



Separating NSAIDs through Aromatic Selectivity

Improve Retention by Using An Allure® Biphenyl HPLC Column

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- Optimize retention and selectivity of non-steroidal anti-inflammatory drugs, for better separations.
- Orthogonal separations with simple mobile phase changes
- Increased retention requires higher organic content, increasing desolvation efficiency in LC/MS.

Non-steroidal anti-inflammatory drugs (NSAIDs), in either prescribed or over-the-counter formulations, are widely used to treat pain, fever, and inflammation. While steroidal anti-inflammatory drugs all share a similar, four-ring chemical structure, NSAIDs have more diverse chemical structures, complicating their analysis. The work we report here is based on three common classes of NSAIDs: arylalkanoic acids, 2-arylpropionic acids (profens), and oxicams.

NSAIDs have a high carbon to heteroatom ratio and, therefore, historically have been separated through reversed phase HPLC on C18 columns. A conventional C18 stationary phase separates compounds based mainly on their overall hydrophobicity. Considering the carbon to heteroatom ratio, this is an effective separation mechanism for NSAIDs. Newer stationary phases are available, however, and we set out to determine if other phases, using other separation mechanisms, such as π - π interactions, could be more effective for assaying NSAIDs.

When selecting a stationary phase, it is advantageous to exploit inherent differences in the target analytes' chemical structures. Among these three classes of NSAIDs, there are some common functional groups, like halogens, amines, and carboxylic acids, but no one group is shared across the entire list of analytes (Figure 1). However, all of the target analytes do share one basic structural component – the six-carbon aromatic ring. Aromatic rings are common components of drug molecules, and they can be targeted using a phenyl-based stationary phase.

As a retention mechanism, phenyl stationary phases employ π - π interactions between the phenyl groups in the stationary phase and any unsaturated bonds in the analyte. The use of conventional phenyl phases has been somewhat limited due to their moderate retention capacity, relative to that of a C18 phase. Figure 2 illustrates the relative retention capacities of NSAID test probes on an Allure® Biphenyl column, a conventional phenyl column and a C18 column. Note that, in all cases, as commonly seen in practice, the conventional phenyl phase yields only moderate retention compared to that of a C18 column. However, the Allure® Biphenyl phase, which is a stationary

Figure 1 Aromatic rings make NSAIDs candidates for separation through π - π interactions.

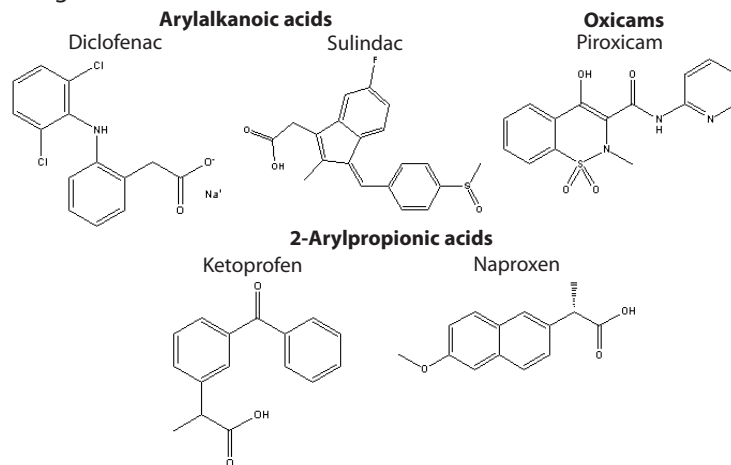
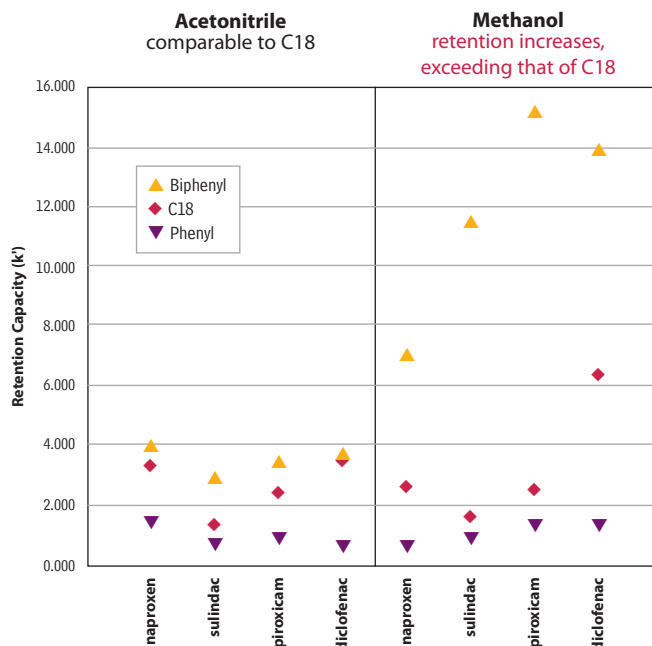


Figure 2 The retention capacity of the Allure® Biphenyl phase far exceeds that of conventional phenyl phases.



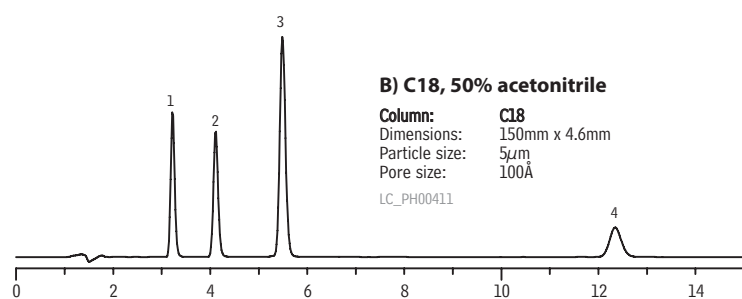
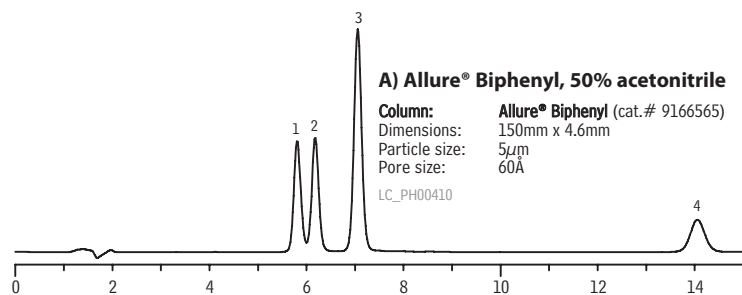
For each analyte all columns were assayed under identical isocratic conditions. The equivalent elutropic strength between acetonitrile and methanol was determined by the relative retention capacities of the C18 phase.

Columns: 5 μ m, 4.6mm x 150mm
 Mobile Phase: 10mM potassium phosphate (pH 2.5): acetonitrile or methanol
 Det.: UV @ 254nm
 Flow: 1.0 mL/min.

Figure 3 The versatility of the Allure® Biphenyl phase makes it a great alternative to conventional phenyl phase columns, especially in method development.

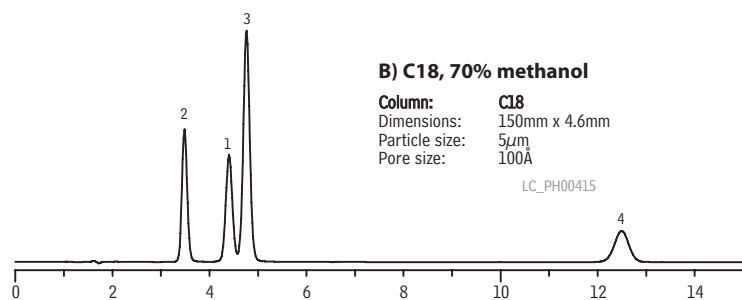
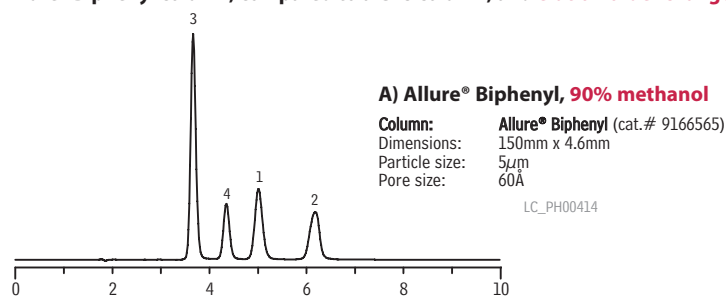
Sample: 1. sulindac
 Inj.: 5µL 2. piroxicam
 Conc.: ~300µg/mL each component 3. ketoprofen
 Sample diluent: mobile phase 4. diclofenac

In acetonitrile, retention of NSAIDs on an Allure® Biphenyl column is comparable to retention on a C18 column and 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

In methanol, retention capacity & selectivity of NSAIDs are much greater on an Allure® Biphenyl column, compared to a C18 column, and 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

phase composed of two phenyl groups bonded end-to-end, easily achieves retention capacities similar to, and even greater than, those of a C18 column when used with a highly organic mobile phase. For this reason, we evaluated the enhanced retention of the Allure® Biphenyl column for assaying NSAIDs through aromatic selectivity.

First, we compared the retention characteristics of a conventional C18 column and an Allure® Biphenyl column, using acetonitrile as the organic modifier. As expected, the Allure® Biphenyl column exhibited similar retention under equivalent analytical conditions (Figure 3). But, when we assayed the same analytes, using methanol as the organic modifier, we found retention on the Allure® Biphenyl column was *greatly increased*. To maintain the same retention capacities (*k'*) between the columns, we had to increase the organic content by 20% (Figure 3). In addition, selectivity between the two columns became dramatically different. Based on these results, we conclude that methanol in the mobile phase enhances π - π interactions between aromatic compounds and the biphenyl stationary phase, leading to greater retention and superior selectivity.

An Allure® Biphenyl column, in combination with a methanol-containing mobile phase, significantly improves separations of NSAIDs, or other aromatic drug compounds. Increased retention capacity creates a need for a higher percentage of organic solvent in the mobile phase, to elute the analytes in a timely manner. Increasing the organic content, in turn, increases sensitivity in LC/MS methods, because it optimizes the desolvation efficiency in electrospray interfaces. And this, in turn, makes an Allure® Biphenyl column the best choice for separating aromatics.

Allure® Biphenyl Columns (USP L11)

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, 4.6mm	cat. #
150mm	9166565

For other dimensions of these columns, visit our website at www.restek.com

Allure® Guard Cartridges

Allure Biphenyl	qty.	cat. #
10 x 2.1mm	3-pk.	916650212
10 x 4.0mm	3-pk.	916650210
20 x 2.1mm	2-pk.	916650222

