RESTEK Pure Chromatography

The Development of a Virtual Liquid Chromatography Method Development Tool

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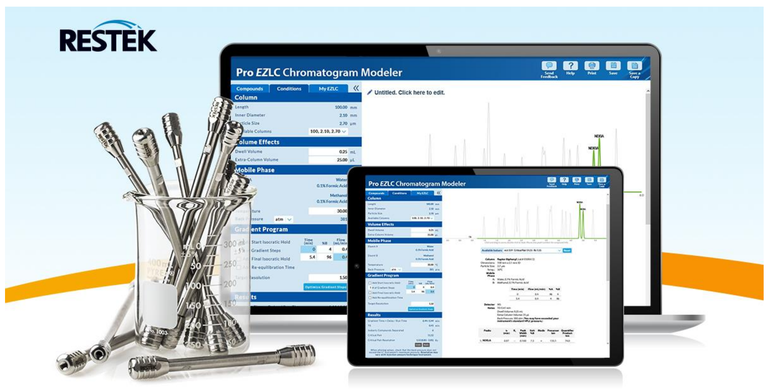
Introduction and Background

The development and optimization of a Liquid Chromatography (LC) method can be time consuming and costly. Often this requires several steps including literature research, column selection, method scouting, development, and optimization. To mitigate the burden of sacrificing instrument-uptime, reduce cost, and to save time and materials, an instrument-free software modeling tool was developed. This software modeling tool enables users to select compounds from a comprehensive database and model separations in real-time. It maintains critical pair resolution by adjusting parameters such as column dimensions, mobile phase compositions, gradient profiles, instrument/system effects, and other variables.



Due to the number of parameters in LC, the initial software build focused on only six variables with additional levers to be added at a later date. To ensure a robust tool, focus was placed on the most used variables of LC method development:

- Column Chemistries
- Column Dimensions and Lengths
- Organic Modifiers
- Gradient Slopes
- Column Temperatures

Build

Prior to collecting library data, lot check tests were completed on three separate lots of both Raptor Biphenyl and Raptor C18 (50 x 2.1 mm, 2.7 µm). Retention time data was collected using a set of nine compounds, referred to as “meld compounds”, that span the chromatographic space (Tables I and II). These compounds were later analyzed alongside each new collected library to ensure a match to the base library. With all three lots in agreement, the base library was created.

Raptor Biphenyl 50 x 2.1 mm, 2.7 µm, 0.4 mL/min			
Lot Number:	190134E	200415P	201001P
	Time (min.)	Time (min.)	Time (min.)
trans-3-Hydroxycotinine	0.41	0.39	0.41
Methylephedrine	1.34	1.40	1.39
Diphenhydramine	3.46	3.48	3.50
Methaqualone	4.19	4.26	4.30
Phenazepam	4.65	4.72	4.76
Norketamine	2.00	2.06	2.07
Levetiracetam	1.19	1.25	1.28
JWH-073	7.10	7.24	7.24

Table I: Retention time data for three unique lots of Raptor Biphenyl

Raptor C18 50 x 2.1 mm, 2.7 µm, 0.8 mL/min			
Lot Number:	210712Q	210543E	210409B
	Time (min.)	Time (min.)	Time (min.)
trans-3-Hydroxycotinine	0.14	0.14	0.14
Methylephedrine	0.35	0.33	0.33
Diphenhydramine	1.53	1.48	1.48
Methaqualone	1.96	1.96	1.98
Phenazepam	2.29	2.30	2.31
Norketamine	0.73	0.70	0.70
Levetiracetam	0.34	0.35	0.36
JWH-073	3.76	3.75	3.77

Table II: Retention time data for three unique lots of Raptor C18

The base library consisted of 50 compounds plus the meld compounds. Retention times were collected using three different gradient conditions and three different temperatures. After the base library was created, approximately 180 drugs of abuse (DoA) were systematically added to the database. To account for the separation of isobars and to generate the optimal points per peak during instrument analysis, compounds were divided into small groups of approximately 30 compounds including meld compounds. Retention times were collected and added to the base library.

Verification

Stage 1: Use a different column dimension from initial library collection and build

- A simple gradient condition and ~ 30 analytes outside of library compounds and different lots of Raptor Biphenyl 50 x 3.0 mm, 2.7 µm column. Data was used to develop correction factors.

Stage 2: Use different flow rates, temperatures, and gradient slopes compared to initial library collection and build

- Raptor Biphenyl 50 x 2.1 mm, 2.7 µm column, data used for modeler adjustments and correction factors. Moved to the next step once retention times were in agreement.
  1. Simple gradient supplied by modeler
  2. Different flow rates holding temperature and gradient constant
  3. Different temperatures holding flow rate and gradient constant
  4. Different gradient slope while holding flow rate and temperature constant
  5. Repeat steps 1 – 4 on a Raptor Biphenyl 100 x 3.0 mm, 2.7 µm column

Stage 3: Use the modeler as a method development tool

- Re-ran full set of data using both stationary phases (C18 & Biphenyl), multi-step gradients (shallow, step gradients, and isocratic hold), used multiple column dimensions, mobile phases (ACN and MeOH), and different temperatures (30 ºC 60 ºC and a 45 ºC verification run).
- Library created results were used to compare modeler to validation experiments.

Validation

To determine sustainability and transferability to different instrument platforms, a new set of compounds were used along with the following:

- Stationary Phases: Raptor Biphenyl 2.7 µm and Raptor C18 2.7 µm
- Column Dimensions: 50 x 2.1 mm, 50 x 3.0 mm, and 100 x 2.1 mm
- Temperature: 40 ºC (Note: both 50 x 2.1 mm also analyzed at 35 ºC and 50 ºC)
- Mobile Phases: ACN and MeOH , with 0.1% Formic Acid
- Gradients:

Gradient 1: Linear	
Time	%B
0.00	5
10.00	98
10.01	5
12.00	5

Gradient 2: Isocratic Hold	
Time	%B
0.00	6
1.00	6
10.00	99
10.01	6
12.00	6

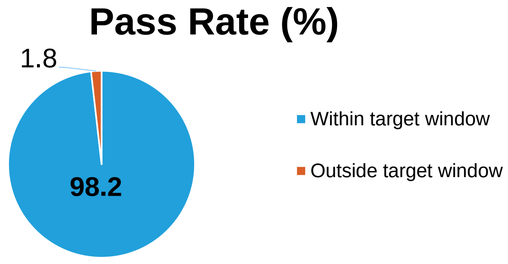
Gradient 3: Multistep	
Time	%B
0.00	7
1.00	30
5.00	45
8.00	80
10.00	95
10.01	7
12.00	7

Performance targets for data collection:

1. Retention time comparison between modeled and experimental runs cannot exceed more than 50% of a standard MRM window (±15 seconds) or no more than 10% of the analytical run time.
2. Data is easily normalized from column-to-column variability and different instrument platforms.

Validation Results

Of the 14 variables analyzed, 704 data points were collected. Only 13 compounds exceeded the target of ±15 second window.



Expanded Software Features

Library Expansion

- Drugs of Abuse (DoA) – over 300+ analytes
- Per- and polyfluoroalkyl substances (PFAS)
- Cannabinoids

Parameter Expansion

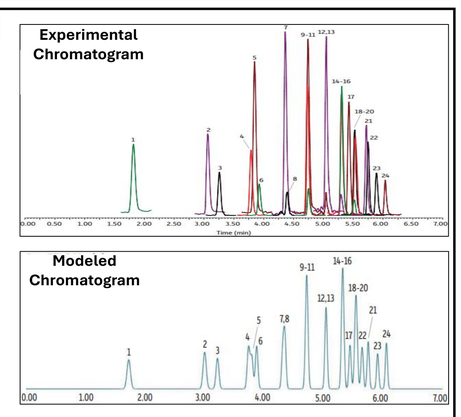
- UV detection
- Mobile phase composition
- Mobile phase additives
- Particle Types : fully porous particles (FPP) and superficially porous particles (SPP)
- Isocratic analysis support
- Multi-step optimization

Languages

- English, Italian, French, German, Spanish, Portuguese, Dutch, Chinese, Japanese

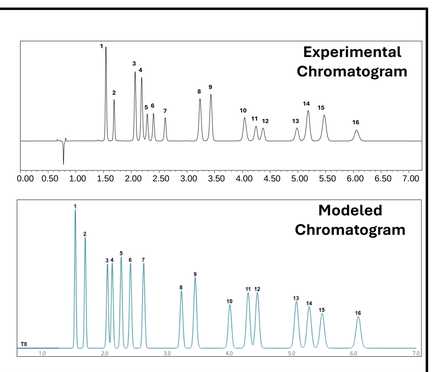
Evaluation

EPA 8327 Conditions		
Column	Force C18 50 x 2.1 mm, 1.8 µm	
Diluent	Methanol	
Inj. Vol.	1 µL	
MP A	Water, 5 mM ammonium acetate	
MP B	Methanol	
Flow	0.4 mL/min	
Detector	LC/MS	
Temp	40 °C	
Gradient	Time (min)	(%) B
	0.00	20
	6.00	95
	6.01	20
	8.00	20



Peak	Compound	Synonym	Experimental t_r (min)	Modeler t_r (min)	Difference (sec)
1	Perfluorobutanoic acid	PFBA	1.82	1.74	4.80
2	Perfluoropentanoic acid	PFPeA	3.07	3.01	3.60
3	Perfluoro-1-butanesulfonic acid	PFBS	3.25	3.23	1.20
4	1H, 1H, 2H, 2H-perfluorohexane sulfonic acid	4:2 FTS	3.79	3.75	2.40
5	Perfluorohexanoic acid	PFHxA	3.84	3.80	2.40
6	Perfluoro-1-pentanesulfonic acid	PFPes	3.92	3.89	1.80
7	Perfluoroheptanoic acid	PFHpA	4.36	4.32	2.40
8	Perfluoro-1-hexanesulfonic acid	PFHxS	4.40	4.36	2.40
9	1H, 1H, 2H, 2H-perfluorooctane sulfonic acid	6:2 FTS	4.72	4.72	0.00
10	Perfluorooctanoic acid	PFOA	4.75	4.72	1.80
11	Perfluoro-1-heptanesulfonic acid	PFHpS	4.75	4.73	1.20
12	Perfluorononanoic acid	PFNA	5.05	5.05	0.00
13	Perfluoro-1-octanesulfonic acid	PFOS	5.05	5.05	0.00
14	1H, 1H, 2H, 2H-perfluorodecane sulfonic acid	8:2 FTS	5.29	5.34	3.00
15	Perfluoro-1-nonanesulfonic acid	PFNS	5.30	5.32	1.20
16	Perfluorodecanoic acid	PFDA	5.30	5.32	1.20
17	N-methylperfluoro-1-octanesulfonamidoacetic acid	NMeFOSAA	5.43	5.45	1.20
18	Perfluoroundecanoic acid	PFUnA	5.52	5.54	1.20
19	Perfluoro-1-decanesulfonic acid	PFDS	5.53	5.55	1.20
20	N-ethylperfluoro-1-octanesulfonamidoacetic acid	NEtFOSAA	5.54	5.57	1.80
21	Perfluorododecanoic acid	PFDoA	5.71	5.76	3.00
22	Perfluoro-1-octanesulfonamide	FOSA	5.75	5.65	6.00
23	Perfluorotridecanoic acid	PFTriA	5.89	5.91	1.20
24	Perfluorotetradecanoic acid	PFTreA	6.04	6.06	1.20

16 Cannabinoid Conditions		
Column	Raptor ARC-18 150 x 4.6 mm, 2.7 µm	
Diluent	Acetonitrile	
Inj. Vol.	5 µL	
MP A	Water, 4 mM ammonium formate, 0.1% formic acid	
MP B	Acetonitrile, 0.1% formic acid	
Flow	1.7 mL/min	
Detector	UV/vis @ 228 nm	
Temp	35 °C	
Gradient	Time (min)	(%) B
	0.00	77
	7.00	77



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

Results show the virtual tool surpasses performance targets, indicating it can be used to develop methods quickly and accurately with improved turnaround times, offer an on-demand consultative user experience, and provide a greener solution for method development.

Future Work

- **Library Expansions**
  - Analytes
  - Column Chemistries
  - Solvents
  - Mobile Phase Additives
- **New Libraries**
  - Pesticides
  - Mycotoxins
- **Additional Languages**
- **Parameter Expansions**
  - Guard Columns

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