RESTEK Pure Chromatography

Visit us at Booth 14!

Towards greener workflows with clever method development

S. K. Ruiz Perez[a], E. Ceccon[b], F. Rodriguez Carrero[c], D. Bell[d], P. Conolly[d];

[a] Restek GmbH, Bad Homburg, DE; [b] Restek Italia, Cernusco sul Naviglio, IT , [c] Restek S.r.l. Sucursal en España, Madrid, ES [d] Restek Corporation, Bellefone, PA, USA

Introduction

Greener workflows can be achieved through energy-efficient practices, reducing or eliminating organic and toxic solvents, minimizing sample preparation, and using multi-method approaches. Many of these improvements can be implemented **without additional costs.**

Multi-methods enable analysis of more components in a single run, saving instrument time and reducing solvent waste and energy consumption. However, the growing range of analytes, especially **polar compounds**, increases demands on the stationary phase. The traditional C18 phase is reaching its limits, making versatile alternatives essential.

A **Biphenyl phase** offers greater flexibility with multiple retention mechanisms and **100% water** compatibility.

Free virtual tools like the ***Pro EZLC Chromatogram Modeler*** and *Method Translator* further reduce lab time, energy use, and instrument wear by enabling method development without lab work.

Versatile Biphenyl Phase for multi-method development

The **versatile Biphenyl** phase can undergo different separation mechanisms (Figure 1):

- π-π interactions for
  - aromatic analytes (polar and nonpolar)
  - Condensed aromatics with e⁻-withdrawing groups
  - Unsaturated and conjugated analytes
  - Dipoles
  - Lewis acids
- hydrophobic interactions (C18 like) for
  - nonpolar analytes

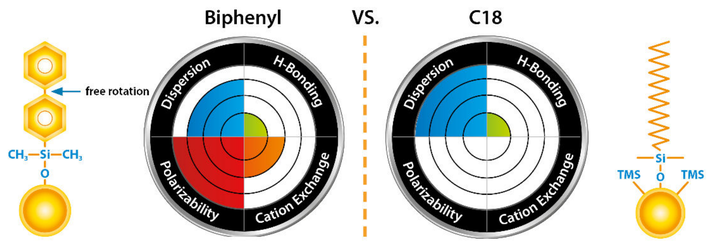


Figure 1 – The Biphenyl phase provides a wider range of interaction mechanisms with the analyte than a C18 phase.

This duality in separation mechanism and the 100% water compatibility makes it particularly useful for **isomeric isobars, polar compounds, MS-detection.**

The **retention mechanism** can be **tuned** by different mobile phases (Figure 2). Due to its triple bond, acetonitrile suppresses π-π interactions of the stationary phase with the analytes. With methanol more retention mechanism can be accessed.

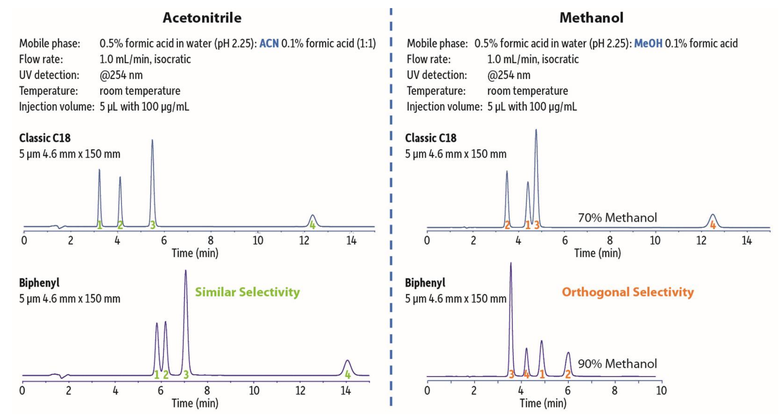


Figure 2 – Choice of organic modifier can be used to alter selectivity and elution order.

Examples for Multi-Methods

The Biphenyl phase is ideal for green method development, offering broad analyte coverage in **a single column**—eliminating the need for multiple C18 modifications (e.g., polar embedded, sterically hindered, polymeric)—for a simpler, more sustainable approach.

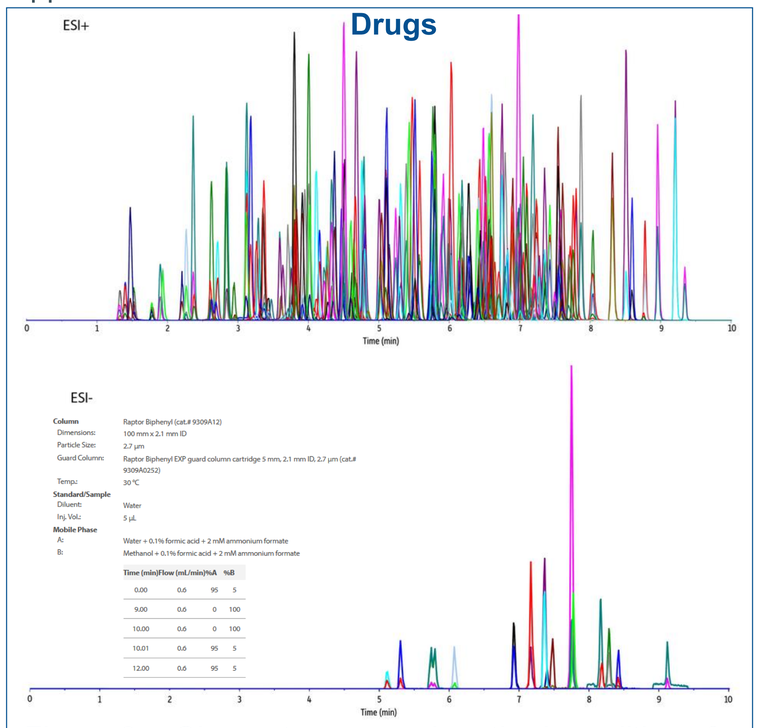


*Figure 3 – Chromatogram and method conditions for our Multiclass Drug Panel with **231 drugs** resolved in < 13 minutes. The Biphenyl phase is especially suited for the separation of isomeric isobars on Raptor Biphenyl by LC-MS/MS.*

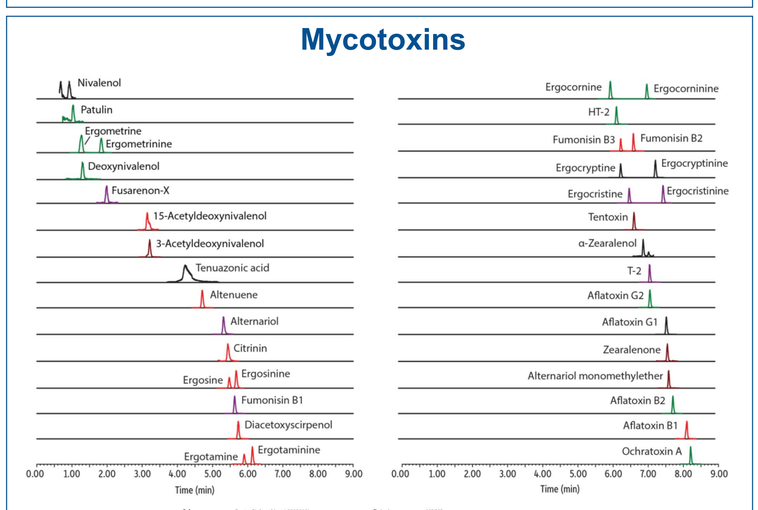
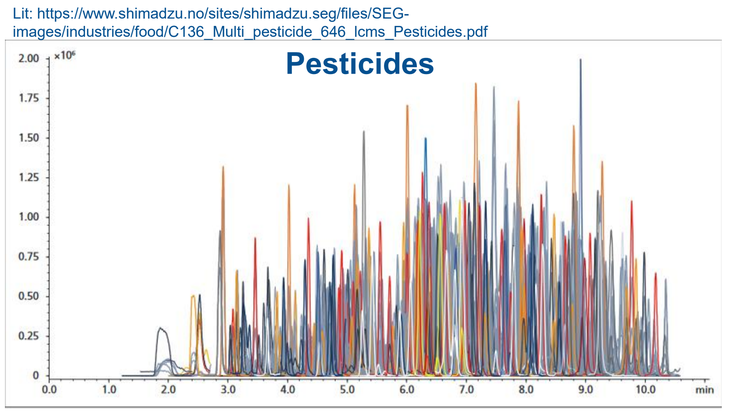


*Figure 4 – Chromatogram and method conditions for our **Ergot alkaloids, Alternaria toxins and other major Mycotoxins** in fortified flour sample on Raptor Biphenyl by LC-MS/MS.*

Lit: https://www.shimadzu.no/sites/shimadzu.seg/files/SEG-images/industries/food/C136_Multi_pesticide_646_lcms_Pesticides.pdf



Liquid chromatography		Mass spectrometry	
UHPLC	Nexera LC system	LC/MS/MS	LCMS-8060
Analytical column	Restek Raptor Biphenyl (2.1 mm I.D. x 100 mm L., 2.7 µm)	Ionisation mode	Heated electrospray
Column temperature	35 °C	Polarity switching time	5 msec
Flow rate	0.4 mL/min	Pause time	1 msec
Solvent A	2 mmol/L ammonium formate + 0.002 % formic acid - Water	Total MRM transitions	1,919 (1,819 positive; 100 negative)
Solvent B	2 mmol/L ammonium formate + 0.002 % formic acid - Methanol	MRM Dwell	4 msec (target ion); 1 msec (reference ion)
Binary Gradient B.Conc.	3 % (0 min) - 10 % (1.00 min) - 55 % (3.00 min) - 100 % (10.50 - 12.00 min) - 3 % (12.01 - 15.00 min)	Interface temperature	350 °C
Injection volume	2 µL sample (plus 40 µL water)	Heating block	300 °C
		Desolvation line	150 °C
		Heating gas	10 L/min
		Drying gas	10 L/min
		Nebulizer gas	3 L/min

*Figure 5 – Chromatogram and method conditions for multiresidue pesticides analysis with **646 pesticides** developed by **Shimadzu** on a Raptor Biphenyl using Shimadzu LC-MS 8060.*

Virtual Method Development

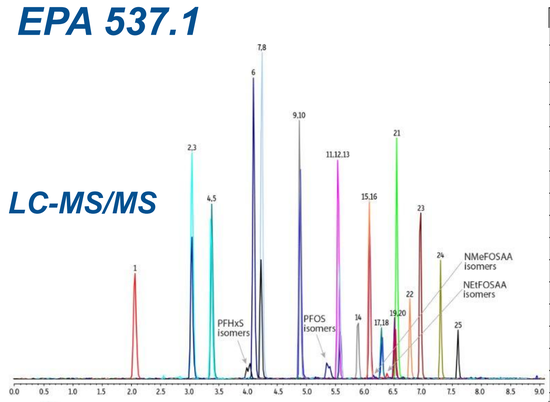
A **no-cost virtual method development** tool was developed and makes the sacrifice of valuable instrument uptime for method development obsolete. The so-called "EZLC Chromatogram Modeler" contains a comprehensive library with **311 DoA, 60 PFAS, and 16 nitrosamines, 58 cannabinoids and 243 pesticides**. This tool allows users to obtain optimized separations while maintaining critical pair resolution by adjusting parameters such as column dimension, mobile phase, gradients, and more **without setting foot into the lab.**

Figure 6 – From left to right: 1. Search your compounds in the libraries and see the isobars of your method, 2. adapt the conditions to your equipment and methods, 3. save your method development for futures alterations, 4. See if your critical isobars are separated or not.

Model *vs.* Reality

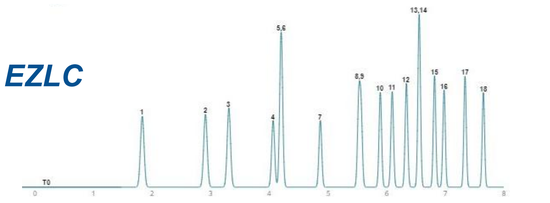
EPA 537.1

LC-MS/MS



Compound	LC_EV0560	Modeled Result	
Name	RT (min)	RT (min)	Diff (min)
PFBS	2.06	1.84	-0.22
PFHxA	3.04	2.91	-0.13
HFPO-DA	3.38	3.31	-0.07
PFHpA	4.09	4.07	-0.02
PFHxS	4.22	4.20	-0.02
ADONA	4.24	4.21	-0.03
PFOA	4.90	4.87	-0.03
PFNA	5.54	5.53	-0.01
PFOS	5.58	5.57	-0.01
9Cl-PF3ONS	5.88	5.89	0.01
PFDA	6.08	6.10	0.02
NMeFOSAA	6.30	6.34	0.04
NEtFOSAA	6.52	6.56	0.04
PFUnA	6.55	6.56	0.01
11Cl-PF3OUdS	6.77	6.82	0.05
PFDoA	6.95	6.98	0.03
PFTrDA	7.30	7.34	0.04
PFTeDA	7.60	7.65	0.05
	Average Difference (min):		-0.014

EZLC



Peak Heights are not representative

Conclusion

Greener workflows can be implemented in any lab with minimal effort and cost. Free method development tools allow you to optimize methods virtually, saving instrument time, energy, and solvent waste. Analyzing more components in less time with a single method (multi-method) increases both efficiency and sustainability. Choosing the right stationary phase is key—columns with multiple retention mechanisms, like the Biphenyl phase, offer greater versatility and greener performance.

Contact: Fernando.R.Carrero@restek.com

www.restek.com