

# RESOLUTION EVOLUTION



**BIPHENYL** Next Generation  
of Phenyl Columns



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# RESOLUTION EVOLUTION



## The Next Generation of Phenyl Column Chemistry

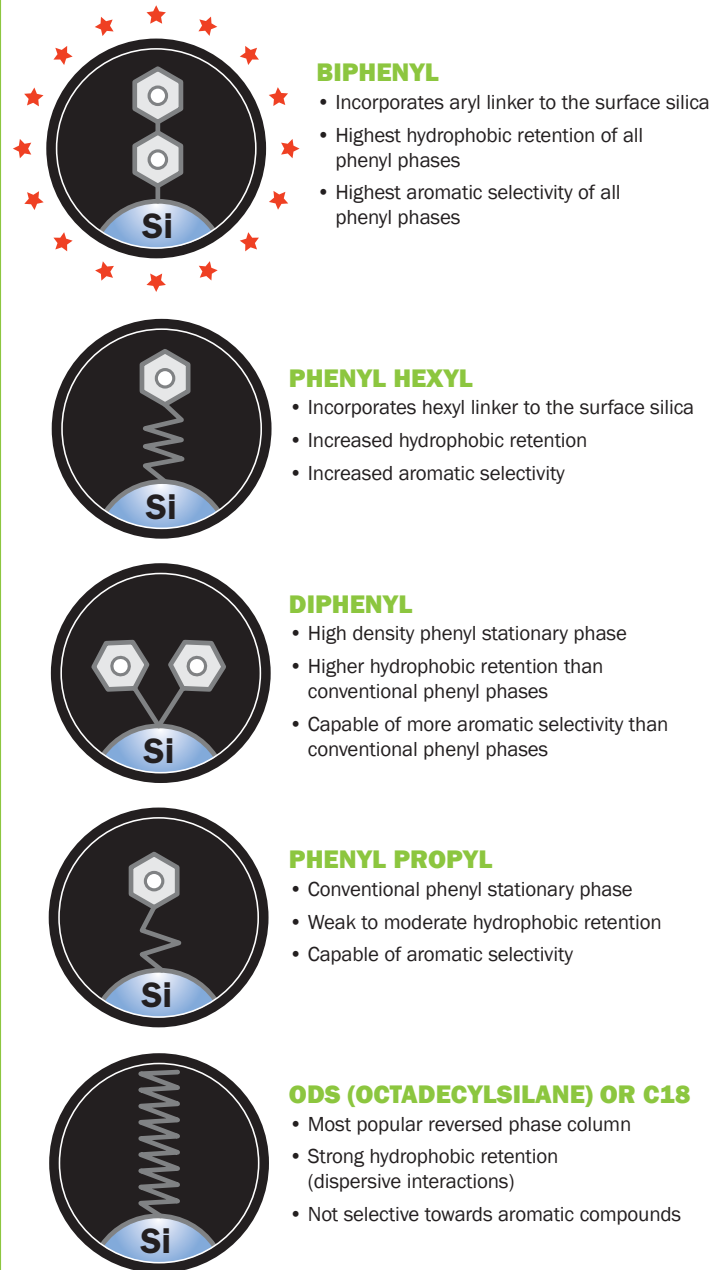
*The Biphenyl phase is the next generation of phenyl column chemistries, providing both outstanding aromatic selectivity and increased hydrophobic retention in a single column.*

Reversed phase HPLC analyses are performed predominantly on straight chain alkyl phases, such as C18 or C8 columns. While this is a very effective technique for nonpolar analytes, often these phases do not provide the desired selectivity for other compounds. In such cases, phenyl columns are the primary alternative to alkyl phases. Phenyl phases undergo pi-pi interactions and, therefore, can produce alternate selectivity to alkyl phases. The conventional phenyl phase, a phenyl ring bonded through a propyl spacer (Figure 1, phenyl propyl), produces only moderate retention for hydrophobic compounds when compared to a C18 column. More recently, longer chain spacers, most commonly hexyl (Figure 1, phenyl hexyl), have been bonded to phenyl rings to increase hydrophobic retention.

Restek chemists have extensively researched phenyl stationary phases and developed the Biphenyl phase—the next generation of phenyl column. The Biphenyl phase, composed of two phenyl groups bonded end-to-end, is unique among phenyl phases in both its structure and performance (Figure 1). Rather than using a straight chain hexyl linker, the Biphenyl phase incorporates an aryl linker, making the phase both more hydrophobic than conventional phenyls and providing a larger electron cloud than single phenyl ring phases. The result is a phase that offers the highest degree of aromatic selectivity and hydrophobic retention of any phenyl phase.

### The Evolution of Resolution

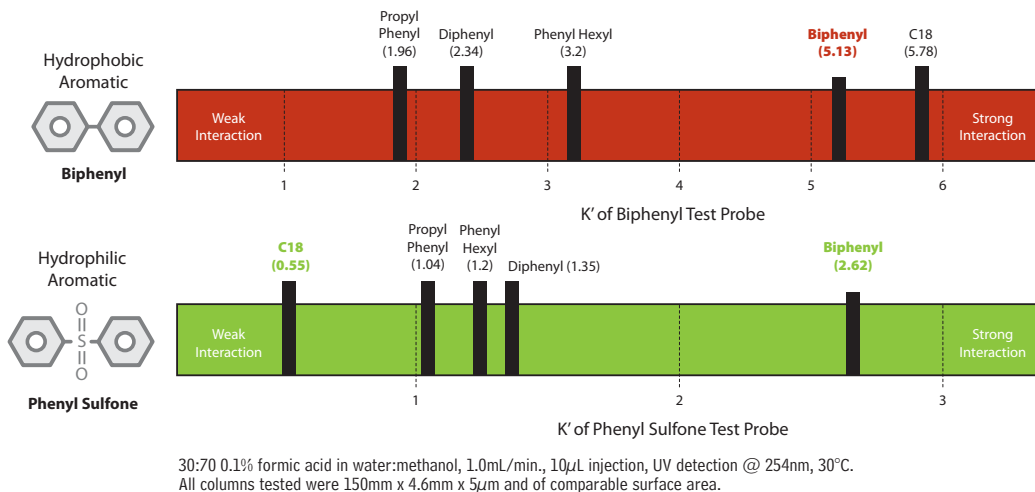
**Figure 1** The Biphenyl phase provides higher retention of both hydrophobic and hydrophilic aromatic compounds and is capable of stronger pi-pi interactions than other phenyl phases.



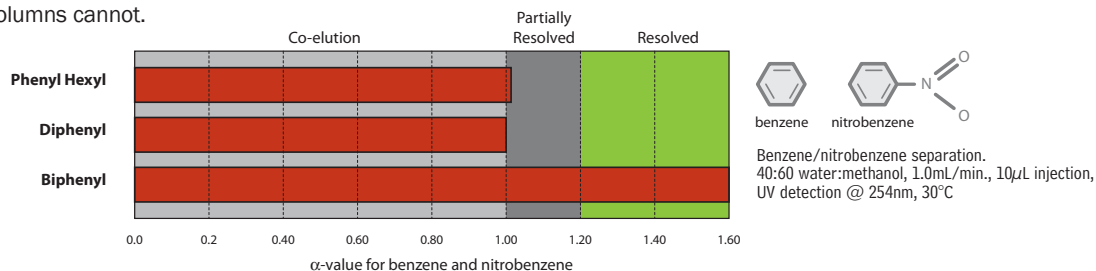
## Comparing Phenyl Stationary Phases

Comparison to other modified phenyl phases demonstrates the superior performance of the Biphenyl phase for drug compounds and residues. When comparing columns of equivalent dimensions and surface areas, the Biphenyl exhibited a much higher overall retention capacity for both hydrophobic and hydrophilic aromatics (Figure 2). More importantly, markedly better aromatic selectivity was observed. For example, higher selectivity was achieved between benzene and nitrobenzene, a compound containing a strongly electron withdrawing ring substituent which changes the electron cloud (Figure 3). When looking at a common test procedure for aromatic selectivity (pi-acidity)<sup>2</sup>, the Biphenyl again shows the greatest selectivity and the densest aromatic character of competitive phenyl phases (Figure 4). These experiments demonstrate that the Biphenyl phase is capable of stronger pi-pi interaction than other commercially available phenyl phases. Enhanced aromatic selectivity is beneficial for applications in drug development and testing where compounds commonly contain rings, conjugation, and ring substituents.

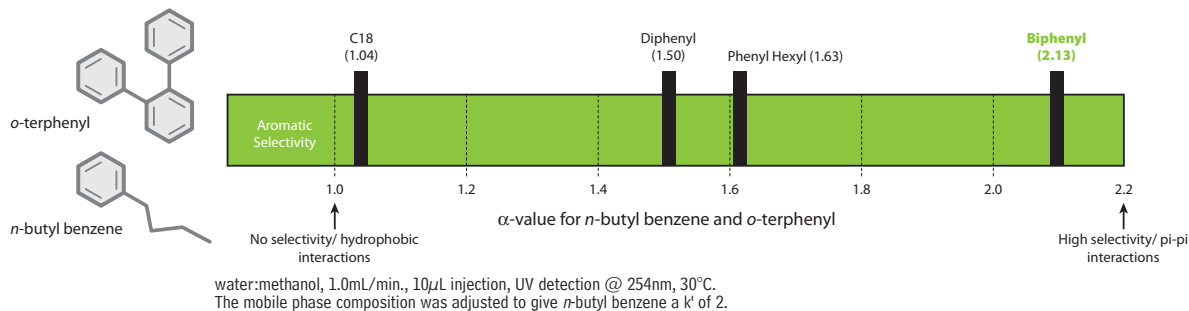
**Figure 2** The Biphenyl phase offers **exceptional retention** for both hydrophobic and hydrophilic aromatic compounds.



**Figure 3** Biphenyl columns exhibit greater **aromatic selectivity** for electron withdrawing groups, easily resolving compounds that competitive columns cannot.



**Figure 4** The Biphenyl phase offers the **greatest aromatic selectivity** among phenyl phases.



# Streamline Method Development with Versatile Biphenyl Columns

One of the biggest challenges of method development is finding the optimal stationary phase for a particular separation. The Biphenyl phase is ideal for reversed phase method development as it offers the best of both worlds—a high degree of hydrophobic interaction, much like that of a C18, plus heightened aromatic selectivity. Easy control of these two distinct separation mechanisms through mobile phase choice makes columns with a Biphenyl stationary phase ideal for method development.

## Advantages include:

- ★ Enhanced selectivity for drug compounds.
- ★ Increased retention of target pharmaceutical analytes.
- ★ Tunable selectivity—easy control of hydrophobic and aromatic separation mechanisms through mobile phase choice.
- ★ High versatility, wide variety of silicas and dimensions.

## Enhanced Selectivity for Drug Compounds

The main advantage to using phenyl phases is the ability to resolve compounds using pi-pi interactions, commonly referred to as aromatic selectivity. Phenyl phases are often used to provide alternate selectivity to a C18 column, but they also offer an effective way of resolving drug substances and impurities, which commonly contain aromatic rings or conjugated bonds, and often differ by levels of unsaturation or electron withdrawing ring substituents. The unique end-to-end bonding of the Biphenyl phase both maximizes hydrophobic retention and also increases the area available for pi-pi interactions, providing the highest level of aromatic selectivity available (Figures 5-6).

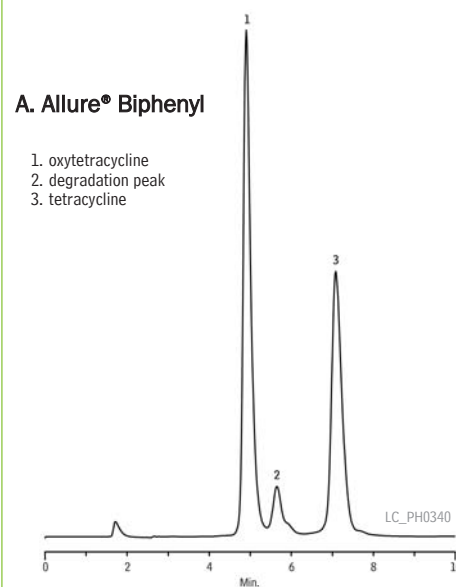
## Increased Retention of Target Pharmaceutical Analytes

Achieving adequate retention of target pharmaceutical compounds and metabolites is often a challenge, particularly for phenyl phases. Conventional phenyl phases are capable of only moderate hydrophobic retention, resulting in a lower retention capacity than that of C18 columns, significantly limiting their practical application. In comparison, the Biphenyl phase shows an overall improvement in retention capacity. In the example shown in Figure 7, only the Biphenyl phase provides the retention generally needed to separate target analytes from unretained matrix.

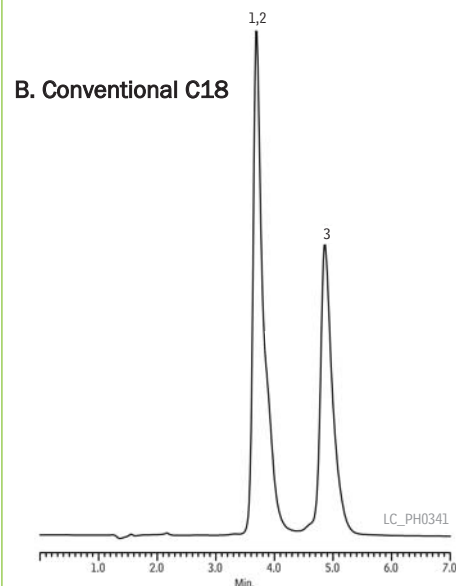
Figure 8 compares the relative retention capacities of nonsteroidal anti-inflammatory drug (NSAID) test probes on C18, conventional phenyl, and Biphenyl columns. In all cases—as is commonly seen in practice—the conventional phenyl phase yields only moderate retention compared to that of a C18 column. However, the Biphenyl phase easily achieves retention capacities similar to a C18 column—or even greater, when used with a methanolic mobile phase. The use of methanol has been noted to greatly increase the retention and selectivity of phenyl phases.<sup>2</sup> This benefit is highly pronounced when using a Biphenyl column and allows column selectivity to be “tuned” for specific separations by using mobile phase composition to control the separation mechanism.

★ *Biphenyl columns are more retentive for drug compounds than conventional phenyl and C18 phases, and are an excellent choice for LC/MS or the analysis of complex biological matrices.* ★

**Figure 5** The Biphenyl stationary phase, is more selective than a conventional C18 for tetracycline antibiotics.



**Better selectivity than a C18 in comparable analysis times.**

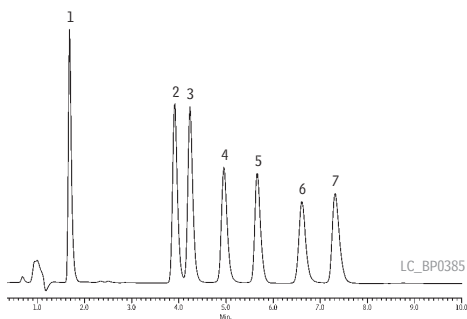


**Sample:** Inj.: 20µL; Conc.: 100µg/mL each component; Sample diluent: methanol; **Column:** Dimensions: 150 x 4.6mm; Particle size: 5µm; **Conditions:** Mobile phase: 20mM ammonium phosphate; (pH 2.5): acetonitrile, 80:20; Flow: 1mL/min.; Temp.: ambient; Det.: UV @ 254nm

**Figure 6** A Biphenyl column resolves **steroid hormones** in a simple, isocratic analysis, a separation not possible using a conventional C18.

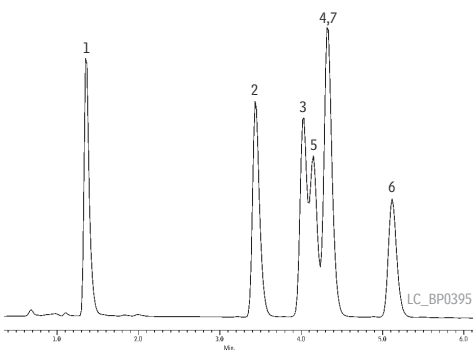
**Easily resolve target analytes.**

**A. Allure® Biphenyl**



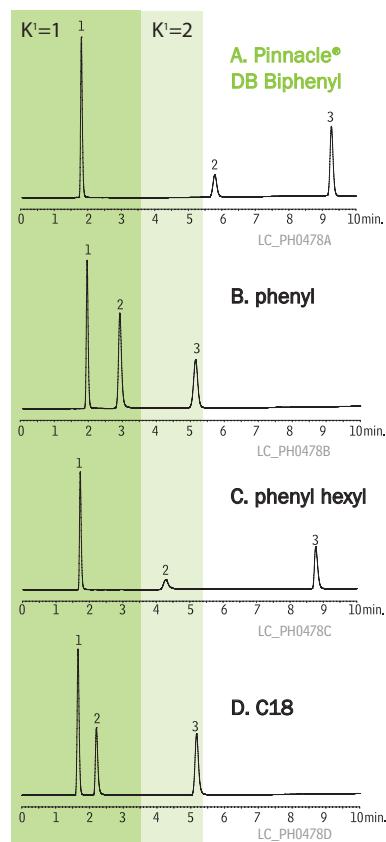
1. estriol
2. 17β-estradiol
3. 17α-estradiol
4. 17α-ethynyl estradiol
5. testosterone
6. estrone
7. norethindrone

**B. Conventional C18**



**Sample:** Inj.: 10µL; Conc.: 50µg/mL each component; Sample diluent: acetonitrile:methanol, 4:1 (v/v); **Column:** Dimensions: 150 x 4.6mm; Particle size: 3µm; Pore size: 100Å; **Conditions:** Mobile phase: water:acetonitrile, 50:50 (v/v); Flow: 1.5mL/min.; Temp.: ambient; Det.: UV @ 254nm

**Figure 7** Only the Biphenyl phase retains both **sulfur-containing drug compound** test probes to  $k' > 2$ , the level generally required for separation from unretained matrix contaminants.



**Better retention with <sup>1/2</sup> the carbon load of a phenyl hexyl.**

- Peak List:**
1. uracil (void marker)
  2. tenoxicam
  3. sulfapyrazone

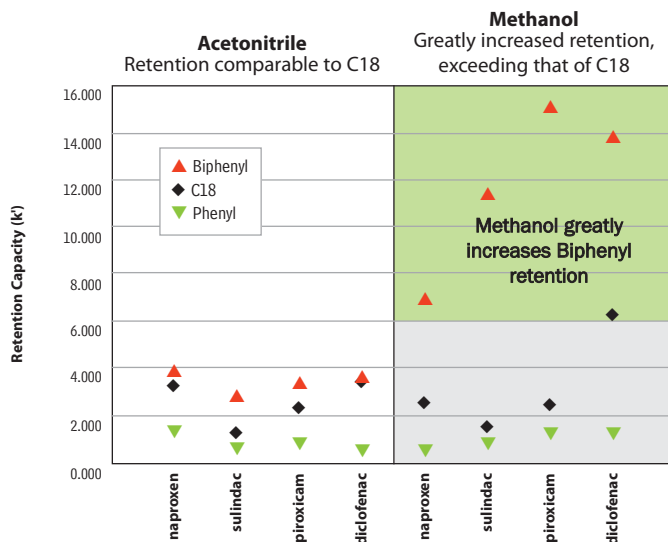
**Sample:** Inj.: 10µL; Conc.: 100µg/mL each component; Sample diluent: 40:60 water:0.1% formic acid:methanol; Dimensions: 150mm x 4.6mm; Particle size: 5µm; Pore size: 140Å

**Conditions:**  
 Mobile phase: A: water w/ 0.1% formic acid  
 B: methanol

Time (min.)	Flow (mL/min.)	%B
0.0	1.0	60
2.0	1.0	60
8.0	1.0	90
20.0	1.0	90
20.1	1.0	60

Temp.: 30°C  
 Det.: Shimadzu PDA (SPD-M20A) @ 254nm

**Figure 8** Retention on the Biphenyl phase equals or exceeds C18 and conventional phenyl phases, and is easily controlled with mobile phase choice.



For each analyte all columns were assayed under identical isocratic conditions. The equivalent elutropic strength between acetonitrile and methanol was determined by the relative retention capacities of the C18 phase.

**Columns:** 5µm, 4.6mm x 150mm; **Conditions:** Mobile phase: 10mM potassium phosphate (pH 2.5): acetonitrile or methanol; Det.: UV @ 254nm; Flow: 1.0 mL/min.

## Tunable Selectivity: Easy Control of Hydrophobic and Aromatic Separation Mechanisms

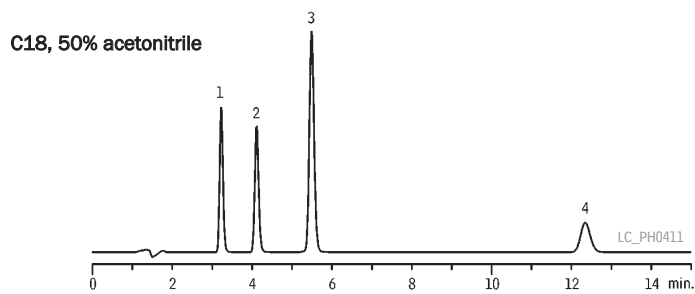
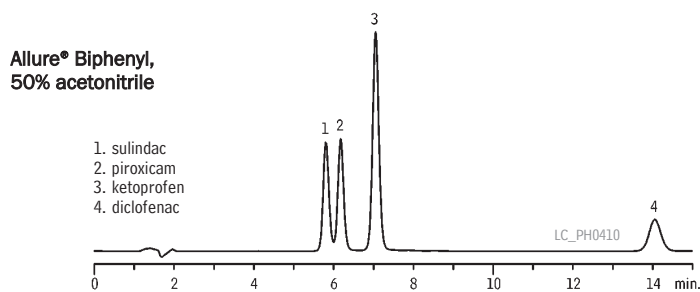
In HPLC, the mobile phase can be altered to enhance a separation or to obtain a desired resolution. With the Biphenyl phase, this can be achieved more easily through tunable selectivity, the fine control of separations attainable with simple mobile phase changes. The choice of organic solvent used in the mobile phase can alter the selectivity by switching between two separation mechanisms: dispersive (hydrophobic) interactions and pi-pi interactions. For example, as was seen in Figure 8, using acetonitrile in the mobile phase makes a Biphenyl column more C18-like in its retention and selectivity, while methanol induces aromatic selectivity. By controlling the desired levels of dispersive and pi-pi interactions—or simply mixing methanol and acetonitrile to the appropriate percentages—markedly better selectivity for molecules that differ only in degree of unsaturation, position of double bonds, or electron withdrawing groups can be achieved.

The example separation of NSAID drug compounds shown in Figure 9 illustrates the effect of increased retention and tunable selectivity, and the dramatically different selectivities that can be obtained. Using just simple mobile phase changes, orthogonal separations are easily achieved—an invaluable tool for method development. Higher organic percentages, or mixed organics, can also improve the sensitivity of MS methods by increasing desolvation efficiencies. The tunable selectivity achievable with Biphenyl phases is a powerful new tool for method development.

★ *The versatility of a Biphenyl column makes it an invaluable tool for the practicing method developer and a great addition to column screening systems.* ★

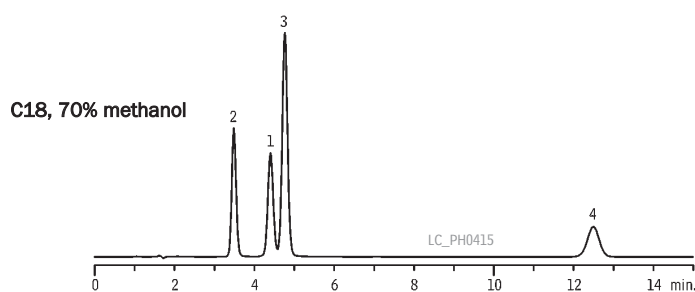
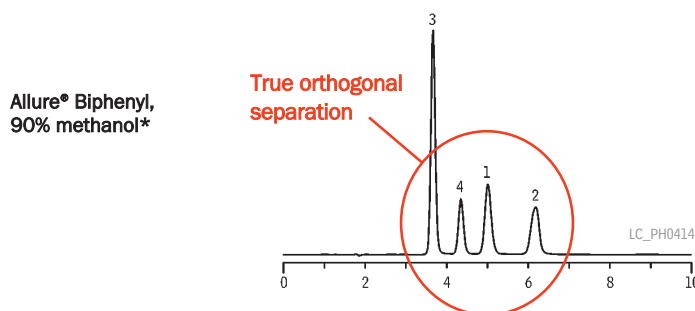
**Figure 9** Orthogonal separations are easily achieved with simple mobile phase changes, making the versatile Biphenyl phase ideal for method development.

**A. In acetonitrile, selectivity is C18-like—elution order is the same.**



Conditions: Mobile phase: 0.5% formic acid in water (pH 2.25):0.1% formic acid in acetonitrile, 50:50 (v/v); Flow: 1.0mL/min.; Temp.: ambient; Det.: UV @ 254nm.

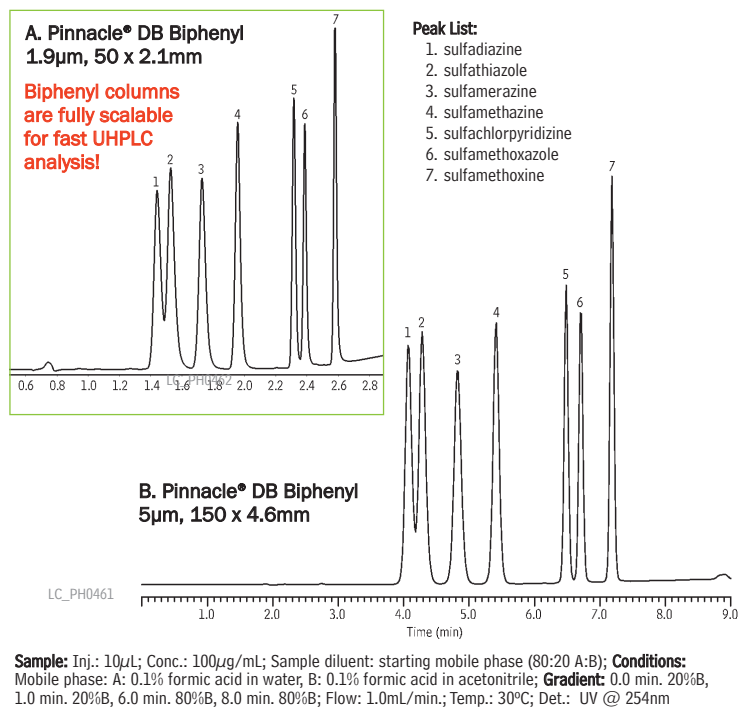
**B. In methanol, selectivity is orthogonal to a C18—elution order changes.**



Conditions: Mobile phase: 0.5% formic acid in water (pH 2.25):0.1% formic acid in methanol, 30:70 or 10:90 (v/v); Flow: 1.0mL/min.; Temp.: ambient; Det.: UV @ 254nm.

\* To maintain acceptable retention capacity organic content was increased 20%. This has the added benefit of increasing desolvation efficiency and improving sensitivity for MS applications.

**Figure 10** Using a 100% Restek manufactured 1.9µm Pinnacle® DB Biphenyl column creates a fast and selective analysis of sulfonamides that can be **easily scaled between HPLC and UHPLC**.



## High Versatility: Wide Variety of Silicas and Dimensions

Restek's Biphenyl phase is available in a wide range of silicas and sizes—choose standard or specialty options, specifically for your applications. Offered on 1.9 to 5µm silicas, and in capillary, standard, and preparative dimensions, Biphenyl columns allow you to easily scale and transfer methods (Figure 10).

The Biphenyl column offers versatility in both format and performance, with tunable selectivity designed to give optimal control of separations. Bring every advantage to method development—streamline your process with a Biphenyl column.

### References

1. M. R. Euerby, P. Petersson, W. Campbell, W. Roe, J. Chromatogr. A 1154 (2007) 138.
2. M. Yang, S. Fazio, D. Munch, P. Drumm, J. Chromatogr. A 1097 (2005) 124.



## free literature

Systematic Transfer of HPLC Methods to UHPLC

Lit. cat.# GNAN1033

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# Product Listing

## Pinnacle® DB Biphenyl (USP L11)

- ★ Restek manufactured base-deactivated silica
- ★ Available from 1.9µm to 5µm
- ★ Optimized for UHPLC, fully scalable to HPLC

### Physical Characteristics:

particle size: 1.9µm, 3µm, or 5µm, spherical      pore size: 140Å      pH range: 2.5 to 7.5  
carbon load: 8%      temperature limit: 80°C  
endcap: yes

1.9µm Column, 2.1mm	cat. #	5µm Column, 2.1mm	cat. #
30mm	9409232	30mm	9409532
50mm	9409252	50mm	9409552
100mm	9409212	100mm	9409512
3µm Column, 2.1mm	cat. #	150mm	9409562
30mm	9409332	200mm	9409522
50mm	9409352	250mm	9409572
100mm	9409312	5µm Column, 3.2mm	cat. #
150mm	9409362	30mm	9409533
3µm Column, 3.2mm	cat. #	50mm	9409553
30mm	9409333	100mm	9409513
50mm	9409353	150mm	9409563
100mm	9409313	200mm	9409523
150mm	9409363	250mm	9409573
3µm Column, 4.6mm	cat. #	5µm Column, 4.6mm	cat. #
30mm	9409335	30mm	9409535
50mm	9409355	50mm	9409555
100mm	9409315	100mm	9409515
150mm	9409365	150mm	9409565
		200mm	9409525
		250mm	9409575

★ ★ ★ ★ ★ ★ ★

Restek manufactures the silica for select column lines, giving us total control over quality and reproducibility.

★ ★ ★ ★ ★ ★ ★

# Product Listing

## Pinnacle® II Biphenyl (USPL11)

- ★ Restek manufactured silica
- ★ Ideal for reproducible analysis of acidic and neutral compounds

### Physical Characteristics:

particle size: 5µm, spherical      pH range: 2.5 to 7.5  
 pore size: 110Å                      temperature limit: 80°C  
 endcap: yes

5µm Column, 4.6mm	cat. #
150mm	9209565
250mm	9209575

## Allure® Biphenyl (USP L11)

- ★ High purity, high surface area silica
- ★ Excellent choice for maximum retention and LC/MS

### Physical Characteristics:

particle size: 5µm, spherical      endcap: yes  
 pore size: 60Å                      pH range: 2.5 to 7.5  
 carbon load: 23%                  temperature limit: 80°C

5µm Column, 2.1mm	cat. #
30mm	9166532
50mm	9166552
100mm	9166512
150mm	9166562
200mm	9166522
250mm	9166572
5µm Column, 3.2mm	cat. #
30mm	9166533
50mm	9166553
100mm	9166513
150mm	9166563
200mm	9166523
250mm	9166573
5µm Column, 4.6mm	cat. #
30mm	9166535
50mm	9166555
100mm	9166515
150mm	9166565
200mm	9166525
250mm	9166575

★ ★ ★ ★ ★ ★ ★ ★

Many other dimensions available—from capillary to prep, from UHPLC to HPLC. Visit [www.restek.com/biphenyl](http://www.restek.com/biphenyl) for a complete product listing.

★ ★ ★ ★ ★ ★ ★ ★

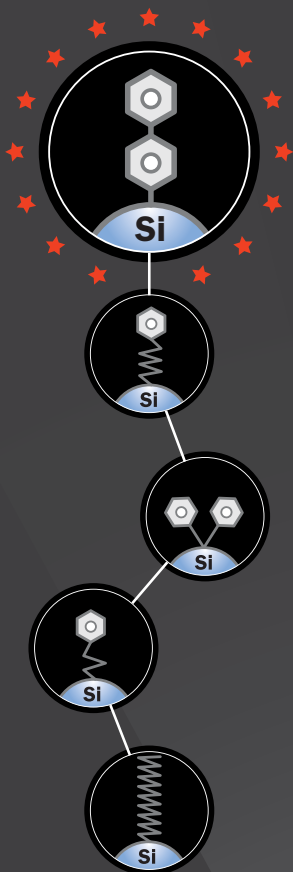
## Viva Wide Pore Biphenyl (USP L11)

- ★ Restek manufactured wide-pore silica
- ★ Designed for optimal separation of biomolecules and other large molecules

### Physical Characteristics:

particle size: 5µm                      endcap: yes  
 pore size: 300Å                      pH range: 2.5 to 7.5  
 carbon load: 6.7%                  temperature limit: 80°C

5µm Column, 2.1mm	cat. #
30mm	9516532
50mm	9516552
100mm	9516512
150mm	9516562
200mm	9516522
250mm	9516572
5µm Column, 3.2mm	cat. #
30mm	9516533
50mm	9516553
100mm	9516513
150mm	9516563
200mm	9516523
250mm	9516573
5µm Column, 4.6mm	cat. #
30mm	9516535
50mm	9516555
100mm	9516515
150mm	9516565
200mm	9516525
250mm	9516575



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