RESTEK Pure Chromatography

Method Development of Cannabinoids for LC-UV Analysis Using a Virtual Method Development Tool

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Introduction

In 1996, the state of California approved the legalization of cannabis for medicinal use. Since then, 37 additional states have also legalized cannabis for medicinal use. With that number likely to increase, there is an ever-growing need for accurate, reliable, and affordable cannabis testing. While regulations vary from state-to-state, these tests often include microbial, mycotoxin, residual solvents, pesticides, heavy metals, and potency testing.

Traditional method development of cannabinoid separations can be time consuming and costly. The emergence of new cannabinoids often necessitate a re-optimization of current methods. To reduce costs and save time, a freely available tool can be used to perform virtual method development. In this work, compounds were selected from a database containing over 40 cannabinoids. Methods were developed using the chromatogram modeler by adjusting parameters such as instrument/system effects (dwell and extra column volume), temperature, flow rate, and mobile phase additives. The methods were then transferred to an HPLC-UV system. Experimental retention times from the in-lab work were compared against retention times generated from the virtual method development tool.

Cannabinoid Library Build

Prior to collecting data, a lot check test was completed on three separate 150 x 3.0 mm Raptor ARC-18 2.7 µm columns. Retention time data was collected using a designated set of five compounds that span the chromatographic space. Data was tabulated in Excel. With all lots in agreement, the base library was created using one of the columns tested.

A cannabinoid design space was built based on results of the following method conditions:

- ❖ Isocratic Compositions: 30:70 , 25:75, and 20:80
- ❖ Column Temperature: 30 ºC and 45 ºC
- ❖ Mobile Phase: Acetonitrile
- ❖ Aqueous Modifier: 0.5, 5, 10, 15, and 20 mM ammonium formate

To build a base library, 16 cannabinoid compounds were selected. A subset of five compounds (meld compounds) were selected to verify the instrument performance from day-to-day and injection-to-injection. These compounds were then used to add additional compounds to the base library for a total of 46 cannabinoid compounds. Data collected from the conditions listed above were input into the software to complete the library build.

Validation

To determine the sustainability of the modeler, a new set of compounds were used in addition to the meld compounds under the following conditions:

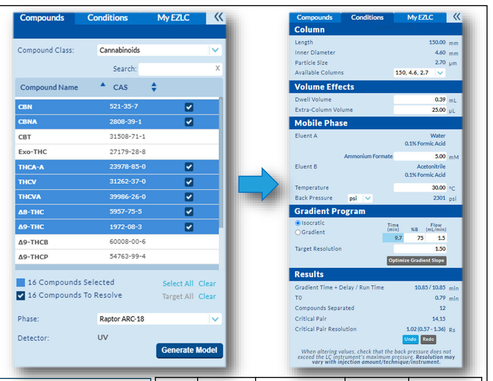
- ❖ Stationary Phase: ARC-18
- ❖ Column Dimensions: 150 x 3.0 mm 2.7 µm, 50 x 3.0 mm 1.8 µm, and 150 x 4.6 mm 2.7 µm
- ❖ Temperature: 30 ºC (150 x 3.0 mm also analyzed at 45 ºC)
- ❖ Mobile Phase: Acetonitrile
- ❖ Aqueous Modifier: 3.5 and 7 mM ammonium formate
- ❖ Gradient Program: Isocratic and Gradient
- ❖ Flow Rate: 0.8 and 1.5 mL/min

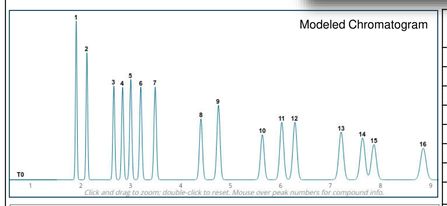
Performance Targets for Data Collection: Retention time comparison between modeled and experimental data cannot exceed more than ± 15 seconds (or no more than 10% of the analytical run time).

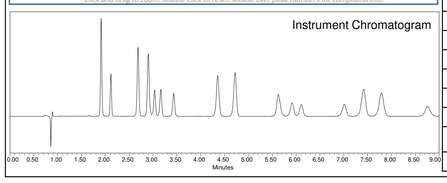
Experiments and Results

Method Build

1. Select Analytes
2. *Target All* for separations
3. Select phase chemistry
4. Generate model
5. Set method conditions
6. Transfer virtual method to instrument

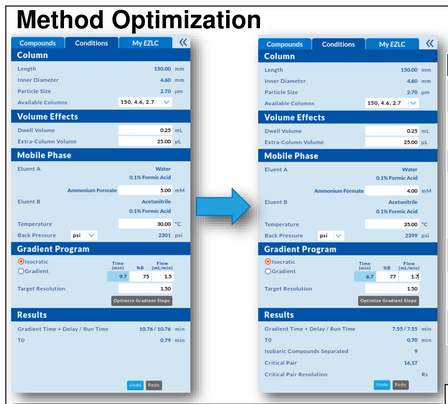




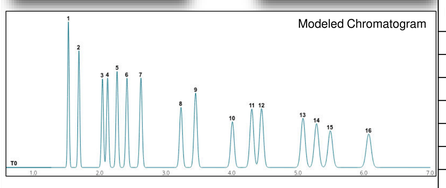


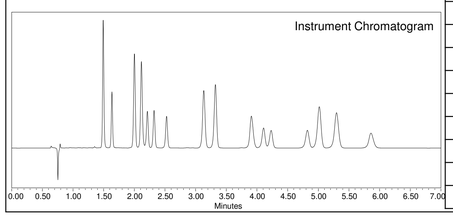
Peak	Analyte	Experimental RT (min)	Modeled RT (min)	Difference (sec)
1	CBDVA	1.91	1.91	0.00
2	CBDV	2.12	2.12	0.00
3	CBDA	2.68	2.66	1.20
4	CBGA	2.90	2.83	4.20
5	CBG	3.03	3.00	1.80
6	CBD	3.16	3.20	2.40
7	THCV	3.43	3.49	3.60
8	THCVA	4.36	4.40	2.40
9	CBN	4.72	4.75	1.80
10	CBNA	5.63	5.63	0.00
11	Δ9-THC	5.92	6.02	6.00
12	Δ8-THC	6.11	6.28	10.20
13	CBL	7.01	7.21	12.00
14	CBC	7.42	7.64	13.20
15	THCA-A	7.79	7.86	4.20
16	CBCA	8.75	8.85	6.00

Method Optimization



Conditions	Published	Optimized
Column:	Raptor ARC-18 150 x 4.6 mm , 2.7 µm	Raptor ARC-18 150 x 4.6 mm , 2.7 µm
Flow (mL/min):	1.5	1.7
Temp (° C):	30	35
% MP A:	25 (Water, 5mM ammonium formate, 0.1 % formic acid)	23 (Water, 3mM ammonium formate, 0.1 % formic acid)
% MP B:	75 (Acetonitrile, 0.1 % formic acid)	77 (Acetonitrile, 0.1 % formic acid)
Solvent Usage (mL):	15	12
Cycle Time (min):	10	7





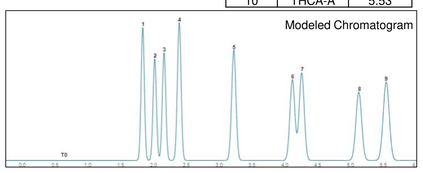
Peak	Analyte	Experimental RT (min)	Modeled RT (min)	Difference (sec)
1	CBDVA	1.49	1.52	1.80
2	CBDV	1.64	1.68	2.40
3	CBDA	2.00	2.04	2.40
4	CBGA	2.12	2.12	0.00
5	CBG	2.21	2.26	3.00
6	CBD	2.32	2.41	5.40
7	THCV	2.53	2.62	5.40
8	THCVA	3.14	3.23	5.40
9	CBN	3.33	3.45	7.20
10	CBNA	3.91	4.01	6.00
11	Δ9-THC	4.11	4.30	11.40
12	Δ8-THC	4.24	4.45	12.60
13	CBL	4.83	5.07	14.40
14	CBC	5.02	5.28	15.60
15	THCA-A	5.30	5.49	11.40
16	CBCA	5.87	6.07	12.00

Additional Compound Monitoring

Many regulations and methods require monitoring for select cannabinoids. However, it is important to know if other analytes are co-eluting with analytes of interest.

Requirements:
- 9 Analytes
- Resolution ≥ 1.3

Peak	Analyte	RT (min)
1	CBDA	1.82
2	CBG	2.01
3	CBD	2.15
4	THCV	2.38
5	CBN	3.21
6	Δ9-THC	4.10
8	Δ8-THC	4.24
9	CBC	5.11
10	THCA-A	5.53

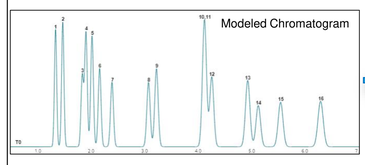


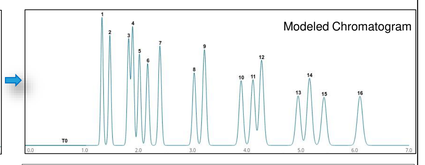
Conditions	Department of Cannabis Control (DCC) Published Method		
Column:	Raptor ARC-18 150 x 2.1 mm , 2.7 µm		
Temp (ºC):	35		
% MP A:	Water, 0.05 % formic acid		
% MP B:	Acetonitrile, 0.05 % formic acid		
Gradient:	Flow (mL/min)	Time (min)	%B
	0.4	0.00	75
	0.4	7.00	75
	0.4	7.01	100
	0.4	9.00	100
	0.4	9.01	75
	0.4	12.00	75

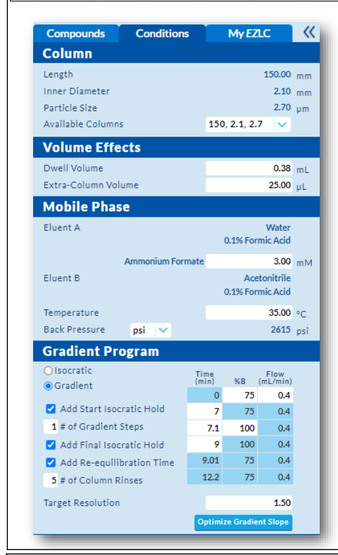
To check if this method contained any coelutions, seven additional analytes were added. Two compounds, CBNA and CBGA, both showed coelutions under the current conditions.

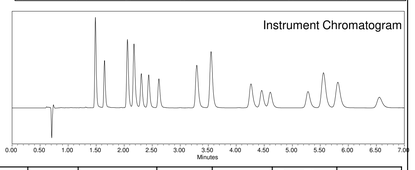
Experiments and Results Cont.

Using the modeler, a method was developed such that all 16 analytes resolved with resolution ≥ 1.3 through the addition of ammonium formate and increased formic acid.



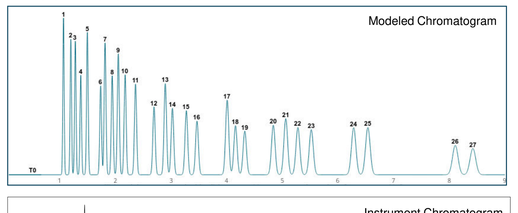


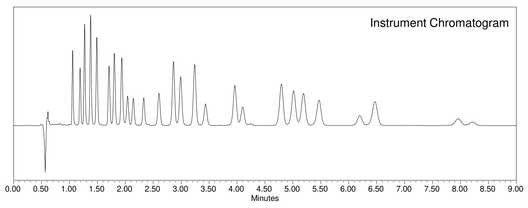


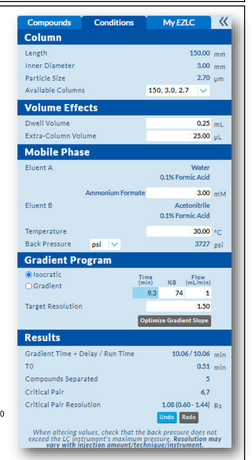


Peak	Analyte	Experimental RT (min)	Modeled RT (min)	Difference (sec)	Run Time Difference (%)	USP Resolution
1	CBDVA	1.47	1.32	9.12	2.2	
2	CBDV	1.63	1.46	10.26	2.4	3.2
3	CBDA	2.03	1.81	13.26	3.2	7.1
4	CBGA	2.14	1.89	15.24	3.6	1.7
5	CBG	2.27	2.01	15.78	3.8	1.9
6	CBD	2.40	2.16	14.64	3.5	1.9
7	THCV	2.59	2.39	11.82	2.8	2.6
8	THCVA	3.25	3.02	13.92	3.3	8.1
9	CBN	3.50	3.21	17.28	4.1	2.6
10	CBNA	4.20	3.89	18.42	4.4	6.6
11	Δ9-THC	4.39	4.11	16.74	4.0	1.6
12	Δ8-THC	4.54	4.27	16.20	3.9	1.3
13	CBL	5.21	4.95	15.30	3.6	5.2
14	CBC	5.47	5.16	18.78	4.5	1.9
15	THCA-A	5.73	5.43	17.88	4.3	1.7
16	CBCA	6.45	6.09	21.54	5.1	4.4

27 Cannabinoids







Peak	Compound	Experimental RT (min)	Modeled RT (min)	Difference (sec)	Run Time Difference (%)
1	Cannabidiocin (CBDO)	1.06	1.06	0.00	0.0
2	Cannabidiethanol (CBDE)	1.20	1.27	4.20	0.7
3	Cannabidivarinic acid (CBDVA)	1.28	1.38	6.00	1.0
4	Cannabigerovarinic Acid (CBGVA)	1.37	1.49	7.20	1.2
5	Cannabidibutolic Acid (CBDBA)	1.49	1.71	13.20	2.2
6	Cannabidibutol (CBDB)	1.73	1.80	4.20	0.7
7	Cannabidiolic acid (CBDA)	1.81	1.94	7.80	1.3
8	Cannabigerolic acid (CBGA)	1.94	2.04	6.00	1.0
9	Cannabigerol (CBG)	2.05	2.14	5.40	0.9
10	Cannabidiol (CBD)	2.17	2.33	9.60	1.6
11	Tetrahydrocannabivarin (THCV)	2.36	2.60	14.40	2.4
12	Cannabigerohexol (CBGH)	2.69	2.86	10.20	1.7
13	Cannabichromevarin (CBCV)	2.89	2.99	6.00	1.0
14	Tetrahydrocannabivarinic acid (THCVA)	3.02	3.24	13.20	2.2
15	Cannabinol (CBN)	3.27	3.44	10.20	1.7
16	Cannabigerophorol (CBGP)	3.46	3.69	13.80	2.3
17	Cannabinolic acid (CBNA)	4.01	3.96	3.00	0.5
18	Δ9-Tetrahydrocannabinol (Δ9-THC)	4.16	4.10	3.60	0.6
19	Δ8-Tetrahydrocannabinol (Δ8-THC)	4.32	4.24	4.80	0.8
20	(6aR,9S)-Δ10 - THC	4.84	4.80	2.40	0.4
21	9(R)- $\Delta^{6a,10a}$ tetrahydrocannabinol (9R-$\Delta^{6a,10a}$ - THC)	5.06	5.01	3.00	0.5
22	Cannabichromene (CBC)	5.28	5.19	5.40	0.9
23	Tetrahydrocannabinolic acid A (THCA-A)	5.52	5.47	3.00	0.5
24	Cannabichromenic acid (CBCA)	6.28	6.20	4.80	0.8
25	Cannabicyclolic Acid (CBLA)	6.54	6.47	4.20	0.7
26	Δ9-Tetrahydrocannabiphorol (Δ9-THCP)	8.11	7.96	9.00	1.5
27	Cannabicitran (CBT)	8.42	8.22	12.00	2.0

Conclusions

Results show the virtual tool can assist in the optimization of existing methods for the addition of new cannabinoids, offer an on-demand consultative user experience, and lead to greener solutions for method development.

Future Work:

- ❖ Additional Stationary Phases
- ❖ Expanded Mobile Phase Modifiers
- ❖ New Compound Libraries – *Pesticides*

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