

Superior Separations of Unsaturated Compounds by HPLC

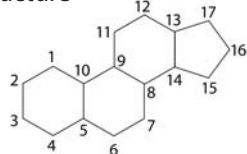
Separating Steroids by π - π Interactions Using the New Allure™ Biphenyl Column

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- Greater retention and specificity for compounds with small differences in double bonding.
- Better resolution, efficiency, and specificity for steroids, compared to C18 phases.
- Excellent choice for stability-indicating methods.

Steroids owe their broad range of medicinal properties largely to the diversity in their chemical structures. The basic structure consists of a phenanthrene ring linked to a cyclopentane ring (Figure 1), but various levels of unsaturation (double and triple bonds) and differing ring substituents (functional groups) create great diversity. Because the diversity in steroid composition consists of variations from one structure, we chose these compounds to illustrate the superiority of the Allure™ Biphenyl stationary phase for analyzing unsaturated compounds.

Figure 1 Basic Steroid Structure



Steroids are hydrophobic molecules that typically are analyzed using a reversed phase column, such as a C18 column. The

hydrophobic surface of this phase interacts with the hydrophobic portions of analyte molecules. This provides adequate separation for steroids that have differing hydrophobicity and differing functional groups. As Figures 2-4 show, however, the hydrocarbon ring system also presents structural variations. A separation mechanism based primarily on hydrophobic characteristics has limited effectiveness for resolving unsaturated compounds that differ only in the location of double bonds in a carbon ring.

In contrast, the new Allure™ Biphenyl stationary phase offers a unique separation mechanism that is more selective for separating compounds with slight differences in saturation: π - π (π - π) interactions. These interactions can occur when the ring moieties of the steroids and the biphenyl phase overlap.

We conducted three separate analyses, using simple isocratic conditions. Hydrocortisone, cortisone, and prednisone, which differ in C1-C2 double bonding and exhibit slight differences among position 17 and 11 functional groups, are almost completely resolved by an Allure™ Biphenyl column (Figure 2). The C18 stationary phase is unable to resolve hydrocortisone and prednisone.

Figure 2 Greater retention and resolution of corticosteroids, using an Allure™ Biphenyl column.

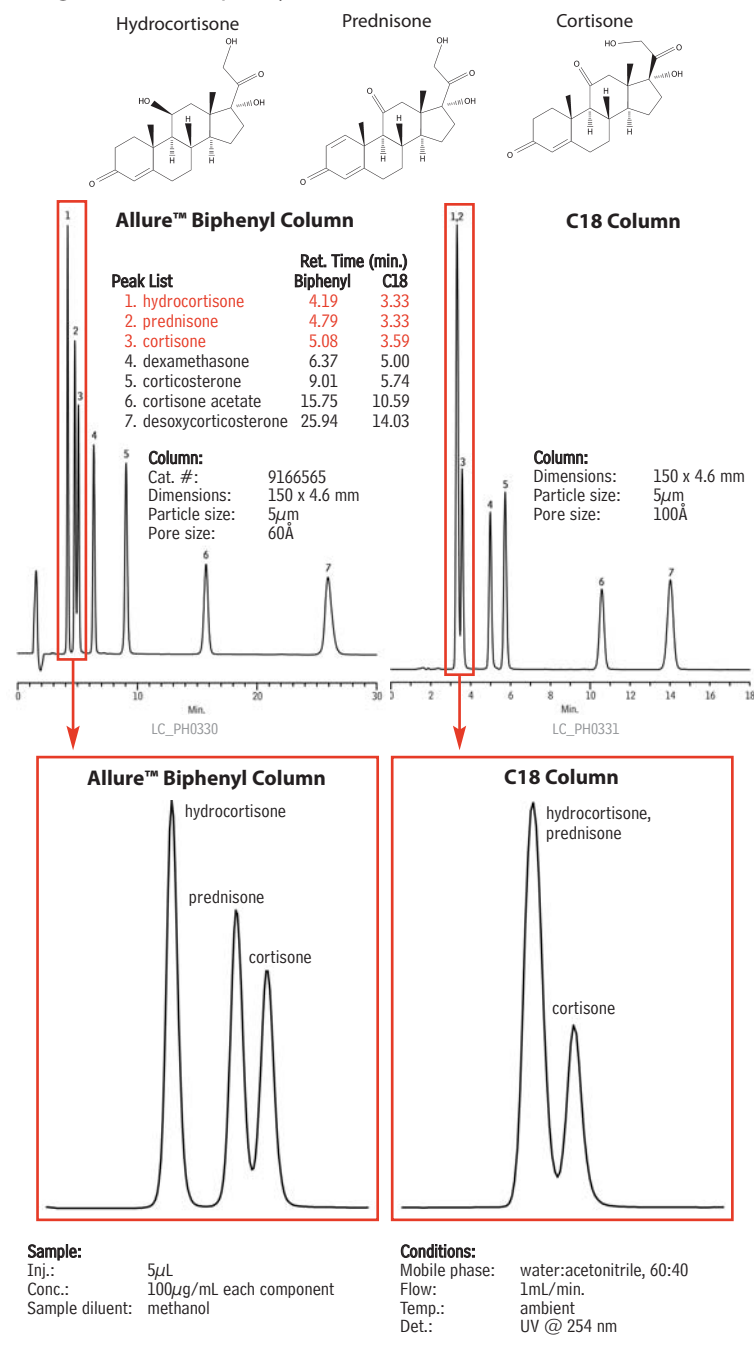
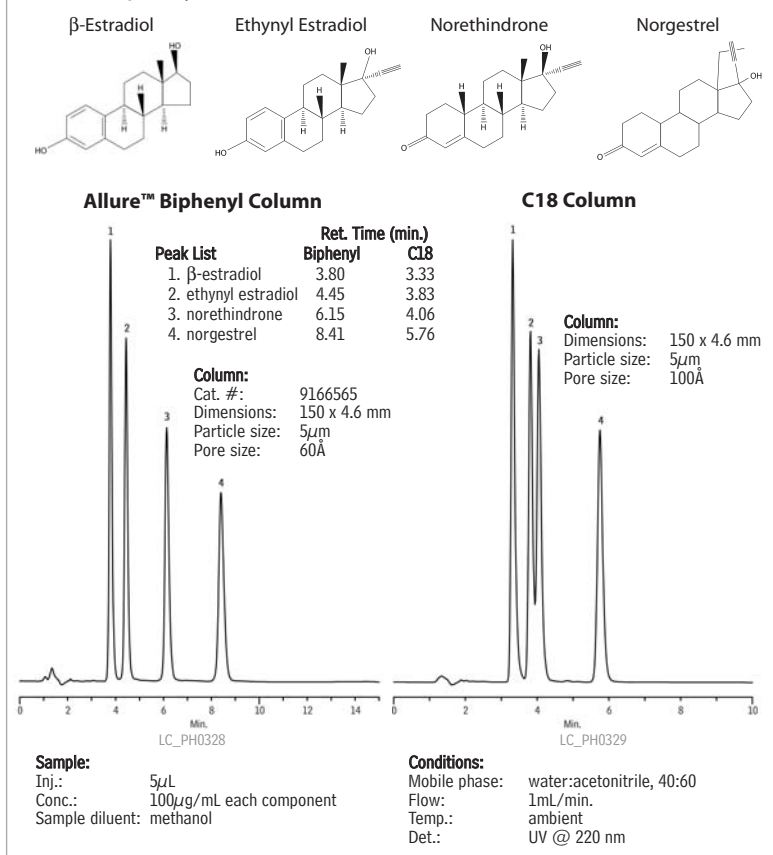


Figure 3 Baseline resolution of contraceptive steroids on an Allure™ Biphenyl column.



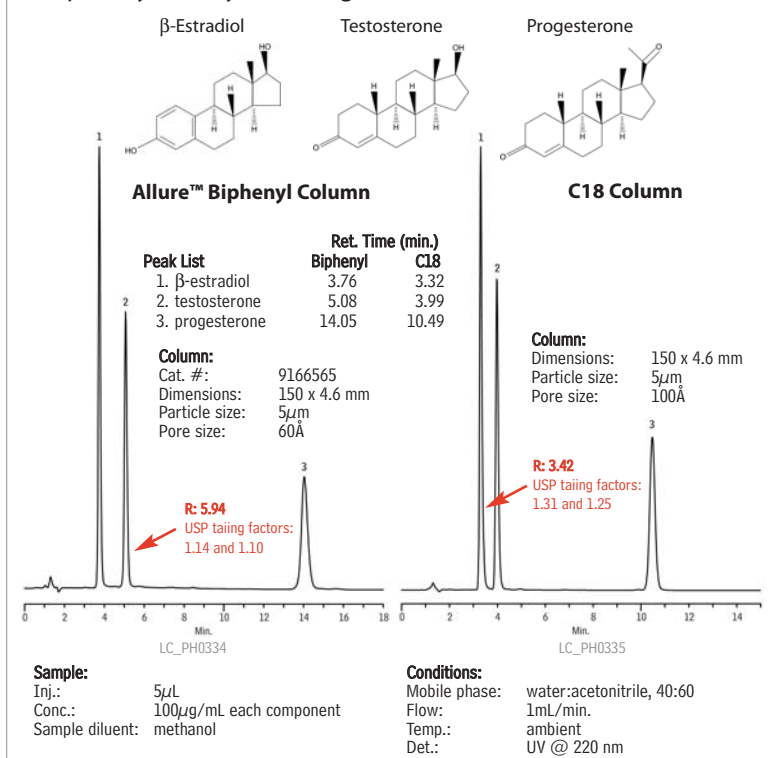
The minor differences in ring structure are sufficient to enable the Allure™ Biphenyl phase, but not the C18 phase, to elute these steroids selectively.

Contraceptive hormones also illustrate the Allure™ Biphenyl phase's superior retention and selectivity for steroids (Figure 3). As expected, the C18 phase resolves β-estradiol and ethynyl estradiol, which have differing functional groups, but it cannot resolve ethynyl estradiol and norethindrone, which have differing ring structures.

To verify the selectivity of the Allure™ Biphenyl phase, and to investigate possible enhanced system suitability criteria, we also analyzed endogenous hormones. β-estradiol and testosterone are structurally very similar, differing primarily in ring structure (Figure 4). By comparing resolution of these two compounds, we can make a correlation between hydrocarbon ring variation and resolution. The C18 column produced a resolution of 3.42, with USP tailing factors of 1.31 and 1.25, respectively; the Allure™ Biphenyl column provided a resolution of 5.94 — a 43% increase — and superior tailing factors of 1.14 and 1.10.

In all these analyses, the Allure™ Biphenyl column provided superior retention factors, relative to the C18 column. By increasing retention, we increase selectivity — the most effective way to improve resolution among analytes. When selecting a column for steroids, or for any other analysis, the stationary phase that provides greater retention ultimately will allow more control in choosing other method parameters and, thus, more control over the analysis.

Figure 4 An Allure™ Biphenyl column provides greater resolution and peak symmetry for endogenous steroid hormones.



Overall, these analyses demonstrate that π-π interactions are an excellent mechanism for resolving compounds with saturation differences in their hydrocarbon structures. The Allure™ Biphenyl stationary phase offers excellent retention, selectivity, and efficiency for unsaturated compounds with or without unsaturated functional groups. The greater selectivity and efficiency exhibited by the Allure™ Biphenyl phase, relative to a C18 phase, make it well suited for developing stability-indicating methods. Greater selectivity can mean better resolution between an analyte and its degradation products; greater efficiency will provide the capability to institute more accurate system suitability parameters.

Allure™ Biphenyl Column

5µm Column, 4.6mm
150mm

cat. #
9166565

for **more info**

For more information, and a complete list of Allure™ Biphenyl columns, request lit. cat.# 580015.