Beyond C18—Increase Retention of Hydrophilic Compounds Using Biphenyl Columns

*Searching for a better way to retain hydrophilic aromatic drug compounds? Biphenyl phases, such as the **Pinnacle® DB Biphenyl** column, provide greater retention than alkyl phases. Use a Biphenyl column to separate difficult-to-retain polar aromatics from unretained matrix contaminants.*

By Amanda Rigdon, Pharmaceutical Innovations Chemist and Rick Lake, Pharmaceutical Market Development Manager

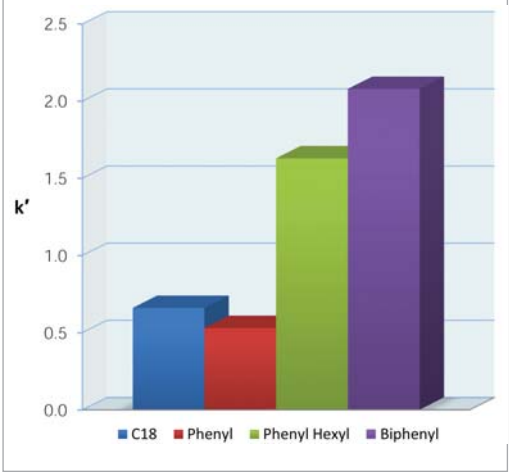
Many drug classes include compounds with aromatic ring structures, some of which also contain a sulfone or sulfoxide group. Both sulfur groups have dipole moments, adding a hydrophilic character to compounds containing these functional groups. The analysis of hydrophilic compounds on a traditional alkyl column (e.g., C18) can be problematic, since alkyl columns depend on hydrophobic (dispersive) interactions for retention. Since the sulfone and sulfoxide groups contain π bonds, the Biphenyl column's affinity toward compounds containing these bonds makes it a logical choice when increased retention of compounds containing these groups is desired.

To explore the selectivity of the biphenyl phase towards sulfur-containing aromatic compounds, phenyl sulfone, a simple probe, was analyzed on alkyl (C18), phenyl, phenyl hexyl, and Biphenyl columns to determine the relative retention of each phase, as measured by capacity factor (k'). In order to ensure separation of analytes from unretained contaminants, a minimum k' value of 2 is recommended for most analyses, however in cases where there is little to no matrix interference, a k' of 1 may be acceptable. The data in Figure 1 show that phenyl sulfone is retained to a much greater degree on the Pinnacle® DB Biphenyl column, than on the other phases tested (k' = 2.08). This is due to the unique retention mechanism of the biphenyl stationary phase, which can interact with both the hydrophobic aromatic ring and the hydrophilic sulfone group through π-π interactions. Although the phenyl stationary phase also allows for the use of π-π interactions, the biphenyl phase has a larger electron cloud and is significantly more retentive.

To further test the retention of the Biphenyl column, a second set of probes, consisting of compounds in the NSAID family, was analyzed. Tenoxicam, which contains a sulfone group, and sulfinpyrazone, which contains a sulfoxide group, were analyzed along with a void marker (uracil). Although these compounds are more complex than the probe used in the first experiment, the same pattern of retention was observed (Figure 2). The Pinnacle® DB Biphenyl column exhibited the greatest retention for tenoxicam. With k' values of 0.33 on the C18 and 0.49 on the phenyl columns, tenoxicam shows almost no retention on these stationary phases. The phenyl hexyl phase performed slightly better with a k' value of 1.52 for tenoxicam. However, when tenoxicam was analyzed on the Biphenyl column under the same conditions, the k' value increased to 2.22, a value much more likely to provide adequate resolution from matrix components. Sulfinpyrazone, a less polar compound, also followed the same pattern of retention (Table I).

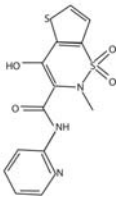
The improved retention for hydrophilic aromatics shown here is due to the unique π-π interaction retention mechanism of the Biphenyl phase. This mechanism is particularly useful for analysis of sulfone- and sulfoxide-containing drug compounds, which are not easily retained on alkyl or phenyl phases. The Biphenyl phase provides greater retention than alkyl and phenyl phases and is ideal for separating difficult-to-retain polar aromatics from unretained matrix contaminants.

Figure 1 The Biphenyl phase is more retentive for phenyl sulfone than other alkyl and phenyl phases.

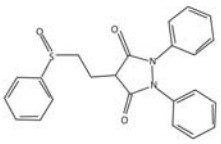


Biphenyl columns are much more effective than alkyl, phenyl, or phenyl hexyl phases when increased retention of hydrophilic aromatics is desired.

Figure 2 Only the Biphenyl phase retains both test probes to k' > 2, the level recommended to ensure separation from unretained matrix contaminants.



2. tenoxicam



3. sulfinpyrazone

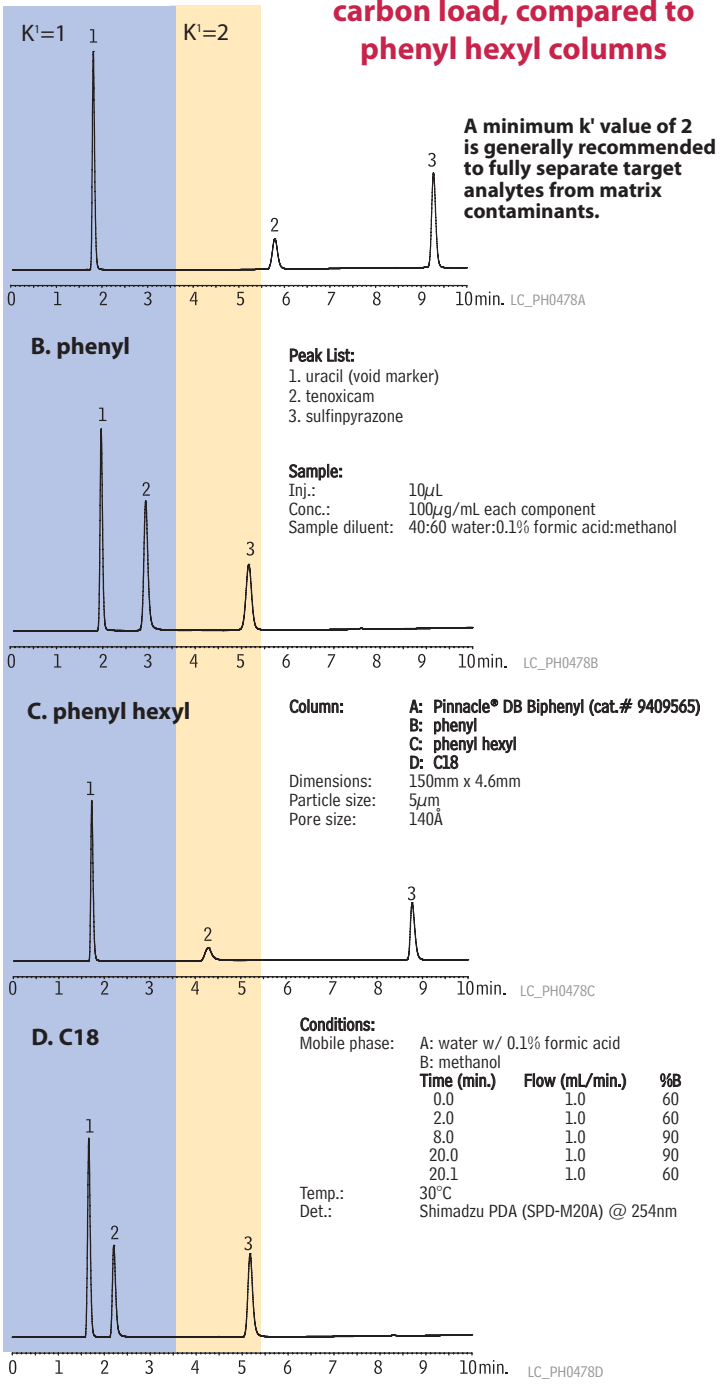


Table I Biphenyl columns show improved retention of sulfone- and sulfoxide-containing aromatic drugs.

K' Value	Biphenyl	Phenyl hexyl	Phenyl	C18
Tenoxicam	2.23	1.39	0.637	0.235
Sulfinpyrazone	4.18	3.90	1.88	1.89

Product Listing

Pinnacle® DB Biphenyl Columns (USP L11)

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

3µm Column, 1.0mm	cat. #	price
30mm	9409331	
50mm	9409351	
100mm	9409311	
150mm	9409361	
3µm Column, 2.1mm	**cat. #**	**price**
30mm	9409332	
50mm	9409352	
100mm	9409312	
150mm	9409362	
3µm Column, 3.2mm	**cat. #**	**price**
30mm	9409333	
50mm	9409353	
100mm	9409313	
150mm	9409363	
3µm Column, 4.6mm	**cat. #**	**price**
30mm	9409335	
50mm	9409355	
100mm	9409315	
150mm	9409365	
5µm Column, 1.0mm	**cat. #**	**price**
30mm	9409531	
50mm	9409551	
100mm	9409511	
150mm	9409561	
200mm	9409521	
250mm	9409571	
5µm Column, 2.1mm	**cat. #**	**price**
30mm	9409532	
50mm	9409552	
100mm	9409512	
150mm	9409562	
200mm	9409522	
250mm	9409572	
5µm Column, 3.2mm	**cat. #**	**price**
30mm	9409533	
50mm	9409553	
100mm	9409513	
150mm	9409563	
200mm	9409523	
250mm	9409573	
5µm Column, 4.6mm	**cat. #**	**price**
30mm	9409535	
50mm	9409555	
100mm	9409515	
150mm	9409565	
200mm	9409525	
250mm	9409575	

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