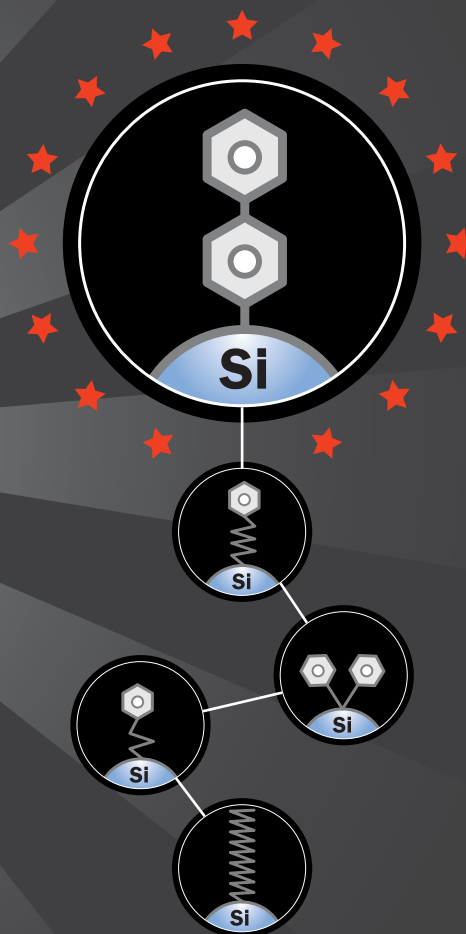


RESOLUTION EVOLUTION



BIPHENYL Next Generation
of Phenyl Column



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RESOLUTION EVOLUTION



The Next Generation of Phenyl Column Chemistry

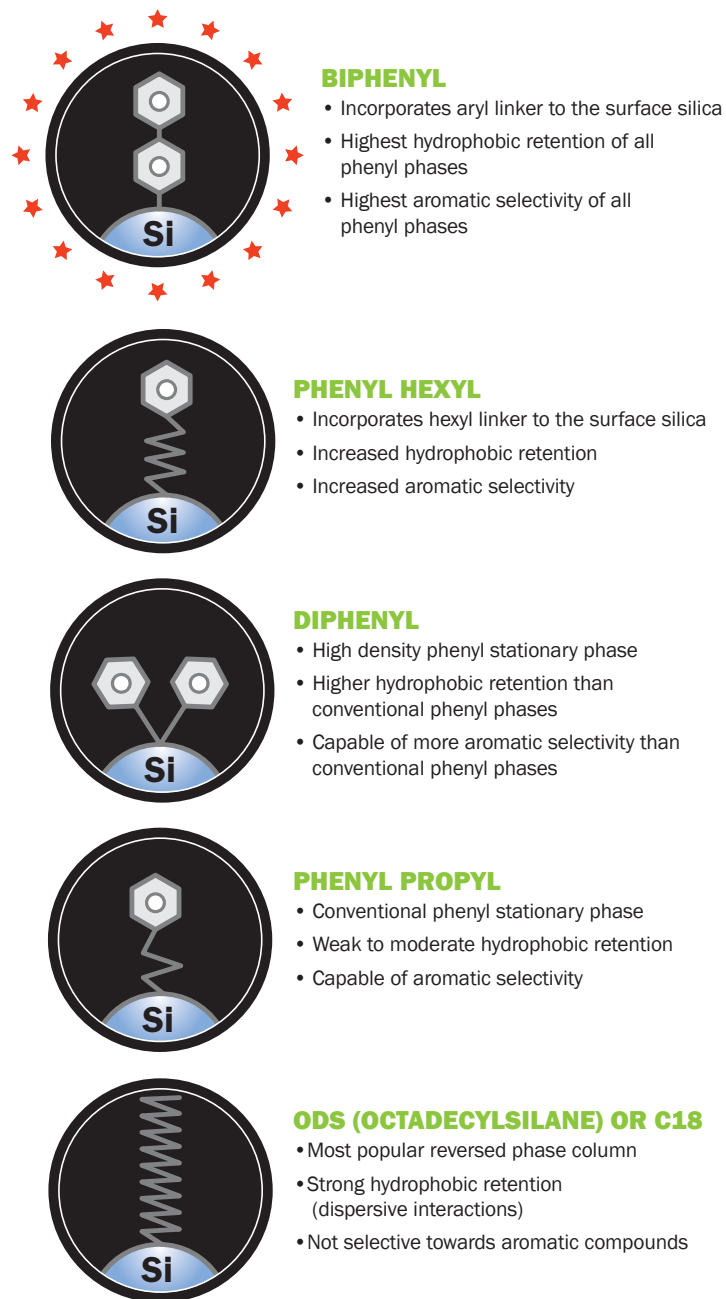
The Biphenyl phase is the next generation of phenyl column chemistries, providing both outstanding aromatic selectivity and increased hydrophobic retention in a single column.

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 for reversed phase analyses. 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, produces only moderate retention for hydrophobic compounds when compared to a C18 column. More recently, longer chain spacers, most commonly 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 (phenyl propyl, diphenyl, and phenyl hexyl), 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, under identical conditions, between benzene and nitrobenzene, a compound containing a strongly electron withdrawing ring substituent (Figure 3). When looking at a common test procedure for aromatic selectivity (pi-acidity)¹, 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 the **highest retention of phenyl phases** for both hydrophobic and hydrophilic aromatic compounds.

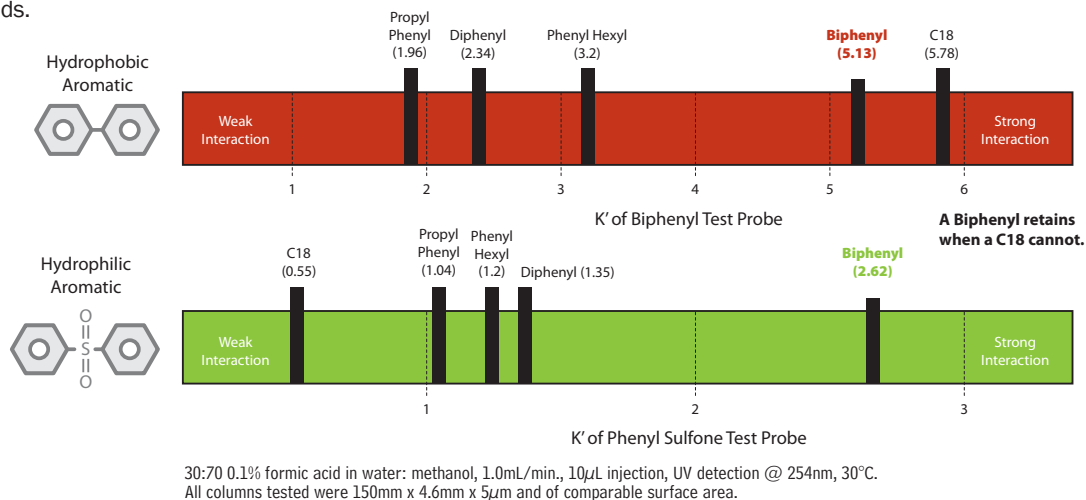


Figure 3 Biphenyl columns exhibit greater **aromatic selectivity** for electron withdrawing groups, fully resolving compounds that competitive columns cannot.

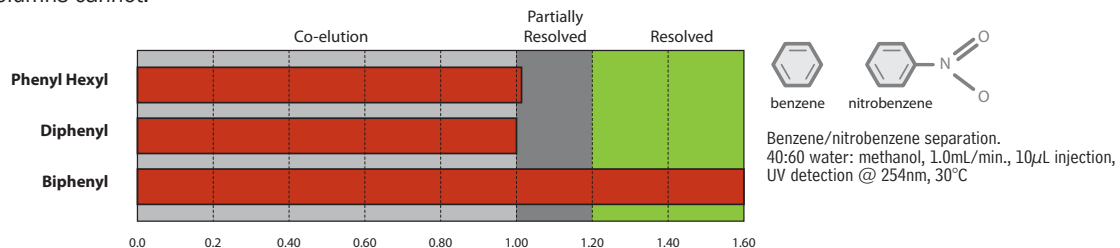
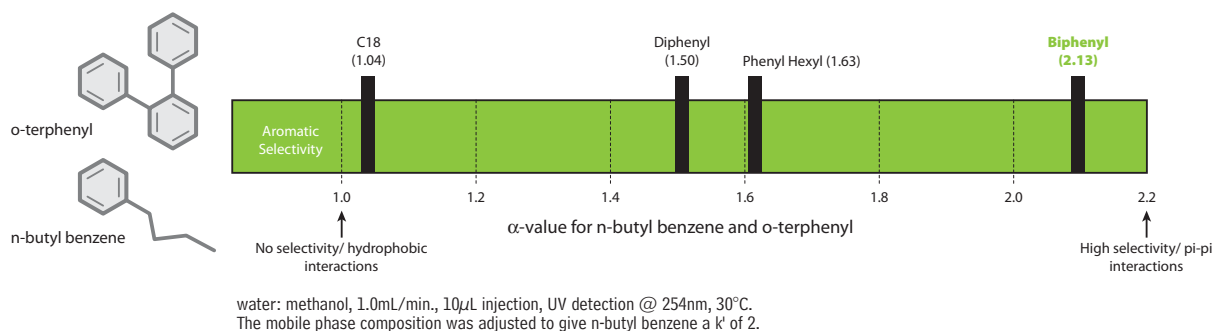


Figure 4 The Biphenyl phase offers the greatest **aromatic selectivity** among phenyl phases.



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 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.
- ★ 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).

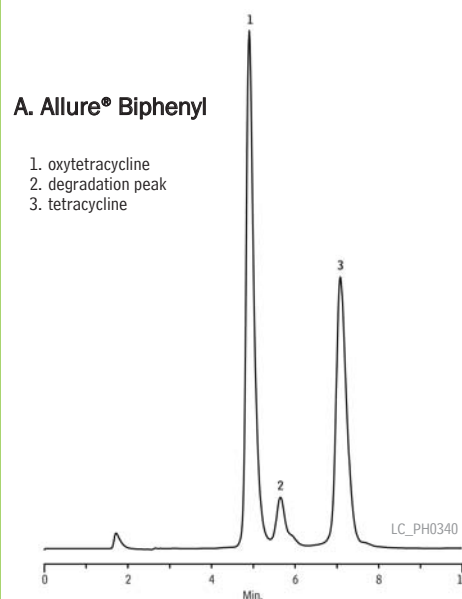
Increased Retention of Target Pharmaceutical Analytes

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.

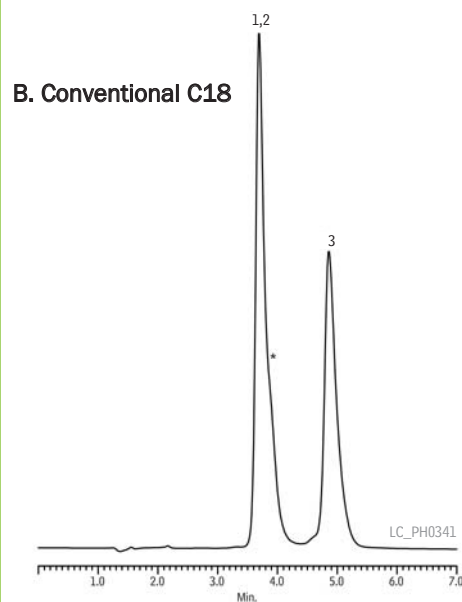
Figure 8 compares the relative retention capacities of nonsteroidal anti-inflammatory drug (NSAID) test probes on C18, conventional phenyl, and Biphenyl columns. In all cases—as is commonly seen in practice—the conventional phenyl phase yields only moderate retention compared to that of a C18 column. 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. 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 work 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.

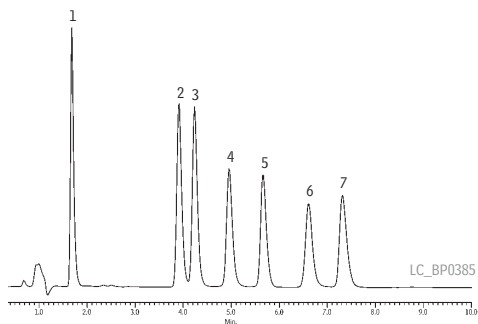


Sample: Inj.: 20µL; Conc.: 100µg/mL each component; Sample diluent: methanol; **Column:** Dimensions: 150 x 4.6 mm; Particle size: 5µm; **Conditions:** Mobile phase: 20mM ammonium phosphate; (pH 2.5): acetonitrile, 80:20; Flow: 1mL/min.; Temp.: ambient; Det.: UV @ 254 nm

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

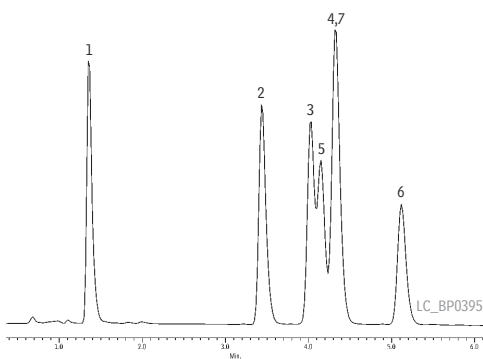
Easily resolve target analytes.

A. Allure® Biphenyl



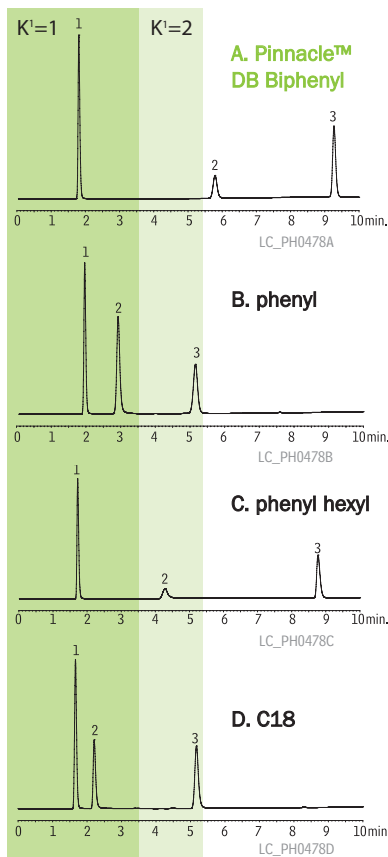
1. estriol
2. 17β-estradiol
3. 17α-estradiol
4. 17α-ethynyl estradiol
5. testosterone
6. estrone
7. norethindrone

B. Conventional C18



Sample: Inj.: 10µL; Conc.: 50µg/mL each component; Sample diluent: acetonitrile:methanol, 4:1 (v/v); **Column:** Dimensions: 150 x 4.6 mm; 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 ^{1/2} the carbonload 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

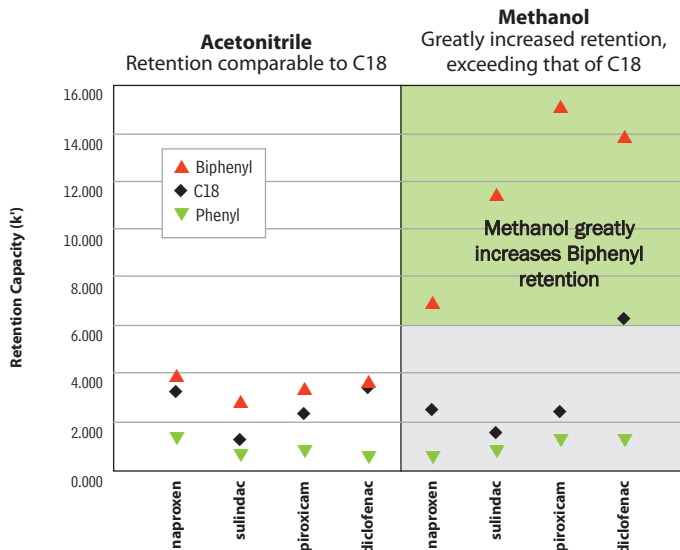
Conditions:

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

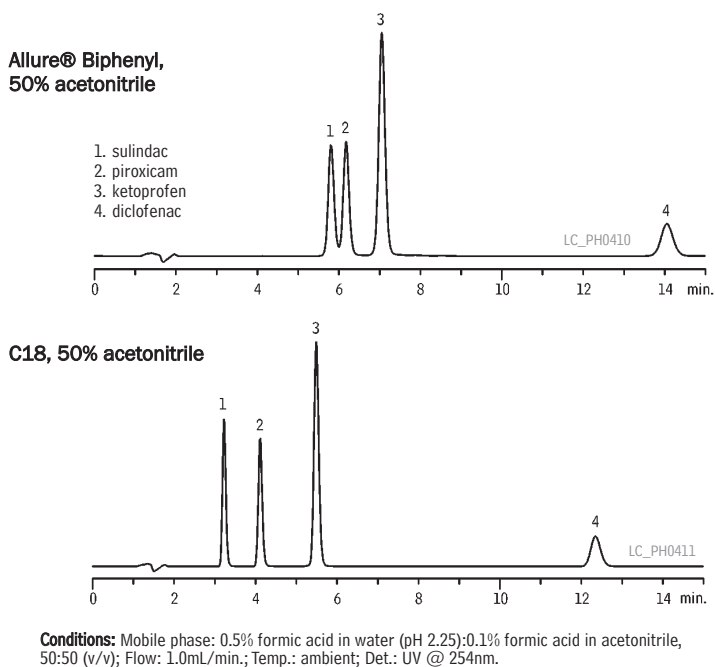
Often in HPLC, the mobile phase can be altered to enhance a separation or to obtain a desired resolution. With the Biphenyl phase, this can be achieved more easily through tunable selectivity, the fine control of separations attainable with simple mobile phase changes. The choice of organic used in the 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.

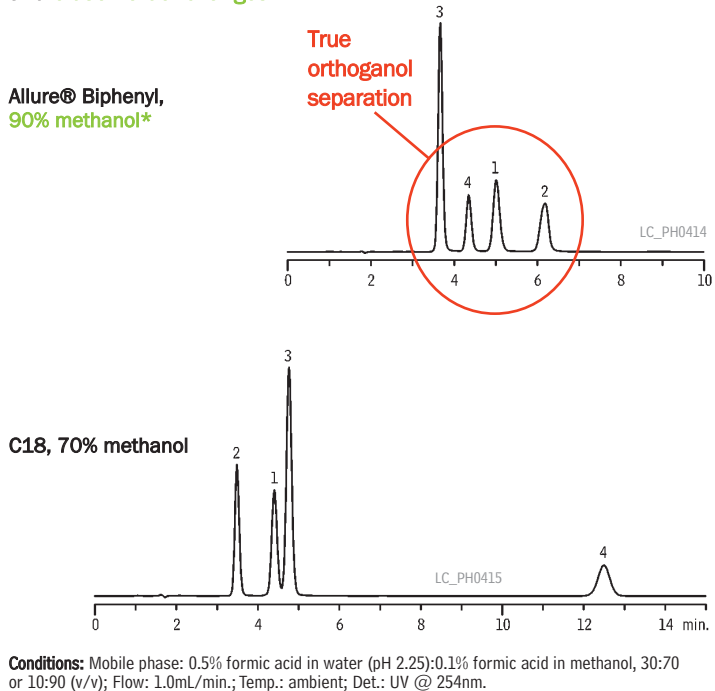
★ *The versatility of a Biphenyl column makes it an invaluable tool for the practicing method developer and a great addition to column screening systems.* ★

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

A. In acetonitrile, elution order is the same.

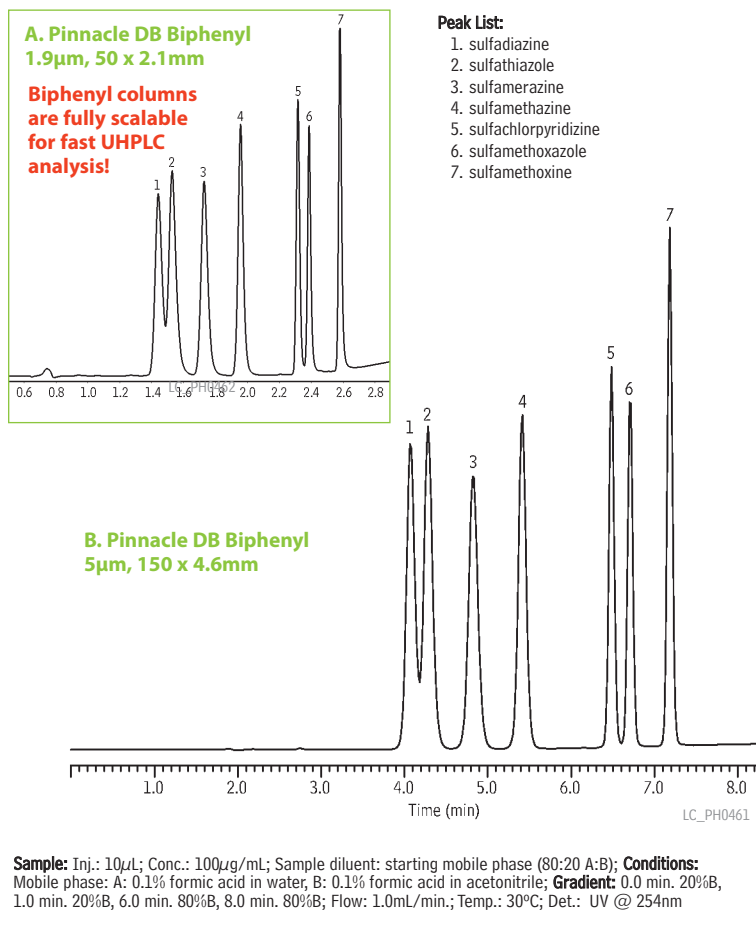


B. In methanol, retention is greater on an Allure® Biphenyl column, and elution order changes.



* 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.

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Systematic Transfer of HPLC Methods to UHPLC

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