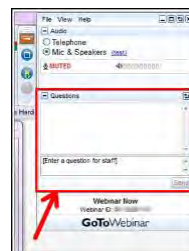


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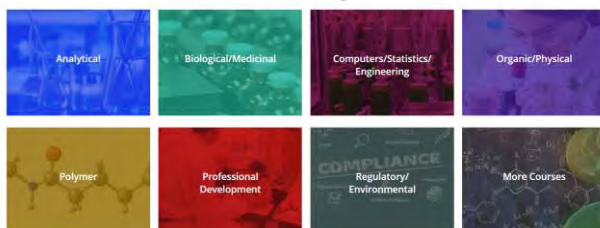
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CANNABINOIDS STUMBLING THROUGH CHALLENGING SEPARATIONS

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THIS ACS WEBINAR WILL BEGIN SHORTLY...

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Cannabinoids: Stumbling Through Challenging Separations



Lee Polite
President and Founder,
Axion Analytical Labs



Bryan Tweedy
Senior Manager, ACS Learning and Career
Development and Professional Resources

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Cannabinoids: Stumbling Through Challenging Separations

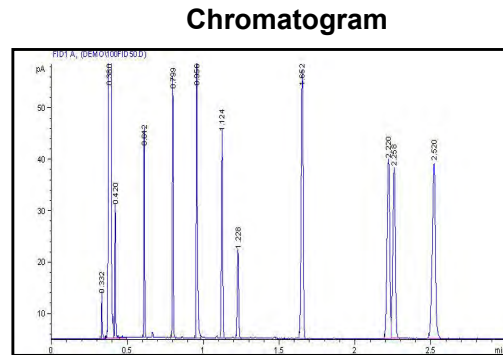
Lee N. Polite, MBA, PhD
Axion Analytical Labs, Inc
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info@axionlabs.com



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Chromatography – The World’s Most Popular Analytical Tool

Technology: Separates complex mixtures into pure compounds



Audience Challenge Question

ANSWER THE QUESTION ON BLUE SCREEN IN ONE MOMENT



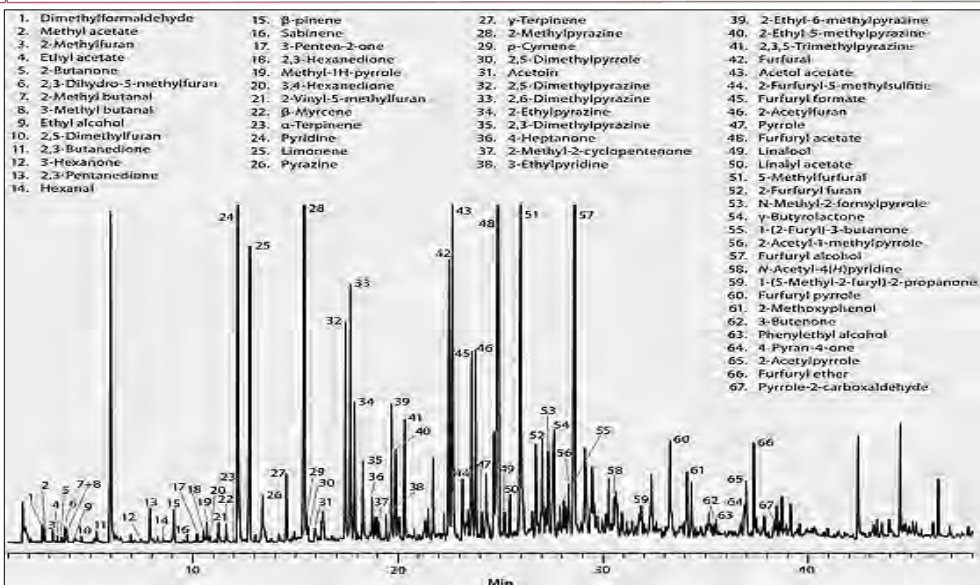
How many different chemicals are in the aroma of coffee?

- One (coffee)
- Three (including cream and sugar)
- Approximately 15
- Approximately 24
- Approximately 67

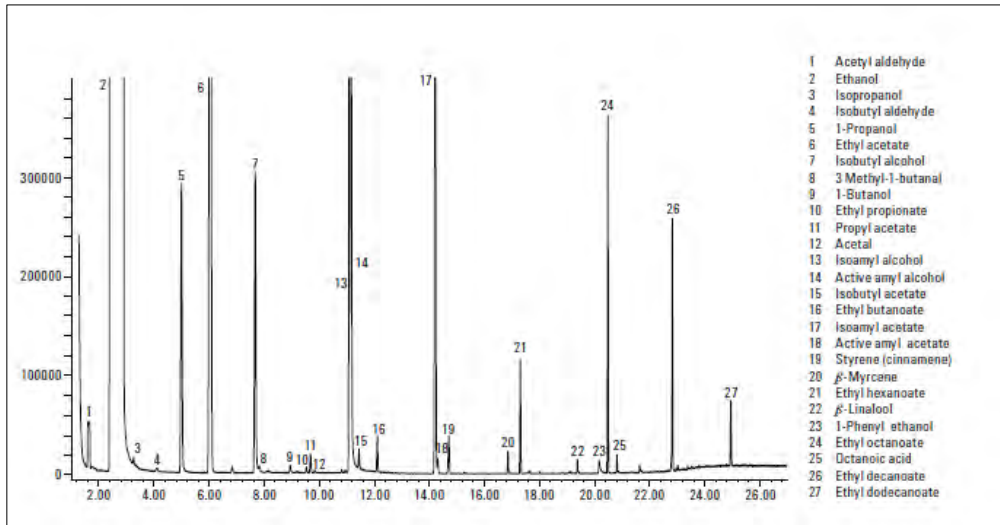


** If your answer differs greatly from the choices above tell us in the chat!*

Coffee Analysis by Static Headspace GC

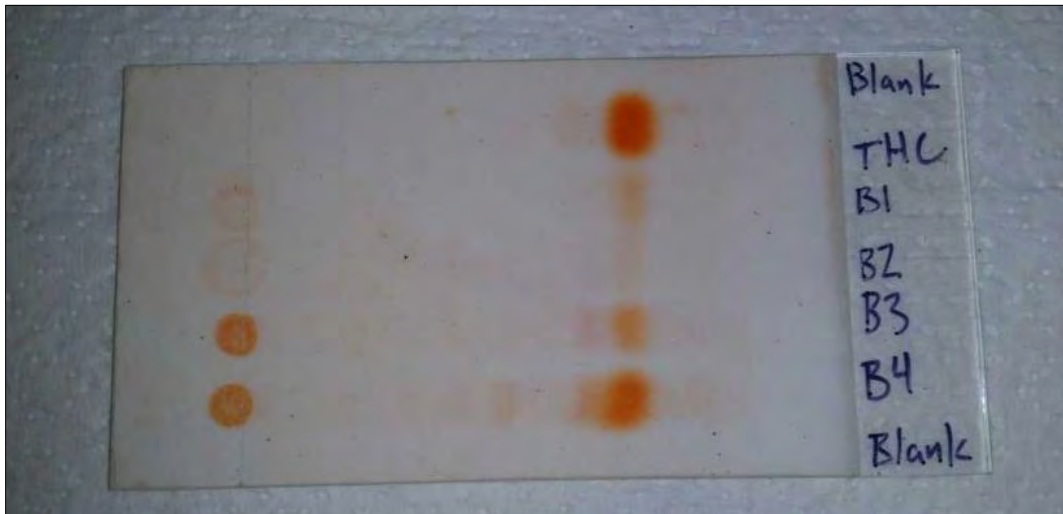


Volatiles in Beer by GC



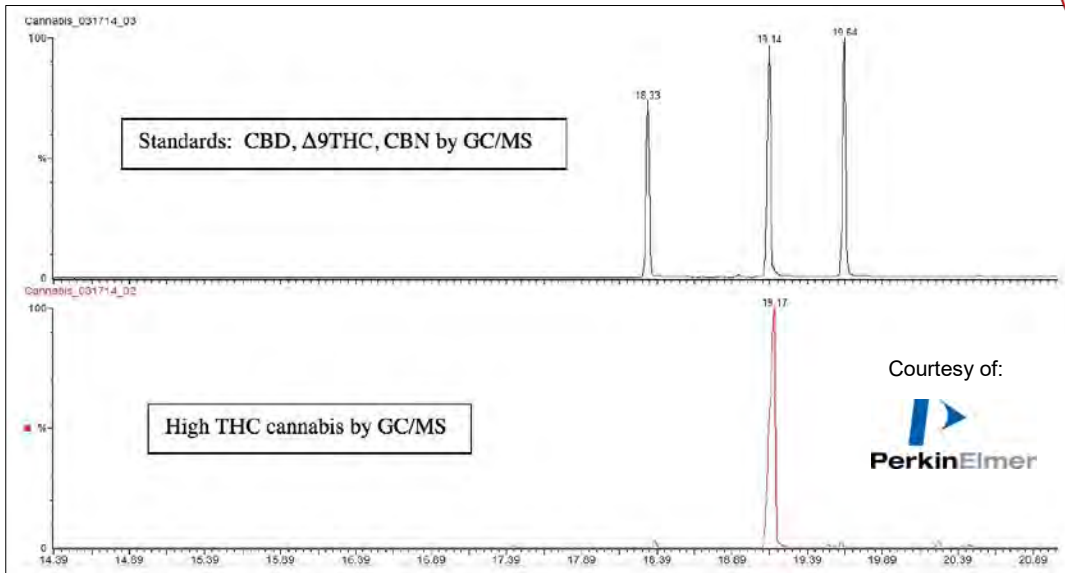
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Forensic Analysis of Cannabinoids by TLC



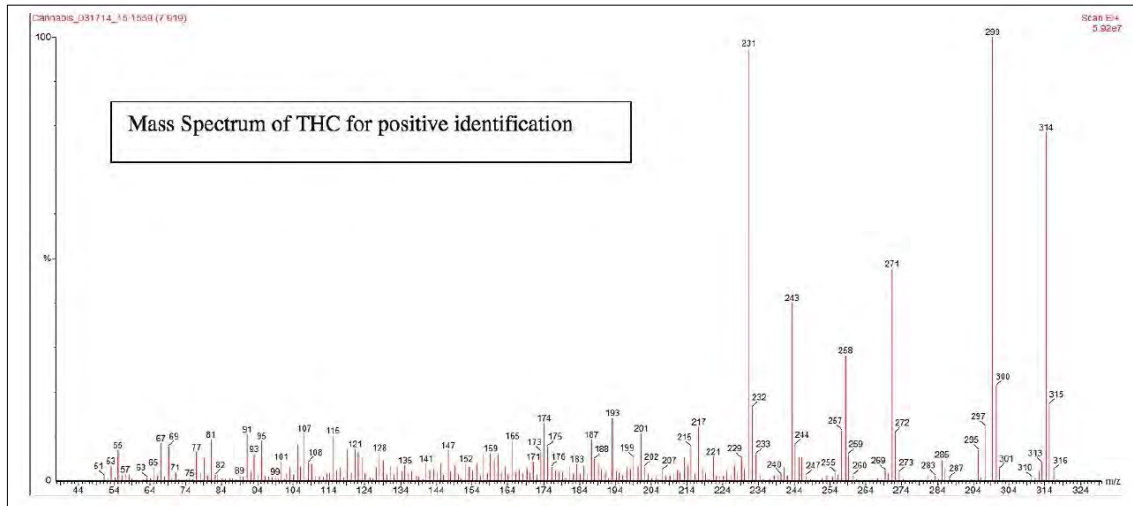
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Cannabinoids by GC-MS

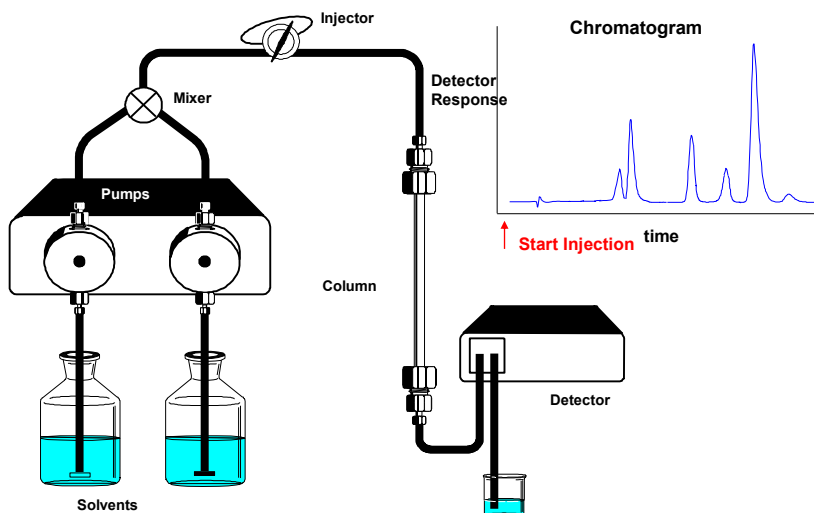


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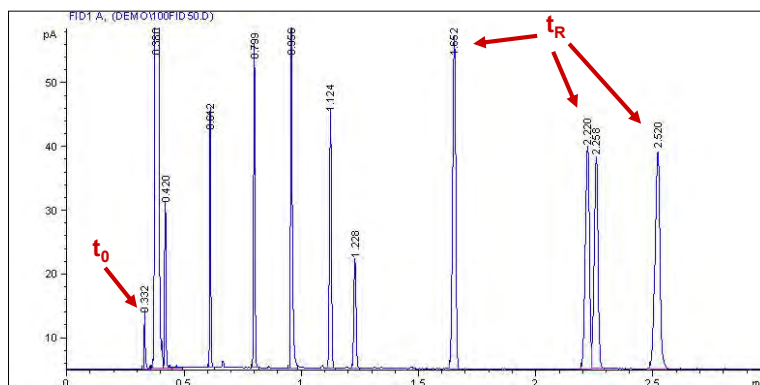
GC/MS of THC



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Typical Chromatogram



Retention Time (t_R):

Used to identify a sample component (Qualitative Analysis).
Characteristic of a compound, **but NOT unique!**

Peak Area:

Used to measure the quantity of the sample component (Quantitative Analysis).

Kool Aid Experiment

Separation of Color Compounds by Liquid Chromatography



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Kool Aid Experiment

Separation of Color Compounds by Liquid Chromatography



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Separation of Color Compounds by Liquid Chromatography



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Separation of Color Compounds by Liquid Chromatography



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Separation of Color Compounds by Liquid Chromatography



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Kool Aid Experiment

Separation of Color Compounds by Liquid Chromatography



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Kool Aid Experiment



Separation of Color Compounds by Liquid Chromatography



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Audience Challenge Question

ANSWER THE QUESTION ON BLUE SCREEN IN ONE MOMENT



How many parameters do you need to set correctly in order to guarantee a separation?

- One
- Two
- Three
- Four
- 420



** If your answer differs greatly from the choices above tell us in the chat!*

HPLC Method Development: Coming Up with the Recipe!



Two Requirements

- Find the correct chromatographic conditions (k , α , N)
- Find the correct detector wavelength (Diode Array)

Choose the Correct Conditions: HPLC Master Resolution Equation



$$R_s = \left(\frac{k}{1+k} \right) x \left(\frac{\alpha-1}{\alpha} \right) x \frac{\sqrt{N}}{4}$$

Resolution	Capacity / Retention Factor	Selectivity	Efficiency ("Peak Skinniness")
$R > 1.50$	$1 < k < 5$	$\alpha > 1.2$	Avg ~ 7,000 Max ~ 30,000
	$k = (t_r - t_0) / t_0$	$\alpha = k_B / k_A$	$N = 5.545 \times \left(\frac{t_r}{W_h} \right)^2$
	Weaken Mobile Phase ↑ %H ₂ O by 10% Increases $k \sim 2X$	Chemistry of Mobile Phase Stationary Phase pH, Temp, buffer, additive, etc.	↑ Column Length (L_c) ↓ Particle Diameter (D_p) Optimize Flow Rate (μ) Min. Extra Col. Volume

Method Development Step 1



Find the Correct Capacity Factor (Solvent Strength)

$$R_s = \left(\frac{k}{1+k} \right) \cdot \left(\frac{\alpha-1}{\alpha} \right) \cdot \frac{\sqrt{N}}{4}$$

Capacity
Factor Selectivity Efficiency

- How do you find the correct mobile phase strength?
- Try all of the strengths... and see where your peaks elute!
- Scouting Run: Gradient from weakest to strongest mobile phase
- Listen to your sample. The peaks will elute at their desired %B

HPLC Scouting Run Conditions

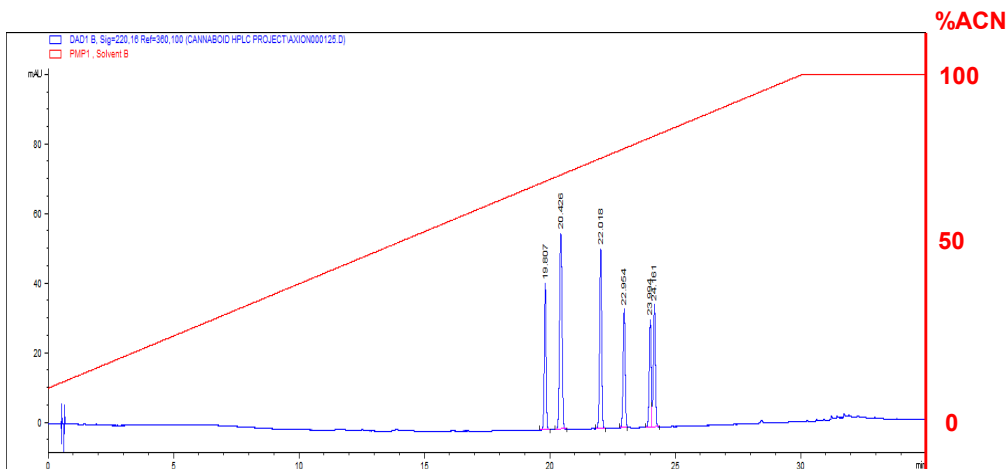


- 1) Agilent Zorbax Eclipse Plus C18 (5 cm x 4.6 mm x 1.8 um)
- 2) Flow 1.5 mL/min
- 3) Gradient from 10% ACN to 100% ACN in 30 min.
- 4) Hold 100% for 10 minutes
- 5) Evaluate Chromatogram
- 6) The more shallow the gradient ramp, the better the separation.

Scouting Run for 7 Cannabinoids



10-100% Acetonitrile in 30 min with a 5 min Hold



Method Development Step 2



Efficiency (Column Dimensions)

$$R_s = \left(\frac{k}{1+k} \right) \cdot \left(\frac{\alpha-1}{\alpha} \right) \cdot \frac{\sqrt{N}}{4}$$

Capacity **Selectivity** **Efficiency**

- Start with the highest efficiency column that you can buy.
- Try a 15 cm with 3.5 um particles (~20,000 plates) or
- 10 cm with 1.8 um particles (~28,000 plates) – Requires high pressure
- Note: During method optimization, we may opt for a shorter column.
- Column length is proportional to the efficiency.

Method Development Step 3



Find the Correct Selectivity (Column Chemistry)

$$R_s = \left(\frac{k}{1+k} \right) \cdot \left(\frac{\alpha-1}{\alpha} \right) \cdot \frac{\sqrt{N}}{4}$$

Capacity Selectivity Efficiency

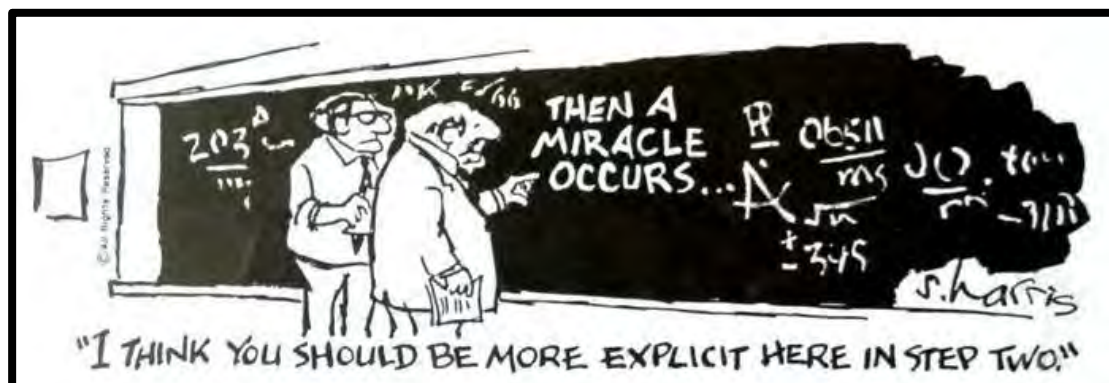
Choose reversed phase because...

Approximately 85% of all HPLC separations are carried out in the reversed phase mode!
(Acetonitrile or methanol blended with water on a good, base-deactivated C18 column)

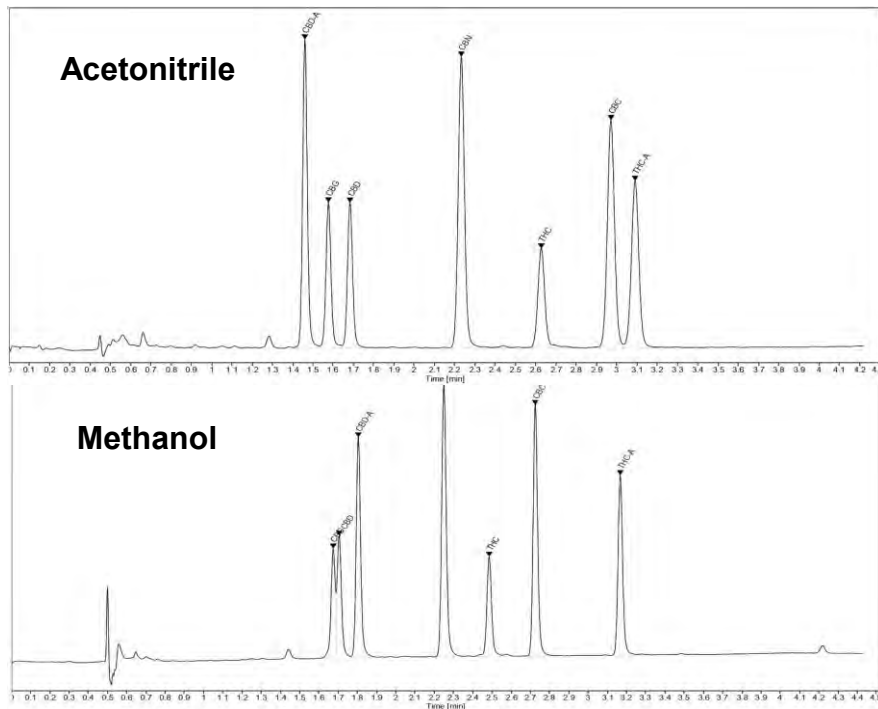
Choose Reversed Phase Because...



It Just seems to work for most applications!



**Mobile Phase Effect
on Selectivity (a)**

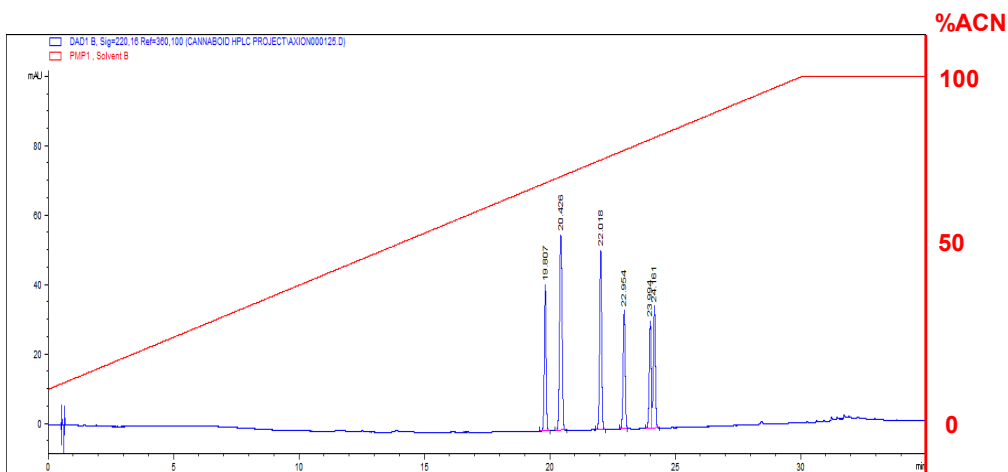


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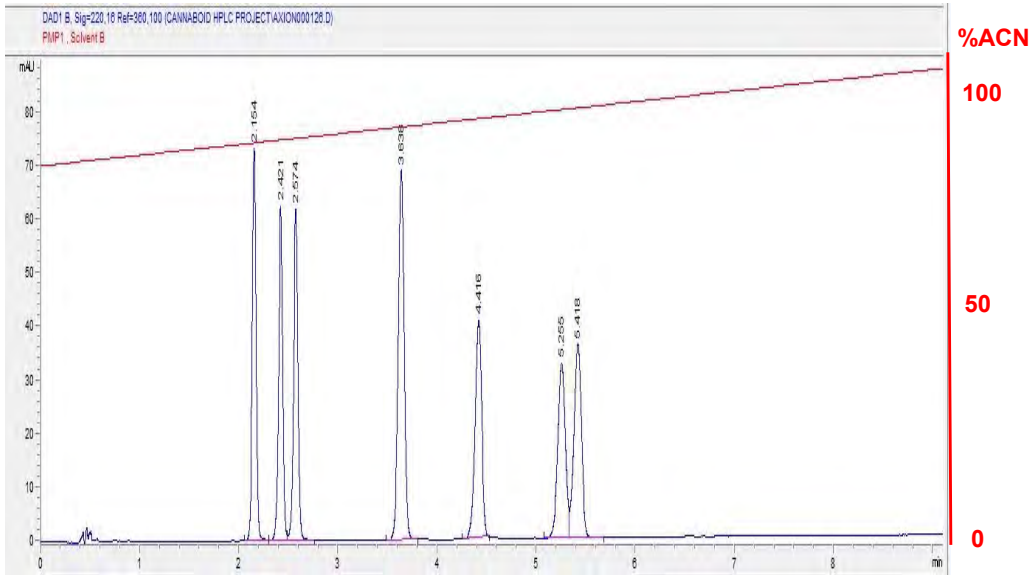
Scouting Run for 7 Cannabinoids



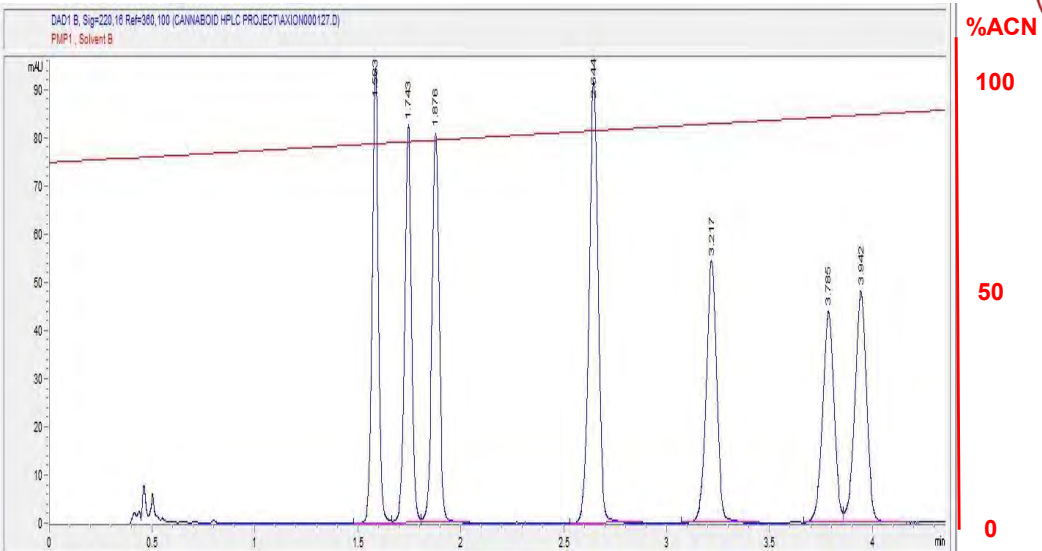
10-100% Acetonitrile in 30 min with a 5 min Hold



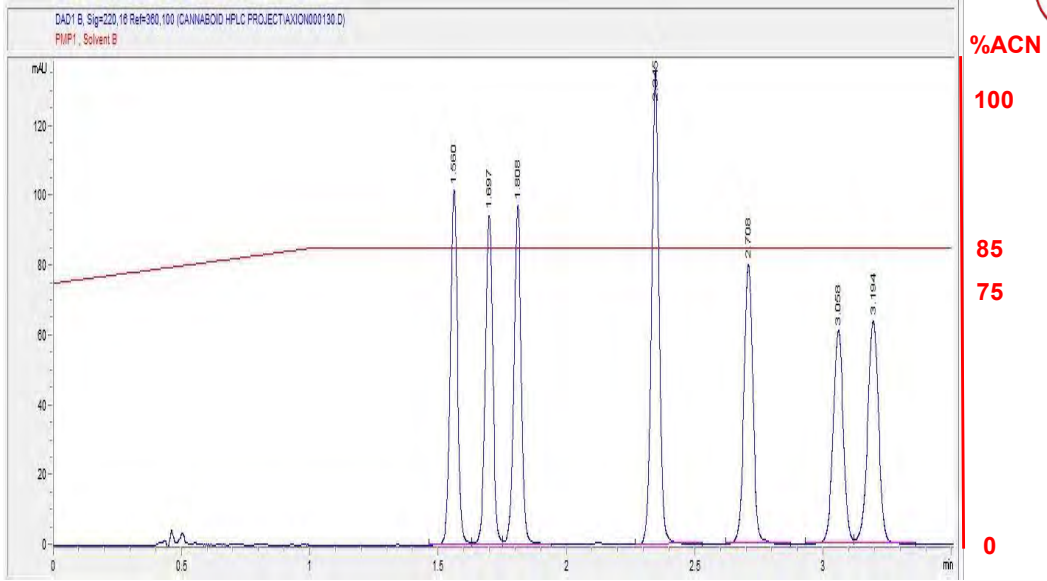
70-100% ACN in 15 min



75-100% ACN in 10 min

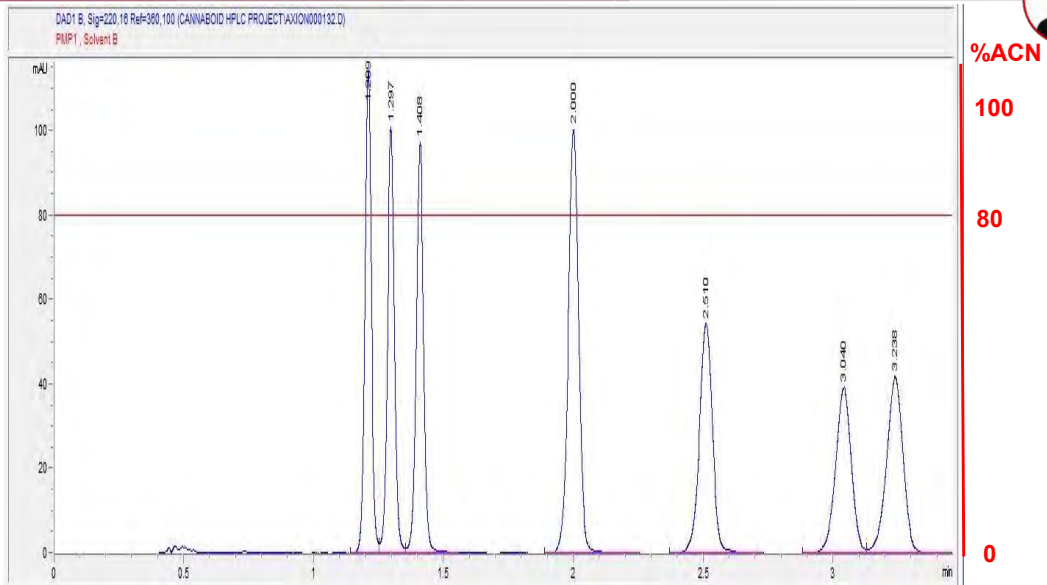


75%-85% ACN in 1min



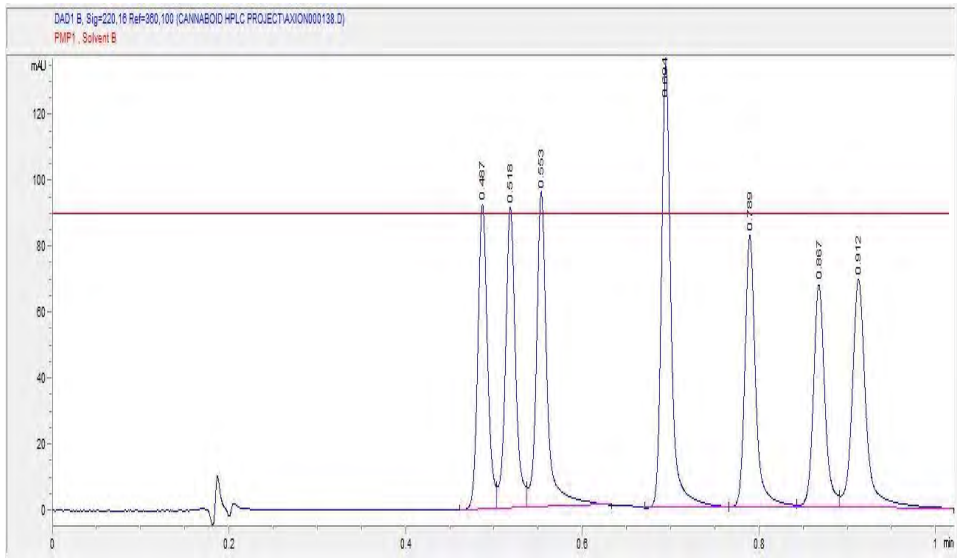
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80% ACN Isocratic

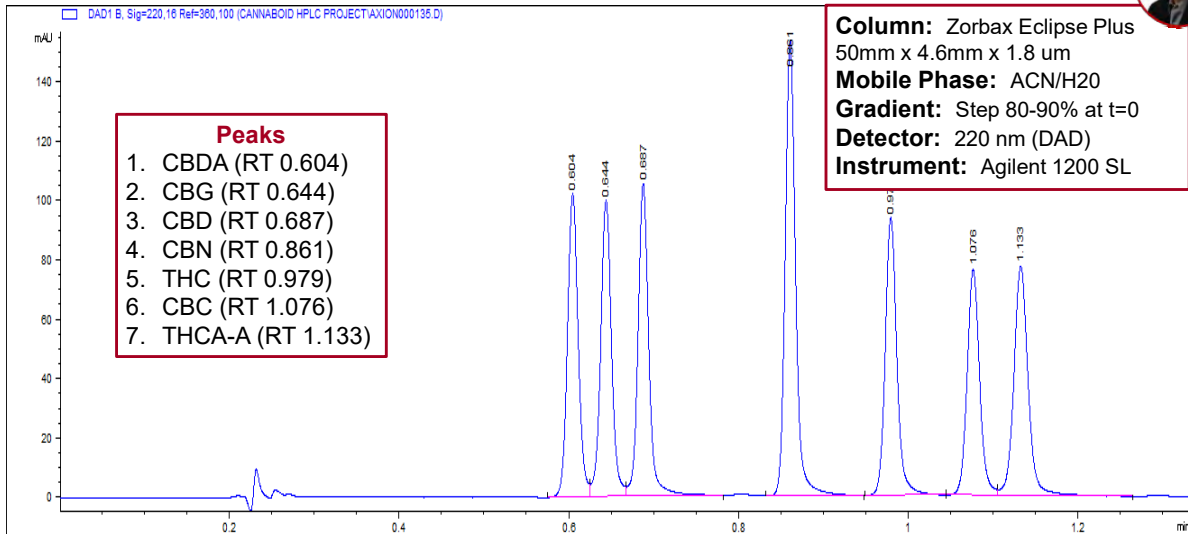


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OK...That's Too Fast!



80-90% ACN Step Gradient with Flow = 2ml min





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Cannabinoids: Stumbling Through Challenging Separations



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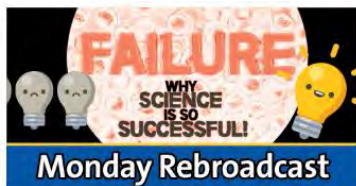
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