Using a Virtual Chromatography Tool to Develop Methods for Novel Psychoactive Substances

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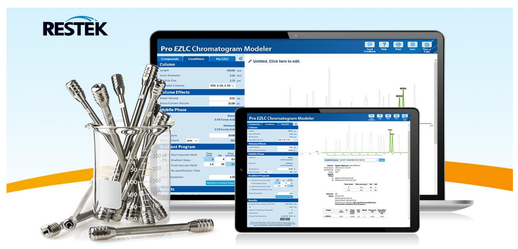
Disclosure: I have (or a member of my immediate family has) a financial relationship with a company as defined in the AACC policy on potential bias or conflict of interest

Introduction

Novel psychoactive substances (NPS) have created a challenge for toxicology laboratories. New NPS are constantly disappearing as fast as they emerge, making it difficult to stay on top of which compounds are necessary to add to laboratory testing scopes.

The development and optimization of liquid chromatography (LC) separations is time consuming and costly. To alleviate the burden of sacrificing instrument-uptime, labor and materials, an instrument-free software modeling tool was developed to include a comprehensive drugs of abuse (DoA) library. With this tool, users can obtain optimized separations while maintaining critical pair resolution by adjusting parameters such as column dimension, mobile phase, gradient programs, and more for almost 300 compounds including the 38 newly added NPS drugs.



NPS Library Build

The NPS library utilized the same design space as the existing DoA library. Retention times were collected using the following method conditions:

- Gradients: Fast (5 minute) and slow (15 minute)
- Column temperature: 30°C and 60°C
- Mobile Phase: ACN and MeOH
- Stationary Phases: Raptor Biphenyl and Raptor C18 (50 x 2.1 mm, 2.7 µm)

The 38 NPS compounds were divided into three small groups to account for the separation of isobars to generate optimal points per peak for instrument analysis. A set of 8 compounds, referred to as “meld compounds”, were then added to each group. These meld compounds spanned the chromatographic space and were used to verify instrument performance from injection to injection. Data was collected and input into the platform.

Results of retention times between experimental and modeled data were compared. To be considered passing, modeled and experimental retention time could not exceed more than 50% of a typical MRM window (±15 seconds). All analytes passed criteria.

Development of LC-MS/MS Methods for NPS

Three methods were developed for the following classes of NPS using the virtual chromatography modeler: 1) synthetic opioids and toxic adulterants, 2) synthetic cathinones and synthetic cannabinoids, 3) designer benzodiazepines.

The generated methods were transferred to a Shimadzu 8045 LC-MS. The modeled chromatography gradients are shown below. Conditions such as column chemistry and dimension, column temperature, and mobile phases were the same for all methods.

Synthetic Opioids and Toxic Adulterants Gradient		
Time (min)	**%A**	**%B**
0.00	60	40
5.00	13	87
6.00	13	87
6.01	60	40
8.20	60	40

Designer Benzodiazepines Gradient		
Time (min)	**%A**	**%B**
0.00	55	45
8.00	25	75
9.00	25	75
9.01	55	45
11.00	55	45

Synthetic Cathinones and Cannabinoids Gradient		
Time (min)	**%A**	**%B**
0.00	85	15
8.00	49	51
12.00	0	100
13.00	0	100
13.01	85	15
15.80	85	15

Chromatography Conditions For All Methods	
Column	Raptor Biphenyl, 100 x 2.1 mm, 2.7 µm
Column Temperature	40°C
Mobile Phase A	0.1% formic acid in water
Mobile Phase B	0.1% formic acid in methanol
Flow Rate	0.6 mL/min
Diluent	0.1% formic acid in water (MPA)
Injection Volume	5 µL
Sample Concentration	500 ng/mL

Synthetic Opioids and Toxic Adulterants

Figure 1. Modeler Predicted Chromatogram

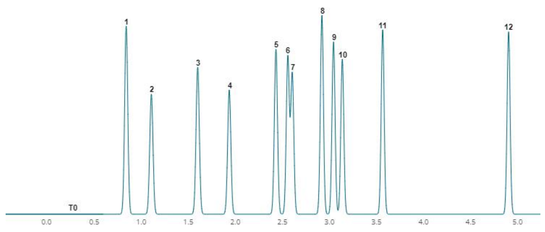
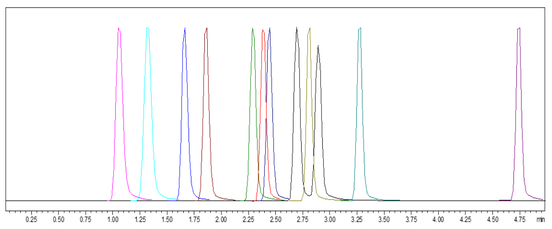


Figure 2. Experimental Chromatogram



Retention Time Comparison				
Peak	***Analyte***	***Predicted RT (min)***	***Experimental RT (min)***	***Difference (sec)***
1	4’-Hydroxy Nitazene	0.84	1.05	12.6
2	Xylazine	1.11	1.32	12.6
3	2-methyl AP-237	1.60	1.63	1.8
4	N-pyrrolidino metonitazene	1.93	1.87	3.6
5	N-pyrrolidino etonitazene	2.43	2.28	9.0
6	para-Fluorofentanyl	2.56	2.39	10.2
7	N-Desethyl isotonitazene	2.60	2.44	9.6
8	Isotonitazene	2.92	2.70	13.2
9	N-pyrrolidino protonitazene	3.04	2.80	14.4
10	Protonitazene	3.13	2.90	13.8
11	*iso*-Butonitazene	3.56	3.31	15.0
12	Phenylbutazone	4.90	4.75	9.0

Designer Benzodiazepines

Figure 3. Modeler Predicted Chromatogram

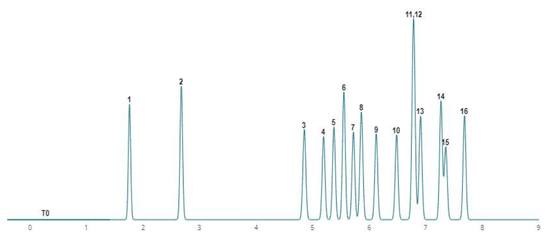
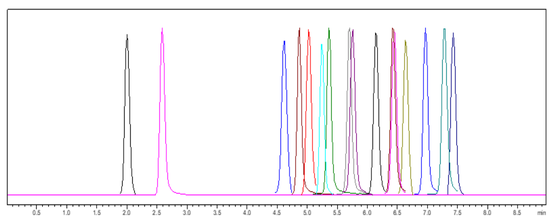


Figure 4. Experimental Chromatogram



Retention Time Comparison				
Peak	***Analyte***	***Predicted RT (min)***	***Experimental RT (min)***	***Difference (sec)***
1	Adinazolam	1.73	1.98	15.0
2	8-Aminoclonazolam	2.63	2.59	2.4
3	Pyrazolam	4.78	4.62	9.6
4	Desalkylflurazepam	5.12	4.87	15.0
5	Clonazolam	5.30	5.05	15.0
6	Flubromazepam	5.47	5.26	12.6
7	Delorazepam	5.64	5.39	15.0
8	Desalkylgidazepam	5.79	5.71	4.8
9	Flualprazolam	6.05	5.80	15.0
10	Flubromazolam	6.41	6.16	15.0
11	4'-chloro deschloroalprazolam	6.70	6.46	14.4
12	Phenazolam	6.71	6.46	15.0
13	Bromazolam	6.83	6.64	11.4
14	Diclazepam	7.19	6.97	13.2
15	Deschloroetizolam	7.27	7.29	1.2
16	Etizolam	7.60	7.45	9.0

Synthetic Cathinones and Synthetic Cannabinoids

Figure 5. Modeler Predicted Chromatogram

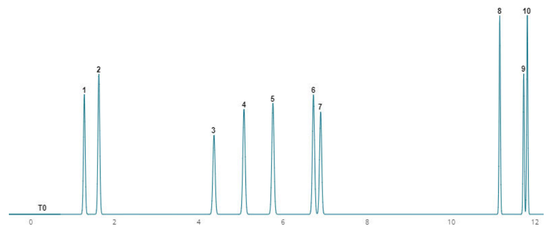
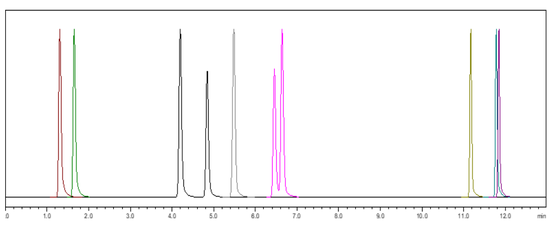


Figure 6. Experimental Chromatogram



Retention Time Comparison				
Peak	***Analyte***	***Predicted RT (min)***	***Experimental RT (min)***	***Difference (sec)***
1	Cathinone	1.27	1.37	6.0
2	Methcathinone	1.62	1.71	5.4
3	Eutylone	4.35	4.20	9.0
4	Pentylone	5.07	4.86	12.6
5	N-Ethyl Pentylone	5.76	5.51	15.0
6	α-PiHP	6.72	6.47	15.0
7	α-PHP	6.89	6.65	14.4
8	ADB-BINACA	11.15	11.17	1.2
9	MDMB-4en-PINACA	11.72	11.81	5.4
10	CH-PIATA	11.80	11.85	3.0

Discussion

Results

The modeler predicted and experimental retention times were compared for each method. All methods maintained elution order and resolution when transferred to the instrument. For the Synthetic Opioids and Toxic Adulterants method, there was an average difference of 10.4 seconds between the modeled and experimental retention times. For the Synthetic Cathinones and Synthetic Cannabinoids method, there was an average difference of 11.4 seconds. For the Designer Benzodiazepines method, there was an average difference of 8.7 seconds. Based on the acceptance criteria of ±15 seconds, each NPS method was successfully transferred from the virtual model to an LC-MS/MS instrument.

Advantages of Virtual Method Development

1. Time: Steps like column selection, method scouting, and gradient optimization can be time consuming. Performing these steps using the virtual modeler can significantly reduce the time required to develop a new methods. The three methods depicted here were developed using the modeler in under ten minutes per method.
2. Cost: Method development can quickly use up consumables and solvents. Because developing methods using the virtual chromatography tool require no hands-on instrument time, solvents and consumable use is significantly decreased.
3. Instrument Up-Time: Optimizing LC-MS/MS methods can require hours to days of time on an instrument. By virtually developing methods, instruments are freed up for other use.

In-Lab Method Development

- Confirm instrument availability.
- Select compounds and prepare standard solutions.
- Use compound infusion to establish mode and ion transitions.
- Run experiments to select column.
  - Stationary phase
  - Dimensions
- Run experiments to select mobile phases.
  - Organic solvent
  - Buffers
  - Additives
- Run experiments to optimize chromatographic conditions.
  - Flow rate
  - Gradient
  - Temperature

Pro EZLC Method Development

- Select analytes and critical pairs from list.
- Choose a column phase to try.
- Click “Generate Model”.
- Use the model in lab or optimize further to meet specific goals.

Conclusion and Future Directions

This no-cost virtual chromatography tool is easy to use for LC method developers, both novice and expert. Those who lack the expertise, or the time can develop separations quickly and accurately, improving turnaround time and increasing throughout of existing methods. This is especially pertinent when it comes to keeping up with the ever-changing landscape of NPS compounds. The development of these three methods shows that the tool can be used to effectively optimize LC-MS/MS methods for NPS. As NPS continue to evolve, the modeler will be routinely updated to include newly emerging compounds.

Try it out!

References

“Accelerate LC-MS/MS Method Development Using the Pro EZLC Chromatogram Modeler”. https://www.restek.com/en/articles/accelerate-lc-msms-method-development-using-the-pro-ezlc-chromatogram-modeler (accessed September 23rd, 2023).

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