RESTEK Pure Chromatography

Method Development of PFAS Compounds Using a Virtual Method Development Tool

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Introduction

Per- and polyfluoroalkyl substances (PFAS) are a class of compounds used to make fluoropolymer coatings and products. Often referred to as “forever chemicals”, they are applied to surfaces for heat, oil, stain, grease, and water resistance. PFAS do not break down in the environment, can move through soil to contaminate drinking water, and can accumulate in fish and wildlife. Recent studies have also shown adverse health effects on humans. According to the infrastructure bill and a recent public release from the Biden/Harris Administration, the EPA has approximately $9 billion allocated to PFAS testing. The understanding of PFAS toxicity and bioaccumulation requires advancements in testing methodologies to enhance sensitivity, specificity, and efficiency for detection and quantification of not only current compounds, but also emerging ones. PFAS testing is poised to be an environmental and public health concern and monitoring will continue for the foreseeable future.

In this work, methods from organizations were selected for evaluation. Using the modeler tool, methods were developed virtually, tested experimentally, and retention times were compared to the results predicted by the modeler.

Background PFAS Library Build

Prior to collecting data, a lot check test was completed on three separate 50 x 2.1 mm Raptor C18 2.7 µm columns and three separate 50 x 2.1 Force C18 3 µm columns. Retention time data was collected using a designated set of eight compounds that span the chromatographic space. Data was tabulated in Excel and the percent difference, median, and ±% difference calculated. With all lots in agreement, the base library was created using one of columns tested.

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

- Gradients: Fast (5 minute) and Slow (15 minutes)
- Column Temperature: 30 ºC and 60 ºC
- Mobile Phases: Methanol and Acetonitrile
- Stationary Phases: Raptor C18 (50 x 2.1 mm, 2.7 µm) and Force C18 (50 x 2.1, 3.0 µm)

To build a base library, 28 PFAS compounds were selected. A subset of eight PFAS 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 57 PFAS compounds and three bile acids. Data collected from 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 using the following conditions:

- Stationary phases: Raptor C18 2.7 µm and Force C18 3µm
- Column Dimensions: 50 x 2.1 mm , 50 x 3.0 mm and 100 x 2.1 mm
- Temperature: 40 ºC (both 50 x 2.1 mm also analyzed at 35 ºC and 50 ºC)
- Mobile Phases: Acetonitrile, Methanol, and Acetonitrile:Methanol (50:50)
- Gradients: Linear, Isocratic hold, and Multi-step

Performance Targets for Data Collection: 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.

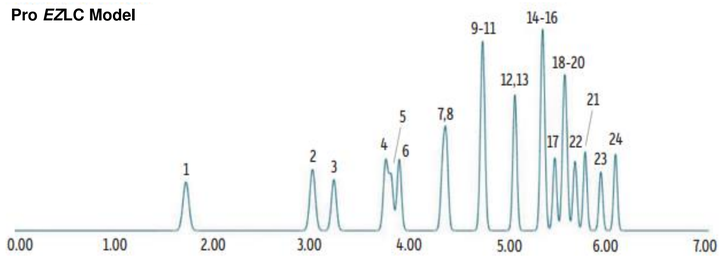
Experiments and Results

Published methods from the International Organization for Standards (ISO) and the Environmental Protection Agency (EPA) were selected for evaluation. Using the modeler tool, methods were developed virtually, transferred to the instrument, tested experimentally, and retention times compared.

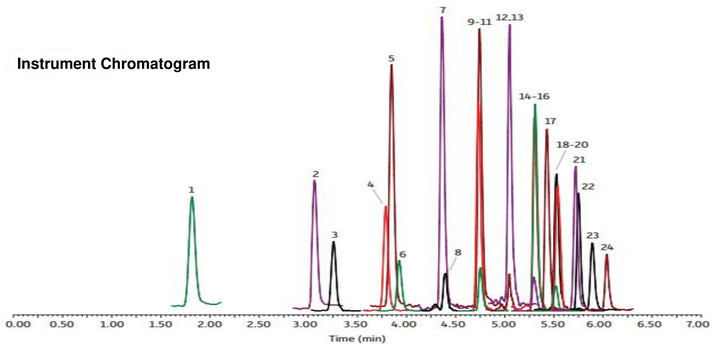
EPA 8327 Method Conditions			
Column:	Force C18, 50 mm x 2.1 mm, 1.8 µm		
Temp:	40 ºC		
Diluent:	Methanol		
Detector:	MS/MS		
MP A:	Water, 5 mM ammonium acetate		
MPB:	Methanol		
Conditions:	Time (min)	Flow (mL/min)	% B
	0.00	0.4	20
	6.00	0.4	95
	6.01	0.4	20
	8.00	0.4	20

Analyte	Experimental t_r (min)	Modeler t_r (min)	Difference (sec)
PFBA	1.82	1.74	4.80
PFPeA	3.07	3.01	3.60
PFBS	3.25	3.23	1.20
4:2 FTS	3.79	3.75	2.40
PFHxA	3.84	3.80	2.40
PFPes	3.92	3.89	1.80
PFHpA	4.36	4.32	2.40
PFHxS	4.40	4.36	2.40
6:2 FTS	4.72	4.72	0.00
PFOA	4.75	4.72	1.80
PFHpS	4.75	4.73	1.20
PFNA	5.05	5.05	0.00
PFOS	5.05	5.05	0.00
8:2 FTS	5.29	5.34	3.00
PFNS	5.30	5.32	1.20
PFDA	5.30	5.32	1.20
NMeFOSAA	5.43	5.45	1.20
PFUnA	5.52	5.54	1.20
PFDS	5.53	5.55	1.20
NEtFOSAA	5.54	5.57	1.80
PFDoA	5.71	5.76	3.00
FOSA	5.75	5.65	6.00
PFTriA	5.89	5.91	1.20
PFTreA	6.04	6.06	1.20

Pro *EZ*LC Model



Instrument Chromatogram



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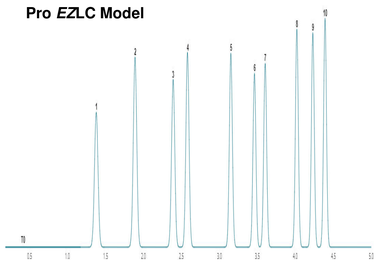

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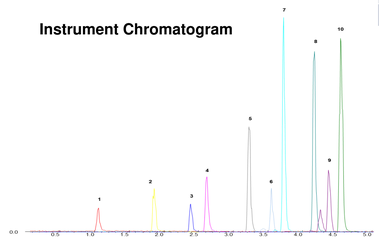


DIN 38407-42 Method Conditions			
Column:	Raptor C18, 100 mm x 2.1 mm, 1.8 µm		
Temp:	35 ºC		
Diluent:	Methanol		
Detector:	LC-MS/MS		
MP A:	Water, 5 mM ammonium acetate		
MP B:	Methanol:Acetonitrile (50:50)		
Conditions:	Time (min)	Flow (mL/min)	%B
	0.00	0.5	30
	5.00	0.5	90
	5.01	0.5	30
	7.00	0.5	30

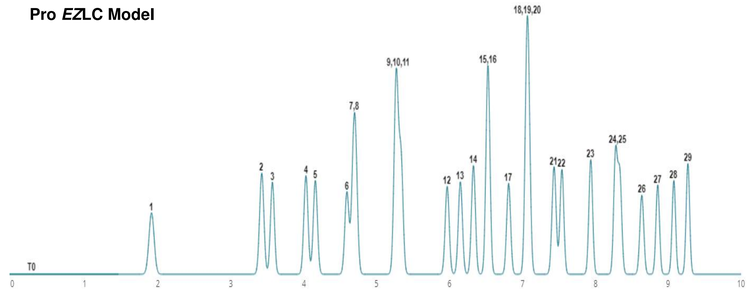
Analyte	Experimental t_r (min)	Modeler t_r (min)	Difference (sec)	% Run Time
PFBA	0.99	1.38	23.4	7.8
PFPeA	1.79	1.89	6.0	2.0
PFBS	2.31	2.39	4.8	1.6
PFHxA	2.55	2.58	1.8	0.6
PFHpA	3.15	3.15	0.0	0.0
PFHxS	3.48	3.46	1.2	0.4
PFOA	3.65	3.60	3.0	1.0
PFNA	4.08	4.02	3.6	1.2
PFOS	4.30	4.23	4.2	1.4
PFDA	4.47	4.39	4.8	1.6

Results Continued

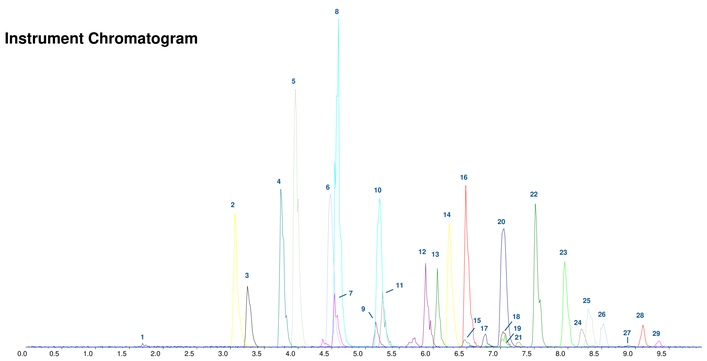
ISO 21675:2019 Method Conditions			
Column:	Raptor C18, 50 mm x 2.1 mm , 1.8 µm		
Temp:	30 ºC		
Diluent:	Methanol		
Detector:	LC-MS/MS		
MP A:	Water, 5 mM ammonium acetate		
MP B:	Methanol		
Conditions:	Time (min)	Flow (mL/min)	%B
	0.00	0.3	10
	2.00	0.3	50
	10.00	0.3	100
	14.00	0.3	100
	14.01	0.3	10
	17.00	0.3	10

Analyte	Experimental t_r (min)	Modeler t_r (min)	Difference (sec)	% Run Time
PFBA	1.7	1.91	12.6	4.2
PFPeA	3.07	3.43	21.6	7.2
PFBS	3.25	3.57	19.2	6.4
PFHxA	3.75	4.03	16.8	5.6
HFPO-DA	3.96	4.16	12	4.0
PFHpA	4.48	4.59	6.6	2.2
PFHxS	4.54	4.68	8.4	2.8
ADONA	4.6	4.71	6.6	2.2
6:2 FTS	5.15	5.26	6.6	2.2
PFOA	5.22	5.28	3.6	1.2
PFHpS	5.25	5.34	5.4	1.8
PFOS	5.89	5.97	4.8	1.6
8:2 FTUCA	6.07	6.15	4.8	1.6
9Cl-PF3ONS	6.24	6.33	5.4	1.8
8:2 FTS	6.47	6.53	3.6	1.2
PFDA	6.49	6.53	2.4	0.8
NMeFOSAA	6.78	6.81	1.8	0.6
PFUnA	7.06	7.06	0	0.0
NEtFOSAA	7.06	7.06	0	0.0
PFDS	7.03	7.09	3.6	1.2
FOSA	7.27	7.43	9.6	3.2
PFDoA	7.52	7.54	1.2	0.4
PFTrDA	7.95	7.94	0.6	0.2
NMeFOSA	8.21	8.28	4.2	1.4
PFTeDA	8.36	8.34	1.2	0.4
NEtFOSA	8.53	8.64	6.6	2.2
PFHxDA	8.89	8.86	1.8	0.6
8:2 diPAP	9.11	9.08	1.8	0.6
PFODA	9.35	9.27	4.8	1.6

Pro *EZ*LC Model

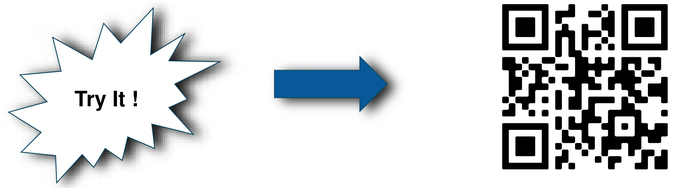


Instrument Chromatogram



Conclusions

Results show this virtual tool can be used to develop PFAS methods quickly and accurately. Performance targets, ± 15 seconds or %10 of the analytical run time, were achieved using three test methods. This indicates the virtual tool can improve turnaround time, optimize existing methods for the addition of new PFAS compounds, offer on-demand consultative user experience, and a greener solution for method development.



References

The United States Government. (2024, April 9). *Fact sheet: Biden-Harris Administration takes critical action to protect communities from pfas pollution in drinking water.* The White House. https://www.whitehouse.gov/briefing-room/statements-releases/2024/04/10/fact-sheet-biden-harris-administration-takes-critical-action-to-protect-communities-from-pfas-pollution-in-drinking-water/