

HPLC Stationary Phase Selection for the Analysis of Steroids

Steroids are an important class of pharmaceuticals. Of the top 200 prescribed drugs in 1998, 29 are classified as steroids.¹ Their uses range from contraception and hormone therapy to treatment of bronchial asthma. Steroids of differing sub-classes frequently are used in combinations to generate a desired therapeutic effect.

High performance liquid chromatography (HPLC) analysis of steroids can be performed with many types of stationary phases. Restek's Ultra C18, Ultra C4, Ultra Cyano, Allure™ C18, and Allure™ Basix columns can provide the selectivity and retention characteristics needed to develop rapid and robust analytical methods for a wide range of steroids. Therefore, the choice of column phase essentially is dictated by the sample matrix and sensitivity concerns.

¹ RxList, www.rxlist.com

Hydrophobic Interaction

C18 phases may provide excellent separation of steroid components but can produce added retention for certain hydrophobic steroids or lipophilic matrix components, resulting in longer analysis times. The Ultra C4 stationary phase provides excellent stability and selectivity for steroids. Its reduced hydrophobic nature can significantly reduce retention time while maintaining the desired selectivity. Reducing the retention time of an analyte increases its signal-to-noise response for better detection. Retention and resolution of two estrogens and a progestogen using Ultra C4 and Ultra C18 columns with the same mobile phase conditions show that both columns offer similar resolution of these compounds. The retention of other more hydrophobic components should be significantly reduced on the Ultra C4 (Figure 1).

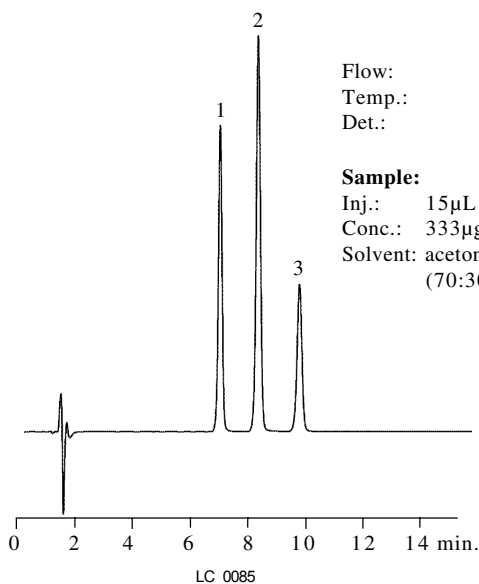
Figure 1

The Ultra C4 column's reduced hydrophobic tendencies provide excellent resolution and reduced analysis time for two estrogens and a progestogen when compared to the Ultra C18 phase.

Peak List:
 1. B-estradiol
 2. norethindrone
 3. ethynyl estradiol

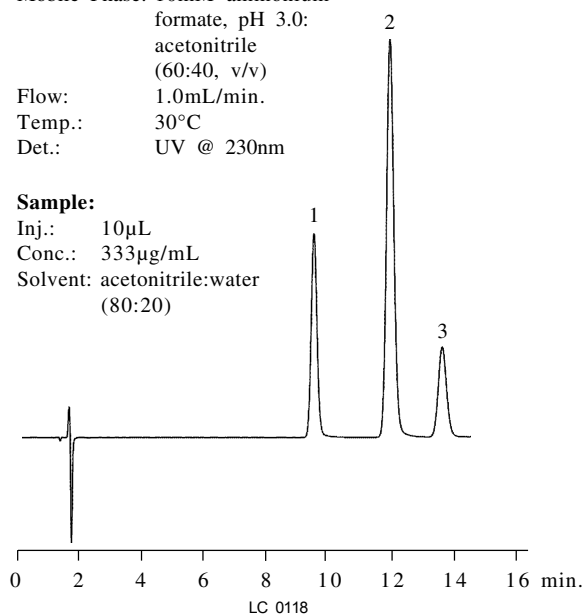
Column: Ultra C4
 Catalog #: 9102365
 Dimensions: 150 x 4.6mm
 Particle Size: 3µm
 Pore Size: 100Å
Conditions:
 Mobile Phase: 10mM ammonium formate, pH 3.0: acetonitrile (60:40, v/v)
 Flow: 1.2mL/min.
 Temp.: 30°C
 Det.: UV @ 230nm

Sample:
 Inj.: 15µL
 Conc.: 333µg/mL
 Solvent: acetonitrile:water (70:30)



Column: Ultra C18
 Catalog #: 9174565
 Dimensions: 150 x 4.6mm
 Particle Size: 5µm
 Pore Size: 100Å
Conditions:
 Mobile Phase: 10mM ammonium formate, pH 3.0: acetonitrile (60:40, v/v)
 Flow: 1.0mL/min.
 Temp.: 30°C
 Det.: UV @ 230nm

Sample:
 Inj.: 10µL
 Conc.: 333µg/mL
 Solvent: acetonitrile:water (80:20)



When analyzing steroids by LC/mass spectrometry (MS), the extra retention offered by the Ultra C18 column may prove advantageous. LC/MS sensitivity can be enhanced by increasing the organic content of the mobile phase. The added retention of the Ultra C18 phase allows the use of mobile phases with higher organic content to speed elution of the analytes and increase LC/MS sensitivity. Keep in mind that increasing the organic solvent content of the mobile phase may alter the elution order of the components.

Polar Interaction

Hydrophobic interaction is only one mechanism for resolving related compounds. Phases that can introduce a polar mechanism of retention can sometimes provide selectivity

not offered by C18 phases. The Ultra Cyano and Allure™ Basix phases have shown excellent utility in the analysis of steroids and provide an alternative selectivity to the C18 phase.

When measuring impurities by analytical chromatography or separating impurities by preparative chromatography, it often is better to have the impurity elute before the major analyte peak. If the major analyte peak tails badly or must be injected at high concentration, an impurity residing in the tail of the primary analyte peak may reduce fractional purity and analytical reproducibility. The Ultra Cyano and Ultra C18 phases demonstrate the outstanding peak shape and selectivity toward beclomethasone and des-chlorobeclo-methasone (Figure 2). The elution order of

Figure 2

The elution order change exhibited by the Ultra Cyano column can aid in the separation of beclomethasone from its des-chlorinated impurity.

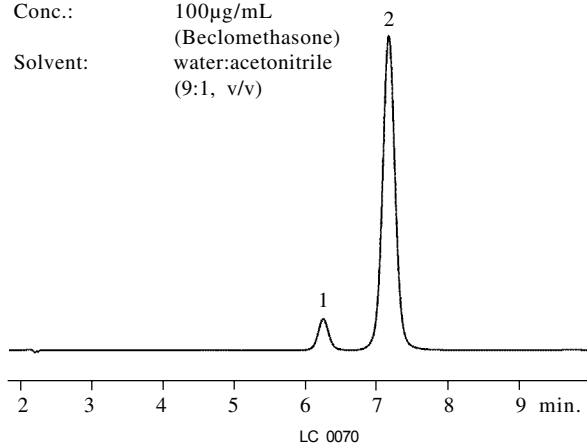
Peak List:

1. des-chlorobeclo-methasone
2. beclomethasone

Column: Ultra Cyano
Catalog #: 9106565
Dimensions: 150 x 4.6mm
Particle Size: 5µm
Pore Size: 100Å

Conditions:
Mobile Phase: water:acetonitrile (70:30, v/v)
Flow: 1.0mL/min.
Temp.: ambient
Det.: UV @ 238nm

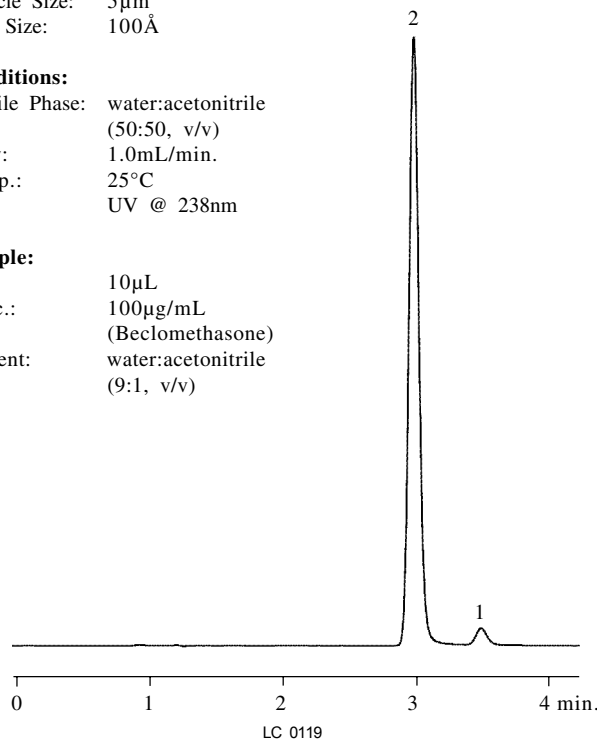
Sample:
Inj.: 10µL
Conc.: 100µg/mL (Beclomethasone)
Solvent: water:acetonitrile (9:1, v/v)



Column: Ultra C18
Catalog #: 9174565
Dimensions: 150 x 4.6mm
Particle Size: 5µm
Pore Size: 100Å

Conditions:
Mobile Phase: water:acetonitrile (50:50, v/v)
Flow: 1.0mL/min.
Temp.: 25°C
Det.: UV @ 238nm

Sample:
Inj.: 10µL
Conc.: 100µg/mL (Beclomethasone)
Solvent: water:acetonitrile (9:1, v/v)



beclo­methasone and des-chlorobeclo­methasone is reversed on the Ultra Cyano column compared to the Ultra C18 column, thereby allowing the impurity to elute before the major component.

The analysis of the contraceptive mixture of ethynyl estradiol and norgestrel using Allure™ C18 and Allure™ Basix columns demonstrates excellent peak shape and selectivity towards these compounds, although the elution order is reversed (Figure 3). Because either column is capable of separating these compounds, sensitivity and sample matrix concerns will dictate the selection of the most appropriate column.

Conclusion

Factors such as sensitivity, purity, selectivity, and reproducibility influence column selection when creating a chromatographic method. No one column can provide all the necessary factors for every analysis. The Ultra and Allure™ column series provide a wide range of retention and selectivity characteristics to create rugged chromatographic methods. The choice of the best stationary phase must be determined during the course of method development to insure the appropriate analytical factors have been optimized.

Figure 3

The Allure Basix column offers an alternative elution order along with excellent peak shape and selectivity for a common contraceptive mixture when compared to the Allure C18 column.

Peak List:

1. uracil (marker)
2. norgestrel
3. ethynyl estradiol

Column: Allure™ Basix
Catalog #: 9161565
Dimensions: 150 x 4.6mm
Particle Size: 5µm
Pore Size: 60Å

Conditions:

Mobile Phase: water:methanol (40:60, v/v)
Flow: 1mL/min.
Temp.: 25°C
Det.: UV @ 220nm

Sample:

Inj.: 5µL
Conc.: 100µg/mL
Solvent: mobile phase

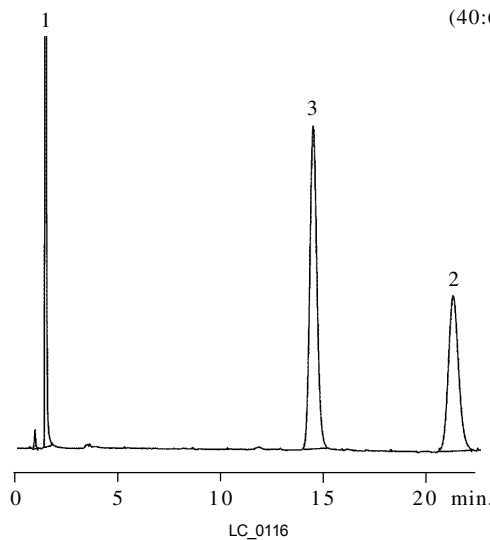
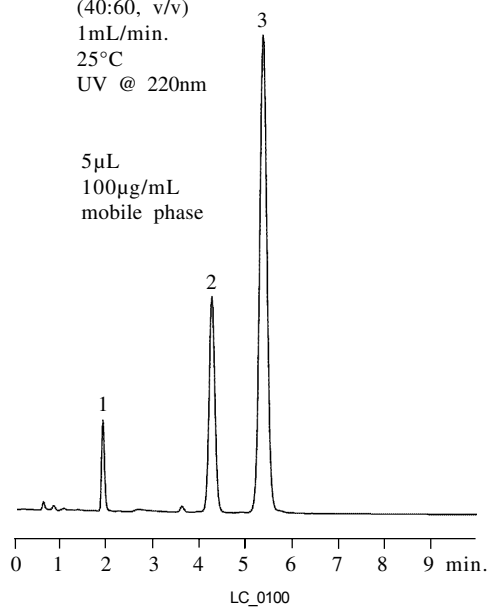
Column: Allure™ C18
Catalog #: 9164565
Dimensions: 150 x 4.6mm
Particle Size: 5µm
Pore Size: 60Å

Conditions:

Mobile Phase: water:methanol (25:75, v/v)
Flow: 1.0mL/min.
Temp.: 25°C
Det.: UV @ 220nm

Sample:

Inj.: 5µL
Conc.: 100µg/mL
Solvent: water:methanol (40:60)



■ Allure™ Basix

Particle Size: 5µm	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9161531	9161532	9161533	9161535
50mm length	9161551	9161552	9161553	9161555
100mm length	9161511	9161512	9161513	9161515
150mm length	9161561	9161562	9161563	9161565
200mm length	9161521	9161522	9161523	9161525
250mm length	9161571	9161572	9161573	9161575

■ Allure™ C18

Particle Size: 5µm	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9164531	9164532	9164533	9164535
50mm length	9164551	9164552	9164553	9164555
100mm length	9164511	9164512	9164513	9164515
150mm length	9164561	9164562	9164563	9164565
200mm length	9164521	9164522	9164523	9164525
250mm length	9164571	9164572	9164573	9164575

■ Ultra C4

Particle Size: 3µm	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9102331	9102332	9102333	9102335
50mm length	9102351	9102352	9102353	9102355
100mm length	9102311	9102312	9102313	9102315
150mm length	9102361	9102362	9102363	9102365
200mm length	9102321	9102322	9102323	9102325

■ Ultra C18

Particle Size: 5µm	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9174531	9174532	9174533	9174535
50mm length	9174551	9174552	9174553	9174555
100mm length	9174511	9174512	9174513	9174515
150mm length	9174561	9174562	9174563	9174565
200mm length	9174521	9174522	9174523	9174525
250mm length	9174571	9174572	9174573	9174575

■ Ultra Cyano

Particle Size: 5µm	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9106531	9106532	9106533	9106535
50mm length	9106551	9106552	9106553	9106555
100mm length	9106511	9106512	9106513	9106515
150mm length	9106561	9106562	9106563	9106565
200mm length	9106521	9106522	9106523	9106525
250mm length	9106571	9106572	9106573	9106575

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