RESTEK Pure Chromatography

Towards greener workflows with clever method development

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

[a] Restek GmbH, Bad Homburg, D; [b] Restek Italia, Cernusco sul Naviglio, IT , [c] Restek Corporation, Bellefone, PA, USA

Introduction

Greener workflows can be accomplished by various parameters: working more energy efficiently, using less or no organic and toxic solvents, reducing sample treatment, or using multi-methods.

Several of these approaches may be easily implemented in each lab without extra costs. In a multi-method more components can be analyzed in one run, which not only saves valuable instrument uptime, but also minimizes organic waste and energy costs. One problem is, that the scope of analytes is always growing, and polar analytes come to the fore. Consequently, the requirements for the stationary phase increase. The ubiquitous C18 is increasingly reaching its limits and more versatile alternatives are needed. A Biphenyl phase can be advantageous because its multiple separation mechanisms and 100% water compatibility make it much more versatile than a C18 phase.

Such method developments can be accomplished using no-cost virtual method development tools called “Pro EZLC Chromatogram Modeler” and “Pro EZLC Method Translator”. Both utilities save time, energy, and instrument uptime as you can develop or adapt your method without setting foot in the lab.

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

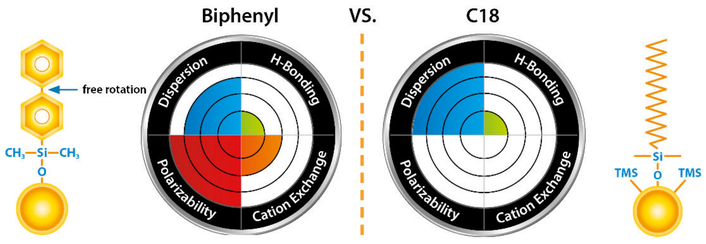


Figure 1 – The Biphenyl phase provides a wider range of interaction mechanisms with the analyte than a C18 phase.

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.

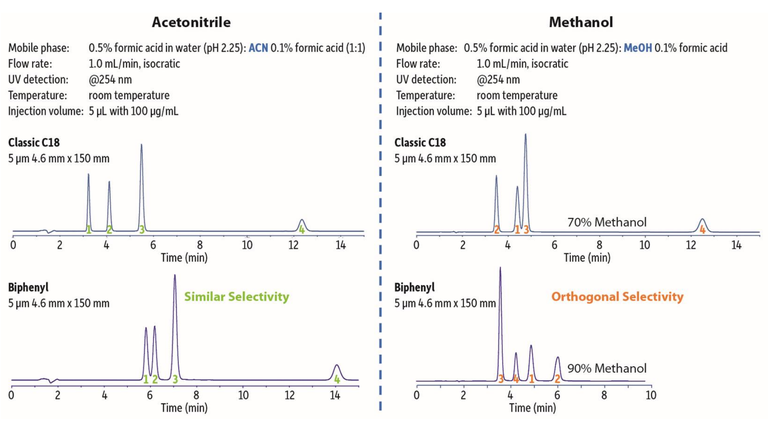


Figure 2 – Choice of organic modifier can be used to alter selectivity and elution order.

Examples for Multi-Methods

The Biphenyl phase is the perfect phase for green method development as this **one column** can separate **many** very broad **panels** that would otherwise require many different modifications of a C18 phase (polar embedded, sterically hindered, polymeric…) – greener approach.

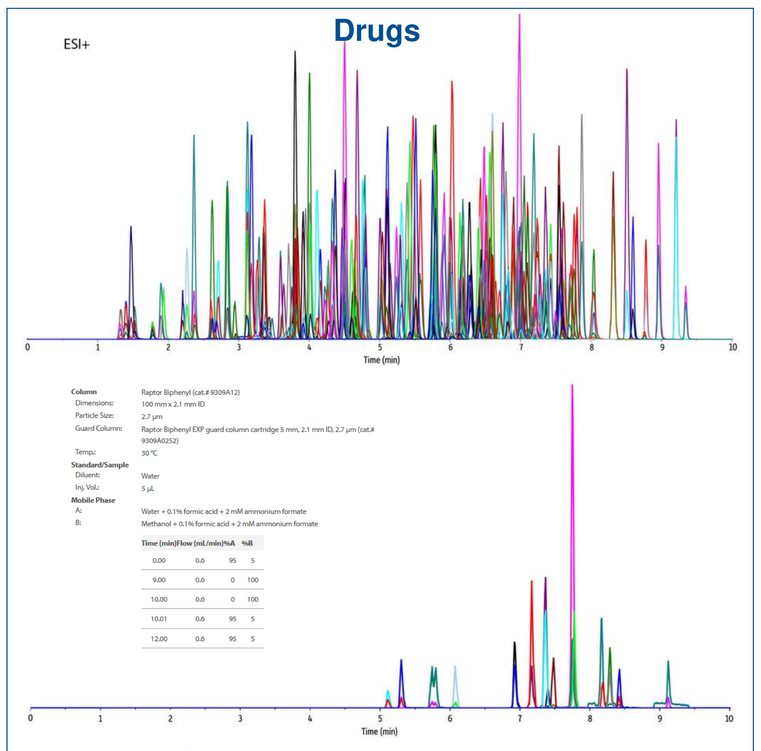


*Figure 3 – Chromatogram and method conditions for our Multiclass Drug Panel with **231 drugs** resolved in < 13 minutes. The Biphenyl phase is especially suited for the separation of isomeric isobars on Raptor Biphenyl by LC-MS/MS.*

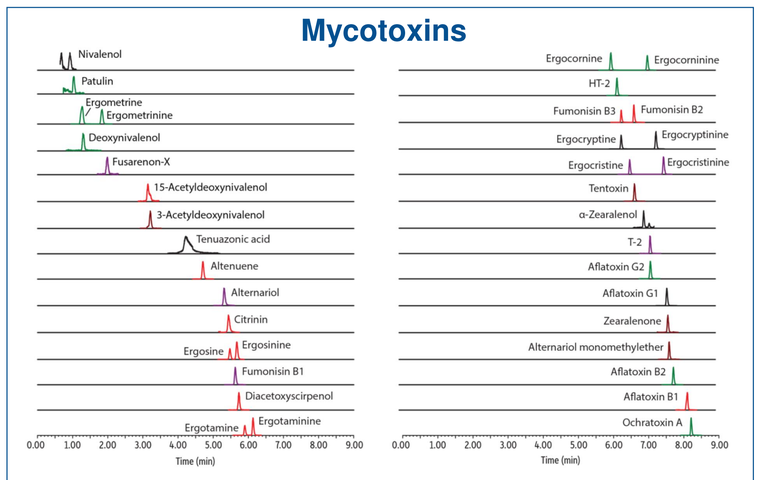
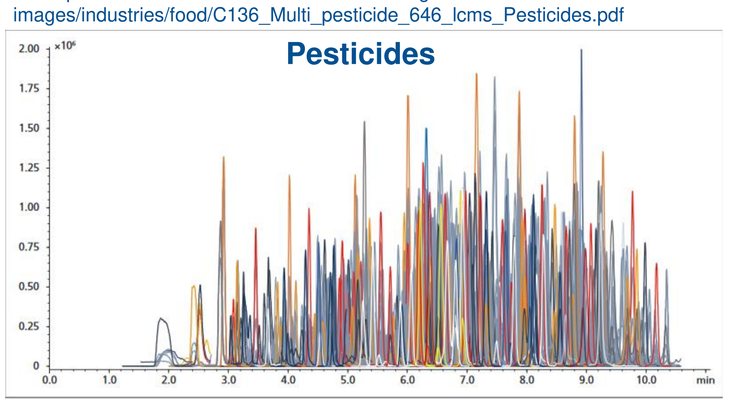


*Figure 4 – Chromatogram and method conditions for our **Ergot alkaloids, Alternaria toxins and other major Mycotoxins** in fortified flour sample on Raptor Biphenyl by LC-MS/MS.*

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
		Heating block	300 °C
		Desolvation line	150 °C
		Heating gas	10 L/min
		Drying gas	10 L/min
Injection volume	2 µL sample (plus 40 µL water)	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 291 drugs of abuse and 16 nitrosamines. 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.

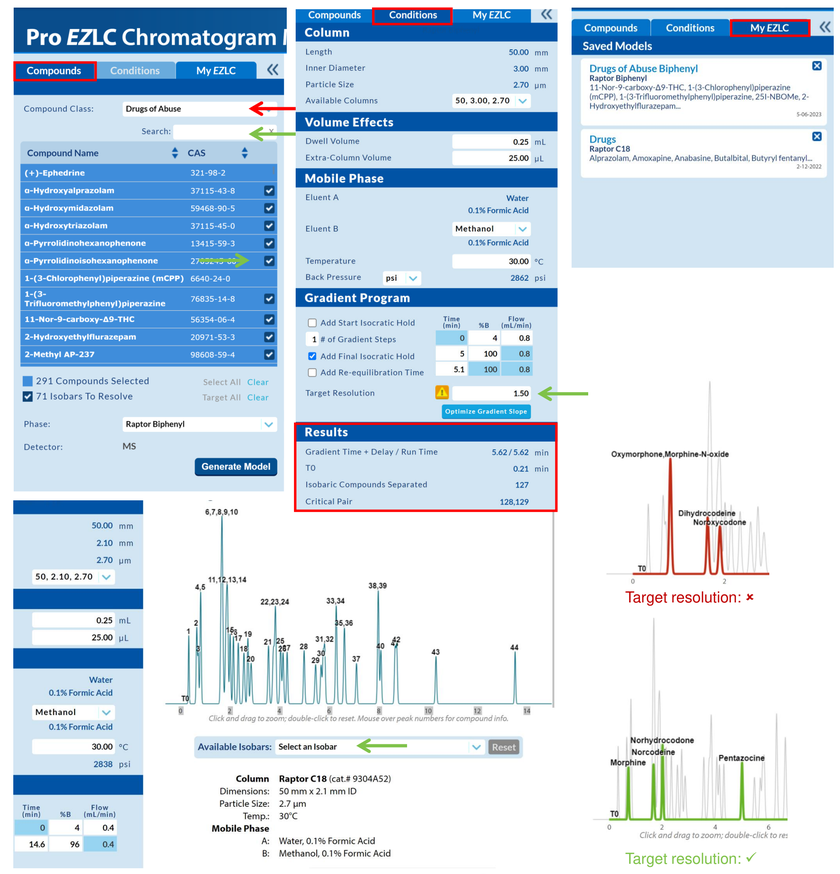
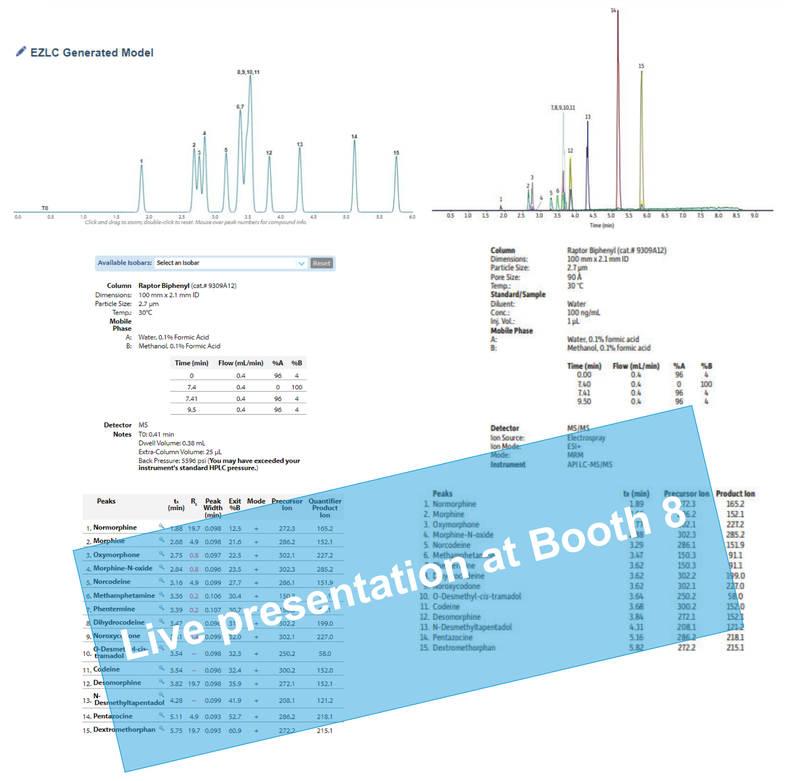


Figure 6 – From left to right: 1. Search your compounds in the libraries and see the isobars of your method, 2. adapt the conditions to your equipment and methods, 3. save your method development for futures alterations, 4. See if your critical isobars are separated or not.

Model *vs.* Reality



Conclusion

Greener workflows can be accomplished in each and every lab with low effort and minimal costs. With no-cost method development tools, your method development can be done without setting foot in the lab saving valuable instrument uptime, energy costs and organic waste. If you manage to analyze as many components as possible in least time in one method (multi-method), your method is more cost-effective and environmentally-friendly. Here for, the right choice of stationary phase is essential - the more retention mechanisms can be accessed, the more versatile the column gets. The Biphenyl stationary phase is a good example for such a column.