

# Optimizing Difficult Separations of Steroids

## Using an Allure™ Biphenyl HPLC Column

By Rick Lake, Pharmaceutical Innovations Chemist

- Increase resolution while using simple, isocratic conditions.
- Achieve separations not possible on a C18 column.
- Rugged and reproducible analyses.

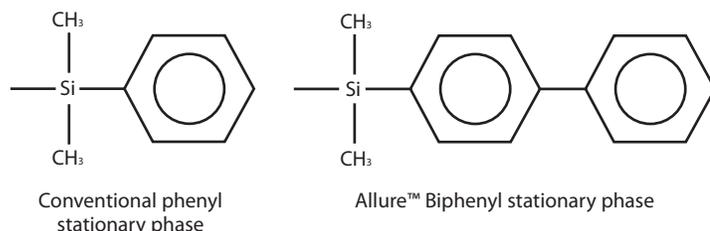
Steroids are an unusual class of compounds, in that all structural variation is centered on a common conjugated ring system, with differences in double bonding and ring constituents producing chemical diversity. Because of the consistency in their chemical structures, it can be difficult to achieve adequate separation of steroids on an alkyl (e.g., C18) HPLC stationary phase. An optimized stationary phase can be the key to these analyses.

When choosing a stationary phase, a separation mechanism that utilizes inherent differences in the chemical structures of the target analytes should be used. For analyses in which the target analytes are structurally very similar, this is especially critical. For steroids, this includes separations based on pi-pi ( $\pi$ - $\pi$ ) interactions between aromatic or unsaturated moieties: a stationary phase containing phenyl groups forms  $\pi$ - $\pi$  bonds as the phenyl group on the stationary phase overlaps with the aromatic rings or double bonds in the analytes.

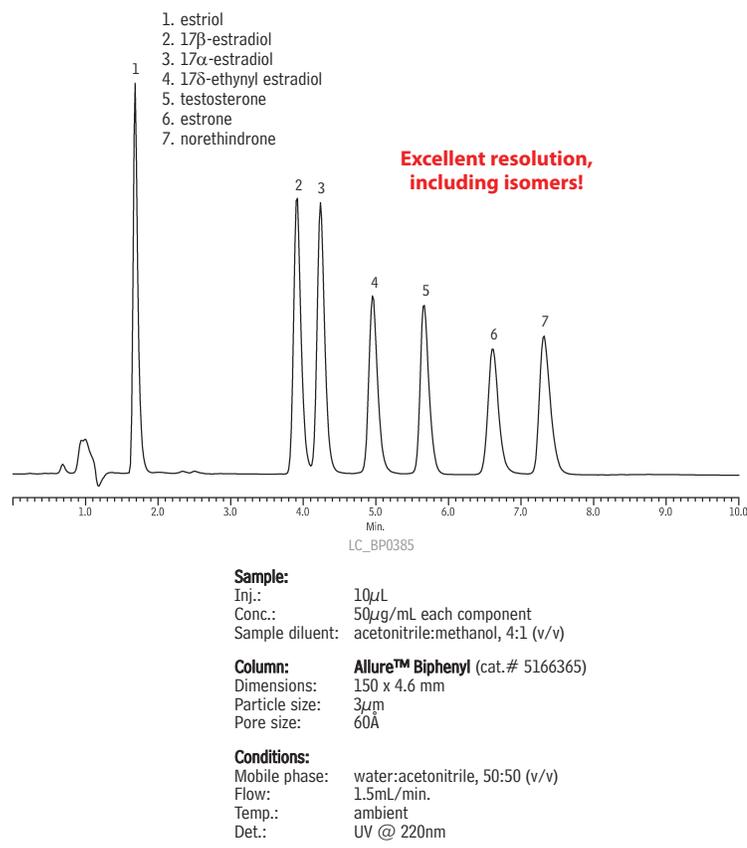
Restek chemists have made significant advancements in phenyl stationary phase chemistry, to increase retention of unsaturated compounds in reversed phase HPLC applications, while enhancing selectivity. The Allure™ Biphenyl stationary phase is a product of these advancements. A typical silica-based phenyl stationary phase consists of a single phenyl group bonded to a silica backbone (Figure 1). By developing a phase that consists of two phenyl groups bonded end-to-end, the Allure™ Biphenyl offers a more concentrated arrangement of phenyl groups, in a sterically favorable positioning (Figure 1). This phase shows markedly better selectivity for unsaturated compounds and shows a high retention capacity, similar to that of a C18 phase.<sup>1-3</sup>

We assayed two groups of steroids, hormones and corticosteroids, on an Allure™ Biphenyl column, to determine if separation can be enhanced by exploiting differences in  $\pi$ - $\pi$  interactions. First, we compared performances by the Allure™ Biphenyl column and a conventional C18 column of the same dimensions, using a complex mix of steroid hormones. Under identical isocratic analytical conditions, the Allure™ Biphenyl column resolved all target compounds (Figure 2), but the C18 column showed very limited resolving power (Figure 3).

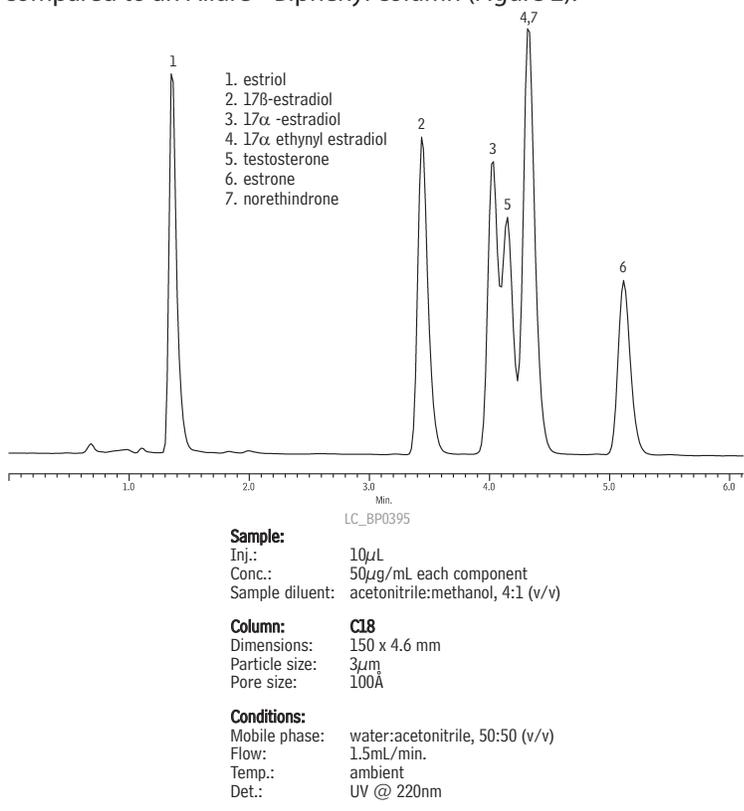
**Figure 1** The unique chemistry of the Allure™ Biphenyl stationary phase creates a more concentrated phenyl arrangement in a sterically favorable positioning.



**Figure 2** An Allure™ Biphenyl column resolves steroid hormones in a simple, isocratic analysis.



**Figure 3** A C18 column shows less resolving capability for steroids, compared to an Allure™ Biphenyl column (Figure 2).



The Allure™ Biphenyl column also provided an overall increase in analyte retention – a very useful improvement relative to conventional phenyl phases.

The Allure™ Biphenyl column also showed enhanced selectivity in a second analysis, using corticosteroids. Under simple isocratic conditions, the Allure™ Biphenyl column provided baseline separation of hydrocortisone and prednisone and, more important, resolved isomers betamethasone and dexamethasone (Figure 4).

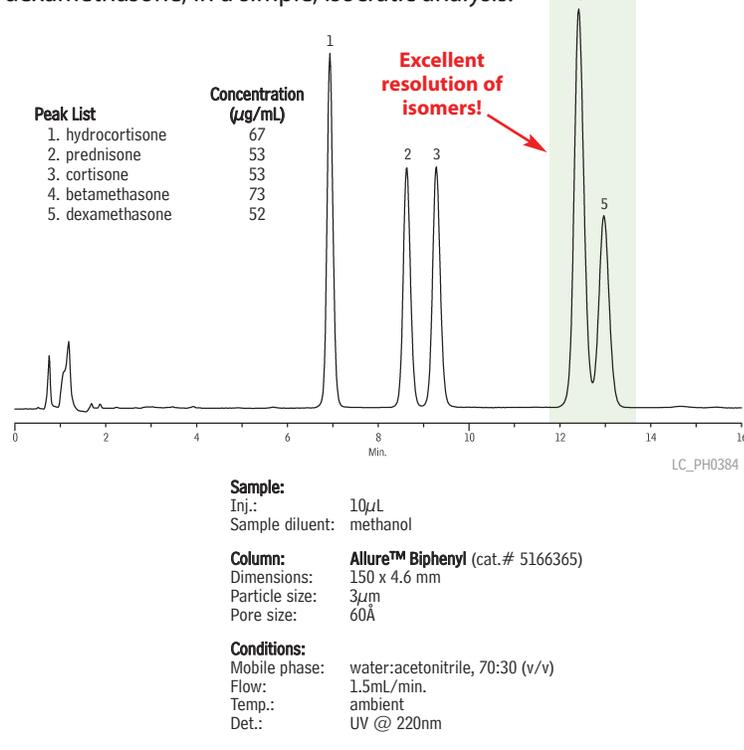
These analyses show that markedly better selectivity for steroids easily can be achieved, by using an Allure™ Biphenyl column under simple isocratic conditions. High retention capacity, similar to that of an ODS phase, also is demonstrated; a useful feature unavailable from conventional phenyl phases. By increasing π-π interactions, the Allure™ Biphenyl stationary phase offers a unique and more effective alternative to hydrophobic alkyl phases for resolving chemically similar unsaturated compounds, such as steroids.

#### References

1. *Superior Separations of Unsaturated Compounds by HPLC* Restek Advantage 2005v4 (lit. cat.# 580022).
2. *Improved HPLC Analysis of Steroids* Restek Application Note (lit. cat.# 580020).
3. Lake, R., and Wittrig, R., *Increasing HPLC Retention and Selectivity for Unsaturated Compounds, Using π-π Interactions* Pharmaceutical Canada, June 2006.

References 1&2 available on request.

**Figure 4** An Allure™ Biphenyl column resolves all target corticosteroids, including isomers betamethasone and dexamethasone, in a simple, isocratic analysis.



#### Allure™ Biphenyl Columns

3μm Column, 2.1mm	cat. #	price
30mm	9166332	\$364
50mm	9166352	\$364
100mm	9166312	\$390
3μm Column, 3.2mm		
30mm	9166333	\$364
50mm	9166353	\$364
100mm	9166313	\$390
3μm Column, 4.6mm		
30mm	9166335	\$364
50mm	9166355	\$364
100mm	9166315	\$390
150mm	5166365	\$405
3μm Column, 2.1mm		
30mm (with Trident™ Inlet Fitting)	9166332-700	\$379
50mm (with Trident™ Inlet Fitting)	9166352-700	\$379
100mm (with Trident™ Inlet Fitting)	9166312-700	\$405
3μm Column, 3.2mm		
30mm (with Trident™ Inlet Fitting)	9166333-700	\$379
50mm (with Trident™ Inlet Fitting)	9166353-700	\$379
100mm (with Trident™ Inlet Fitting)	9166313-700	\$405
3μm Column, 4.6mm		
30mm (with Trident™ Inlet Fitting)	9166335-700	\$379
50mm (with Trident™ Inlet Fitting)	9166355-700	\$379
100mm (with Trident™ Inlet Fitting)	9166315-700	\$405
Allure™ Biphenyl Guard Cartridges		
10 x 2.1mm	916650212	\$131
10 x 4.0mm	916650210	\$131
20 x 2.1mm	916650222	\$131
20 x 4.0mm	916650220	\$131