LAB TESTING

DR. RICH RICHINS, PHD

Rio Grande Analytics
Las Cruces, NM

Validation of a Gas Chromatography Method for Analysis of Medicinal-Quality Cannabis.

by Two Students

2008, Oakland, CA

Why was this Study Necessary?

Since the passage of California's Compassionate Use Act (Proposition 215) in 1996, *Cannabis* products have become available for medical applications. Scientific inquiries into the quality, safety and medical use of *Cannabis* are re-emerging with renewed acceptance of this botanical medicine (Pertwee 2004; Stott and Guy 2004; Ben Amar 2006; Wright 2007). Understanding of the roles of genetics and plant management h dramatically increased the potency of medicinal strains (de Meijer et al. 1992, 2003, 2005; Hazekamp 2007; Pacifico et al. 2008), and marked divergence of potency fron historical norms and international collections is now common (Turner et al. 1979; Lo

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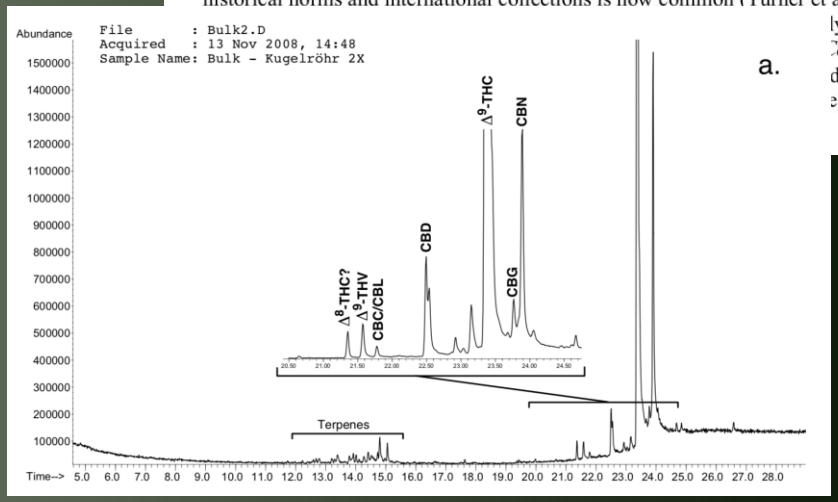
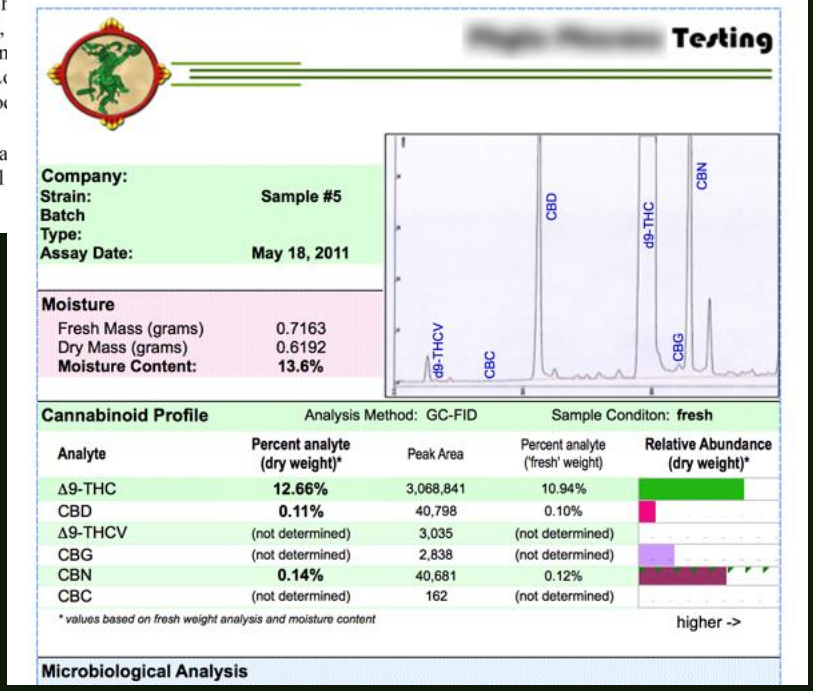
Cannabis
d genetics a
e medicinal





riogrande analytics.net/RGA_Resources.html

Toastmasters | XFINITY WiFi | iCloud | digital_astro: Management | Fitness | Research | Solar | Weather | Astronomy | >>

Rio Grande Analytics - Resources

Rio Grande Analytics
Medicinal Cannabis Testing Lab

Home | Prices | Contact Us

About Us | Testing 101 | Reliability | Resources

Resources

This page will be continually updated to provide useful links and resources for New Mexico medical cannabis community. This may include unpublished research efforts.

PDF (6 pages) of Powerpoint Slides from Hemp Testing Talk (click image to right)

Presentation given Friday, May 24, 2019 at the Farm & Ranch Heritage Museum in Las Cruces, NM

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http://riograndeanalytics.net/RGA_Resources.html

Quality Assurance Testing of Hemp Products

Is it Safe?
Does it Work?
Is it Legal?

Q/A testing helps to minimize the risk

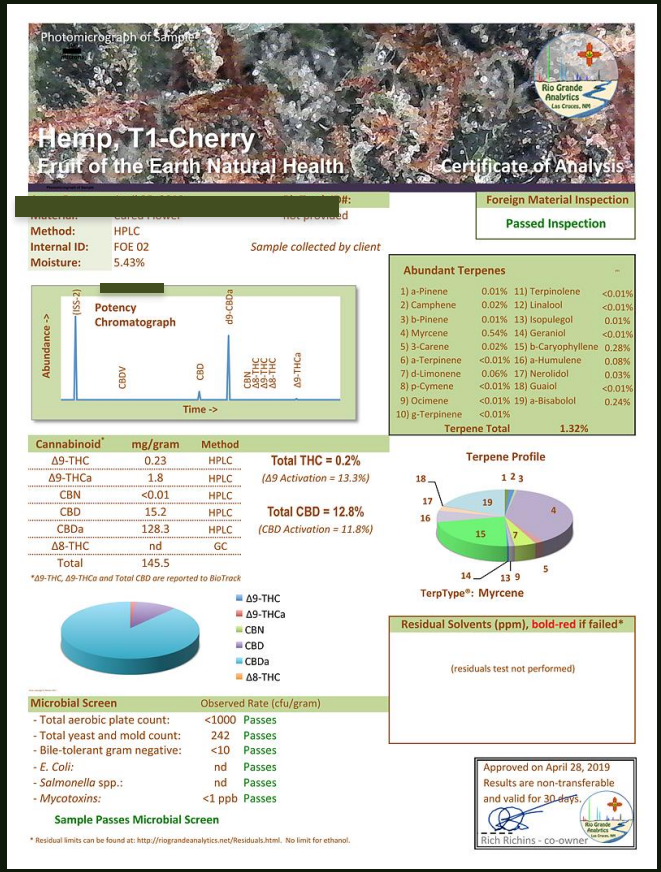
Quality Assurance incorporates two principles:

"Fit For Purpose" - the product should be suitable for the intended purpose

"Right First Time" - mistakes should be eliminated

Section Objectives

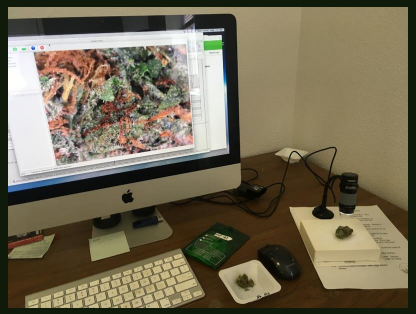
A. Review Salient Regulations for Hemp Testing
B. Explain how and why Q/A tests are performed
C. R&D Opportunities
D. Answer any testing-related questions you may have



Hemp Production Testing Overview

- Supervised samples taken periodically. Securely packaged.
- Material transported (by producer or approved carrier) to approved testing lab. Producer responsible for transportation and testing fees.
- Lab determines (minimally) concentration of CBD, THC
- Lab reports findings to producer, NMDA
- Samples must be ≤ 0.3% (w/w) to be considered hemp
- Samples exceeding the limit are considered cannabis, and must be destroyed.

Welcome to the Lab!



Sample Drying / Moisture Analysis

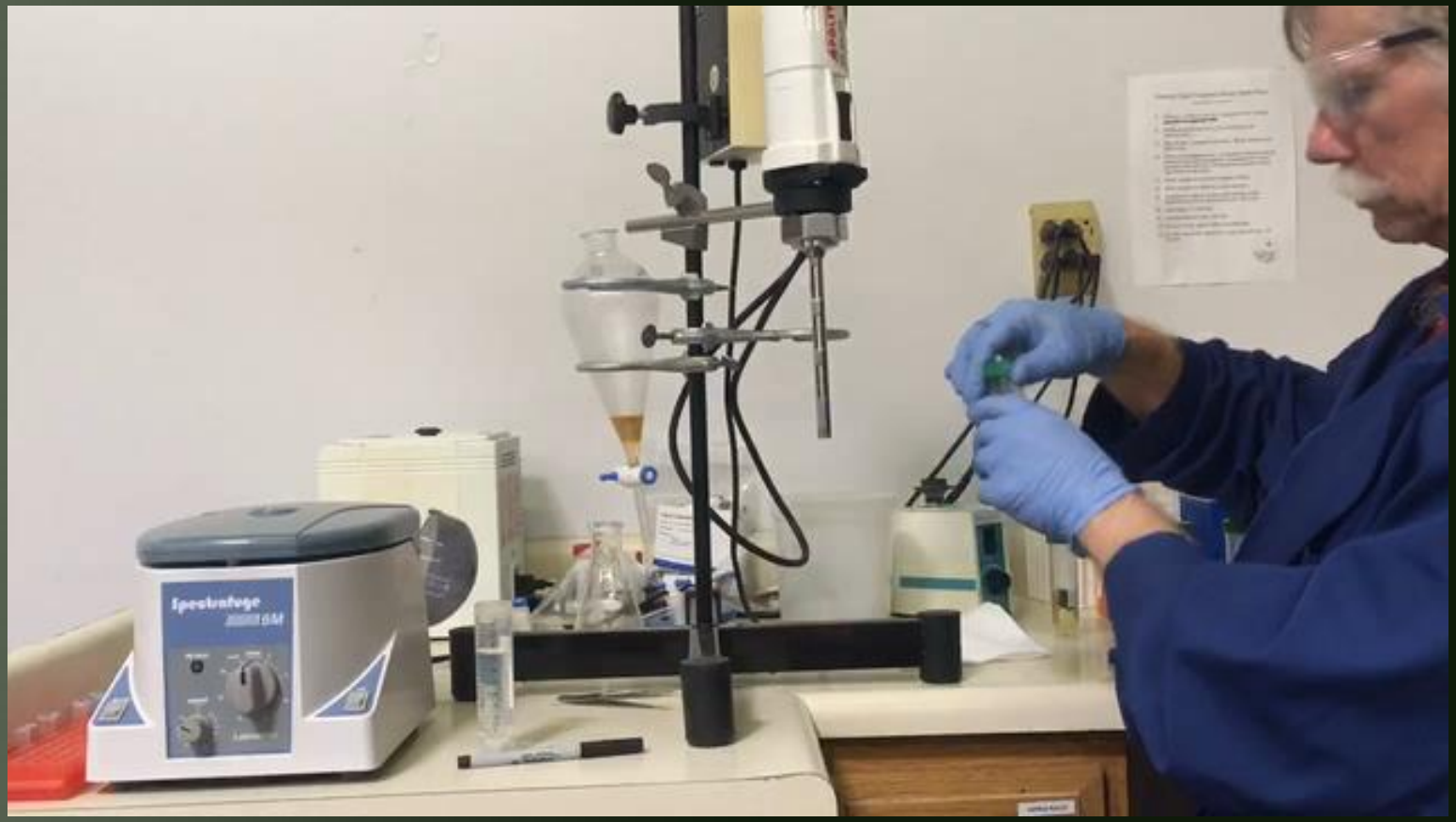
Extraction works much better with dried material

Dry Sample Overnight

Moisture analysis available (if desired)

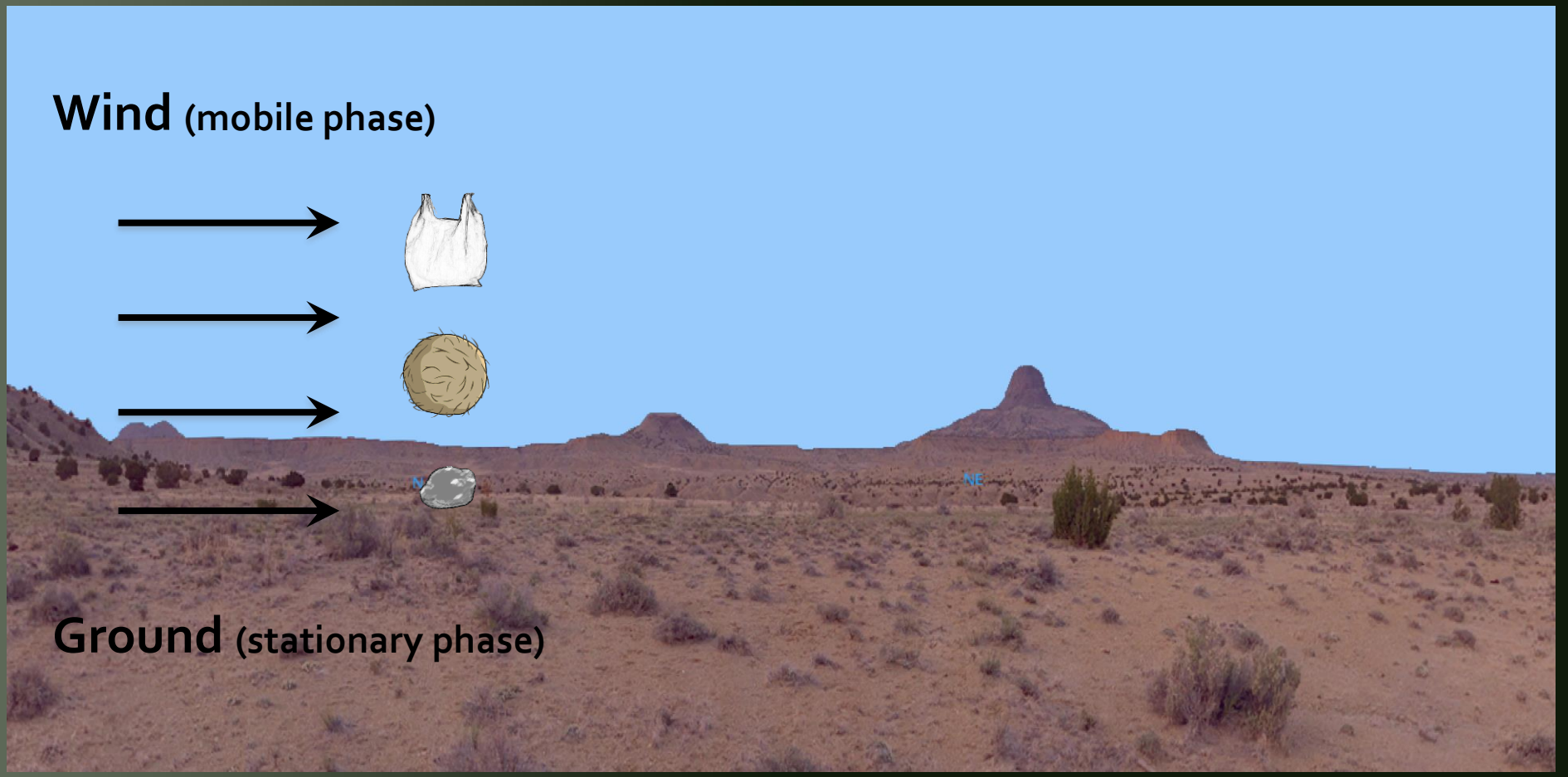
Not a proper ‘cure’. Terpenes will be lost during drying.

Sample Extraction

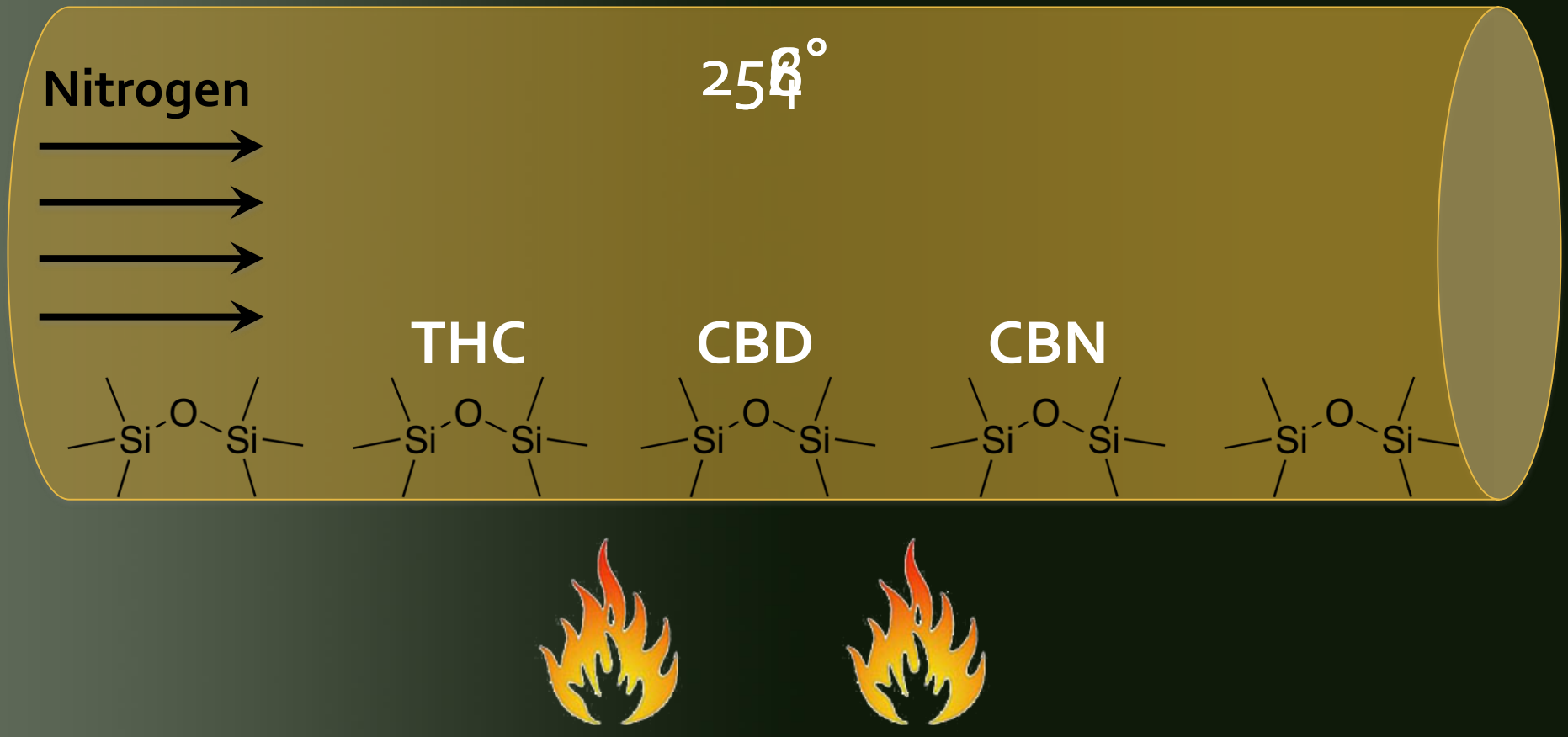


Chromatography 101

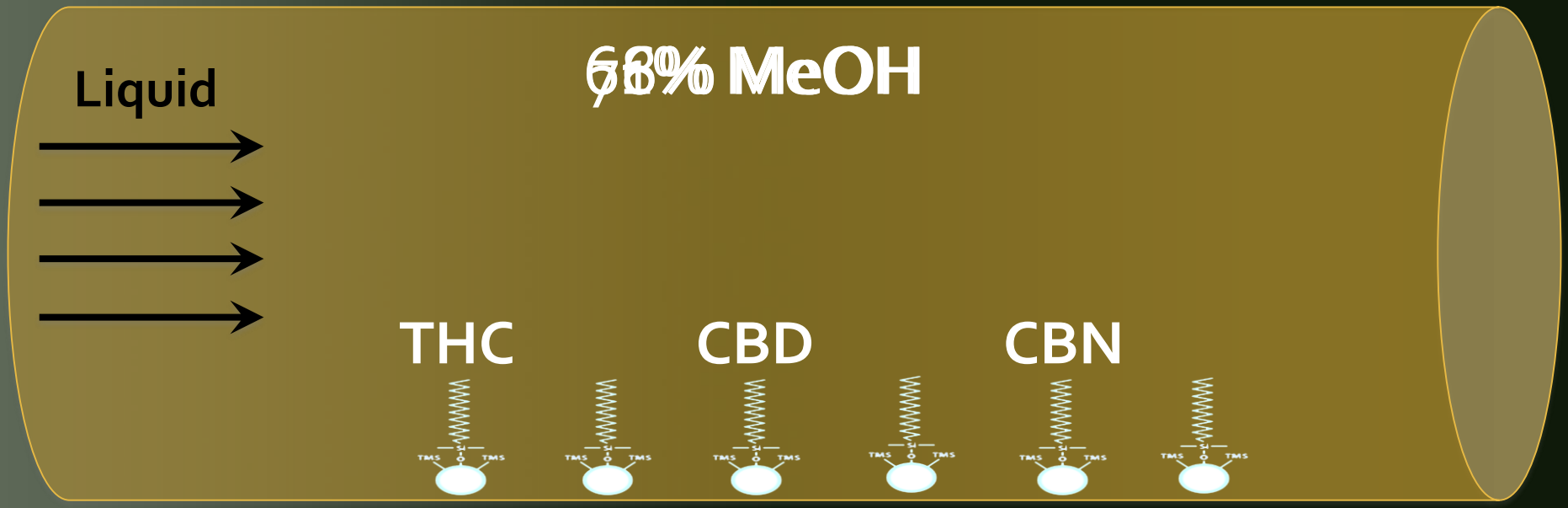
(aka – a spring day in New Mexico)



Gas Chromatography (GC)

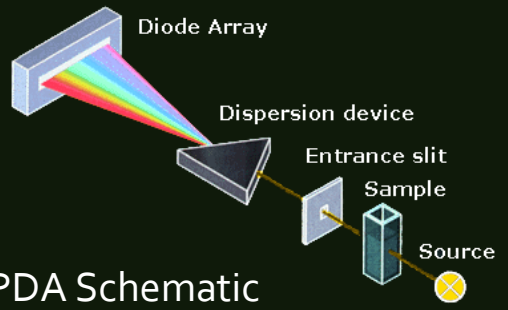


Liquid Chromatography (LC, HPLC, UPLC)



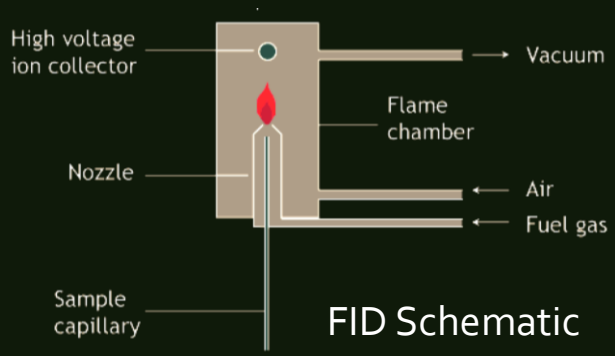
Detecting What's There

- Photodiode array (PDA) measures color intensity.
  - More intense color means more chemical
  - It's not a color that humans can see (ultraviolet)
  - The 'color' (spectrum) is unique for each compound



PDA Schematic

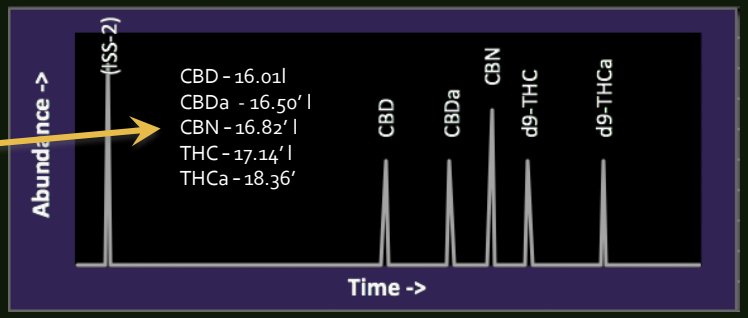
- Flame ionization detector (FID) 'burns' material coming off the column
  - Akin to throwing gas on a fire (but a lot safer)
  - Bigger 'flame' means more chemical
  - Picogram sensitivity; huge dynamic range

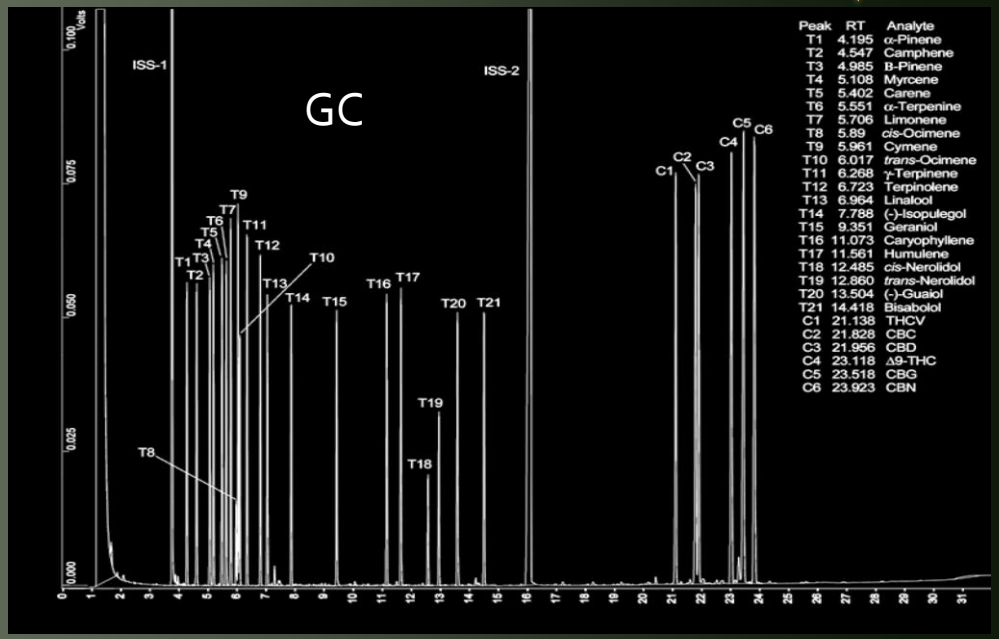


FID Schematic

Identifying What's There

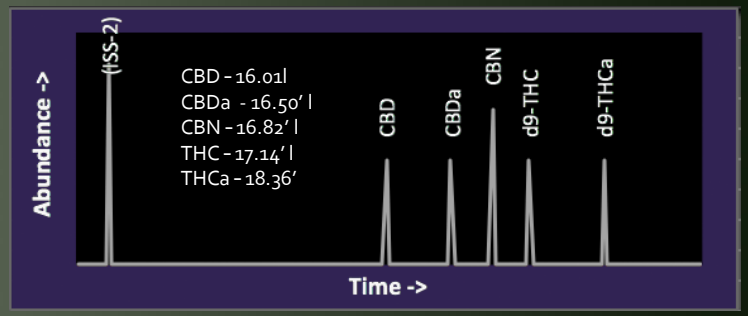
Retention Time (RT) - Time it takes for compound to travel from injector to detector (very consistent)





2 Internal Standards; 3+ External Standards

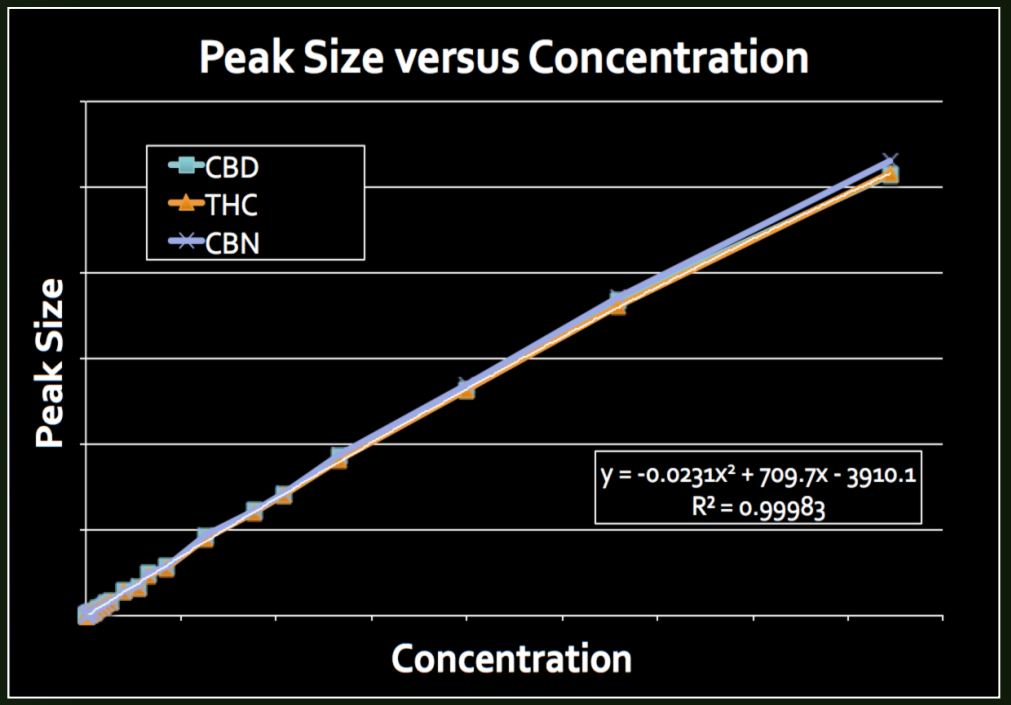
Identifying What's There



Quantifying What's There

Standard Curve – Δ9-THC

Concentration	Peak Size
0.00 mg/ml	0
0.0020 mg/ml	1,052
0.00632 mg/ml	4,859
0.020 mg/ml	12,975
0.0632 mg/ml	43,440
0.20 mg/ml	142,030
0.632 mg/ml	452,137
2.0 mg/ml	1,314,988
3.73 mg/ml	2,577,530

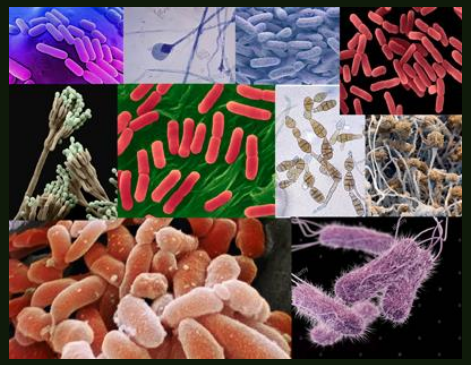


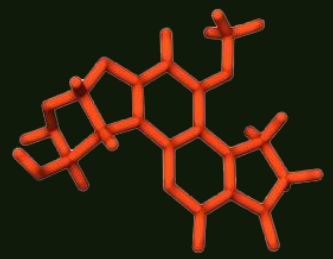
We routinely do curves and checks for every cannabinoid, terpene, toxin, and residual solvent that we report.

Microbial & Mycotoxin Testing

Why you should care

- Some microbes can make you sick.
- Some make chemicals (mycotoxins) that can make you sick.
- Immunocompromised individuals often need to limit exposure to microbes
- Potential allergens
- Affects visual appeal, flavor and aroma
- May indicate poor grow or handing practices





Microbial & Mycotoxin Testing

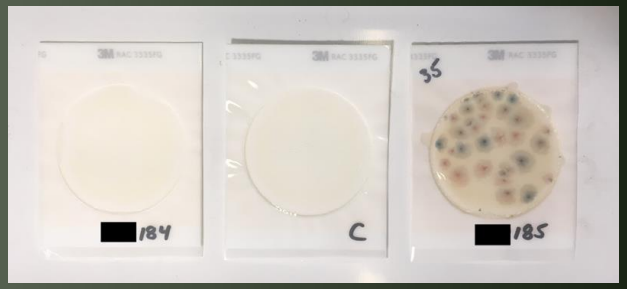
Why some of you maybe shouldn't care

- According to the Center of Disease Control (CDC), bacteria cannot grow in pure oil. Water is required.
- Oils and cannabinoids are actually antimicrobial.
- Growing hemp/cannabis plants are a poor host for the microorganisms that produce mycotoxins
- Still, testing of oils is often performed (or required)

Microbial Testing

Basic Flow…

- Package opened in sterile hood. Sample removed.
- Sample extracted with sterile media
- Dilutions prepared and plated on selective media
- Enrichment for *E. coli* and *Salmonella*
- Additional plating for *E. coli* and *Salmonella*



Check-in & Quality Assurance Bench Sheet			Component:	Lot # / Date:	Component:	Lot # / Date:
			3-Pt GC Std	A0134042	RAC media	3334gm
	Version	4.3.5	5-Pt HPLC Std	n/a	RYM media	3334rr
	Rec. by	Barry	Scale Check	10.000	EB media	3334ny
	Date	7/18/18	5 ppb Myco Spike	6/1/18	EC media	333537
	Photos	rr	Butterfields	5/17/18	R-V broth	6/17/18
Client: [redacted]			5X YTB	5/17/18	MacConkey	6/17/18

Sample ID	Form (cured flower, oil/wax, chocolate, popcorn, etcetera)	Micro Weight (grams)	Potency Weight (grams)	GC or HPLC?	Wet Weight (grams)	Dry (=Myco) Weight (grams)	Residual Weight (grams)	Potency -> SS by	Residual -> SS by	Myco -> SS by	Micro Results: RAC	RYM	EB	EC	*Salmonella*	Micro -> SS by	Results Checked / Sent By
[redacted]	Cured Flower	1.003	0.659	GC	1.035	0.971		rr		rr	0	271	0	0	0	bd	rr
	Cured Flower	1.036	0.646	GC	1.005	0.935		rr		rr	0	9	0	0	0	bd	rr
	Cured Flower	1.014	0.544	GC	1.022	0.943		rr		rr	0	9	0	0	0	bd	rr
	Cured Flower	1.021	0.597	GC	1.006	0.941		rr		rr	35	250	0	0	0	bd	rr
	Cured Flower	1.013	0.581	GC	1.001	0.921		rr		rr	0	7	0	0	0	bd	rr
	Cured Flower	1.014	0.551	GC	1.006	0.950		rr		rr	53	17	0	0	0	bd	rr

Microbial Testing

What we look for	Limit (cfu/g[2])
• Rapid Aerobic Count (RAC)	100,000[3]
• Rapid Yeast & Mold (RYM)	1,000[3]
• Enterobacter (EB)	1,000[3]
• *E. coli*[1] (EC)	0[3]
• *Salmonella*[1] (R-V)	0[3]

[1] multiple enrichment steps

[2] colony forming units per gram

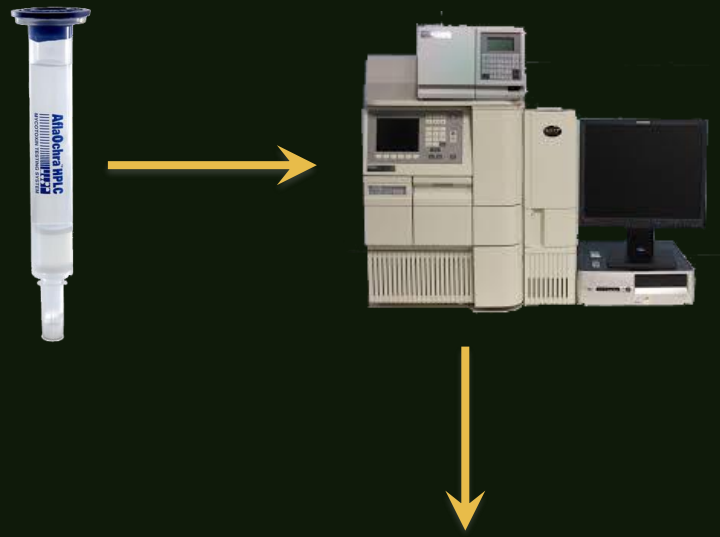
[3] US Pharmacopeia recommendation

Sample Inspection

Mycotoxin Testing

What we look for

- Aflatoxins B1, B2, G1, G2
- Ochratoxin A



Limit – combined toxins cannot exceed 20 parts per billion (ppb) per gram of product.

Potency Determination

Why you should care?

- How strong is your medicine? How much to dose?
- How well 'activated' is your product? Does it matter?
- Federal Limit for THC content (0.3% w/w)
- There's a lot more than THC or CBD in the plant.
- To find (or avoid) similar products

Potency Determination

What we look for

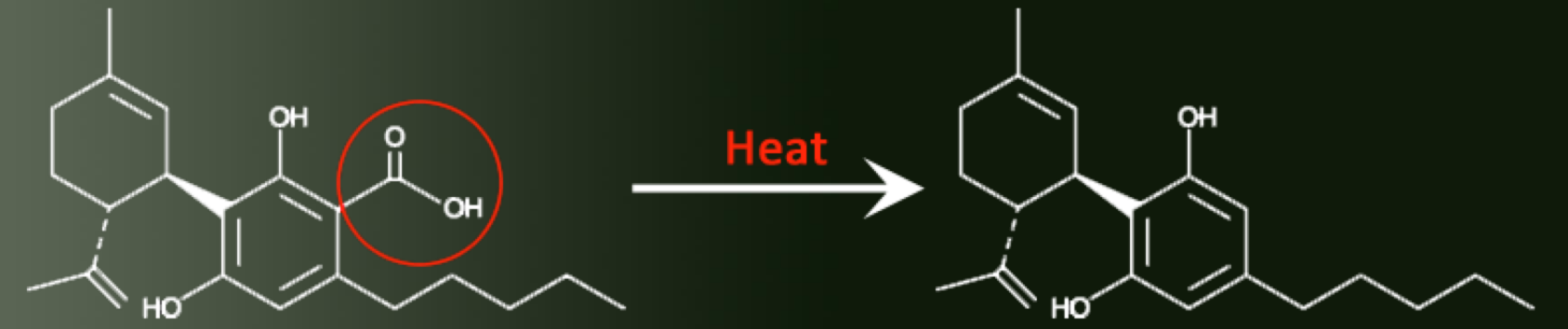
- By Gas Chromatography (GC)
  - Seven cannabinoids (THC, CBD, CBN, CBC, CBG, THCV, CBDV)
  - 19 Terpenes (more coming)
- By Liquid Chromatography (LC)
  - Five cannabinoids (THCa, THC, CBDa, CBD, CBN)
  - Ratio of neutral to total THC (or CBD) indicates 'activation' efficiency.

Potency Determination

Basic Flow…

- Sample weighed, then homogenized or dissolved in appropriate solvent.
- Extract filtered or centrifuged (to remove debris).
- Chromatography performed.
  - Gas chromatography (GC) preferred for leaves, flowers (cleaner, more sensitive)
  - Liquid chromatography (HPLC) used for edibles, tinctures, topicals (products that will not be heated by the end-user)
- Peak sizes compared with standard curves.

Decarboxylation / Activation



Cannabidiolic Acid (MW = 358.478)

Cannabidiol (MW = 314.464)

Note: the 'active' form weighs less

14.2% CBDa is the same potency as 12.4% CBD
0.39% THCa equates to 0.34% THC (passes)

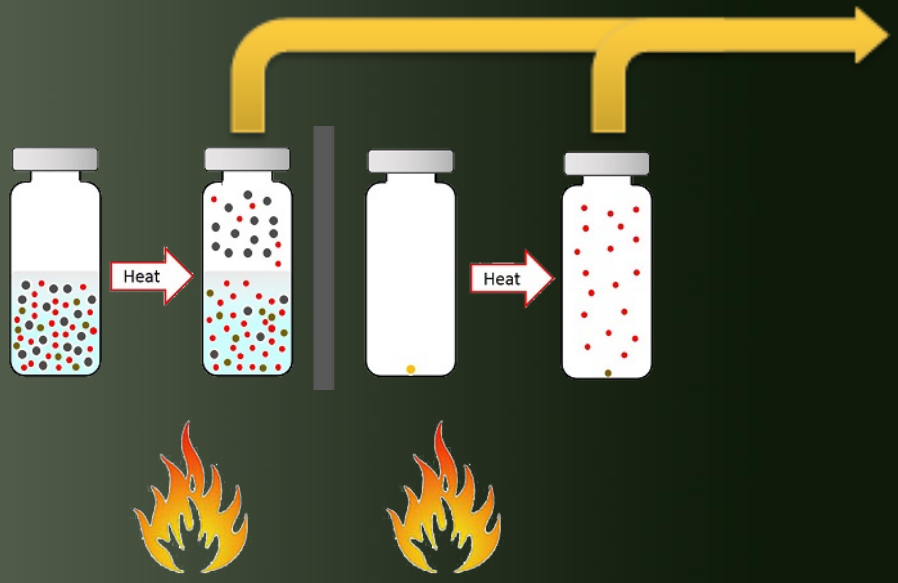
Conversion factor is 0.877
(= 314.48/358.46)

Residual Solvents

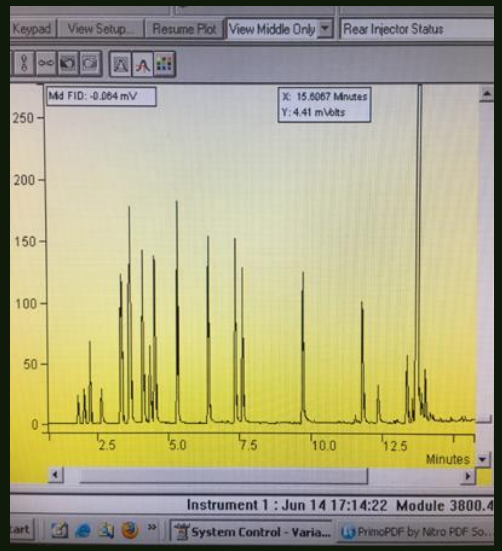
Why you should care?

- High levels of solvents can be toxic
- Some solvents are carcinogenic
- Moderate levels of solvents can be irritants
- An indication of poor manufacturing processes.

Residual Solvents



Collect Vapor



Gas Chromatography

Residual Solvents – Target Analytes and Their Limits

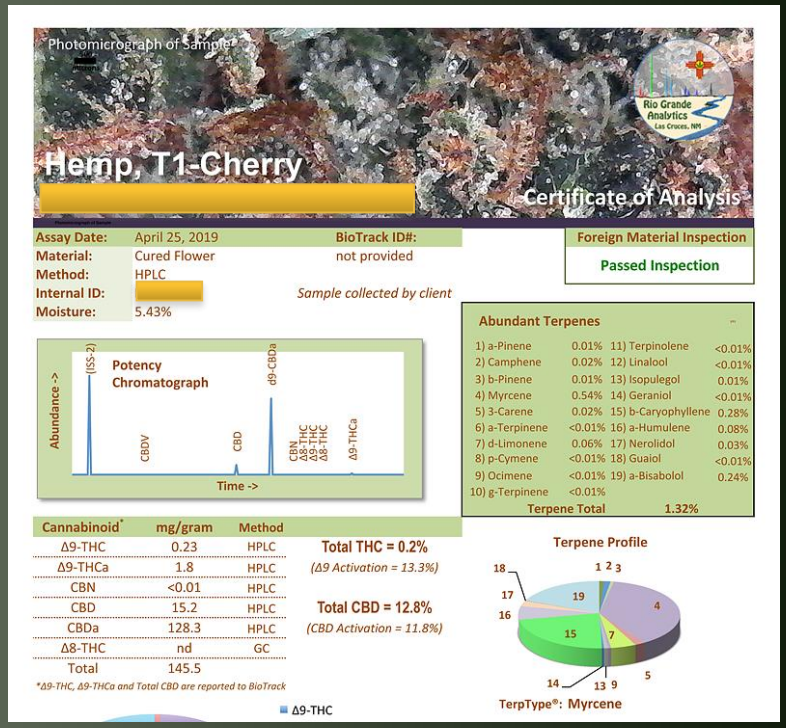
New Mexico Department of Health, Medical Cannabis Program: Residual Solvents Analyte List

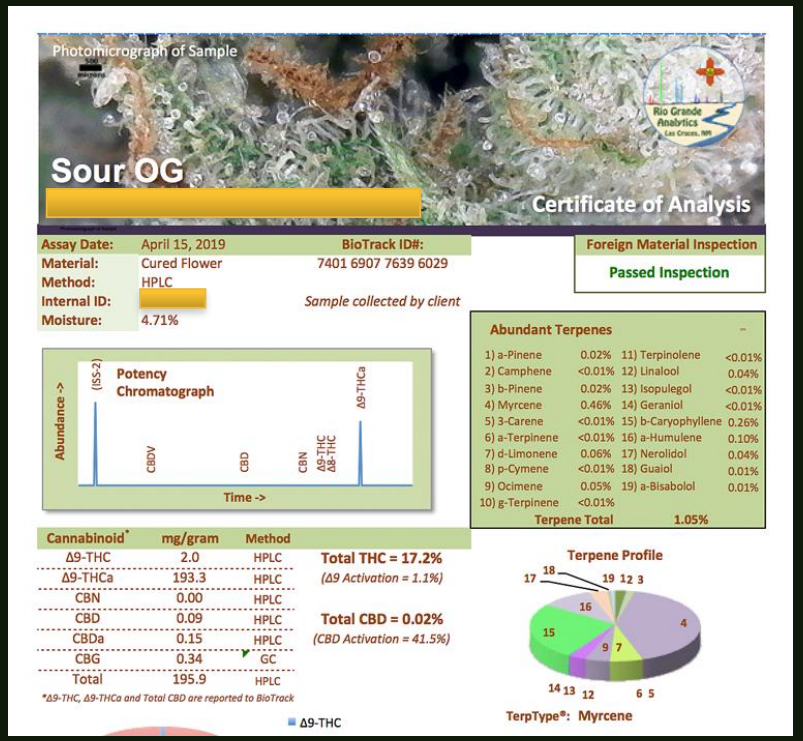
Residual Solvents	CAS Number	Action Level (ug/g) or (ppm)*
Propane	74-98-6	800
Butanes	106-97-8	800
isobutane	75-28-5	800
Pentane	109-66-0	800
Hexane	110-54-3	250
Cyclohexane	110-82-7	1000
Benzene	71-43-2	2
Toluene	108-88-3	800
Heptane	142-82-5	1000

Residual Solvents	CAS Number	Action Level (ug/g) or (ppm)*
Ethylbenzene	100-41-4	2000 (combined)
meta-xylene	108-38-3	
ortho-xylene	95-47-6	
para-xylene	106-42-3	
Methyl Alcohol	67-56-1	1000
Isopropyl Alcohol	67-63-0	2000
Methylene Chloride	75-09-2	500
Acetone	67-64-1	2000

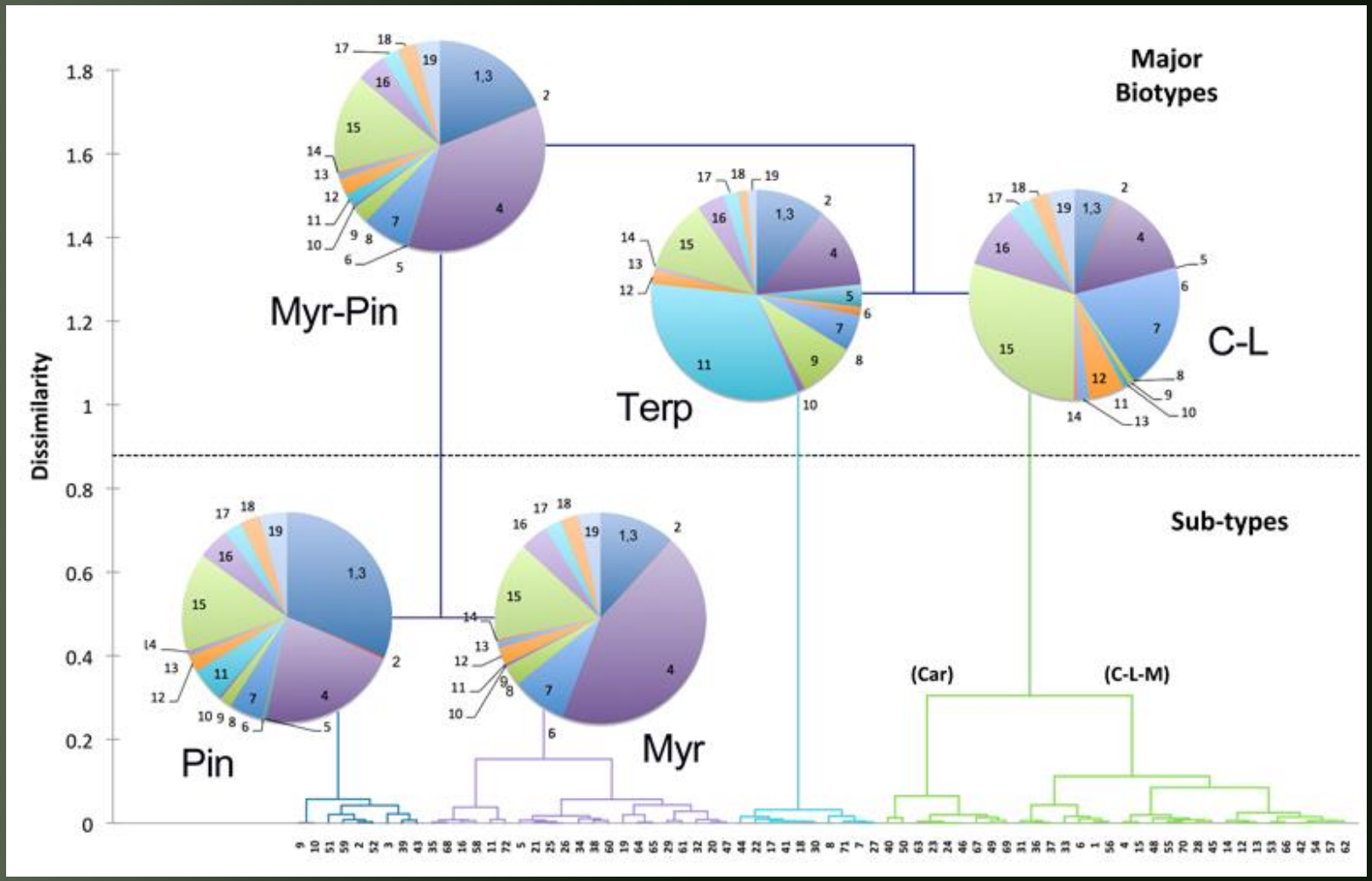
* Micrograms solvent per gram of sample (same as ppm)

Terpenes


Photomicrograph of Sample
Hemp, T1-Cherry
Certificate of Analysis
Rio Grande Analytics
Las Cruces, NM
Assay Date: April 25, 2019
Material: Cured Flower
Method: HPLC
Internal ID:
Moisture: 5.43%
BioTrack ID#: not provided
Sample collected by client
Foreign Material Inspection
Passed Inspection
Potency Chromatograph
Abundance ->
Time ->
(ISS-2)
CBDV
CBD
d9-CBDa
CBN
Δ8-THC
Δ9-THC
Δ9-THCa
Abundant Terpenes
1) a-Pinene 0.01%
2) Camphene 0.02%
3) b-Pinene 0.01%
4) Myrcene 0.54%
5) 3-Carene 0.02%
6) a-Terpinene <0.01%
7) d-Limonene 0.06%
8) p-Cymene <0.01%
9) Ocimene <0.01%
10) g-Terpinene <0.01%
11) Terpinolene <0.01%
12) Linalool <0.01%
13) Isopulegol 0.01%
14) Geraniol <0.01%
15) b-Caryophyllene 0.28%
16) a-Humulene 0.08%
17) Nerolidol 0.03%
18) Guaiol <0.01%
19) a-Bisabolol 0.24%
Terpene Total 1.32%
Cannabinoid* | mg/gram | Method
Δ9-THC 0.23 HPLC
Δ9-THCa 1.8 HPLC
CBN <0.01 HPLC
CBD 15.2 HPLC
CBDa 128.3 HPLC
Δ8-THC nd GC
Total 145.5
*Δ9-THC, Δ9-THCa and Total CBD are reported to BioTrack
Total THC = 0.2%
(Δ9 Activation = 13.3%)
Total CBD = 12.8%
(CBD Activation = 11.8%)
Terpene Profile
TerpType®: Myrcene
Δ9-THC


Photomicrograph of Sample
Sour OG
Certificate of Analysis
Rio Grande Analytics
Las Cruces, NM
Assay Date: April 15, 2019
Material: Cured Flower
Method: HPLC
Internal ID:
Moisture: 4.71%
BioTrack ID#: 7401 6907 7639 6029
Sample collected by client
Foreign Material Inspection
Passed Inspection
Potency Chromatograph
Abundance ->
Time ->
(ISS-2)
CBDV
CBD
CBN
Δ9-THC
Δ8-THC
Δ9-THCa
Abundant Terpenes
1) a-Pinene 0.02%
2) Camphene <0.01%
3) b-Pinene 0.02%
4) Myrcene 0.46%
5) 3-Carene <0.01%
6) a-Terpinene <0.01%
7) d-Limonene 0.06%
8) p-Cymene <0.01%
9) Ocimene 0.05%
10) g-Terpinene <0.01%
11) Terpinolene <0.01%
12) Linalool 0.04%
13) Isopulegol <0.01%
14) Geraniol <0.01%
15) b-Caryophyllene 0.26%
16) a-Humulene 0.10%
17) Nerolidol 0.04%
18) Guaiol 0.01%
19) a-Bisabolol 0.01%
Terpene Total 1.05%
Cannabinoid* | mg/gram | Method
Δ9-THC 2.0 HPLC
Δ9-THCa 193.3 HPLC
CBN 0.00 HPLC
CBD 0.09 HPLC
CBDa 0.15 HPLC
CBG 0.34 GC
Total 195.9 HPLC
*Δ9-THC, Δ9-THCa and Total CBD are reported to BioTrack
Total THC = 17.2%
(Δ9 Activation = 1.1%)
Total CBD = 0.02%
(CBD Activation = 41.5%)
Terpene Profile
TerpType®: Myrcene
Δ9-THC

Terpene Trends



PLoS One. 2018 Jul 23;13(7)

Reporting

- Nominal results reported to client via CoA or simple email/text message.
- Automatic retests for samples > 0.3% THC
  - Retests analyzed on LC.
- Verified samples > 0.3% THC reported to NMDA.
- Protected, searchable web portal for results (for NMDA)

Reporting

To: Randy Marsh

Cc: Brad Lewis

Subject: Hemp Assay Results

From: Barry Dungan – riograndeanalytics@gmail.com

Message Size: 178 KB Image Size: Actual Size

Hello Randy,

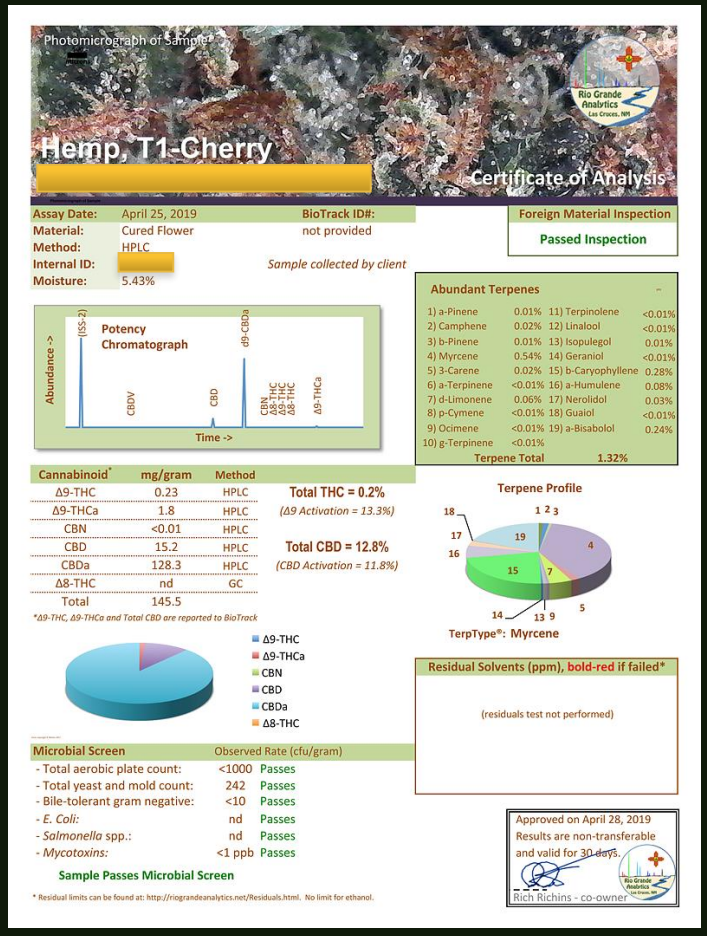
Here are the results for the hemp samples we received on Monday. One sample tested above 0.3% THC. An additional test is warranted. NMDA has been copied.

Client	NMDA Sample ID	Assay Date	Test	[CBD]	[CBG]	[Δ9-THC]	Result	By	Notes
Tegridy Farms	TGDF 1234	14-May-2019	GC-FID	14.5%	0.3%	0.2%	Pass	bd	
Tegridy Farms	TGDF 1235	14-May-2019	GC-FID	11.3%	0.2%	0.1%	Pass	bd	
Tegridy Farms	TGDF 1236	14-May-2019	GC-FID	12.7%	0.3%	0.5%	Fail	bd	
Tegridy Farms	TGDF 1236 (re-test)	14-May-2019	HPLC	12.9%	(nd)	0.54%	Fail	rr	

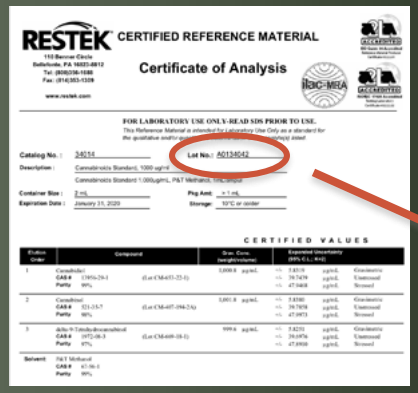
Regards,

rr

Dr. Rich Richins
Rio Grande Analytics
Las Cruces



Maintenance / Calibration / Training / Documentation (aka CYA)


RESTEK CERTIFIED REFERENCE MATERIAL

Certificate of Analysis

FOR LABORATORY USE ONLY-READ SDS PRIOR TO USE.

Catalog No.: 34014 Lot No.: A0134042

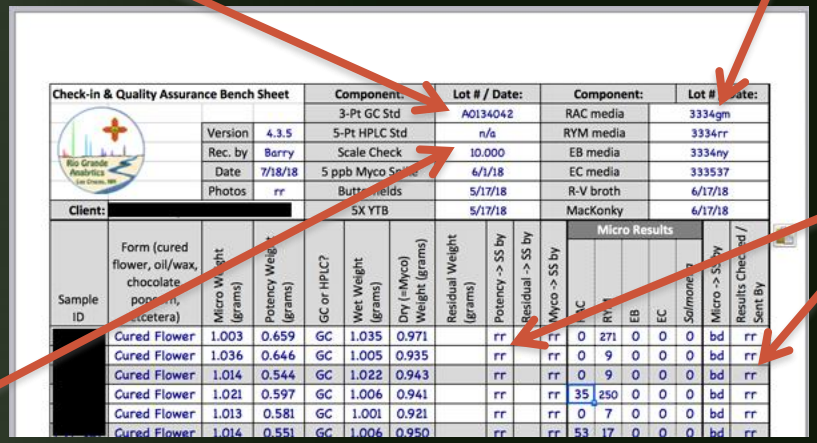
CERTIFIED VALUES

Analytical Standards

PROFICIENCY TESTING

HIPAA
Health Insurance Portability and Accountability Act

Consumables Tracking


Check-in & Quality Assurance Bench Sheet

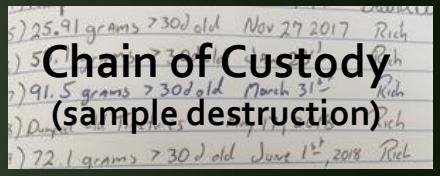
Component:	Lot # / Date:	Component:	Lot # / Date:
3-Pt GC Std	A0134042	RAC media	3334gm
5-Pt HPLC Std	n/a	RYM media	3334rr
Scale Check	10.000	EB media	3334ny
5 ppb Myco Spike	6/1/18	EC media	333537
Buttonfields	5/17/18	R-V broth	6/17/18
5X YTB	5/17/18	MacKonky	6/17/18

Version 4.3.5 | Rec. by Barry | Date 7/18/18 | Photos rr | Client: [redacted]

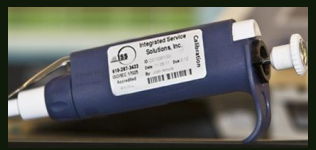
Sample ID	Form (cured flower, oil/wax, chocolate, ponzum, excetera)	Micro Weight (grams)	Potency Weight (grams)	GC or HPLC?	Wet Weight (grams)	Dry (=Myco) Weight (grams)	Residual Weight (grams)	Potency -> SS by	Residual -> SS by	Myco -> SS by	Micro Results: TAC	RYM	EB	EC	Salmonella	Micro -> SS by	Results Checked / Sent By
[redacted]	Cured Flower	1.003	0.659	GC	1.035	0.971		rr		rr	0	271	0	0	0	bd	rr
	Cured Flower	1.036	0.646	GC	1.005	0.935		rr		rr	0	9	0	0	0	bd	rr
	Cured Flower	1.014	0.544	GC	1.022	0.943		rr		rr	0	9	0	0	0	bd	rr
	Cured Flower	1.021	0.597	GC	1.006	0.941		rr		rr	35	250	0	0	0	bd	rr
	Cured Flower	1.013	0.581	GC	1.001	0.921		rr		rr	0	7	0	0	0	bd	rr
	Cured Flower	1.014	0.551	GC	1.006	0.950		rr		rr	53	17	0	0	0	bd	rr

Who did what?

Daily Checks



Chain of Custody
(sample destruction)



Calibration Services

Dataloggers
(monitors incubators, storage areas)

Summary / Questions

- Quailty Assurance testing is important and informative.
  - Certificate of Analysis contains much useful information
- The more you know about the products you grow or purify, the better you can make informed choices.