

Ultra Selective Liquid Chromatography™





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## Ultra Selective Liquid Chromatography™ Technology

Choose Columns Fast. Develop Methods Faster.

What is Ultra Selective Liquid Chromatography<sup>TM</sup> (USLC<sup>TM</sup>) technology? This technique is the directed application of orthogonal selectivity—the most influential factor affecting peak separation, or resolution—to provide the practicing chromatographer with the best tools for choosing columns fast and developing methods faster. Through our extensive study of reversed phase chromatography Restek created the widest range of selectivity in the industry using just 4 unique stationary phases: the USLC<sup>TM</sup> column set. We also defined a simple approach to choosing a column with the appropriate selectivity for any application.

## **Selectivity Drives Separations**

Quickly and effectively resolve analytes by understanding and controlling selectivity through USLC™ technology.

One of the most significant, yet least understood, steps of method development is finding the proper stationary phase for a particular separation. As sample complexity increases, achieving adequate resolution between matrix components and target analytes becomes more difficult. Despite recent advancements in column format, such as sub-2 micron packings and pellicular particles, resolution can still be difficult to obtain because, while these formats can increase chromatographic efficiency and analysis speed, they do not significantly influence resolution. Selectivity, as shown in Equation 1, is the single most powerful factor affecting resolution, and it is largely dependent upon stationary phase composition.

**Equation 1:** Selectivity has the greatest mathematical effect on resolution.

 $R = \frac{1}{4} \sqrt{N} x (k/(k+1)) x (\alpha-1)$ Efficiency Retention Factor Selectivity

## **Real Diversity in Phase Chemistry**

A small set of defined orthogonal columns means faster separations and more robust methods.

While numerous bonded phases are available for reversed phase chromatography, many (e.g., C8 and C18) are similar and offer only moderate changes in retention, rather than significant differences in selectivity. Method development is less laborious and time-consuming when using a full range of column selectivities, including orthogonal phase chemistries like polar embedded, phenyl, and fluorophenyl columns. Restek has led the development of the unique USLC<sup>TM</sup> column set across these phase classes to provide analysts with a more effective range of column selectivities and innovative column chemistries for method development. The USLC<sup>TM</sup> column set (Figure 1) provides the widest range of reversed phase selectivity available with just 4 columns and can be used to guide proper stationary phase selection—the least understood yet most significant part of method development.

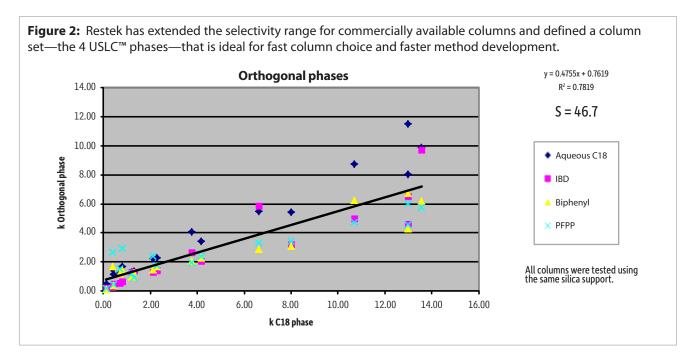
Figure 1: Restek columns offer the widest range of unique and effective phase chemistries to aid the chromatographer in choosing columns fast and developing methods faster. Restek USLC™ Aqueous C18 (alkyl) Biphenyl (phenyl) IBD (polar embedded) **PFP Propyl** (fluorophenyl) Phase (column class) Ligand type Proprietary polar modified and Unique Biphenyl Proprietary polar functional Fluorophenyl functionally bonded C18 embedded alkyl **Properties** • General purpose with a Increased retention for Increased retention for Increased retention for well-balanced retention profile. dipolar, unsaturated, or acids and water-soluble both charged bases and electronegative compounds. · Compatible with 100% conjugated solutes. compounds. · Compatible with 100% aqueous mobile phases. · Enhanced selectivity when Capable of both reversed used with methanolic mobile aqueous mobile phases. phase and HILIC separations. Ideal for multi-component phase. LC-MS analyses. Capable of both reversed Ideal for increasing Ideal for increasing phase and HILIC sensitivity and selectivity sensitivity and selectivity in in LC-MS analyses. separations. LC-MS analyses.

#### **Evaluating and Extending Selectivity**

The Restek USLC™ column set offers the highest range of alternate selectivity available.

The diverse selectivity provided by USLC<sup>TM</sup> columns can be demonstrated empirically using the hydrophobic-subtraction model [1]. This model is a novel procedure for characterizing selectivity that uses test probes to define the solute and stationary phase interactions in reversed phase separations. Restek is leading the commercial application of this model by implementing it in the development of USLC<sup>TM</sup> bonded phases. To evaluate phase selectivity using the hydrophobic-subtraction model, the retention characteristics of the solute probes are compared across different phases relative to a C18 benchmark with all columns using the same silica base.

The resulting scatter plot is an excellent way to visualize selectivity. Stationary phases with similar selectivity show high linearity when graphed. However, stationary phases with alternate selectivity—even orthogonality—produce significant scatter around the regression line. The high degree of scatter shown in Figure 2 shows just how diverse the phases in the USLC<sup>TM</sup> column set are. When we quantify column selectivity based on this correlation by calculating the selectivity (S) statistic [2], the resulting value of 46.7 shows that the USLC<sup>TM</sup> column set truly has the highest range of selectivity available.



### **USLC™ Columns** Making the Right Selectivity Choices Accelerate Method Development Separations

Restek USLC<sup>TM</sup> columns offer the widest range of selectivity available and are an integral part of successful method development (Figure 3). Ideal for column switching systems, these columns provide the orthogonal separations needed to create optimal resolution and robust methods—all in a 4-column set. Combining USLC<sup>TM</sup> phases with a suitable column format (Figure 4) gives practicing chromatographers the most powerful tools available for successful method development.

Column Line									
	Restek		Waters		Phenomenex	Agilent			
Column Type	Pinnacle DB	Ultra	Acquity CSH	Acquity HSS	Acquity BEH	Kinetex	Zorbax RRHD	Poroshell 120	
Alkyl (C18 and C8)	•	•	•	•	•	•	•	•	
Phenyl	•	•	•		•				
Polar Embedded Alkyl	•	•							
Fluorophenyl	•	•	•			•			

**Figure 4:** There's a USLC™ Column for Nearly Every Instrument Platform, Scale, and Application.

Column Line*	Particle Diameter	Use	
Pinnacle DB	1.9 μm	UHPLC	
Ultra	3 and 5 μm	HPLC	
* In addition to USLC™ static	onary phases, Restek also offers C8, C18, a	nd silica.	

Column Class	Column ID	
Capillary	<1.0 mm	
Microbore	1.0 mm	
Narrow bore	2.1–3.0 mm	
Standard bore	3.2–4.6 mm	
Semi-prep	10–21.2 mm	
Prep	30–50 mm	

For detailed analysis of USLC™ column selectivity data, visit **www.restek.com/uslcarticle** 

#### References

- L.R. Snyder, J.W. Dolan, P.W. Carr, The Hydrophobic-Subtraction Model of Reversed-Phase Column Selectivity, J. Chromatogr. A 1060 (2004) 77.
- [2] U.D. Neue, J.E. O'Gara, A. Mendez, Selectivity in Reversed-Phase Separations influence of the Stationary Phase, J. Chromatogr. A 1127 (2006) 161.

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