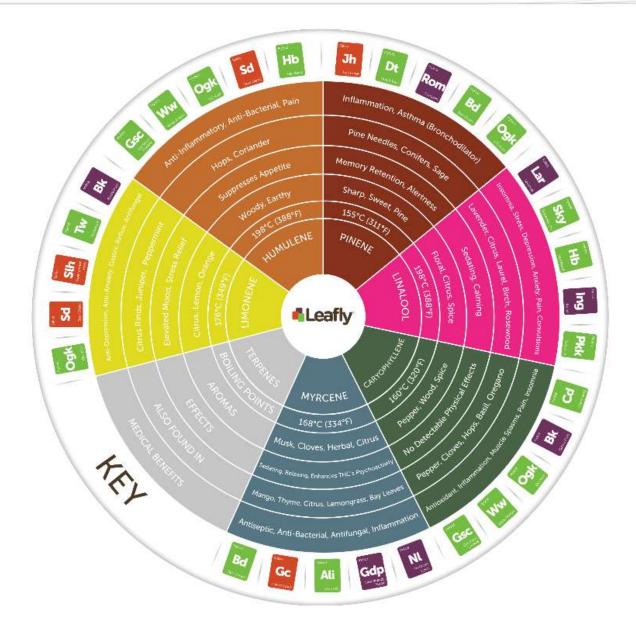






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Terpenoids for Medical Treatment



Cannabinoid & Terpenes in Trichrome



GI Grow Biomedical Farm Video, Available at Shimadzu's GrowYourLab.com cannabis microsite.



Different Cultivar have Different Contents



Purple DanX



Purple Erkle



Super Silver Haze



Trainwreck



Bubba Kush



OG Kush



Purple Kush

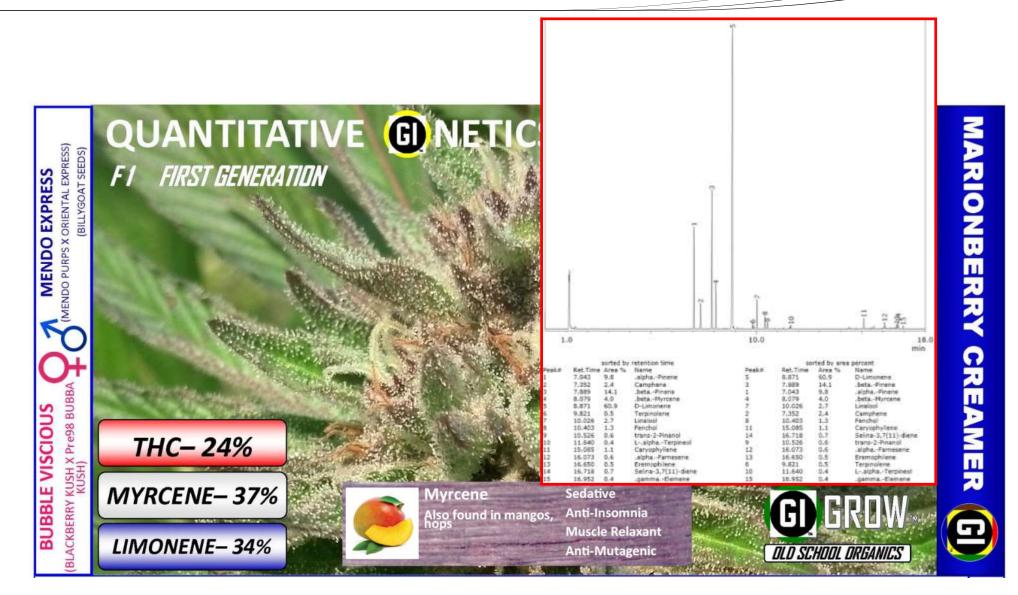


Sour Diesel

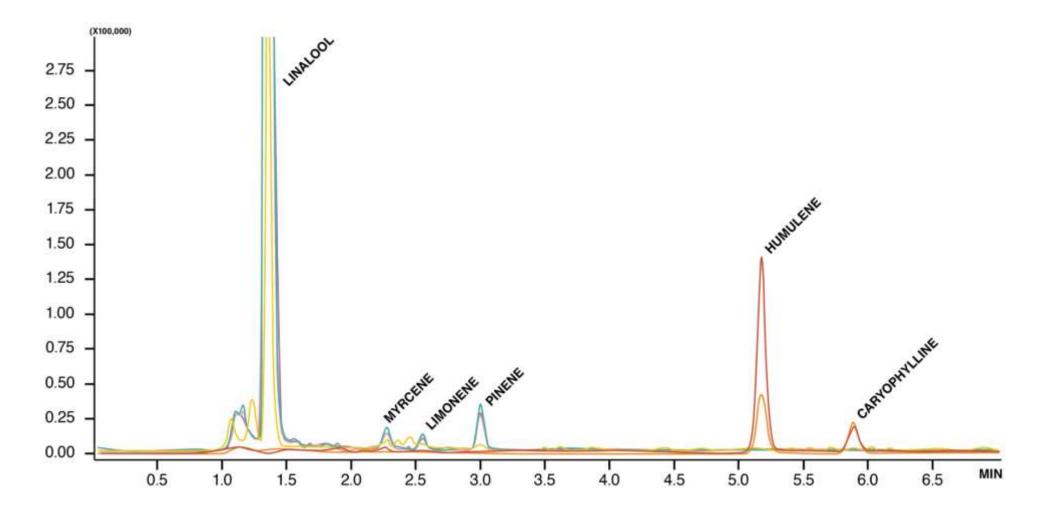
Terpene Profiling



Terpene Profiling

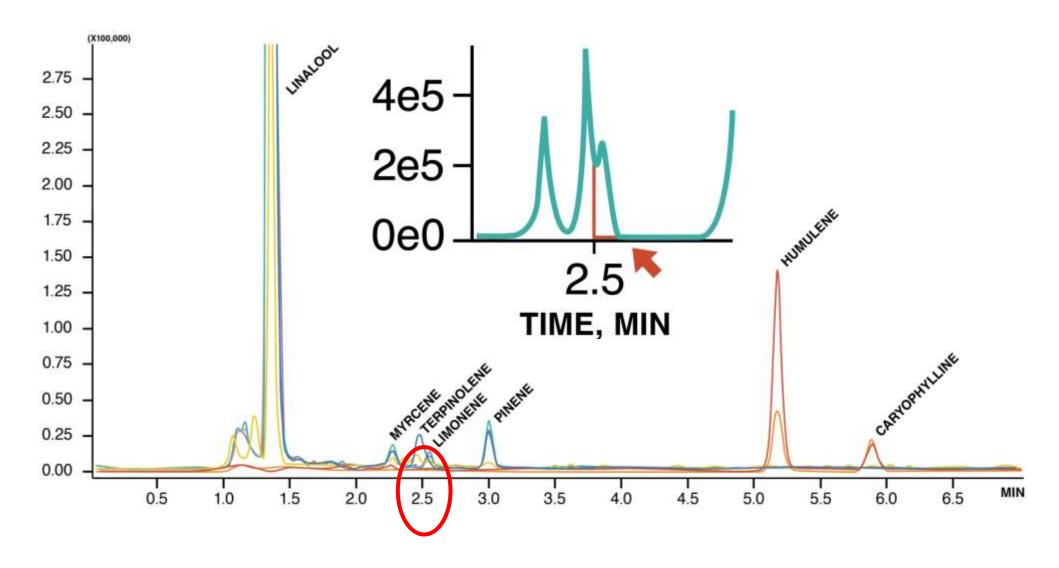


LC-MS/MS Measurement of 6 Terpenes Solvent & Matrix

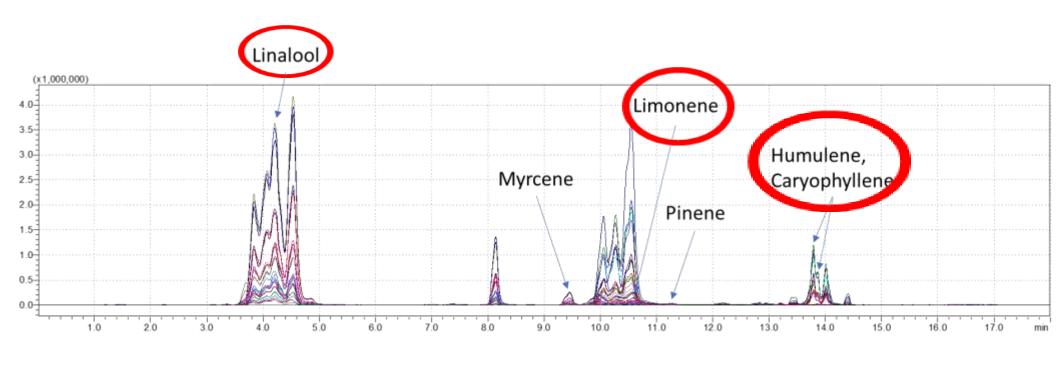


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LC-MS/MS Terpene Analysis with 7 Terpenes



LC-MS/MS Terpene Analysis with 42 Terpene

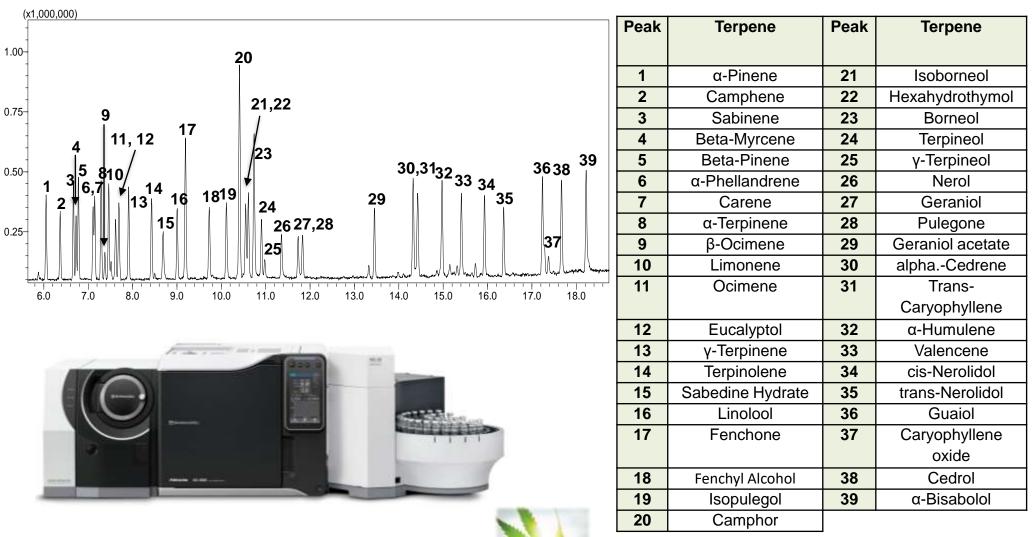




Other Terpenes in cannabis: Caryophyllene Oxide, Nerolidol, Phytol

Russo, Taming THC potential cannabis synergy and phytocannabinoid-terpenoid entourage effects, Br J Pharmacol, 2011

Terpene Analysis by GC-MS



GCMS-2020NX with HS-20 headspace





Cannabis Analytical Testing

Potency profiling

Terpene Profiling

Pesticide screening

Fungicide analyses

Residual solvents

Heavy metals

Mycotoxin/aflatoxin

Moisture content





Nexera UC

- 1. Getting to know the terms? SFE/SFC
- 2. What is a Supercritical fluid?
- 3. A description of SFE and SFC
- 4. Where can I use this technology?
- 5. How has the combined SFE/SFC instrument been received?

Knowing the terms

SFE – Supercritical Fluid Extraction

• SFC – Supercritical Fluid Chromatography

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What is a Supercritical fluid?



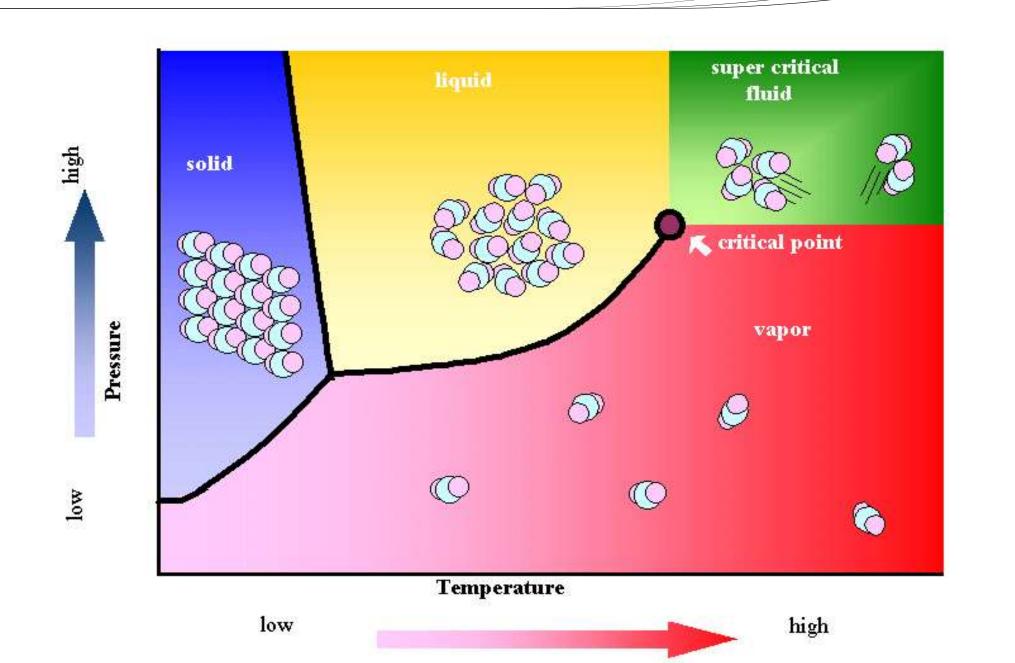
Beer?



Possibly.. However..

• Supercritical fluid for CO2 is: a fluid state of <u>carbon</u> <u>dioxide</u> where it is held at or above its critical temperature and critical pressure. <u>Carbon dioxide</u> usually behaves as a gas in air at standard temperature and pressure (STP), or as a solid called dry ice when frozen

Supercritical Table



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Why do we use Carbon Dioxide for Supercritical fluids?

- Easy to handle due to low critical point (31.1 °C, 7.4 Mpa)
- Chemically inert and non-flammable
- High-Purity carbon dioxide can be obtained relatively inexpensively
- Non-toxic and environmentally friendly
- Low polarity: requires adding a solvent (modifier)

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Where are Supercritical fluids currently used?

 Decaffeination of coffee – Has been performed for over 30 years. The extracted caffeine is on sold to pharmaceutical and food industries.



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Where are Supercritical fluids currently used?

 Removing cork taint from corks... Over a billion corks annually.



Lets talk about.... Supercritical Fluid Extraction SFE

 Supercritical Fluid Extraction – An extraction method using supercritical fluid. It's also as a pretreatment method for solid sample analysis.



SFE features

- Carbon Dioxide is a non-polar molecule, it readily dissolves hydrophobic compounds, similarly to organic solvents such as hexane etc.
- Density can be varied easily by varying pressure. Allows solubility of the solvent to be varied.
- Polarity can be varied by extracting compounds along with an organic solvent additive such as Methanol. It can be used for extracting a wide range of compounds.

SFE features

We can extract for a wide range of analysis

- Dried blood spots
- Trace additives in polymers
- Pesticide analysis in food
- Drugs of abuse
- Cannabinoids in Cannabis leaf

SFE features

- Reduces time for lengthy sample preparation techniques which include centrifugation and homogenization in conjunction with QuEChERS(Quick Easy Cheap Effective Rugged Safe)
- Nexera UC provide sample prep with intervention

(mul

Using SFE



- Nexera UC is capable of online extraction and analysis under supercritical conditions
- SFE is gentle to the analytes it extracts

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How are the samples introduced into the instrument



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SFE Extraction tray with either 0.2 ml or 5ml vessels



SFE Extraction Vessel 0.2 ml

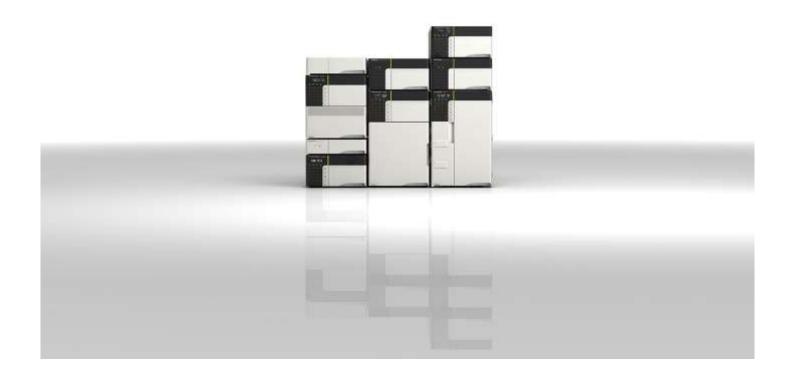


SFE Extraction Vessels 0.2 ml and 5.0 ml



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That's a intro in SFE, lets talk about SFC?



- SFC Supercritical Fluid Chromatography- It's the chromatographic technique using supercritical fluids as mobile phases.
- It has unique properties, It enables high-speed, highresolution analysis and improved efficiency

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SFC - Using a super critical fluid as mobile phase

 Straight CO2 is not the perfect mobile phase but adding a modifier like methanol or ACN, this is performed via the modifier pump.

 Environmentally friendly CO2 is collected from existing fermentation processes and bottled

SFC – Mobile phase benefits

- Very fast separation speed due to relatively low viscosity of supercritical fluid
- Improved separation and detection capabilities from the low viscosity and high diffusion coefficient of supercritical fluid
- Replaces dreaded normal phase chromatography using toxic solvents e.g. hexane

SFC - Benefits

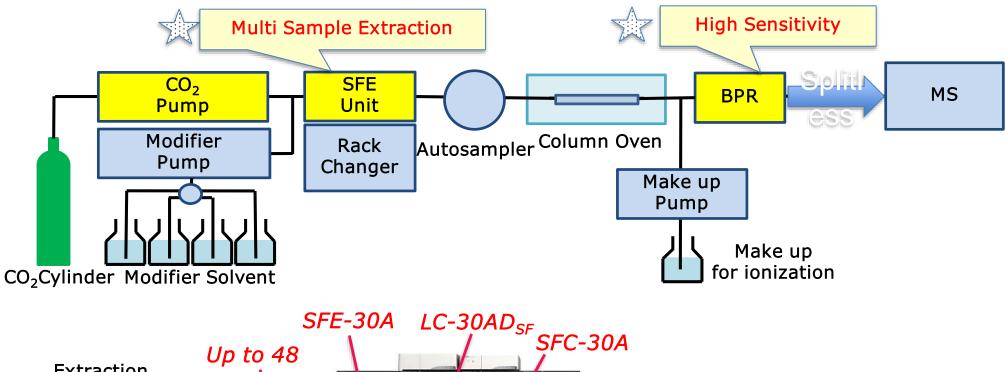
- Supercritical fluid has unique properties different from liquid solvents
- Using SFC as a inlet to a mass spectrometer offers greater sensitivity than achieved by LC/MS/MS
- Reduction of environmental impacts and costs by reducing amount of organic solvents needed

So what is Unified Chromatography?

- It unites the sample preparation and chromatography into one instrument – Nexera UC
- SFE, SFC and LC/MS/MS in one instrument
- Extraction to chromatography performed under supercritical conditions

Nexera UC system configuration

Online SFE-SFC System



Extraction Vessel





Summary: True Comprehensive Cannabis Analysis

Ease of Compound Measurements by Instrument Type (***= Easiest)

		LCMS	GCMS	HPLC	HS-GCMS	HS-GC	ICP-MS
	Potency			***			
	Terpenes		**		***	**	
	Mycotoxins			*			
R	Residual Solvent		**		***	**	
	Imidacloprid	***					
	Myclobutanil	***					
	Malathion	***					
	Pyrethrin1	***	**				
des	Cyfluthrin	***	**				
Pesticides	MGK-264	**	**				
Pes	Cypermethrin	**	***				
	Captan	**	***				
	Methyl parathion	*	***				
	Chlordane	*	***				
	Endosulfan	*	***				
	Heavy Metals						***

Summary: True Comprehensive Cannabis Analysis

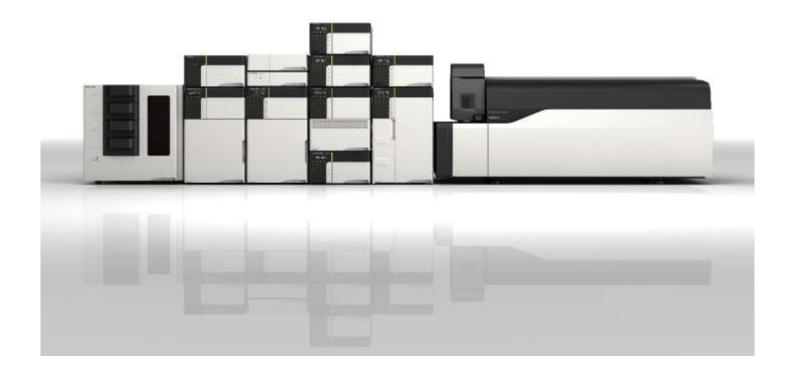
Ease of Compound Measurements by Instrument Type (***= Easiest)

		LCMS	GCMS	HPLC	HS-GCMS	HS-GC	ICP-MS	MALDI-TO
	Potency	*		***				
	Terpenes	*	**		***	**		
	Mycotoxins	***		*				
Re	Residual Solvent		**		***	**		
	Imidacloprid	***						
	Myclobutanil	***						
	Malathion	***						
	Pyrethrin1	***	**					
Pesticides	Cyfluthrin	***	**					
tici	MGK-264	**	**					
Pes	Cypermethrin	**	***					
	Captan	**	***					
	Methyl parathion	*	***					
	Chlordane	*	***					
	Endosulfan	1	***					
	Heavy Metals						***	
Ν	Aicroorganism							***
	CultivarType							***

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Nexera UC – Unified Chromatography



Fraction Collection?



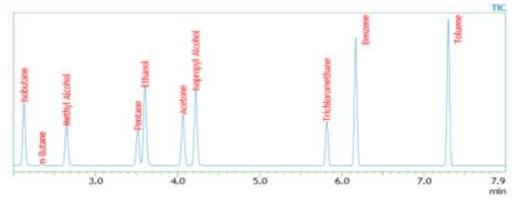
LH-40 Liquid Handler



FRC-40 Fraction Collector

Residual Solvents

- Applicable to cannabis extracts
- Low level analysis pf solvents left over from extraction production
- Relatively inexpensive
- Requires very little sample prep



Residual solvents – Nexus GC-2030

- Nexus GC-2030 Dual FID
- HS-20 Headspace Autosampler
- USP <467> Residual solvent in Pharmaceuticals



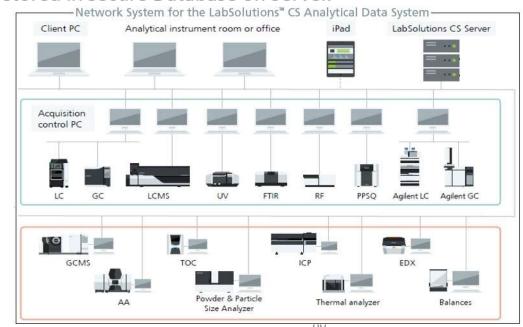
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Types of Compliant LabSolutions Software

Prod	lucts	Form	Data Management	
LabSolutions	File Management edition	Standalone	File Management	
LabSolutions DB	DB edition	Standalone	Database Management	
LabSolutions CS	CS edition	Network	Database Management	

[Network]

Client - Server type. Data and System information are stored in secure Database on Server.

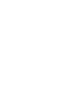


(Standalone) Management on 1 PC



Summary

- Terpene analysis GCMS with HS-20
- Potency Prominence-I HPLC
- Extraction of Cannabinoids Nexera UC for SFE









Summary

- Heavy Metal Analysis ICP-MS
- Residual Solvents GC with Dual FID
- Pesticides GCMS and LCMS
- Mycotoxins LCMS
- ns 🧹
- Moisture content Moisture Balance
- Delivery Yes







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