Choosing the most effective selectivity for an analysis might be more important than you think. Selecting the wrong UHPLC selectivity can waste both time and money for you and your laboratory.

Having a rational selectivity starting point not only facilitates a more effective separation, chromatographically speaking, but provides additional information in relation to potential molecular interactions that occur in the column. These interactions drive observed analyte retention and results from the functionalities of the relative stationary phase interacting with the chemical groups of the analyte(s) and in conjunction with mobile phase convention. Therefore, a rational selectivity starting point provides valuable information, in terms of potential interactions, that can be utilized to adjust an observed analyte retention time. Improved chromatographic selectivity is simple finding the right tool for the job.

The first step in determining the best rational chromatographic starting point is selecting the most appropriate solid support for a given analysis. The bases of a column’s selectivity is correlated to the foundational particle morphology. Where particle surfaces area, packing, and additional physical attributes affect the resulting efficiency of a relative separation. This relationship between a columns morphology and selectivity can be seen in one form of the resolution expression. Core-shell and organo-silica core-shell are unique solid silica core and porous shell that result in faster chromatography and higher efficiencies than the conventional fully porous particles.
How to Improve Your UHPLC Column Selectivity

This support is best used for performance gains on ANY LC system, easier system-to-system and lab-to-lab method transfer, methods were increased sensitivity is required, and for significantly improved productivity of older, established methods.

Fully porous, or thermally modified silica has a unique high efficiency and extremely robust fully porous silica that offers astounding performance and inertness alongside versatile selectivities. It results in astounding HPLC/UHPLC and preparative HPLC performance and efficiencies, greater separation muscle, better peak shape through an inert foundation, and extreme ruggedness and dependability.
Selectivity and efficiency have the greatest impact on observed resolution, when compared to other chromatographic parameters. Often the simplest and most effective way to improve your chromatographic results is to change your column’s phase or solid support.

The next step is to profile the mechanisms of selectivity. Observed selectivity is dictated by several primary molecular interactions. **Figure 1** shows the selectivity parameters used to help characterize reversed phase selectivity mechanisms.

Figure 1
How to Improve Your UHPLC Column Selectivity

**Hydrophobicity**
The ability of a phase to hydrophobically interact with carbon groups

**Steric Interaction**
The ability of a phase to separate compounds based on structural differences

**Hydrogen Bond Donating Capacity**
The ability of a phase to hydrogen-bond with proton accepting groups

**Hydrogen Bond Accepting Capacity**
The ability of a phase to hydrogen-bond with proton donating groups

**Cation Selectivity at pH 2.8**
The ability of a phase to interact with cation groups at acidic pH

**Cation Selectivity at pH 7.0**
The ability of a phase to interact with cation groups at neutral pH
How to Improve Your UHPLC Column Selectivity

See Page 7 in “The Chromatographer’s Guide to Improving UHPLC Column Selectivity” for an outline of relating selectivity to UHPLC stationary phases through color coordination demonstrating the relationship between atomic fragments of compounds and how to relate them to column selectivity profiles.

The guide also lays out a selectivity overview that helps to decipher the correct UHPLC selectivity and column that should be used for alkyl phases, phenyl phases, and polar retentive phases.

Once you have chosen the right selectivity for your analysis, it is time to apply it to your HPLC analysis.

The extent to which resolution depends on UHPLC selectivity becomes evident when a chromatographer takes advantage of technologic advances in polar stationary phases in order to improve analysis. **Figure 2** below is an example of resolution improvements obtained by switching from a traditional C18 to a column with additional selectivity mechanisms.
How to Improve Your UHPLC Column Selectivity

Polar Selectivity of Catecholamines

Polar Modified
Luna® Omega 1.6 μm Polar C18

Dopamine

Traditional C18
Kinetex® 1.7μm C18

Improved Selectivity for Polar Analytes

Improved Separation with Polar C18
The increased hydrogen bond accepting capacity of the Luna Omega Polar C18 column improves polar selectivity for analytes such as dopamine.

Conditions for both columns:
- Column: Luna Omega 1.6μm Polar C18
- Dimensions: 50 x 2.1 mm
- Mobile Phase: A: 10mM Ammonium Formate with 0.1% Formic Acid
  B: Acetonitrile with 0.1% Formic Acid
- Gradient: Time (min) % B
  0 0
  3 90
- Flow Rate: 0.4 mL/min
- Injection Volume: 1 μL
- Temperature: 22°C
- Detection: MS/MS (SCIEX API 4000™)
- Sample: 1. Norepinephrine
  2. Epinephrine
  3. Noradrenaline
  4. Dopamine
  5. Metanephrine
  6. Serotonin

Hydrophobicity
Hydrogen Bond Accepting Capacity
Hydrogen Bond Donating Capacity
Caliper Selectivity at pH 2.8
Caliper Selectivity at pH 7.0
Low
High
While, many may think that traditional C18 phases are the way to go, it may not always be the best option. Instead, trying using a UHPLC column that has both polar and hydrophobic versatility. This will allow for great method development flexibility.

Traditional UHPLC C18 phases can also be prone to peak tailing for high basic compounds. This tailing can become exacerbated when higher loadability is required. Loadability is important when analytical conformation is parallel to purification, when dealing with high API concentrations during stability tests, and during high sample loading, in order to visualize low-level analytes of interest.

A powerful tool in the chromatographer's toolbox is the ability to use 100% aqueous conditions to promote polar selectivity and increase retention. Traditional C18 phases are known to collapse under 100% aqueous conditions, causing a loss of retention and method development headaches.

You also want to make sure to utilize unique selectivities. The elution order of analytes can change depending upon the predominate selectivity characteristics of a UHPLC column and the utilized mobile phase. So, by altering the stationary phase or mobile phase conditions, you can observe elution order and extend retention of more polar analytes.

Working with aromatic based selectivity? Compounds with aromatic ring structures offer a specific type of selectivity associated with pi-pi bond interaction. The compound’s aromaticity provides pi elections that have the potential to interact with pi bonds, which can be found on phenyl-based stationary phases. This provides a unique, orthogonal selectivity compared to a traditional C18 phase.

Have questions? No worries! We are here to help! Live Chat with one of our Technical
How to Improve Your UHPLC Column Selectivity

Experts nearly 24/7—they are there to help with all your method development or analysis needs.

Make sure to download the full UHPLC Column Selectivity Guide for more insight into what was outlined above.

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