RESTEK Pure Chromatography

A Virtual Liquid Chromatography Method Development Tool for Cannabinoid Analysis

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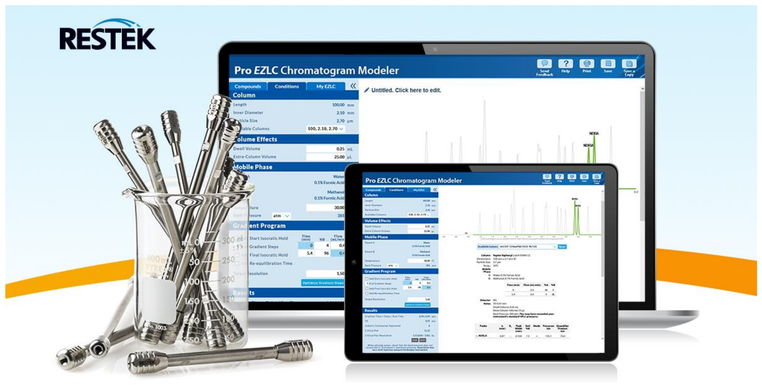
Introduction & Background

As the regulatory landscape of the cannabis industry continues to evolve, it can be difficult to establish or optimize potency methods. With the pressure of low-cost testing and quick turnaround time, laboratories implementing or optimizing new and existing methods for improved profitability and efficiency, struggle with instrument availability and the time needed to do hands on traditional method development work.

The development and optimization of a Liquid Chromatography (LC) method can be time consuming and costly. Often this requires a number of steps including literature research, column selection, method scouting, development and optimization. To alleviate the burden of sacrificing instrument-uptime, labor, and materials, an instrument-free software modeling tool was developed.

Initially launched in Fall of 2022, Pro*EZLC* initially debuted with a Drugs of Abuse (DoA) LC-MS/MS library. In Fall 2023, the release of a cannabinoid LC-UV library is planned. This no-cost tool allows users to obtain optimized separations while maintaining critical pair resolution by adjusting parameters such as temperature, mobile phase buffer, gradients, and more.



Building Database

Prior to data collection, to determine and eradicate column-to-column variability, lot check tests were completed on three separate lots of 150 mm x 3.0 mm Raptor ARC-18 2.7 µm columns. Five cannabinoids were selected as “meld compounds” to analyze alongside each new library collected to ensure a match to the base library. These five compounds were monitored for lot check tests. Data was tabulated in Excel and the percent difference, median, and ± percent difference calculated (Table I). With all three lots in agreement, the base library was created using one of the columns that was lot check tested.

	Raptor ARC-18 150 mm x 3.0 mm, 2.7 µm		
Serial Number:	19052041	19052424	19052411
Lot Number:	200202E	220123E	220117E
	Time (min)	Time (min)	Time (min)
Cannabinolic acid (CBNA)	4.50	4.66	4.68
Δ8-Tetrahydrocannabinol (Δ8-THC)	4.80	5.03	4.98
Δ9-Tetrahydrocannabinol (Δ9-THC)	4.96	5.21	5.16
Cannabichromene (CBC)	6.05	6.35	6.29
Cannabichromenic acid (CBCA)	7.09	7.38	7.38
	% Diff	Median	± % Diff
Cannabinolic acid (CBNA)	4.0%	4.59	2.0%
Δ8-Tetrahydrocannabinol (Δ8-THC)	4.6%	4.92	2.3%
Δ9-Tetrahydrocannabinol (Δ9-THC)	4.8%	5.08	2.4%
Cannabichromene (CBC)	4.9%	6.20	2.4%
Cannabichromenic acid (CBCA)	4.1%	7.24	2.0%

Table I: Results of lot check testing

The base library consisted of 16 cannabinoids. Retention times were collected using three sets of isocratic conditions, two temperatures and five separate buffer strengths collected on a 150 mm x 3.0 mm Raptor ARC-18 2.7 µm column.

Criteria & Performance Targets

Criteria:

1. No cost
2. Instrument free
3. Consultative on-demand method development
4. Improve lab efficiency, data quality, profitability

Performance targets for data collection:

1. Retention time comparison between modeled and experimental runs cannot exceed more ±10% of the total run time.
2. Data is easily normalized from column-to-column variability and different instrument platforms.

Verification

To verify accuracy and robustness of the modeler, a different column dimension was selected, and tested using a different temperature and flow rate (Table II). Retention time data was compared to modeler values and the difference was calculated (Table III).

Column	Raptor ARC-18 (Cat. #9314A65)	
Dimensions:	150 mm x 4.6 mm ID	
Particle Size:	2.7 µm	
Temperature:	35 °C	
Standard/Sample	Acids 7 (cat # 34144) Neutrals 9 (cat # 34132)	
Diluent:	25:75 ACN : Water	
Conc.:	100 µg / mL	
Inj. Vol.:	5 µL	
Detector:	UV-vis @ 228 nm	
Mobile Phase		
A:	Water, 5 mM ammonium formate, 0.1% formic acid	
B:	Acetonitrile, 0.1% formic acid	
Time (min)	Flow (mL/min)	%B
0.00	0.8	75
16.00	0.8	75

Table II: Conditions used for verification run

Verification (cont.)

Peak #	Compound	Experimental t_R (min)	Modeler t_R (min)	Difference (sec)
1	Cannabidivarinic acid (CBDVA)	3.45	3.54	5.40
2	Cannabidivarin (CBDV)	3.86	3.95	5.34
3	Cannabidiolic acid (CBDA)	4.79	4.87	4.50
4	Cannabigerolic acid (CBGA)	5.04	5.07	1.80
5	Cannabigerol (CBG)	5.40	5.53	7.38
6	Cannabidiol (CBD)	5.75	5.85	5.94
7	Tetrahydrocannabivarin	6.21	6.29	4.68
8	Tetrahydrocannabivarinic acid (THCV)	7.75	7.78	1.92
9	Cannabinol (CBN)	8.44	8.54	5.82
10	Cannabinolic acid (CBNA)	9.80	9.76	2.34
11	Δ9- Tetrahydrocannabinol (Δ9- THC)	10.64	10.73	5.22
12	Δ8- Tetrahydrocannabinol (Δ8-THC)	11.01	11.07	3.78
13	Cannabicyclol (CBL)	12.65	12.70	3.24
14	Cannabichromene (CBC)	13.32	13.33	0.24
15	Tetrahydrocannabinolic acid A (THCA-A)	13.78	13.78	0.06
16	Cannabichromenic acid (CBCA)	15.37	15.26	6.36

Table III: Results of verification run

Future Work

Current verification testing is ongoing with results showing differences of ≤ 7 seconds between modeled and experimental results.

Additional verification work:

- Testing the modeler to determine sustainability of UV-vis analysis.
  - ➢ Additional column dimensions
  - ➢ Additional cannabinoids

Conclusions

This no-cost virtual method development tool is easy to use for LC method developers, both novice and expert. The adoption of its use will assist labs in quickly and accurately develop or optimize methods. The software is consultative on-demand, without entering the lab or sacrificing instrument time, labor or consumables.

Planned Release Date: Fall 2023

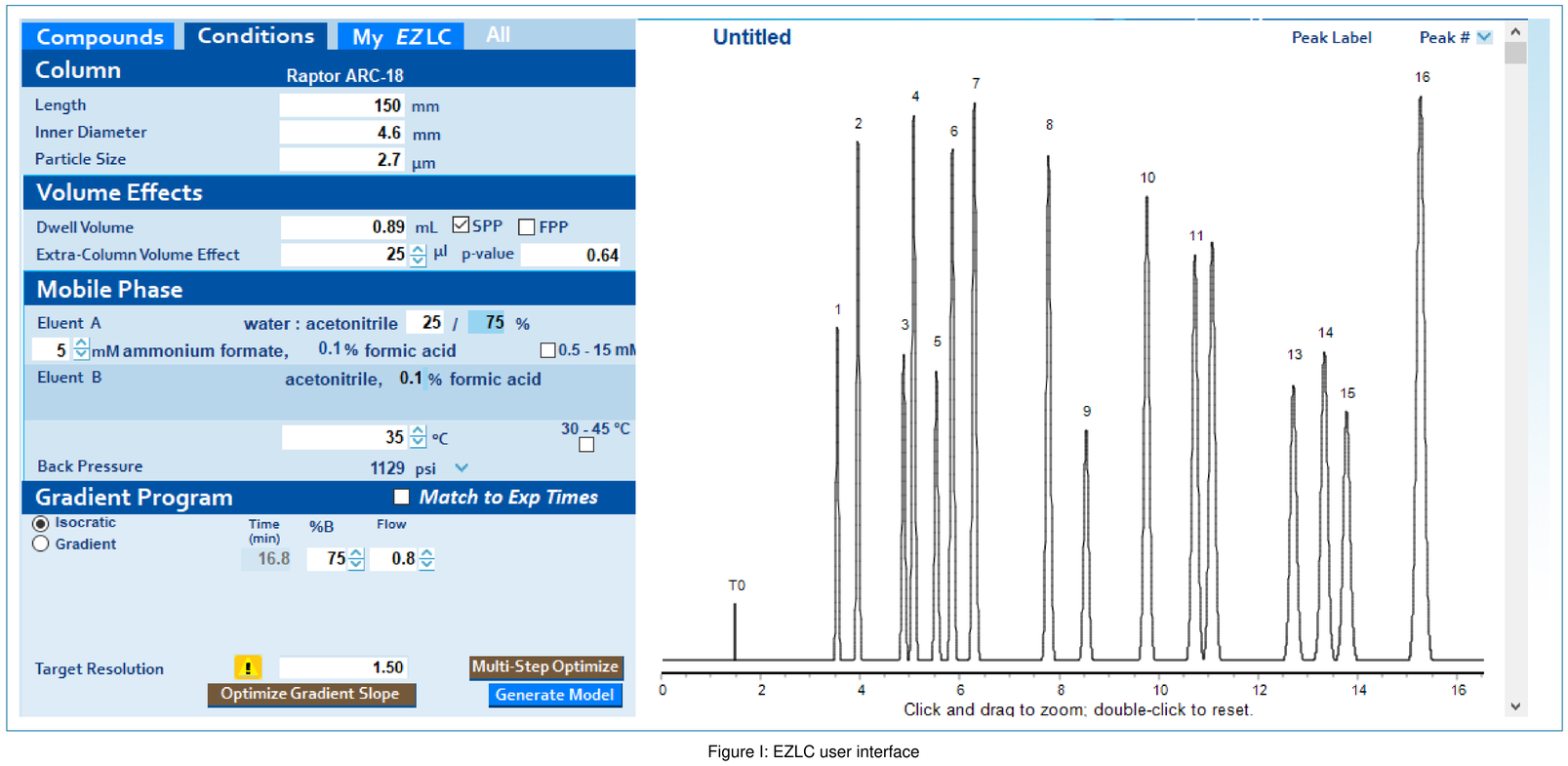


Figure I: EZLC user interface