Selecting the right column for your reversed phase method isn't always easy. We wanted to help in the decision-making process by breaking down the different steps you'll need to consider when selecting your column. For a full in depth look into the selection process see our Ultimate Guide to HPLC/UHPLC Reversed Phase Selectivity.

Selecting the Right Solid Support

To begin the process of choosing the correct column for a reversed phase method, we start with selecting the right solid support. The significance the morphology of the solid support has on the resulting material characteristics and column performance is crucial. That is why we offer a full range of solid supports including core-shell, organo-silica, fully porous, and thermally modified fully porous.

Core-shell and organo-silica core-shell are unique solid silica core and porous shell that results in faster chromatography and higher efficiencies that conventional fully porous particles. This makes it suitable for performance gains on any LC system, easy system-to-system, and lab-to-lab method transfer. Core-shell works best for methods where increased sensitivity is required and also significantly improves the productivity of older, established methods.

Fully porous – thermally modified silica, have high efficiency and are extremely robust fully porous silica that offers astounding performance and inertness alongside versatile selectivities. The thermal modified pore structure eliminates micropores, further improving column efficiency, inertness, and reproducibility. This solid support works best for UHPLC, HPLC, and Preparative HPLC performance and efficiencies. It allows for greater separation muscle, better peak shape through an inert foundation, and extreme ruggedness and dependability.

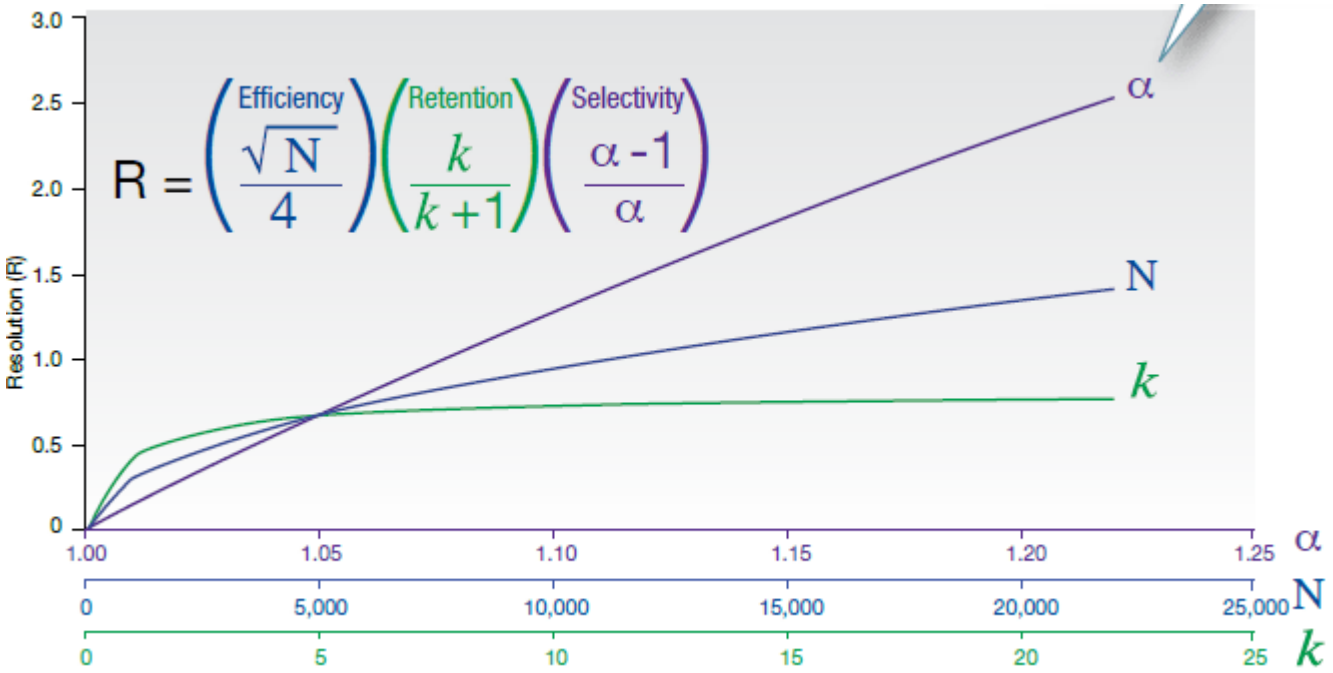
Fully porous- traditional silica particles, have higher surface and provide excellent mechanical strength across a wide range of particle sizes and column dimensions. These traditional particles are best for seamless scale-up from analytical to a preparative or process application. It is also applicable for direct column equivalents to those used in established Pharmacopeia methods.

Fully porous-organo are organic groups grafted into the layers of the silica particle making it more resistant to silica dissolution at higher pHs. If you are running methods at pH extremes, fully porous-organo silica will help extend your columns' lifetime. It is also well suited for premier bulk material products allowing for caustic washes for repeated use.

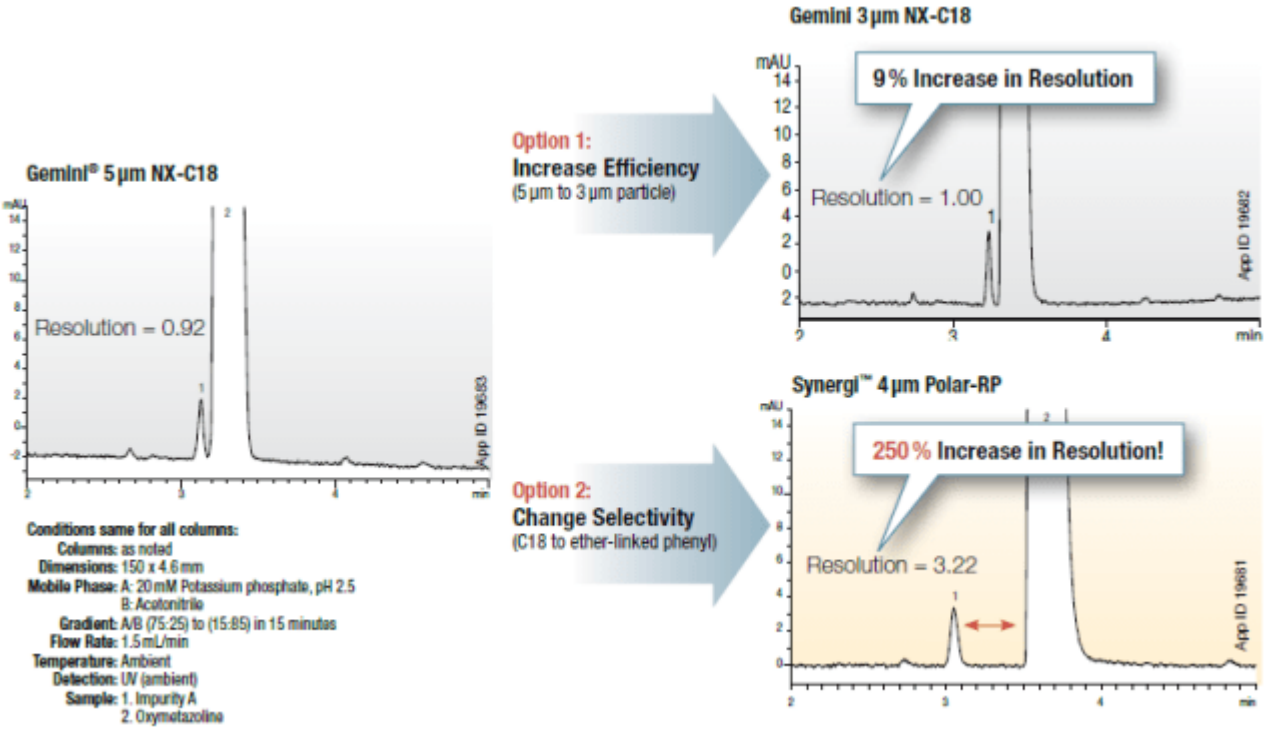
The Importance of Selectivity

Now that solid support is covered, let's go over the importance of column selectivity before diving into choosing a column. Selectivity (α) has the greatest impact on changing resolution (R), as compared to efficiency (N) and retention (k), and the easiest way to change your chromatographic results is to change your column phase. Phenomenex develops a wide breadth of phase chemistries across multiple solid supports for easier and faster method development and optimization.

Impact of Selectivity on Resolution

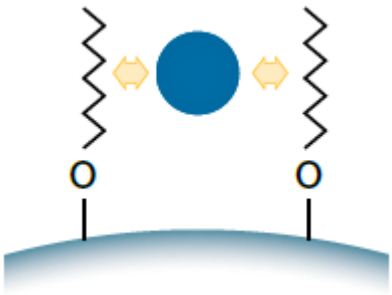


Change Your Selectivity, Dramatically Change Your Results

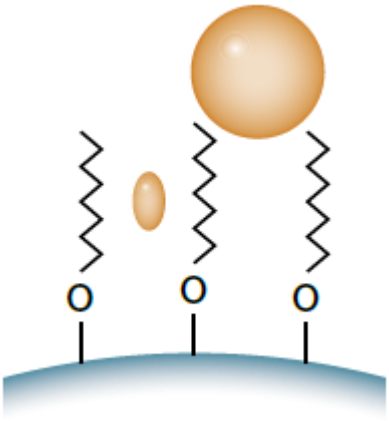


Characterizing Selectivity

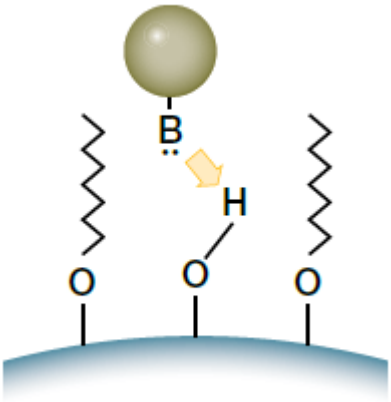
The easiest way to characterize selectivity is to utilize the hydrophobic subtraction model which includes six different parameters of our HPLC and UHPLC columns. Even though hydrophobicity is a dominant retention mechanism in reversed phase chromatography, selectivity is strongly influenced by the other parameters. Those parameters can be found below.



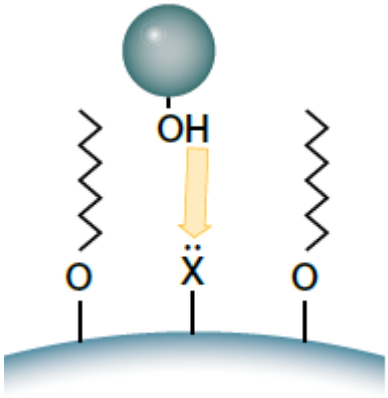
Hydrophobicity interactions occur with all analytes. They are always present and are dominant for neutral compounds.



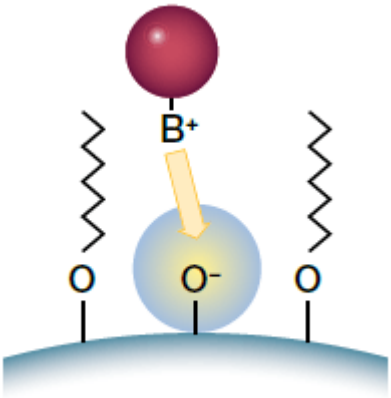
Steric Influences are a measurement of the accessibility of solutes to the stationary phase. Structural differences between compounds can lead to different retention characteristics due to shape selectivity.



Hydrogen Bond (H-bond) donating capacity interaction can be attributed to an exposed silanol or an intentionally added polar functional group. Phenomenex employs the latter technique to create phases that have the ability to hydrogen bond with proton accepting groups like weak bases (amines and amides).



Hydrogen Bond (H-bond) accepting capacity interaction is similar to the hydrogen bond donating capacity parameter, in that the engineered phases have the ability to hydrogen bond and interact with proton donating acidic groups such as carboxylic acids or alcohols.



Cation Selectivity at pH 7.0 is at neutral pH, which means residual silanols on the silica surface will be largely ionized, increasing the cation exchange component of selectivity.
Cation Selectivity at pH 2.8 is at low pH, where most residual silanols are neutral and the cation exchange component will be reduced.
For a deeper look into characterizing column selectivity for reversed phase methods, see our Ultimate Guide to HPLC/UHPLC Reversed Phase Selectivity under Step 2: Selectivity.

Column Selectivity Profiles

The following profiles were developed so that chromatographers would have a dependable approach for comparing the Phenomenex phases and identifying which phase(s) would

provide the best selectivity for their analytes. This section shows how parameters impact column selection based on your compound class.

Hydrocarbon Compounds

Selecting the most appropriate liquid chromatography column for your unique hydrocarbon, or hydrophobic compound is easy! Simply compare the varying degrees of hydrophobicity to determine how much or how little retention you require. An increase in column hydrophobicity typically provides increased retention of hydrophobic compounds. For example, the more hydrophobic Kinetex® Polar C18 chemistry provides a longer retention time which successfully separates a panel of 9 steroids while the less hydrophobic Kinetex C8 column displays coelution of two steroid compounds.

However, a traditional C18 may not always be the best option. A traditional C18 phase is typically recommended as the first choice for the separation of hydrocarbon, or hydrophobic compounds. In some cases though, less hydrophobicity paired with a different selectivity may be required to successfully achieve the separation of your hydrophobic compounds as well as to shorten run times. With so many C18 phases to choose from, it is important to note the hydrophobic properties of each phase. For example, the more hydrophobic Luna® Omega C18 chemistry provides a longer retention time for 10 cannabinoids while the less hydrophobic Luna Omega Polar C18, which contains a polar modified surface, provides less retention and therefore a shorter run time, without negatively affecting the overall separation of the analytes.

High column hydrophobicity values indicate greater retention of carbon-containing analytes. Lower hydrophobicity is recommended for extremely hydrophobic compounds that may be retained too tightly on traditional C18 phases.

Isomers and Isobaric Compounds

Take advantage of multiple interactive mechanisms. The multiple interactive mechanisms of Kinetex® F5 (pentafluorophenyl) column successfully separate methoxybenzene isomers, while the Kinetex C18 column, which has minimal bonding interactions, cannot separate the methoxybenzene isomers. This demonstrates that columns that rely primarily on hydrophobic

interactions may not be the first choice for the separation of isomeric compounds and a column with multiple interactive mechanisms may be required. High column steric interaction values are best suited for the analysis of analytes that require separation based on size and shape differences.

Hydroxyl- or Amine-Containing Compounds

To increase retention it is important to utilize hydrogen bond capacity. Compounds that contain hydroxyl groups, amines, or the combination of those two types of functional groups, typically display the ability to interact with LC stationary phases through hydrogen bonding. This interaction can take place at the silica surface with silanols, endcapping or other functional groups. Additionally, hydrogen bond interactions can take place between these analyte groups and any corresponding polar groups on or within the stationary phase. By utilizing a column selectivity that contains a combined hydrophobic and hydrogen bond capacity, one can gain greater improvement resolution versus just focusing on manipulation of hydrophobic retention. This can be especially true when analyzing compounds that are very polar in nature.

Hydrogen bond accepting groups on the silica surface interact with hydrogen bond donating functionalities on analytes.

Aromatic or Ring Containing Compounds

Every industry in the world that uses chromatography has most likely at some point analyzed compounds that contain carbon based ring structures. While these rings increase the hydrophobicity of a compound, they also provide a source of pi electrons which can directly interact with the pi electrons found within a stationary phase. Column chemistries that contain ring structures interact with aromatic or ring containing compounds via pi-pi interactions. While these aromatic, pi-pi interactions are not as strong as hydrophobic interactions, they can represent an easy way to increase retention and resolution. When choosing a mobile phase to use the aromatic stationary phases that contain a phenyl group, it’s incredibly useful to keep in mind that acetonitrile disrupts pi-pi interactions, while methanol helps to promote them.

Non-ionized Bases and Oxygen- or Halogen-Containing Compounds

Liquid chromatography columns with high hydrogen bond donating capacity provide higher retention of non-ionized bases and oxygen- or halogen-compounds while lower hydrogen bond donating capacity columns will result in less retention. For example, the higher hydrogen bond donating capacity of the Luna Omega Polar C18 column provides longer retention times which successfully separates a suite of 8 acidic, basic, and neutral compounds while the lower hydrogen bond donating capacity of the Kinetex F5 column has less retention and displays coelution of several compounds. Hydrogen bond donating groups on the silica surface interact with accessible functionalities containing a lone pair of electrons.

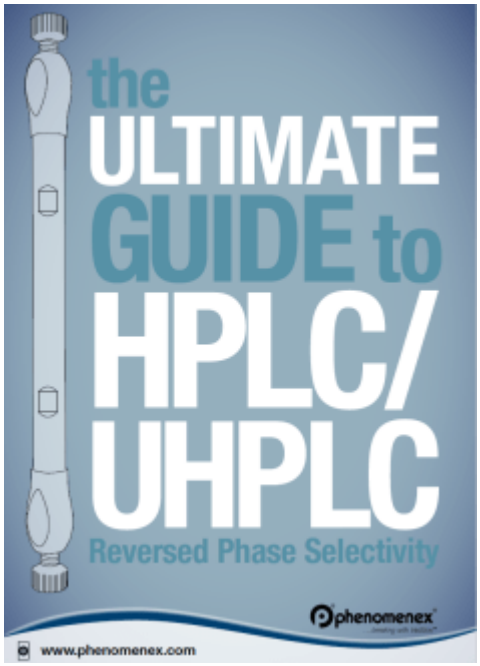
Polar Basic Compounds

A liquid chromatography column's cation selectivity can determine its affinity for ionized bases. High column cation selectivity will provide higher affinity or longer retention of ionized bases while lower column cation selectivity will result in less retention of ionized bases, but may have very good peak shapes. For example, the higher cation selectivity properties of the Kinetex® Biphenyl column provide longer retention of opiates as compared to the Kinetex C18 column which has a lower cation selectivity rating. This can be extremely helpful when needing to move compounds away from early suppression regions.

Polar Acidic Compounds

Charged polar groups on the surface of a particle or within the column's key functional group can play a large role in the separation of polar acidic compounds. Chemistries such as Luna Omega PS C18 have been fine-tuned to provide a mixed mode selectivity that includes positively charged groups on the silica's surface. These groups increase the retention of polar acidic compounds, resulting in improved separation power compared to chemistries that do

not contain these properties. Positive groups on the silica surface or in the column's functional group interact with polar acidic compounds, increasing the retention time.



To see the in depth portfolio for each column go to Step 3: Column Selection in the Ultimate Guide to HPLC/UHPLC Reversed Phase Selectivity.

Click Phenomenex website for more information.

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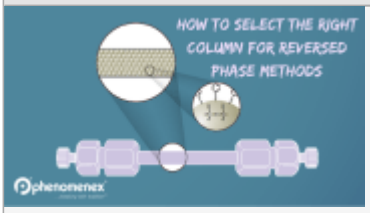
Summary



Article Name

Selecting the Right Column for Your Reversed Phase Method

Description

We break down the selection process when trying to choose the best and right column to use for your reversed phase method