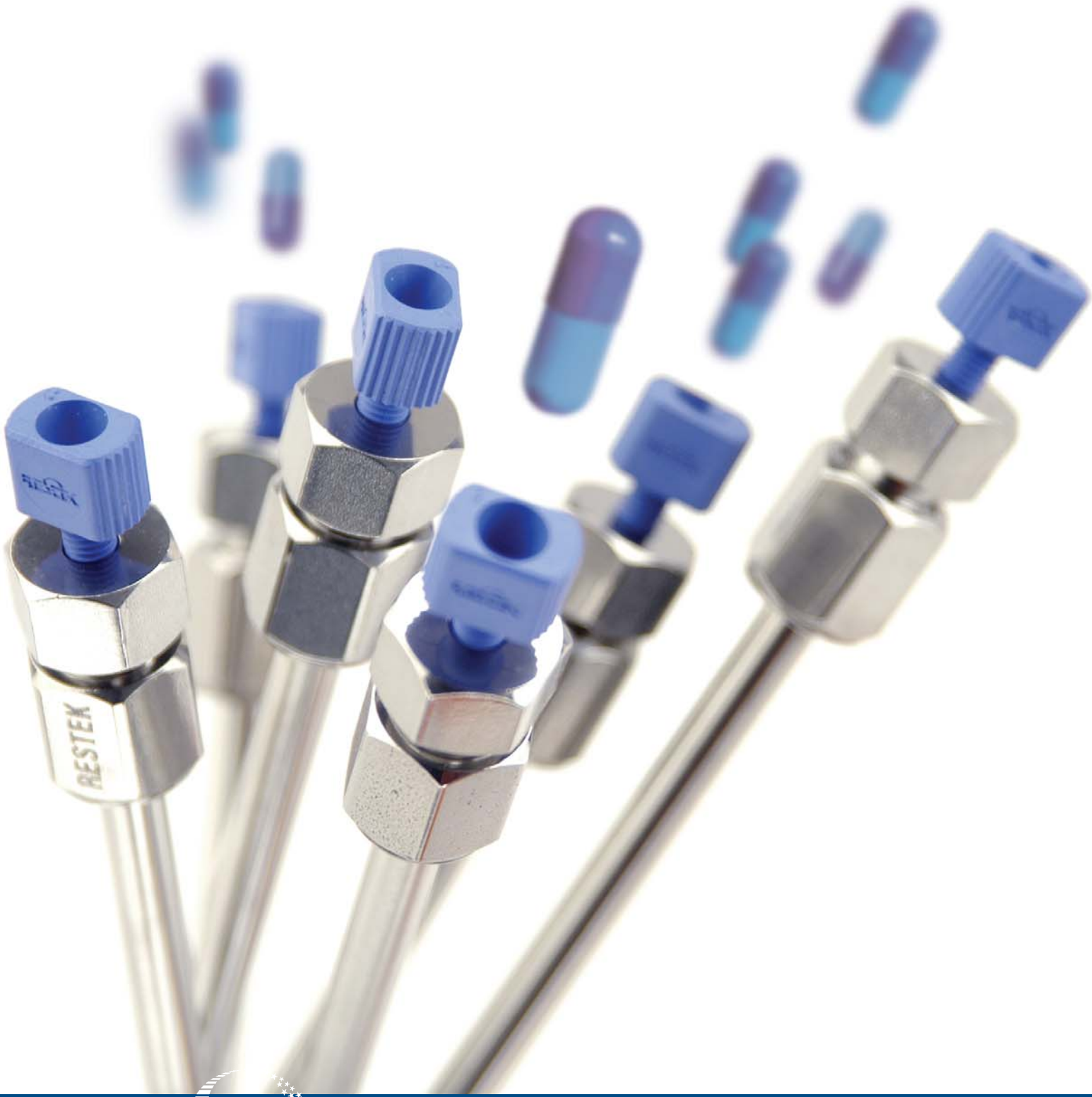
Allure™ Biphenyl HPLC Columns

Enhanced selectivity for unsaturated compounds



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Allure™ Biphenyl HPLC Columns

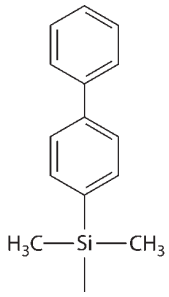
Unique selectivity for unsaturated compounds

- Excellent resolution of stucturally similar unsaturated compounds, compared to alkyl or phenyl phases.
- Rugged, reproducible retention.
- High purity, Type B silica ensures sharp, symmetric peaks.

Pharmaceutical compounds often are analyzed by HPLC, using C18, C8, or phenyl stationary phases. However, for unsaturated compounds, these phases typically do not offer optimum selectivity. To provide enhanced selectivity for such compounds, Restek chemists have developed Allure™ Biphenyl HPLC columns.

The physical arrangement of the Allure™ Biphenyl stationary phase—two phenyl groups bonded end-to-end (Figure 1)—makes the phase distinct from other commercially available phenyl phases, such as diphenyl or phenylhexyl. And, compared to C18 and C8 phases, which separate compounds primarily through hydrophobic interactions, the Allure™ Biphenyl phase offers a unique separation mechanism: π–π interactions. This mechanism provides markedly better selectivity for pharmaceutical or other molecules that differ in degree of unsaturation or position of double bonds within the carbon framework.

Figure 1 Unique structure of the Allure™ Biphenyl stationary phase.



For saturated, hydrocarbon-like analytes, with saturated functional groups, the Allure™ Biphenyl phase has retention and selectivity similar to a C18 phase, but with better retention and selectivity than a phenyl phase. However, when unsaturated compounds, such as corticosteroids, are analyzed on the Allure™ Biphenyl phase and on a C18 phase, selectivity is markedly different. Comparative separations of seven corticosteroids with slight structural differences in the typical steroid ring structure (Figure 2) reveal the unique selectivity of the Allure™ Biphenyl phase, as shown in Figure 3.

An Allure™ Biphenyl column targets even slight differences in saturation in the steroid ring structure, effectively resolving hydrocortisone, cortisone, and prednisone in a simple isocratic analysis (Figure 3A). No other tested column could resolve hydrocortisone and prednisone, which contain nearly identical functional groups, under these simple conditions. In addition, the Allure™ Biphenyl phase shows the greatest retention of the corticosteroids, most likely due to the extent of π–π interactions. It is the only column producing no coelutions in this analysis, as Table I shows (back cover).

Figure 2 Differences in saturation within the carbon rings and among ring substituents (functional groups) create numerous structural variations among steroids.

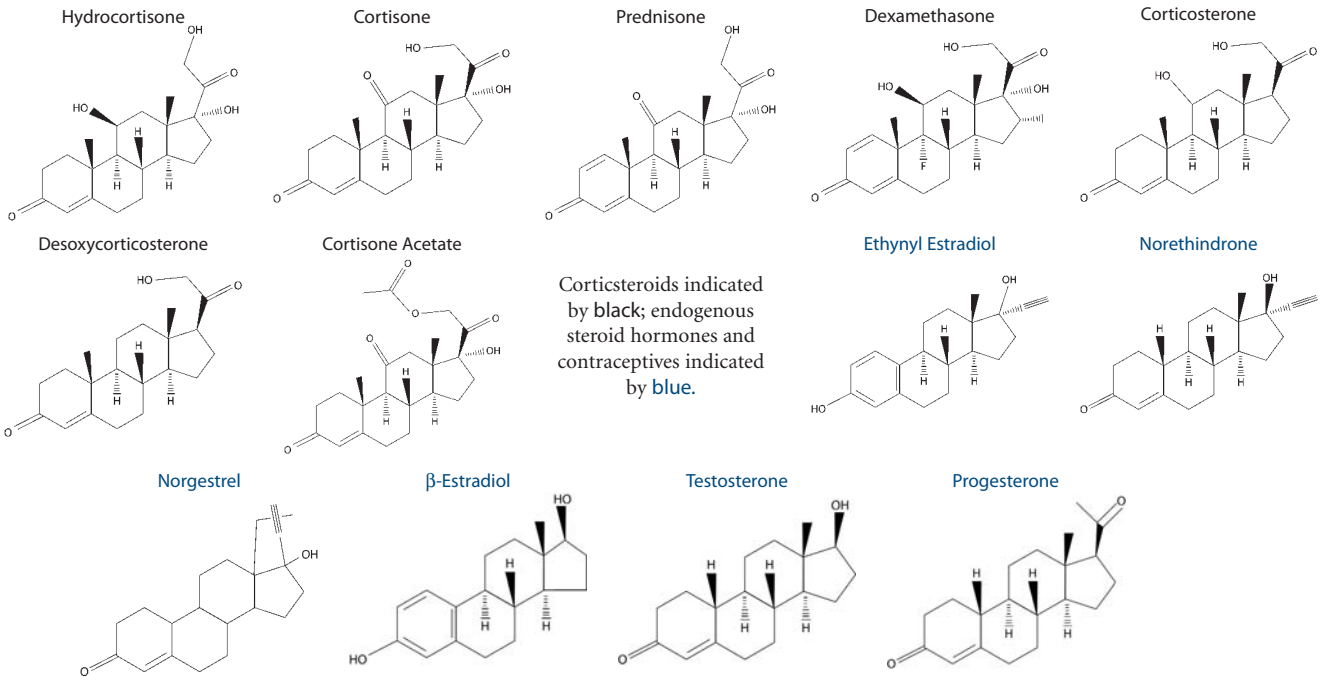
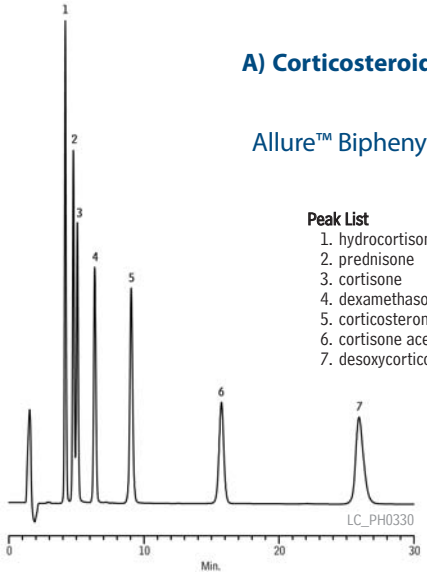


Figure 3 Superior resolution of steroids, using an Allure™ Biphenyl column.

A) Corticosteroids

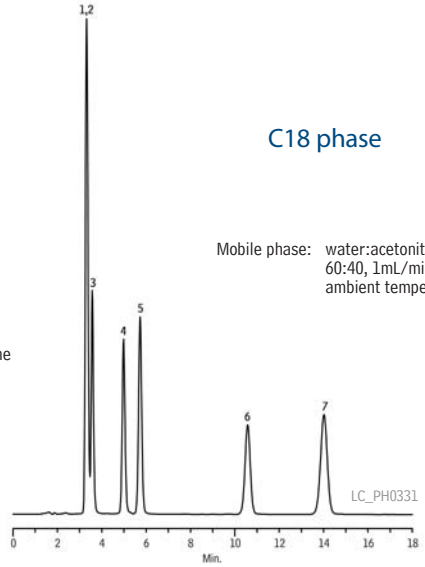
Allure™ Biphenyl

Peak List
1. hydrocortisone
2. prednisone
3. cortisone
4. dexamethasone
5. corticosterone
6. cortisone acetate
7. desoxycorticosterone

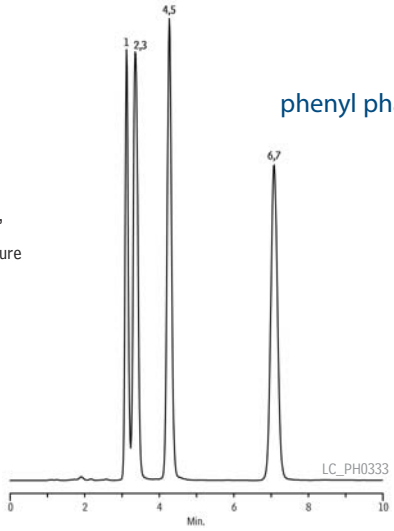


C18 phase

Mobile phase: water:acetonitrile, 60:40, 1mL/min., ambient temperature



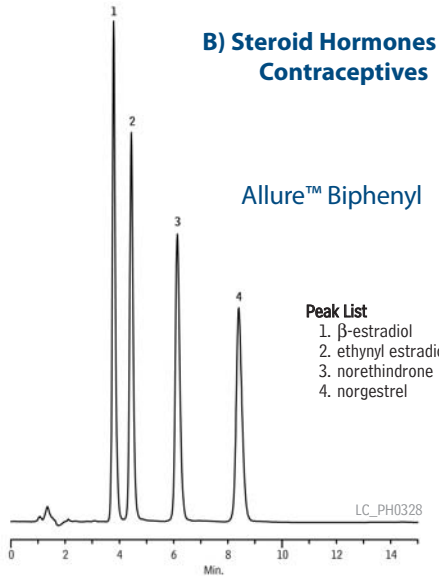
phenyl phase



B) Steroid Hormones and Contraceptives

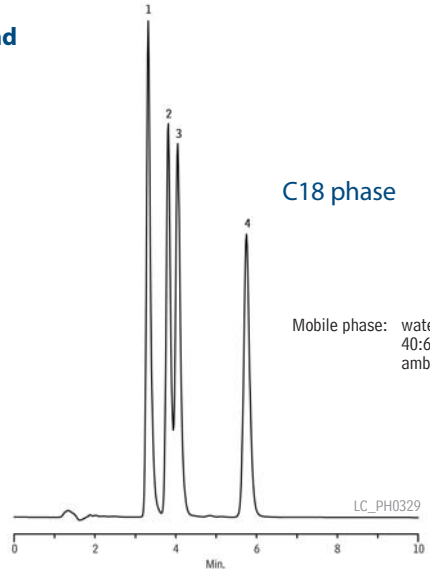
Allure™ Biphenyl

Peak List
1. β-estradiol
2. ethynyl estradiol
3. norethindrone
4. norgestrel



C18 phase

Mobile phase: water:acetonitrile, 40:60, 1mL/min., ambient temperature



Conditions for All Chromatograms

Sample:	5μL, 100μg/mL each component in methanol
Detection:	UV, 220nm
Column:	Allure™ Biphenyl (cat.# 9166565) all columns 150 x 4.6mm, 5μm particles

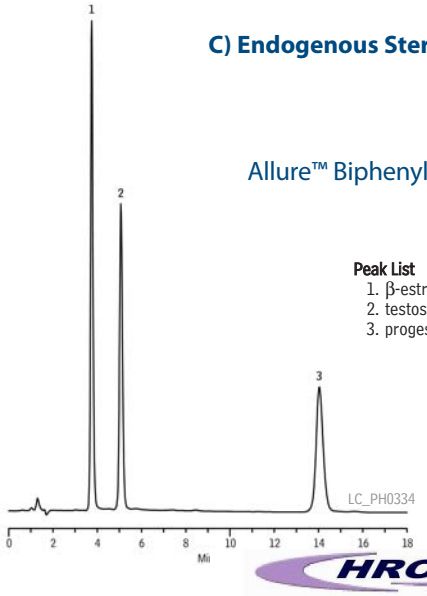
for **more** info

request application note
lit. cat.# 580020

C) Endogenous Steroids

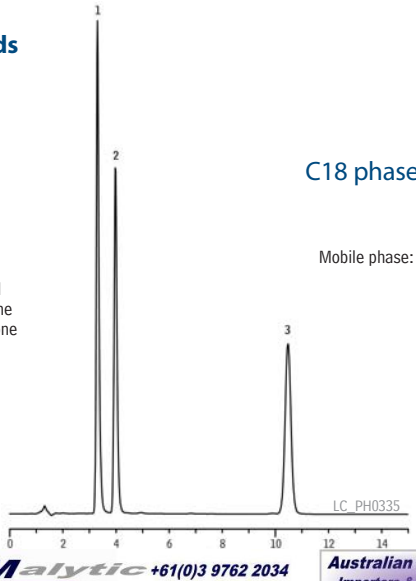
Allure™ Biphenyl

Peak List
1. β-estradiol
2. testosterone
3. progesterone



C18 phase

Mobile phase: water:acetonitrile, 40:60, 1mL/min., ambient temperature



Similarly, when we analyze endogenous steroid hormones and contraceptives (Figure 2) on Allure™ Biphenyl and traditional C18 columns, retention on the Allure™ Biphenyl column is distinctly better (Figures 3B and 3C).

Overall, the Allure™ Biphenyl phase exhibits significantly greater selectivity and retention, relative to traditionally used C18 or phenyl phases. As expected, the C18 phase resolves β-estradiol and ethynyl estradiol, which have differing functional groups, but it can not resolve ethynyl estradiol and norethindrone, which have differing ring structures. In contrast, the Allure™ Biphenyl phase resolves all compounds to baseline. Most important, the Allure™ Biphenyl phase shows greater resolving power for saturation differences in the ring structure, as noted by the superior resolution of ethynyl estradiol and norethindrone.

Table I Coelutions among corticosteroids on conventional HPLC phases.

Phase	Coelutions
C18	hydrocortisone/prednisone
Cyano	cortisone/prednisone
Phenyl	cortisone/prednisone corticosterone/dexamethasone corticosterone acetate/desoxycorticosterone
Allure™ Biphenyl	NONE

The Allure™ Biphenyl stationary phase offers superior selectivity and retention for compounds that exhibit differences in saturation in the hydrocarbon framework or in functional groups attached to the molecules. Further, the Allure™ Biphenyl phase has selectivity similar to a C18 phase for saturated molecules that incorporate saturated, but differing, functional groups. Thus, it is distinctly superior to a C18 phase in many applications, and potentially can replace a C18 phase in many other applications. One Allure™ Biphenyl HPLC column, many applications—an excellent choice!

Allure™ Biphenyl HPLC Columns

Physical Characteristics:

particle size: 3µm or 5µm, spherical
pore size: 60Å
carbon load: 23%
endcap: yes
pH range: 2.5 to 7.5
temperature limit: 80°C

Chromatographic Properties:

Highly retentive and selective phase for aromatic compounds. Increased retention over phenyl phases; uses high-purity, Type B silica.

To order a 2.1mm, 3.2mm, or 4.6mm ID column with a Trident™ Integral Inlet Fitting, add "-700" to the catalog number for the column.
Example: 100mm x 4.6mm ID Allure™ Biphenyl column with Trident™ Integral Inlet Fitting: 9166515-700
Nominal additional charge

Length	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
5µm Columns				
30mm	9166531	9166532	9166533	9166535
50mm	9166551	9166552	9166553	9166555
100mm	9166511	9166512	9166513	9166515
150mm	9166561	9166562	9166563	9166565
200mm	9166521	9166522	9166523	9166525
250mm	9166571	9166572	9166573	9166575

For 3µm columns, please contact Restek Technical Service.

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