

Method Development for the Resolution of Cannabinoids by LC-UV/Vis Using a Virtual Tool

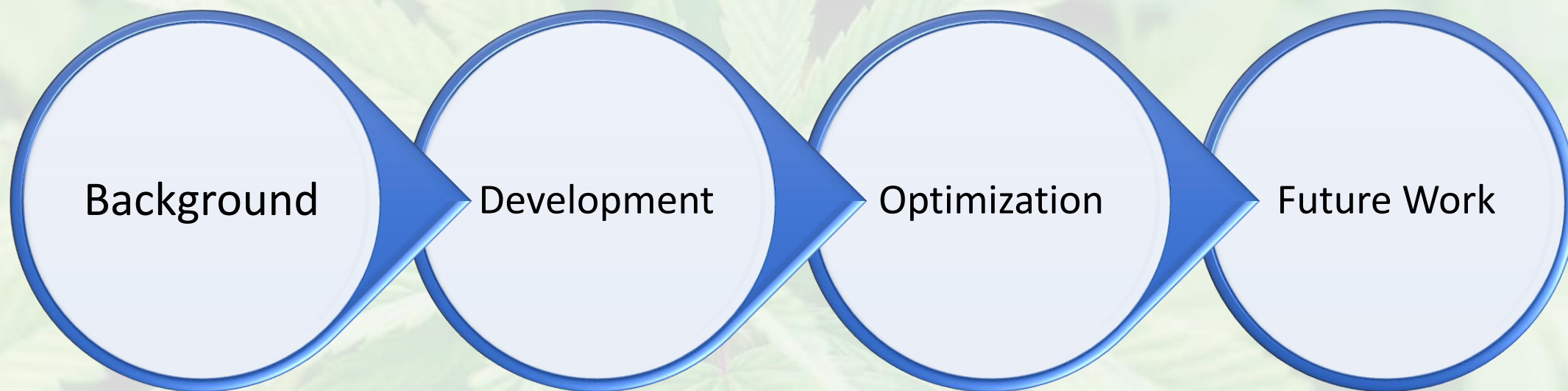
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¹ Restek Corporation, Bellefonte PA

² Analytical Innovations, Dayton OH



Overview

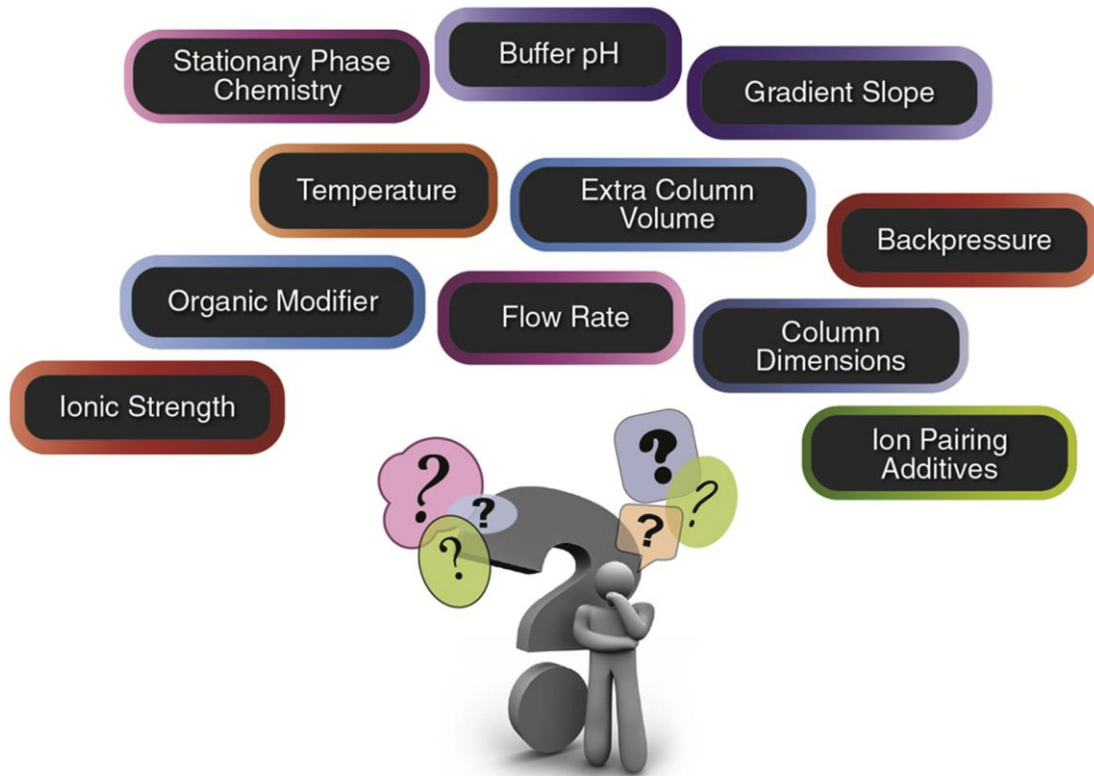


Background

- Constant emergence of new cannabinoids
- Challenging to stay relevant on new additions to testing scopes
- Lack of bandwidth to perform method development



Traditional Method Development



Mattrey, F. T. *Trends in Anal. Chem.* **2017**, 95, 36-46.

Cannabinoids

- Select compound class
- Select analytes
- Select column chemistry
- Click *Generate Model*

The screenshot shows the RESTEK EZLC software interface. The 'Compounds' tab is selected. The 'Compound Class' dropdown is set to 'Cannabinoids'. A search bar is present. Below is a table of cannabinoid compounds with checkboxes for selection. At the bottom, there are options for 'Phase' (set to 'Raptor ARC-18') and 'Detector' (set to 'UV'). A 'Generate Model' button is at the bottom right. Red arrows point to the 'Cannabinoids' dropdown, the 'Target All' button (which is circled in red), and the 'Generate Model' button.

Compound Name	CAS	Select
CBD	55824-13-0	☐
CBDV	24274-48-4	☒
CBDVA	31932-13-5	☒
CBE	52025-76-0	☐
CBG	25654-31-3	☒
CBGA	25555-57-1	☒
CBGB	2468125-69-9	☐
CBGH	2871775-07-2	☐
CBGP	55824-15-2	☐
CBGVA	64924-07-8	☐
CBL	21366-63-2	☒
CBLA	40524-99-0	☐

16 Compounds Selected
16 Compounds To Resolve

Select All Clear
Target All Clear

Phase: Raptor ARC-18
Detector: UV

Generate Model

Method Build

- Select column dimension
- Adjust the dwell and extra-column volume
- Select mobile phase A concentration, column temperature
 - Add gradient steps, change flow rate
- Add re-equilibration time
- Set target resolution

The screenshot displays the 'Method Build' interface with the 'Conditions' tab selected. The interface is organized into several sections:

- Compounds**: Tabbed interface with 'Conditions' and 'My EZLC'.
- Column**: Parameters include Length (150.00 mm), Inner Diameter (4.60 mm), Particle Size (2.70 µm), and Available Columns (150, 4.6, 2.7).
- Volume Effects**: Dwell Volume (0.25 mL) and Extra-Column Volume (25.00 µL).
- Mobile Phase**: Eluent A (Water, 0.1% Formic Acid, Ammonium Formate 5.00 mM) and Eluent B (Acetonitrile, 0.1% Formic Acid). Temperature is 30.00 °C and Back Pressure is 2301 psi.
- Gradient Program**: Isocratic (selected) and Gradient options. A table shows a gradient step at 9.7 min with 75% B and 1.5 mL/min flow. Target Resolution is 1.50.
- Results**: Gradient Time + Delay / Run Time is 10.76 / 10.76 min. T0 is 0.79 min.

Buttons for 'Undo' and 'Redo' are located at the bottom right.

Method Transfer

Compounds Conditions **My EZLC** <<

Column

Length 150.00 mm
Inner Diameter 4.60 mm
Particle Size 2.70 µm
Available Columns 150, 4.6, 2.7 ▾

Volume Effects

Dwell Volume 0.25 mL
Extra-Column Volume 25.00 µL

Mobile Phase

Eluent A Water
0.1% Formic Acid
Ammonium Formate 5.00 mM
Eluent B Acetonitrile
0.1% Formic Acid
Temperature 30.00 °C
Back Pressure psi ▾ 2301 psi

Gradient Program

☒ Isocratic ☐ Gradient

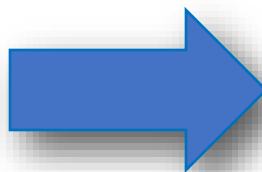
Time (min)	%B	Flow (mL/min)
9.7	75	1.5

Target Resolution 1.50
Optimize Gradient Slope

Results

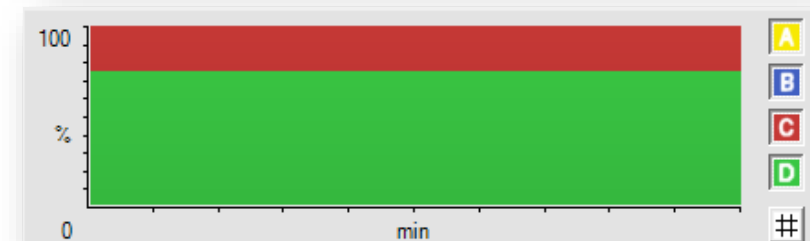
Gradient Time + Delay / Run Time 10.76 / 10.76 min
TO 0.79 min

Undo Redo



Gradient:

	Time	Flow (mL/min)	%A	%B	%C	%D	Curve
1	Initial	1.500	0.0	0.0	25.0	75.0	Initial
2							
3							



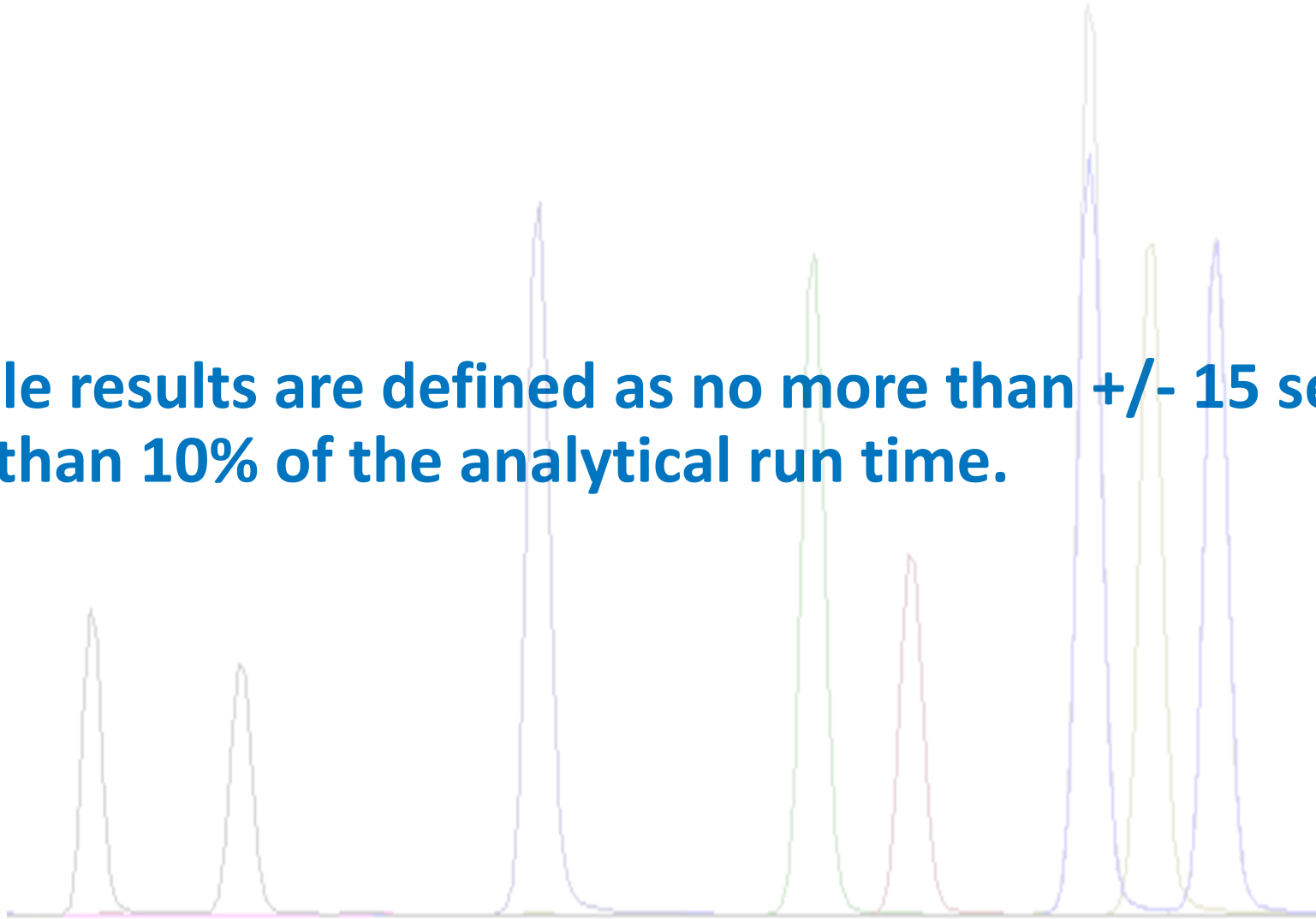
Temperature

Column: 30.0 °C ☒ Alarm Band: ± 1.0 °C ☐ Shutdown all columns

Active Preheater: Enabled ▾

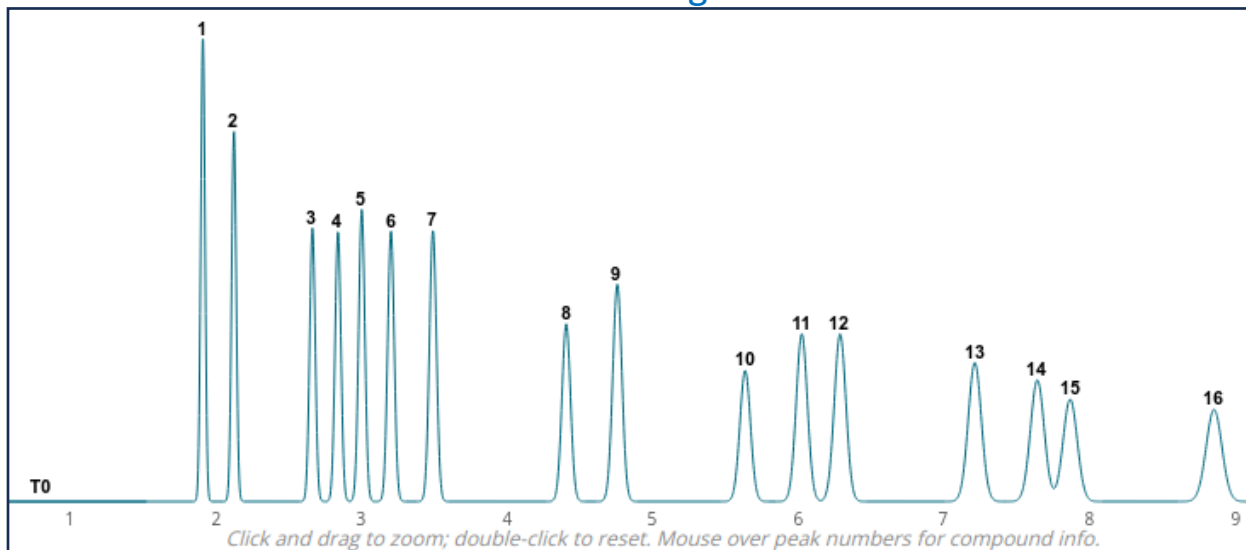
Results

Acceptable results are defined as no more than ± 15 seconds or no more than 10% of the analytical run time.

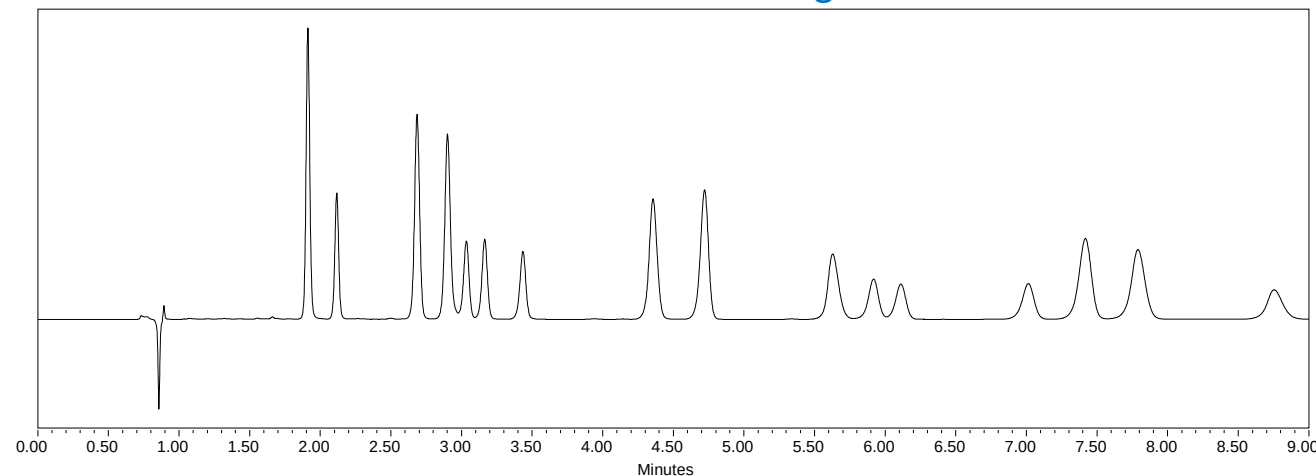


Comparison of Modeled vs. Instrument

EZLC Chromatogram



Instrument Chromatogram



Peak	Analyte	Experimental RT (min)	Modeled RT (min)	Difference (sec)
1	Cannabidivarinic acid (CBDVA)	1.91	1.91	0.00
2	Cannabidivarin (CBDV)	2.12	2.12	0.24
3	Cannabidiolic acid (CBDA)	2.68	2.66	1.44
4	Cannabigerolic acid (CBGA)	2.90	2.83	4.20
5	Cannabigerol (CBG)	3.03	3.00	2.04
6	Cannabidiol (CBD)	3.16	3.20	2.22
7	Tetrahydrocannabivarin (THCV)	3.43	3.49	3.36
8	Tetrahydrocannabivarinic acid (THCVA)	4.36	4.40	2.70
9	Cannabinol (CBN)	4.72	4.75	1.80
10	Cannabinolic acid (CBNA)	5.63	5.63	0.00
11	Δ 9-Tetrahydrocannabinol (Δ 9-THC)	5.92	6.02	6.18
12	Δ 8-Tetrahydrocannabinol (Δ 8-THC)	6.11	6.28	10.14
13	Cannabicyclol (CBL)	7.01	7.21	11.76
14	Cannabichromene (CBC)	7.42	7.64	13.38
15	Tetrahydrocannabinolic acid A (THCA-A)	7.79	7.86	4.20
16	Cannabichromenic acid (CBCA)	8.75	8.85	5.76

Optimization of Published Method

Compounds Conditions **My EZLC** <<

Column

Length 150.00 mm
Inner Diameter 4.60 mm
Particle Size 2.70 μm
Available Columns 150, 4.6, 2.7

Volume Effects

Dwell Volume 0.25 mL
Extra-Column Volume 25.00 μL

Mobile Phase

Eluent A Water
0.1% Formic Acid
Ammonium Formate 5.00 mM
Eluent B Acetonitrile
0.1% Formic Acid
Temperature 30.00 $^{\circ}\text{C}$
Back Pressure psi 2301 psi

Gradient Program

☒ Isocratic
☐ Gradient

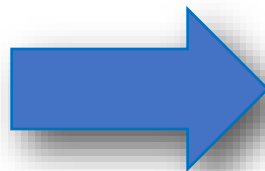
Time (min)	%B	Flow (mL/min)
9.7	75	1.5

Target Resolution 1.50
Optimize Gradient Slope

Results

Gradient Time + Delay / Run Time 10.76 / 10.76 min
T0 0.79 min

Undo Redo



Compounds Conditions **My EZLC** <<

Column

Length 150.00 mm
Inner Diameter 4.60 mm
Particle Size 2.70 μm
Available Columns 150, 4.6, 2.7

Volume Effects

Dwell Volume 0.25 mL
Extra-Column Volume 25.00 μL

Mobile Phase

Eluent A Water
0.1% Formic Acid
Ammonium Formate 4.00 mM
Eluent B Acetonitrile
0.1% Formic Acid
Temperature 35.00 $^{\circ}\text{C}$
Back Pressure psi 2399 psi

Gradient Program

☒ Isocratic
☐ Gradient

Time (min)	%B	Flow (mL/min)
6.7	77	1.7

Target Resolution 1.50
Optimize Gradient Slope

Results

Gradient Time + Delay / Run Time 7.55 / 7.55 min
T0 0.70 min

Undo Redo

Results of Optimization

Compounds

Conditions

My EZLC

<<

Column

Length150.00 mm

Inner Diameter4.60 mm

Particle Size2.70 μm

Available Columns150, 4.6, 2.7

Volume Effects

Dwell Volume0.25 mL

Extra-Column Volume25.00 μL

Mobile Phase

Eluent AWater0.1% Formic Acid

Ammonium Formate4.00 mM

Eluent BAcetonitrile0.1% Formic Acid

Temperature35.00 °C

Back Pressurepsi2399 psi

Gradient Program

Isocratic

Gradient

Time (min)

%B

Flow (mL/min)

6.7

77

1.7

Target Resolution1.50

Optimize Gradient Slope

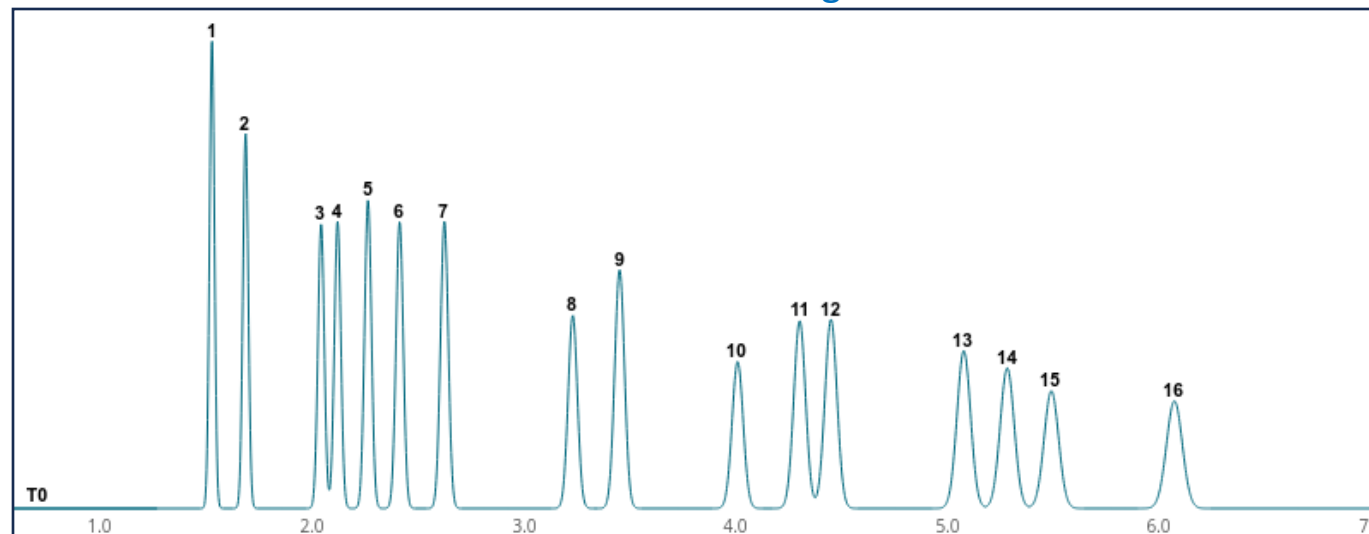
Results

Gradient Time + Delay / Run Time7.55 / 7.55 min

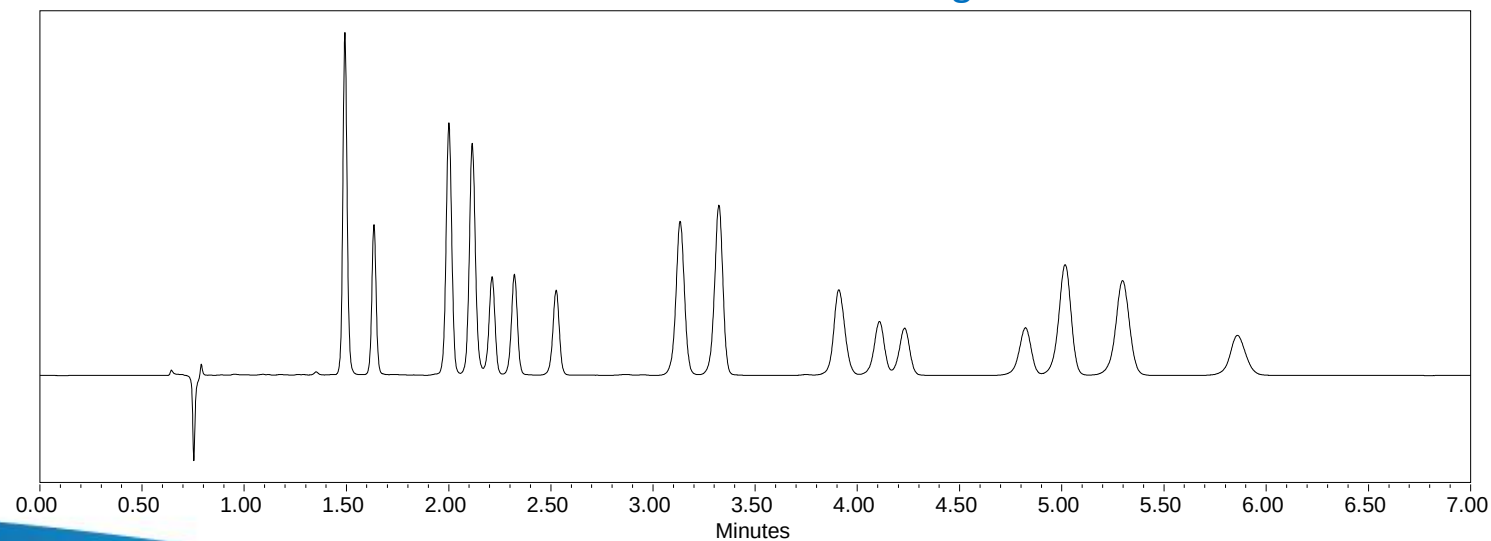
T00.70 min

UndoRedo

EZLC Chromatogram



Instrument Chromatogram



Results of Optimization

Peak	Analyte	Experimental RT (min)	Modeled RT (min)	Difference (sec)
1	Cannabidivarinic acid (CBDVA)	1.49	1.52	1.86
2	Cannabidivarin (CBDV)	1.64	1.68	2.88
3	Cannabidiolic acid (CBDA)	2.00	2.04	2.22
4	Cannabigerolic acid (CBGA)	2.12	2.12	0.00
5	Cannabigerol (CBG)	2.21	2.26	2.88
6	Cannabidiol (CBD)	2.32	2.41	5.22
7	Tetrahydrocannabivarin (THCV)	2.53	2.62	5.64
8	Tetrahydrocannabivarinic acid (THCVA)	3.14	3.23	5.52
9	Cannabinol (CBN)	3.33	3.45	7.44
10	Cannabinolic acid (CBNA)	3.91	4.01	5.58
11	Δ^9 -Tetrahydrocannabinol (Δ^9 -THC)	4.11	4.30	11.28
12	Δ^8 -Tetrahydrocannabinol (Δ^8 -THC)	4.24	4.45	12.72
13	Cannabicyclol (CBL)	4.83	5.07	14.76
14	Cannabichromene (CBC)	5.02	5.28	15.36
15	Tetrahydrocannabinolic acid A (THCA-A)	5.30	5.49	10.98
16	Cannabichromenic acid (CBCA)	5.87	6.07	12.06

Method Comparison

Conditions	Published	Optimized
Analytical Column:	Raptor ARC-18 150 x 4.6 mm , 2.7 μ m	Raptor ARC-18 150 x 4.6 mm , 2.7 μ m
Flow (mL/min)	1.5	1.7
Temperature ($^{\circ}$ C)	30	35
% Mobile Phase A:	25 (Water, 5mM ammonium formate, 0.1 % formic acid)	23 (Water, 3mM ammonium formate, 0.1 % formic acid)
% Mobile Phase B:	75 (Acetonitrile, 0.1 % formic acid)	77 (Acetonitrile, 0.1 % formic acid)
Solvent Usage (mL) :	15	12
Cycle Time (min):	10	7

Department of Cannabis Control (DCC)

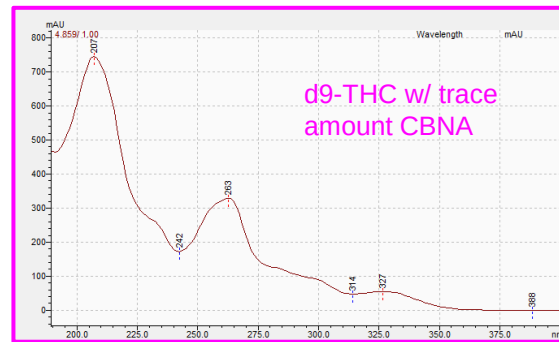
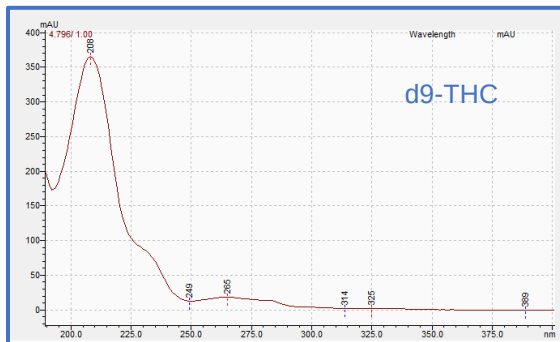
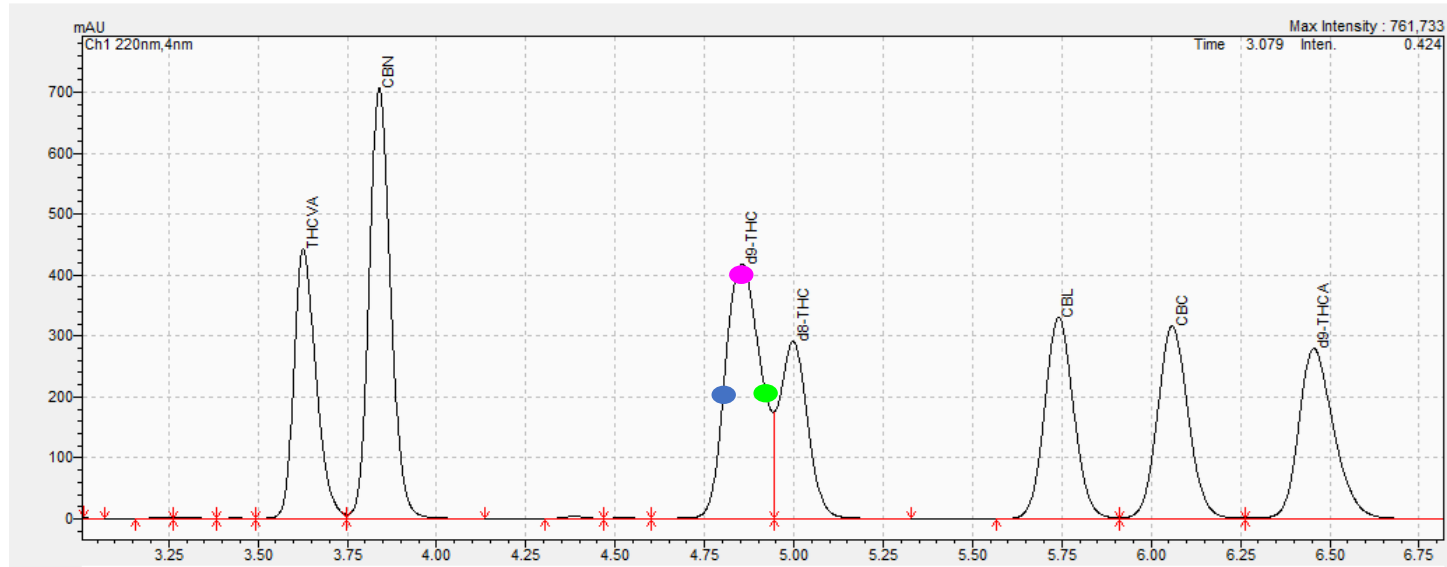
1. HPLC Column: Restek Raptor ARC-18 2.1 x 150 mm, 2.7 μ m or an equivalent column that can separate the cannabinoids of interest to achieve a minimum resolution of 1.3.
2. Mobile Phases:
 - a. Mobile phase A: Water with 0.05 Volume/Volume% formic acid
 - b. Mobile phase B: Acetonitrile with 0.05 Volume/Volume% formic acid
3. Flow Rate 0.4 mL/min
4. Run time: total 12.00 min: 7.00 min + 2.00 min washing period + 3.00 min column re-equilibration
5. Column Temperature: 35 °C

Compounds
CBDA
CBG
CBD
THCV
CBN
Δ 9-THC
Δ 8-THC
CBC
THCA

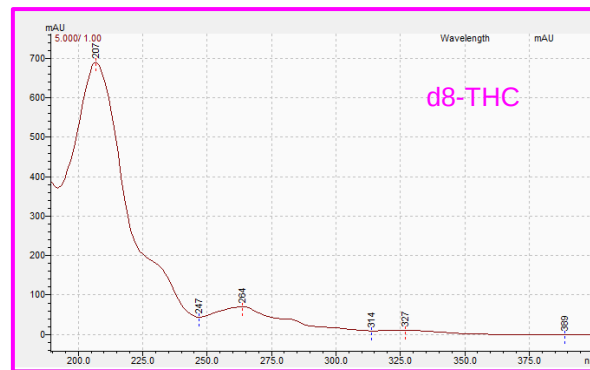
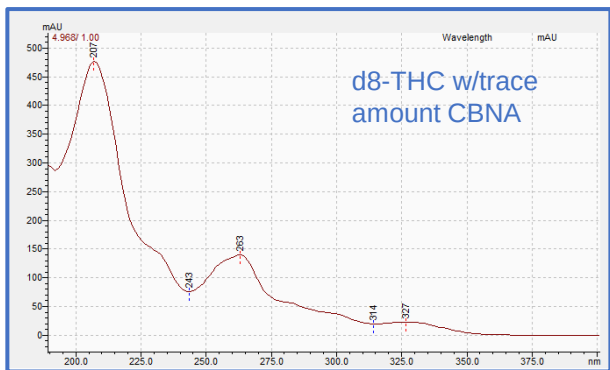
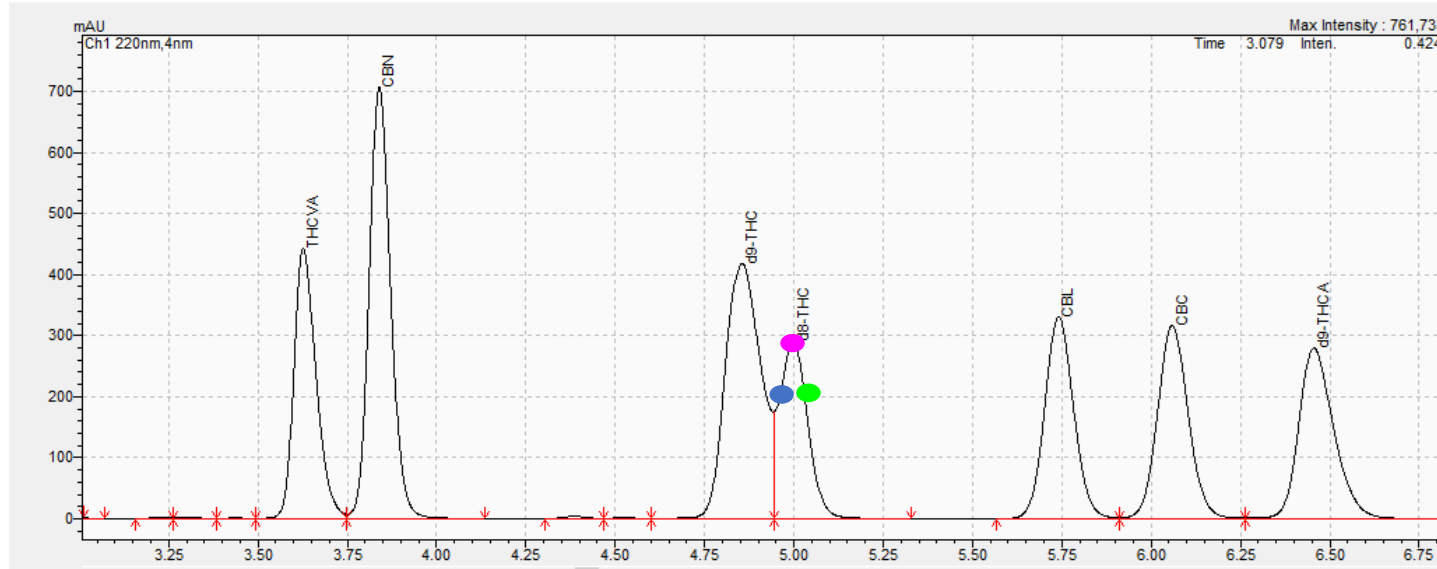
Time (min)	Flow rate (mL/min)	% Mobile Phase A	% Mobile Phase B
0.00	0.4	25	75
7.00	0.4	25	75
7.01	0.4	0	100
9.00	0.4	0	100
9.01	0.4	25	75
12.00	0.4	25	75

Standard Cannabinoids Test Method and ... (n.d.). https://cannabis.ca.gov/wp-content/uploads/sites/2/2023/08/approved_text_standard_test_methods_operating_procedures.pdf

DCC Chromatography



DCC Chromatography



Modified DCC Method

Compounds

Conditions

My EZLC

<<

Column

Length150.00 mm

Inner Diameter2.10 mm

Particle Size2.70 μm

Available Columns150, 2.1, 2.7

Volume Effects

Dwell Volume0.25 mL

Extra-Column Volume25.00 μL

Mobile Phase

Eluent AWater0.1% Formic Acid

Ammonium Formate3.00 mM

Eluent BAcetonitrile0.1% Formic Acid

Temperature35.00 °C

Back Pressurepsi2615 psi

Gradient Program

Isocratic

Gradient

Add Start Isocratic Hold

1 # of Gradient Steps

Add Final Isocratic Hold

Add Re-equilibration Time

5 # of Column Rinses

Time (min)

%B

Flow (mL/min)

0

75

0.4

7

75

0.4

7.1

100

0.4

9

100

0.4

9.01

75

0.4

12.2

75

0.4

Target Resolution

1.50

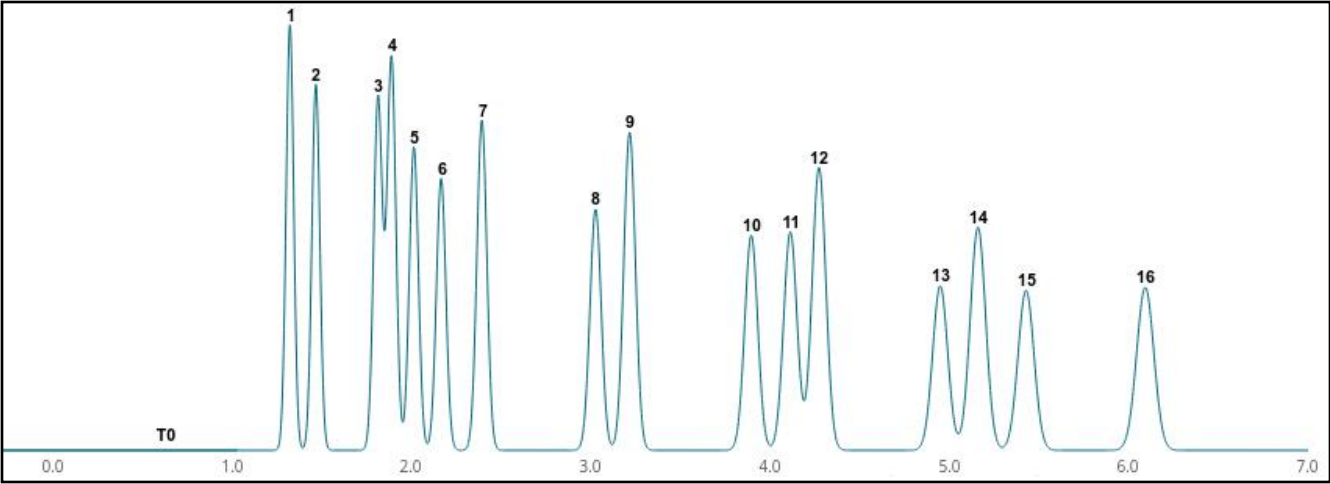
Optimize Gradient Slope

Results

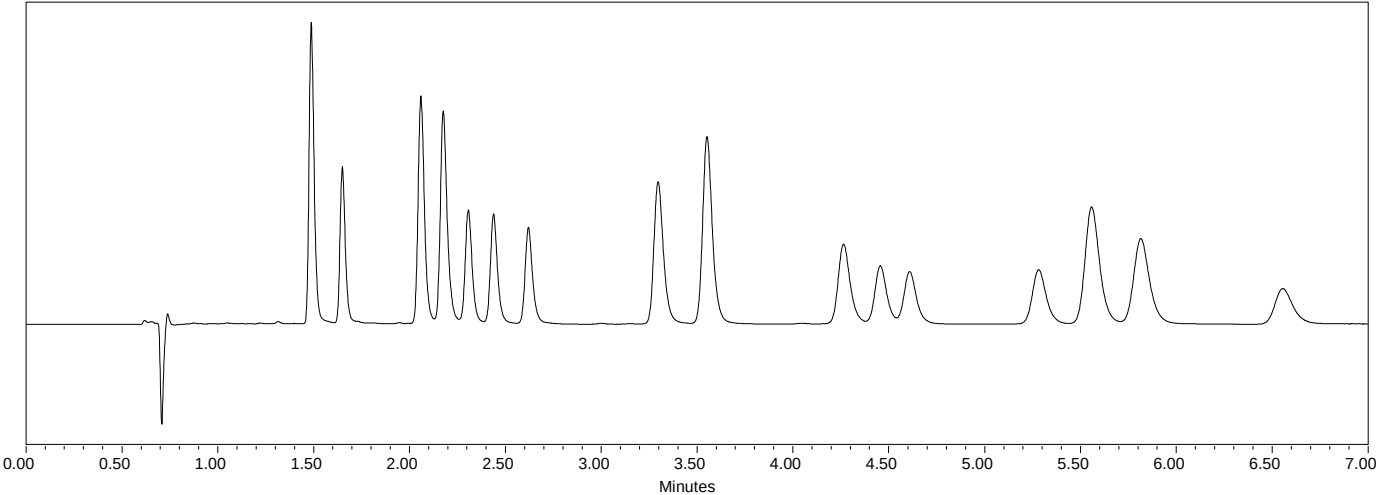
Gradient Time + Delay / Run Time10.25 / 12.16 min

T00.62 min

EZLC Chromatogram



Instrument Chromatogram



Comparison

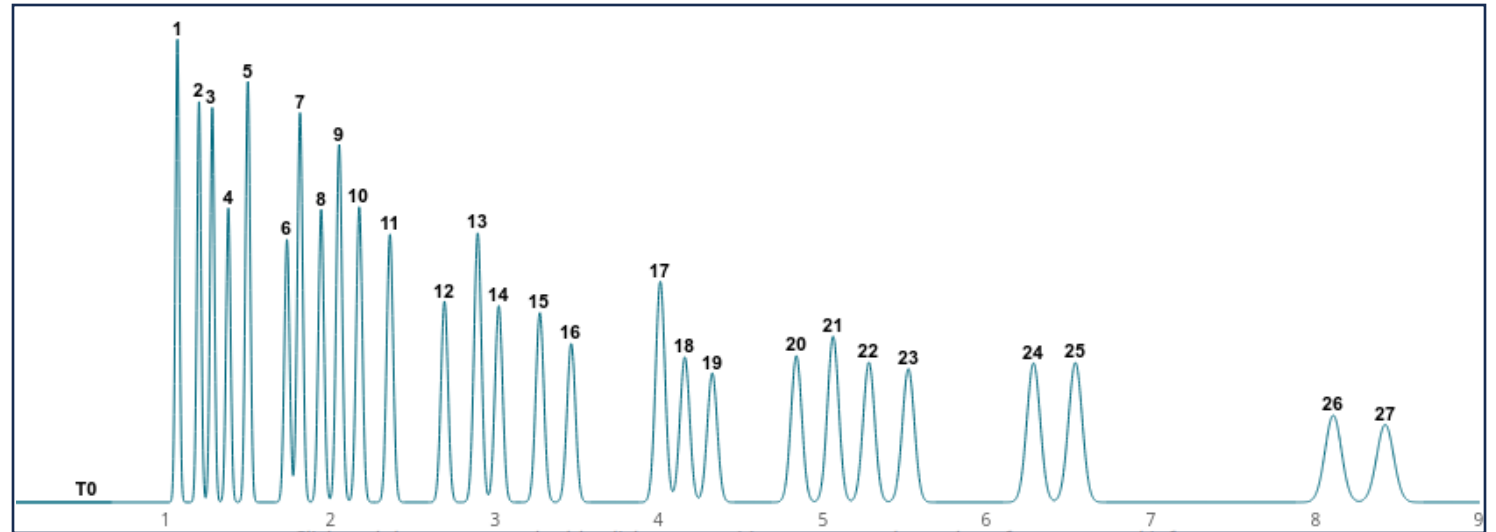
Compound	Experimental RT (min)	Modeled RT (min)	Difference (sec)	Run Time Difference (%)	USP Resolution
CBDVA	1.47	1.32	9.12	2.2	
CBDV	1.63	1.46	10.26	2.4	3.2
CBDA	2.03	1.81	13.26	3.2	7.1
CBGA	2.14	1.89	15.24	3.6	1.7
CBG	2.27	2.01	15.78	3.8	1.9
CBD	2.40	2.16	14.64	3.5	1.9
THCV	2.59	2.39	11.82	2.8	2.6
THCVA	3.25	3.02	13.92	3.3	8.1
CBN	3.50	3.21	17.28	4.1	2.6
CBNA	4.20	3.89	18.42	4.4	6.6
Δ 9-THC	4.39	4.11	16.74	4.0	1.6
Δ 8-THC	4.54	4.27	16.20	3.9	1.3
CBL	5.21	4.95	15.30	3.6	5.2
CBC	5.47	5.16	18.78	4.5	1.9
THCA-A	5.73	5.43	17.88	4.3	1.7
CBCA	6.45	6.09	21.54	5.1	4.4

RESOLUTION?!

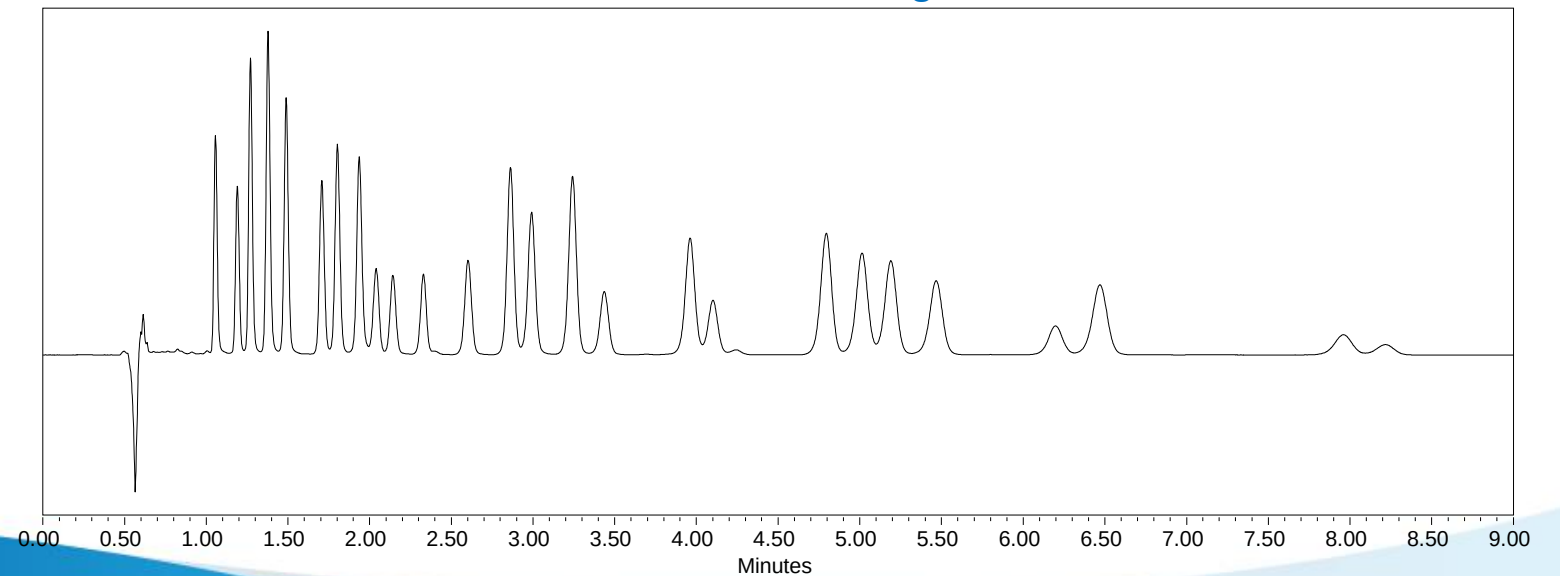


What if

EZLC Chromatogram

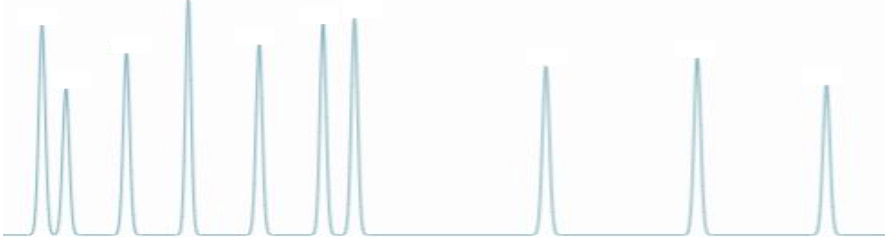


Instrument Chromatogram

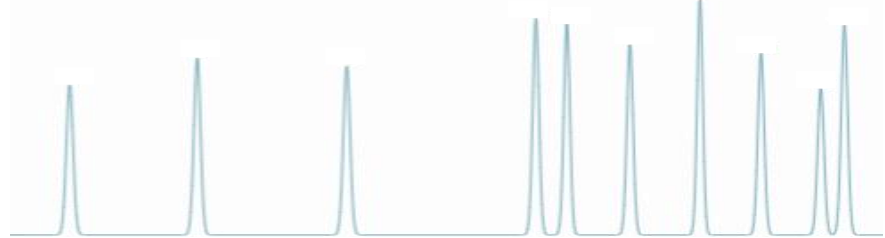


Retention Time Comparison

Compound	Experimental RT (min)	Modeled RT (min)	Difference (sec)
Cannabidiocin (CBDO)	1.06	1.06	0.36
Cannabidiethanol (CBDE)	1.20	1.27	4.56
Cannabidivarinic acid (CBDVA)	1.28	1.38	6.12
Cannabigerovarinic Acid (CBGVA)	1.37	1.49	6.90
Cannabidibutolic Acid (CBDDBA)	1.49	1.71	12.96
Cannabidibutol (CBDDB)	1.73	1.80	4.32
Cannabidiolic acid (CBDA)	1.81	1.94	7.62
Cannabigerolic acid (CBGA)	1.94	2.04	6.06
Cannabigerol (CBG)	2.05	2.14	5.64
Cannabidiol (CBD)	2.17	2.33	9.48
Tetrahydrocannabivarin (THCV)	2.36	2.60	14.70
Cannabigerohexol (CBGH)	2.69	2.86	10.26
Cannabichromevarin (CBCV)	2.89	2.99	5.88
Tetrahydrocannabivarinic acid (THCVA)	3.02	3.24	13.20
Cannabinol (CBN)	3.27	3.44	9.84
Cannabigerophorol (CBGP)	3.46	3.69	13.74
Cannabinolic acid (CBNA)	4.01	3.96	2.70
Δ^9 -Tetrahydrocannabinol (Δ^9 -THC)	4.16	4.10	3.24
Δ^8 -Tetrahydrocannabinol (Δ^8 -THC)	4.32	4.24	4.86
(6aR,9S)- Δ^{10} - THC	4.84	4.80	2.46
9(R)- $\Delta^{6a,10a}$ tetrahydrocannabinol (9R- $\Delta^{6a,10a}$ - THC)	5.06	5.01	2.70
Cannabichromene (CBC)	5.28	5.19	5.28
Tetrahydrocannabinolic acid A (THCA-A)	5.52	5.47	3.06
Cannabichromenic acid (CBCA)	6.28	6.20	5.04
Cannabicyclic Acid (CBLA)	6.54	6.47	4.02
Δ^9 -Tetrahydrocannabiphorol (Δ^9 -THCP)	8.11	7.96	8.94
Cannabicitran (CBT)	8.42	8.22	12.48



Future Plans



- Constantly working on additions to EZLC
 - Column Chemistries
 - Analyte Libraries
 - Languages

What's Next?

Alter method conditions in the Conditions tab to see how changes affect the separation.

Need more help?

If you need further assistance, feel free to [send this compound list](#) and any questions you may have to Restek Technical Service.

Looking for other compounds?

[Tell us](#) what compound libraries you think Restek should focus on next.



Send
Feedback



Help



Print



Save



Save a
Copy



Language ▼

Language



Acknowledgements

Justin Steimling

Jamie York

Tim Yosca

Chris Nelson

John Garrett

Tania Sasaki

EZLC Users

Questions?



Try it out!

