RESTEK USLC™

Ultra Selective Liquid Chromatography™

Choose Columns Fast. Develop Methods Faster.

USLC™ Column Selection & Mobile Phase Adjustment Guide

RESTEK **Innovative Chromatography Products** www.restek.com

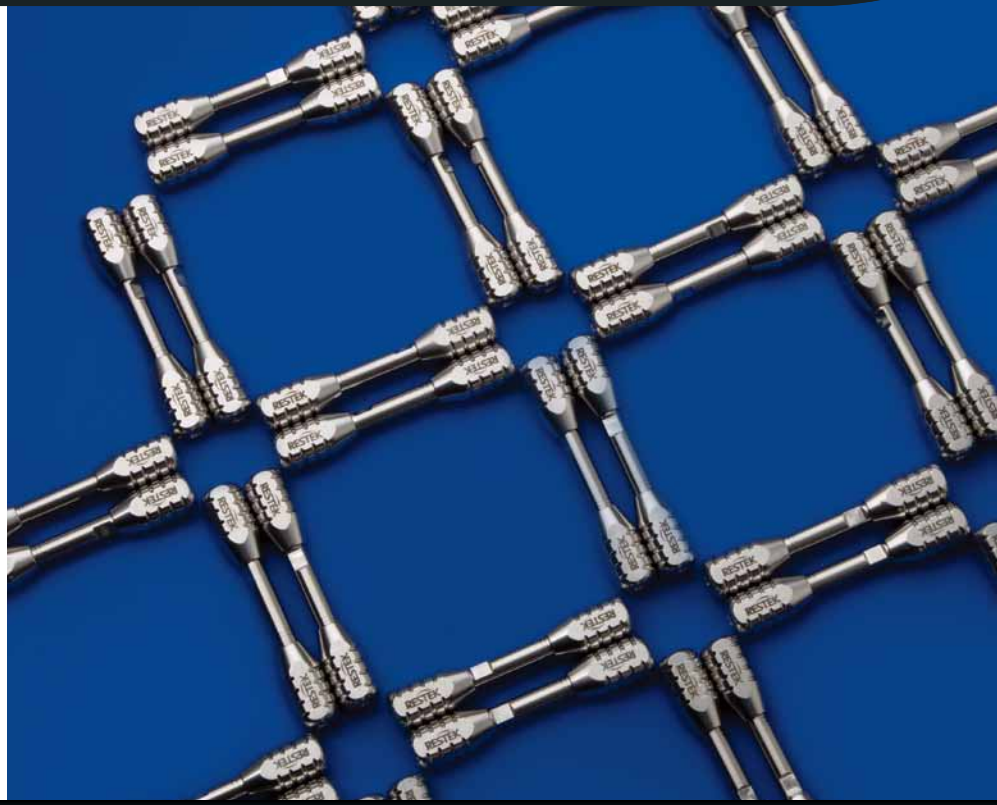
RESTEK USLC™

Ultra Selective Liquid Chromatography™

Ultra Selective Liquid Chromatography™ (USLC™) technology is the directed application of selectivity—the most influential factor affecting peak separation, or resolution—to provide the practicing chromatographer with the best tools available for choosing columns fast and developing methods faster.

Each USLC™ stationary phase is optimized for a different chemical interaction and solute type. More importantly for you, this optimization means that each phase also provides different (or "orthogonal") selectivity. In fact, the well-defined USLC™ 4-column set offers the widest range of selectivity in the market, so you can simply and effectively separate almost any combination of organic analytes.

This guide will help you easily choose the right USLC™ column for nearly any reversed phase or HILIC application. It will also help you properly fine tune your mobile phase based on column choice and analyte type to further improve your results without guesswork or wasted time. When you have USLC™ columns and this guide, you always have the right tools in your method development toolbox!



Using This Guide

To quickly and easily choose your USLC™ column and fine tune your mobile phase, follow these 5 simple steps.

1) Classify Your Target Analyte(s)

It may seem obvious, but in order to choose a column to target specific analytes, you must first define them. Classify your analytes into the following 4 functional groups*:

- **Hydrophobic:** These molecules are often regarded as "water fearing," as they are non-polar and prefer neutral stationary phases and solvents. Hydrocarbons are the ideal example of hydrophobic molecules.

  Note: A simple guideline can be applied here to define the hydrophobicity of the molecule—a molecule with a carbon-to-heteroatom (any atom other than carbon or hydrogen) ratio of 3:1 or higher is often amenable to the hydrophobic interactions of reversed phase analysis. If your molecule contains less than a 3:1 ratio or has limited retention on a C18, focus instead on other applicable function groups.

- **Dipolar:** These molecules are capable of dipole moments or non-uniform distributions of electrons causing positive and negative charges. They can contain either permanent dipoles (polar molecules) or induced dipoles (polarizable molecules).
- **Acidic**:** We can define acids as molecules capable of either donating protons (Brønsted-Lowry definition) or accepting electrons (Lewis definition).
- **Basic**:** We can define bases as molecules capable of either accepting protons (Brønsted-Lowry definition) or donating electrons (Lewis definition).

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* If your analyte fits into more than one functional group, consider routine scouting (e.g., column switching) with all 4 columns to determine your best overall selectivity.

** For acidic and basic compounds, you will also need to identify the pKa of your target analyte(s).

2) Choose Your Column

Each USLC™ column is optimized for a different chemical interaction and solute type. After you have classified your analyte(s), use the column interaction and solute retention profiles on pages 8–15 to choose the best one for your application.

Column Interaction Profile

Put simply, selectivity is the retention of one compound relative to another. Therefore, because solutes will be retained to different degrees by different molecular interactions, we can fundamentally define a column's selectivity based on the molecular interactions it delivers.

The pie chart provided for each USLC™ stationary phase in this guide (Figure 1) identifies the same 4 molecular interactions (color coded to correspond to the retention of a different solute type). The more rings shown for a given interaction, the more significant a role it plays in defining solute retention. These defining interactions and their less-prominent complements are also listed below the chart.

If you know what type of column interaction you need for your analysis, use these charts to select your USLC™ column.

Figure 1. Sample Column Interaction Profile

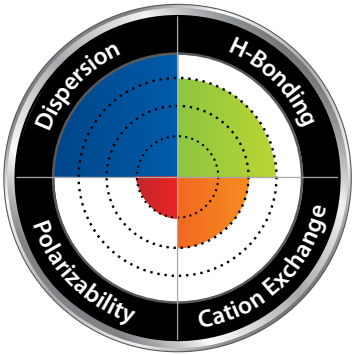
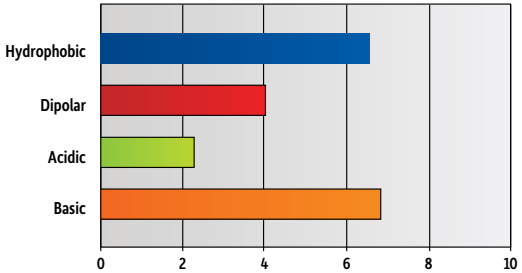


Figure 2. Sample Solute Retention Profile



Solute Retention Profile

Because each USLC™ column has a different interaction profile, it will preferentially retain different solutes. Use the column retention profiles in this guide (Figure 2) to quickly find the column you need based on the type of analyte you want to retain—hydrophobic, dipolar, acidic, or basic. The longer the bar in the graph, the more significant the solute retention. Simply choose the column that delivers heightened selectivity for your analyte type!

3) Scout Your Mobile Phase

Once you've chosen a column, you need to fine tune your mobile phase, but before you can, you must first ensure that your starting mobile phase will yield acceptable data. Choosing a highly customized mobile phase may prove to be unnecessary or even detrimental to data quality, so instead, scout your mobile phase *using a 4–mobile phase system* and the following recommendations:

Aqueous Solutions	**Organic Solvents**
A1) 0.1% Formic Acid in Water	B1) Acetonitrile (aprotic solvent)
A2) 0.1% Formic Acid and 5 mM Ammonium Formate in Water	B2) Methanol (protic solvent)

To scout your mobile phase, run scouting gradients using all A/B combinations above (e.g., A1/B1, A1/B2, A2/B1, A2/B2), then proceed with the combination that yields the best results.

For more information on running a scouting gradient, contact
Restek Technical Service (support@restek.com).

Tech Tip

Many detectors, including mass spectrometers, are not amenable to traditional mobile phase additives like phosphate buffers and ion-pairing agents. That's why we designed the USLC™ column set to work with simple, mass spectrometer–compatible mobile phases (i.e., volatile and acidic).

4) Adjust Your Mobile Phase

If the data quality from your scouting test is unacceptable because of asymmetrical peaks, low retention, etc., you will need to choose an alternate buffer or otherwise change the pH of your mobile phase to correct the problem. Here are some helpful guidelines for changing your mobile phase pH when using a USLC™ column:

- The target mobile phase pH for a USLC™ column is between 2 and 4, but a pH between 2 and 8 is acceptable. A pH above 8 will reduce the lifetime of your column and is not recommended.
- For acidic and basic solutes, mobile phase pH should ideally be at least 1.5 units below your analyte's pKa (which will shift equilibrium to where USLC™ technology is most effective—charged form for bases and neutral form for acids).
- If using an IBD or PFP Propyl column in HILIC mode, increasing the organic percentage in your mobile phase can lead to heightened retention of ionic or very polar analytes. (If you classified your analyte as having less than a 3:1 carbon-to-heteroatom ratio in step 1, we recommend you explore this option.)
- Keep buffer concentrations low (between 5 and 20 mM).
- TFA should be avoided because it reduces sensitivity with electrospray ionization.

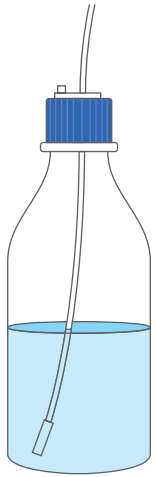
For additional help adjusting the pH for your mobile phase, contact
Restek Technical Service (support@restek.com).

5) Fine Tune Your Mobile Phase

Mobile phases have a greater effect on selectivity when they work in conjunction with a column's interaction and retention profiles. Once you have adjusted your mobile phase pH enough to ensure acceptable chromatography, increase selectivity for your target analytes by fine tuning your mobile phase based on your column choice using the charts on pages 8–15.

Figure 3. Sample mobile phase fine-tuning chart.

If your target analyte is...	Consider...
A Basic or Acidic Moiety	• First, increasing pH to increase retention of bases. • Second, decreasing pH to increase retention of acids. • Third, altering or mixing protic and aprotic solvents to adjust retention and selectivity.
Ionic or Charged	• Increasing buffer strength to decrease retention.
An Aromatic or Alkyl Isomer	• Decreasing temperature to increase selectivity.



Tech Tip

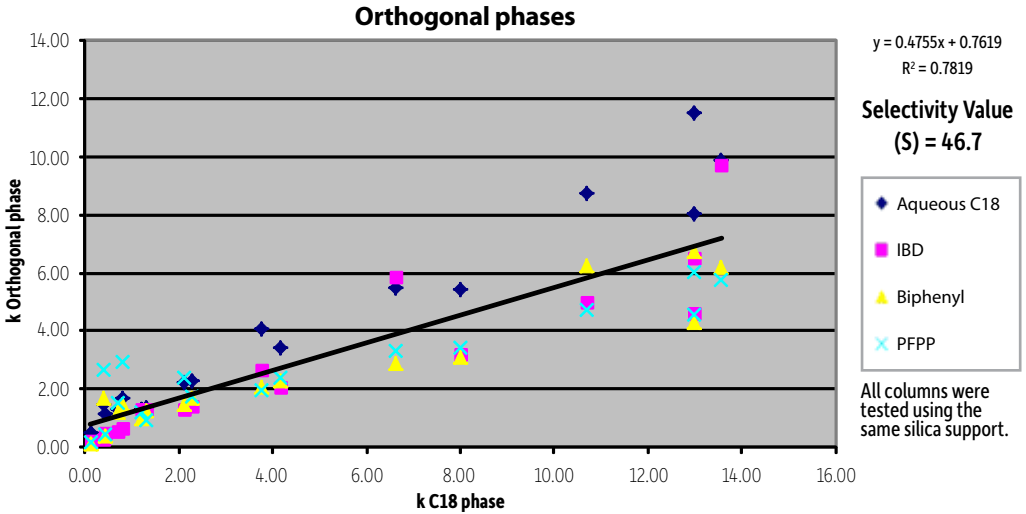
USLC™ columns are a perfect fit with electrospray ionization mass spectrometry because they:

- Retain ionized molecules.
- Create retention that alleviates matrix-induced ion suppression and reduces charge competition.
- Improve ionization and sensitivity with low–surface tension mobile phases.

Defining Selectivity

The hydrophobic-subtraction (H-S) model is a novel procedure for characterizing selectivity [1]. The retention characteristics of a set of solute probes are compared across different stationary phases relative to a C18 benchmark with all columns using the same silica base. In the resulting scatter plot, stationary phases with similar selectivity show high linearity when graphed, but stationary phases with alternate selectivity—even orthogonality—produce significant scatter [2]. The high degree of scatter and resulting selectivity value (S) of 46.7 shows that the USLC™ column set truly has the highest range of selectivity available!

You can learn more about the H-S model and how Restek used it to create the USLC™ column set at **www.restek.com/USLCarticle**



References

[1] 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.

Stationary Phase: Aqueous C18

No practical column set is complete without a C18, but this Restek phase far outperforms your run-of-the-mill C18 column. Our rugged Aqueous C18 has a well-balanced retention profile. It can effectively retain more types of solutes than a conventional C18 and is ideal for multi-component LC-MS analyses. The Aqueous C18 boasts high reproducibility and compatibility with many mobile phase conditions—even 100% aqueous and acidic. And when used with a gradient, it eliminates the all-too-common issue of multiple compounds coeluting near the column void time.

Column Description:

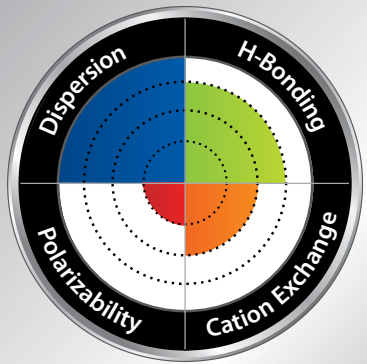
Stationary Phase Category:
C18 (L1)

Ligand Type:
Proprietary polar modified and functionally bonded C18

Properties:
- General purpose with a well-balanced retention profile.
- Compatible with 100% aqueous mobile phases.
- Ideal for multi-component LC-MS analyses.

Switch to an Aqueous C18 when:
- Limited retention or selectivity for polar compounds is observed on a C18.

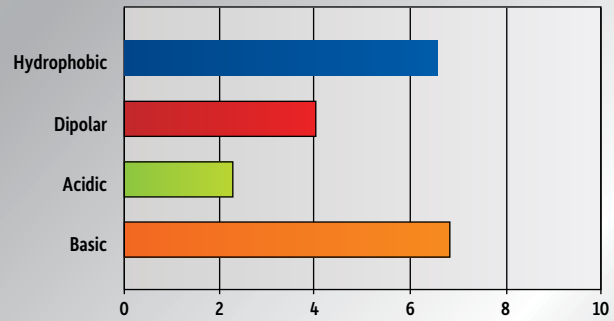
Column Interaction Profile:



Defining Solute Interaction:
- Dispersion

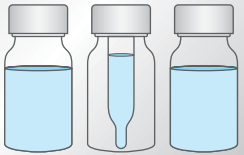
Complementary Solute Interaction:
- Hydrogen bonding

Solute Retention Profile:



Target Analyte Structure:
- Hydrocarbons

Target Analyte Functionalities:
- Organic acids
- Ketones
- Isomeric species



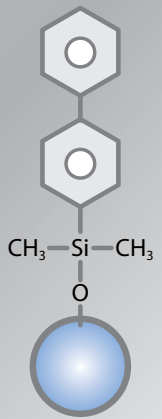
Fine Tuning Your Mobile Phase:

If your target analyte is...	Consider...
A Basic or Acidic Moiety	• First, increasing pH to increase retention of bases. • Second, decreasing pH to increase retention of acids. • Third, altering or mixing protic and aprotic solvents to adjust retention and selectivity.
Ionic or Charged	• Increasing buffer strength to decrease retention.
An Aromatic or Alkyl Isomer	• Decreasing temperature to increase selectivity.

For step-by-step instructions on choosing your USLC™ column and fine tuning your mobile phase, see page 3.

Stationary Phase: Biphenyl

Welcome to the next generation of phenyl columns. The Restek Biphenyl offers a greater degree of dispersion than conventional phenyls and a greater degree of polarizability than phenyl hexyls, creating higher selectivity and a greater range of usability. Because of these heightened interactions, this column shows substantial increases in retention and orthogonal selectivity when using methanol mobile phases.



Column Description:

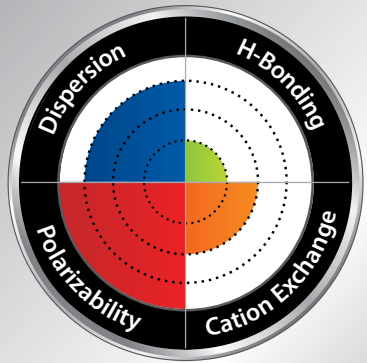
Stationary Phase Category:
Phenyl (L11)

Ligand Type:
Unique Biphenyl

Properties:
- Increased retention for dipolar, unsaturated, or conjugated solutes.
- Enhanced selectivity when used with methanolic mobile phase.
- Ideal for increasing sensitivity and selectivity in LC-MS analyses.

Switch to a Biphenyl when:
- Limited selectivity is observed on a C18.
- You need to increase retention of hydrophilic aromatics.

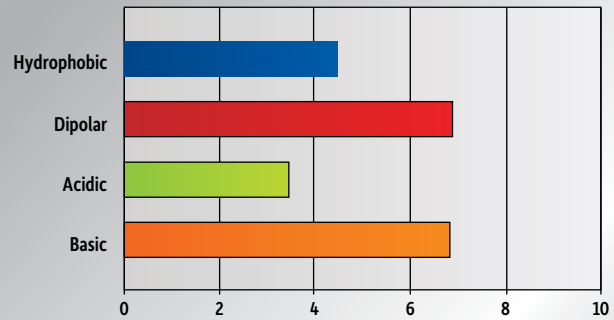
Column Interaction Profile:



Defining Solute Interactions:
- Polarizability
- Dispersion

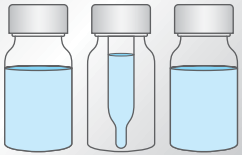
Complementary Solute Interaction:
- Cation exchange

Solute Retention Profile:



Target Analyte Structures:

- Aromatic
- Dipolar



Target Analyte Functionalities:

- Hydrophilic aromatics
- Strong dipoles
- Lewis acids
- Dipolar, unsaturated, or conjugated compounds
- Fused-ring compounds with electron withdrawing groups

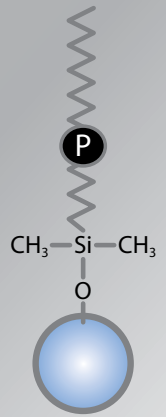
Fine Tuning Your Mobile Phase:

If your target analyte is...	Consider...
Conjugated or Aromatic	• Increasing methanol percentage to alter retention and selectivity.
Dipolar	• Decreasing acid strength and/or concentration to increase retention.
Ionic or Charged	• Decreasing buffer strength to increase retention relative to neutrals.
A Basic or Acidic Moiety	• First, increasing pH to increase retention of bases. • Second, decreasing pH to increase retention of acids.

For step-by-step instructions on choosing your USLC™ column and fine tuning your mobile phase, see page 3.

Stationary Phase: IBD

The IBD is a polar embedded column that acts as a strong hydrogen bonder and may be the most versatile column available today. With a unique polar group, this column is very retentive and selective for acids. It also provides symmetrical peak shape for strong bases. Restek's IBD is compatible with 100% aqueous mobile phases and can be used under HILIC conditions to retain very polar, ionic compounds in highly organic mobile phases.



Column Description:

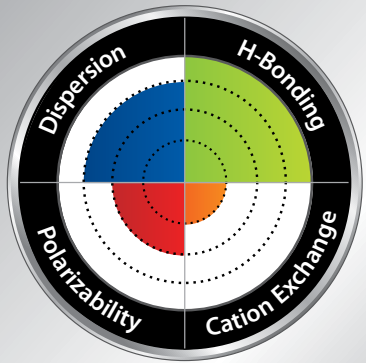
Stationary Phase Category:
Polar Embedded Alkyl (L68)

Ligand Type:
Proprietary polar functional embedded alkyl

Properties:
- Increased retention for acids and water-soluble compounds.
- Compatible with 100% aqueous mobile phases.
- Capable of reversed phase and HILIC separations.

Switch to an IBD when:
- You need improved retention or selectivity of acidic compounds or compounds capable of hydrogen bonding.
- You need improved symmetry for strong bases.

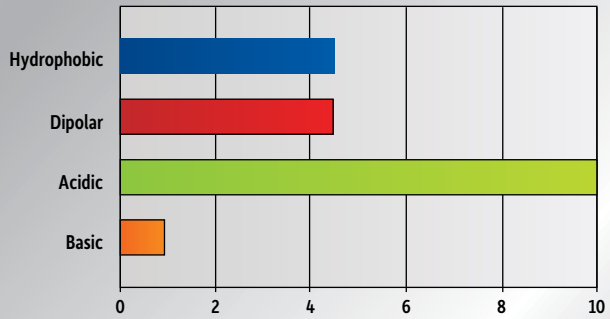
Column Interaction Profile:



Defining Solute Interactions:
- Hydrogen bonding
- Dispersion

Complementary Solute Interaction:
- Polarizability

Solute Retention Profile:

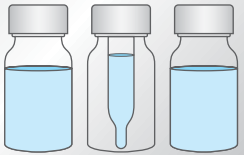


Target Analyte Structure:

- Oxygenated species

Target Analyte Functionalities:

- Alcohols
- Carboxylic acids
- Quaternary ammonium compounds



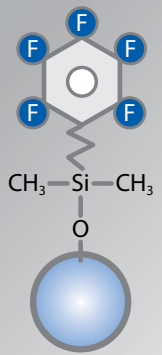
Fine Tuning Your Mobile Phase:

If your target analyte is...	Consider...
Acidic	• Decreasing acid strength and/or concentration to increase retention relative to neutrals and bases.
Ionic or Charged	• Increasing buffer strength to decrease retention.
A Basic or Acidic Moiety	• Altering or mixing protic and aprotic solvents to adjust retention and selectivity.

For step-by-step instructions on choosing your USLC™ column and fine tuning your mobile phase, see page 3.

Stationary Phase: PFP Propyl

Due to its polarity, a cyano stationary phase is often regarded as the most orthogonal to a C18. It is a great choice for the retention and selectivity of bases and amine-containing compounds. Unlike a conventional cyano column, however, the Restek PFP Propyl is much more amenable to LC-MS because it is more reliable and efficient with acidic mobile phases. This versatile column is also compatible with highly aqueous mobile phases and HILIC separations.



Column Description:

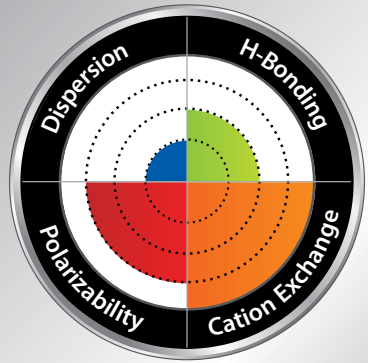
Stationary Phase Category:
Proprietary end-capped pentafluorophenyl propyl (L43)

Ligand Type:
Fluorophenyl

Properties:
- Increased retention for charged bases and electronegative compounds.
- Capable of reversed phase and HILIC separations.
- Ideal for increasing sensitivity and selectivity in LC-MS analyses.

Switch to a PFP Propyl when:
- Limited retention and selectivity are observed on a C18 for basic compounds.
- You need increased retention of hydrophilic compounds.

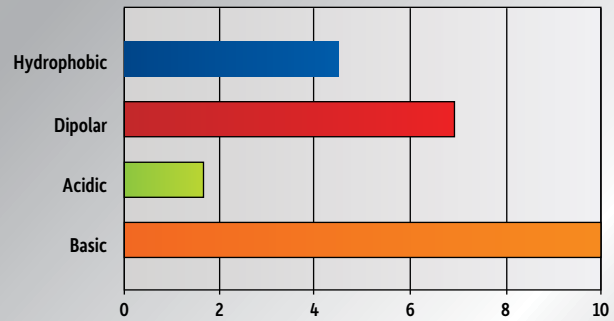
Column Interaction Profile:



Defining Solute Interaction:
- Cation exchange

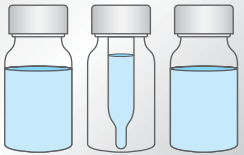
Complementary Solute Interactions:
- Dispersion
- Polarizability

Solute Retention Profile:



Target Analyte Structures:

- Nitrogen and Halogenated Species



Target Analyte Functionalities:

- Protonated amines
- Quaternary ammonium compounds
- Positively charged moieties
- Lewis bases

NOTE: May offer inconsistent results with sulfur-containing compounds. For column recommendations, contact Restek Technical Service.

Fine Tuning Your Mobile Phase:

If your target analyte is...	Consider...
A Basic Moiety	• Decreasing acid strength and/or concentration to increase retention relative to neutrals and acids.
Conjugated or Aromatic	• Altering or mixing protic and aprotic solvents to adjust retention and selectivity.
Ionic or Charged	• Increasing buffer strength to decrease retention.

For step-by-step instructions on choosing your USLC™ column and fine tuning your mobile phase, see page 3.

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™ stationary phases, Restek also offers additional particle diameters on these column lines as well as additional phases.

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 information on choosing column dimensions and setting instrument parameters, please contact **Restek Technical Service (support@restek.com).**

Protect Your LC Columns and Your Separations

Filters and guard cartridges are invaluable for protecting your LC columns and extending their life. Without them, sample impurities, mobile phase contaminants, and even materials from the injector or autosampler can cause particles to collect on the column inlet frit. This buildup can cause an increase in backpressure, split peaks, peak tailing, over-pressure shut-downs, and, ultimately, irreversible column damage.

Adding a filter or guard cartridge as a preventative measure can spare you the significant cost and hassle of frequently replacing your columns. And Restek has a complete line of easy-to-install solutions—even unique combination models with separately replaceable cartridges and filters to make maintenance easier and more cost effective.

Order yours today at **www.restek.com/LCguard**

Looking for Additional Help on Using Your USLC™ Column Set?

Application Note
The information in this selection guide is just the beginning. For a detailed analysis of USLC™ column selectivity data,

visit **www.restek.com/USLCarticle**

Technical Service
We're here to help! If you have any questions about choosing the right USLC™ column, adjusting your mobile phases, selecting the right LC accessories, or anything else, call Restek Technical Service at 800-356-1688, ext. 4 or email them at support@restek.com

ChromaBLOGraphy

Restek's blog is where our renowned experts go to share their thoughts on current trends along with best practices and troubleshooting tips to make your laboratory life easier and more productive. You'll find sneak peeks at tomorrow's applications as they develop today, and best of all, you have the opportunity to weigh in yourself. Join the discussion.

Visit **blog.restek.com** today!

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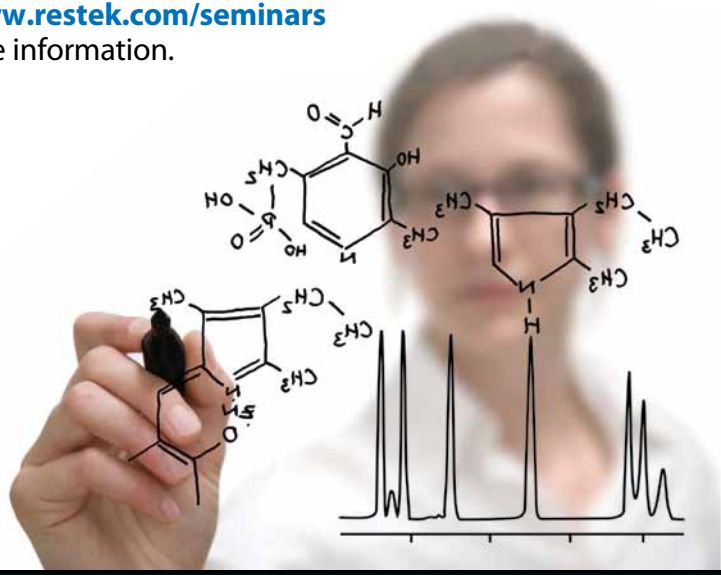

Notes

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