RESTEK Pure Chromatography

The Development of a Virtual Liquid Chromatography Method Development Tool

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Introduction and Background

Laboratories implementing new methods or optimizing 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 with a comprehensive Drugs of Abuse library (DoA). This no-cost tool allows users to obtain optimized separations while maintaining critical pair resolution by adjusting parameters such as column dimension, mobile phase, gradients, and more.

Due to the number of dimensions in LC method development, the software build focused on six variables, with additional levers to be added at a later time.

To ensure a robust tool, focus was placed on the most commonly used variables of LC method development:

- Column Chemistries
- Column Dimensions and Lengths
- Different Organic Modifiers
- Gradients
- Temperature Changes

Build

Prior to collecting data, a lot check test was completed on three separate 50 mm x 2.1 mm Raptor Biphenyl 2.7 µm columns. Retention time data was collected using a set of nine compounds, referred to as “meld compounds”, that span the chromatographic space. These compounds were run alongside each new library collected to ensure a match to the base library. Data was tabulated in Excel and the percent difference, median, and ±% difference calculated (Table 1). With all three lots in agreement, the basis library could be created using one of columns lot check tested.

	Raptor Biphenyl 50 mm x 2.1 mm, 2.7 µm, Acetonitrile		
Column:	A	B	C
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
JWH-018	7.37	7.49	7.49
	% Diff	Median	± % Diff
trans-3-Hydroxycotinine	5.0%	0.40	2.5%
Methylephedrine	4.4%	1.37	2.0%
Diphenhydramine	1.1%	3.48	0.6%
Methaqualone	2.6%	4.25	1.3%
Phenazepam	2.3%	4.71	1.2%
Norketamine	3.4%	2.04	1.7%
Levetiracetam	7.3%	1.24	3.6%
JWH-073	2.0%	7.17	1.0%
JWH-018	1.6%	7.43	0.8%

Table1: Results of lot check testing

The basis library consisted of 50 compounds plus meld compounds. Retention times were collected using three different gradient conditions and three different temperatures.

A list of approximately 180 DoA compounds was systematically added to the database. Compounds were required to be divided into small groups to account for separation of isobars and to generate the optimal points per peak for instrument analysis, approximately 30 compounds per group including meld compounds. Retention times were collected and added to the base library.

Verification

To test the modeler, a three stage verification was completed. Each stage systematically introducing a new source of error. Once retention times were in agreement, advancement to the next stage occurred.

- **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 50 mm x 3.0 mm Raptor Biphenyl 2.7 µm column. Data was used to develop correction factors.
- **Stage 2:** Use different flow rates, temperatures, gradient slopes compared to initial library collection and build.
  - 50 mm x 2.1 mm 2.7 µm Raptor Biphenyl column, data used for modeler adjustments and corrections. Moved to the next step once retention times were in agreement.
- **Stage 3:** Use the modeler as a customer would: “User Experience”
  - 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).

Validation

To test the modeler, 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, 100 x 2.1mm
- 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:

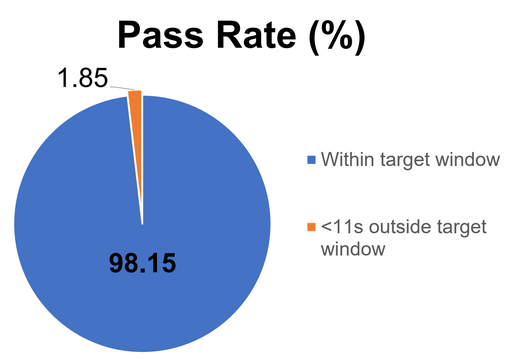
1. Retention time comparison between modeled and experimental runs cannot exceed more than 50% of a standard MRM window (±15 seconds)
2. Data is easily normalized from column-to-column variability and different instrument platforms.

Validation Results

Variables Assessed:

- Two different column dimensions
- Two different column lengths
- Two different mobile phases
- Two different stationary phases
- Three different gradients
- Three different temperatures

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

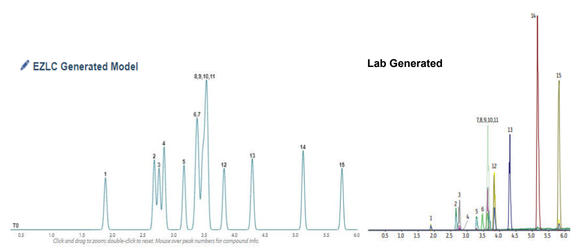


To ensure the modeler performed as expected a set of compounds were chosen to model and test in the lab. Results of the modeled and empirical data show very similar retention times with methamphetamine and phentermine showing improved resolution during empirical conditions (Table 2).

Evaluation and Conclusion

Column	Raptor Biphenyl (cat. #9309A12)		
Dimensions:	100 mm x 2.1 mm ID		
Particle Size:	2.7 µm		
Temp.:	30 °C		
Standard/Sample			
Diluent:	Water		
Conc.:	100 ng/ mL		
Inj. Vol.:	1 µL		
Detector:	MS/MS		
Ion Mode:	ESI^+		
	MRM		
Mobile Phase			
A:	Water, 0.1% formic acid		
B:	Methanol, 0.1% formic acid		
Time (min)	Flow (mL/min)	%A	%B
0.00	0.8	96	4
7.40	0.8	8	92
7.41	0.8	96	4
9.50	0.8	96	4

Table 2: Conditions for evaluation



Peak #	Compound	Experiment t_R (min)	Modeler t_R (min)	Diff. (sec)
1	**Normorphine**	1.89	1.88	0.60
2	**Morphine**	2.66	2.68	1.20
3	**Oxymorphone**	2.77	2.75	1.20
4	**Morphine-N-oxide**	2.88	2.84	2.40
5	**Norcodeine**	3.29	3.16	7.80
6	**Methamphetamine**	3.47	3.36	6.60
7	**Phentermine**	3.62	3.39	13.8
8	**Dihydrocodeine**	3.62	3.47	9.00
9	**Noroxycodone**	3.62	3.51	6.60
10	**O-Desmethyl-*cis*-tramadol**	3.64	3.54	6.00
11	**Codeine**	3.68	3.54	8.40
12	**Desomorphine**	3.84	3.82	1.20
13	**N-Desmethyltapentadol**	4.31	4.28	1.80
14	**Pentazocine**	5.16	5.11	3.00
15	**Dextromethorphan**	5.82	5.75	4.20

Table 3: Results of empirical vs modeled data

This no-cost virtual method tool is easy to use for LC method developers, both novice and expert. Those who lack the expertise or the time to development separations quickly and accurately can improve turnaround time and increase throughput of existing methods.

Newly Released Features

Additional Column Dimension:

- 30 x 2.1 mm, 30 x 3.0 mm, 150 x 2.1 mm, 150 x 3.0 mm

Superficially porous particle (SPP) sizes:

- 4.6 µm and 1.8 µm

Expanded DoA library - Novel Psychoactive Substances (NPS) drugs

Future Work

Updates set for release in 2023:

Fully porous particles (FPP)

Cannabinoid Library

- UV detection

Additional Libraries

Multiple Languages

Try it out!

For more information contact Cyrille Lamboley
cyrille.lamboley@restek.com - EMEA LC Specialist

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