

# Analysis of Estrogens Using a Solid Core HPLC Column

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## Key Words

Accucore Phenyl-X, fused core, superficially porous, estrogens, estrone (E1), estradiol (E2), estriol, ethynylestradiol

## Abstract

This application note demonstrates the use of the Thermo Scientific™ Accucore™ Phenyl-X HPLC column for the analysis of aromatic steroids. When compared with a C18 column the Accucore™ Phenyl-X HPLC column provides high aromatic selectivity, good hydrophobic retention and unique, complementary selectivity.

## Introduction

Accucore HPLC columns use Core Enhanced Technology™ to facilitate fast and high efficiency separations. The 2.6 µm diameter particles are not totally porous, but rather have a solid core and a porous outer layer. The tightly controlled 2.6 µm diameter of Accucore particles results in much lower backpressures than typically seen with sub-2 µm materials. The proprietary Accucore Phenyl-X alkyl aromatic bonded phase provides a unique selectivity when compared to other reversed phase materials such as C18 or Phenyl. The advanced design of the bonded phase makes it robust and compatible with highly aqueous mobile phases.

Aromatic steroids can present a challenge in liquid chromatography as in reversed phase it is difficult to get good separation. The use of a highly selective phase is the key to overcoming this challenge. In this application the Accucore Phenyl-X phase was employed to achieve the separation of four structurally related aromatic steroids classed as estrogens. Estrogens are a group of steroids thus named for their importance in the estrous cycle. They function as the primary female sex hormone. Estrogens are used as part of some oral contraceptives and in estrogen-replacement therapy for postmenopausal women. Three major naturally occurring estrogens in women are estrone (E1), estradiol (E2), and estriol (E3). Estradiol (E2) is the predominant form in nonpregnant females, estrone is produced during menopause, and estriol is the primary estrogen of pregnancy. Ethynylestradiol, a derivative of estradiol, is an orally bioactive estrogen used in almost all modern formulations of combined oral contraceptive pills. While the standard C18 phase fails to separate these four compounds, the



Accucore Phenyl-X HPLC column can baseline resolve them isocratically, providing good retention and unique selectivity.

## Experimental Details

Consumables	Part Number
Fisher Scientific HPLC grade water	W/0106/17
Fisher Scientific HPLC grade acetonitrile	A/0626/17
Fisher Scientific HPLC grade methanol	M/4056/17
Thermo Scientific Premium Autosampler Vial Convenience Kit	A4954-010

### Separation Preparation

1.0 mg/mL stock solution of estriol, estradiol, estrone and ethynylestradiol was prepared separately in methanol. 200  $\mu$ L of each compound was then diluted with 1.20 mL mobile phase to prepare 100  $\mu$ g/mL test mix standard.

Separation Conditions	Part Number	
Instrumentation:	Thermo Scientific HPLC system	
Column:	Accucore Phenyl-X 2.6 $\mu$ m, 100 x 2.1 mm	27926-102130
Column temperature:	40 $^{\circ}$ C	
Injection volume:	1 $\mu$ L	
Flow rate:	0.40 mL/min	
UV detection:	220 nm	
Mobile phase:	15:40:45 (v/v) acetonitrile:methanol:water	
Wash solvent:	Same as mobile phase	

## Results

Figure 1 compares the analysis of the 4 estrogens on the Phenyl-X phase with a C18 phase under the same conditions. The Accucore Phenyl-X HPLC column exhibits greater hydrophobic retention and higher selectivity for all 4 compounds. Accucore Phenyl-X HPLC column also shows greater resolving power, providing unique and complementary selectivity for estrone and ethynylestradiol when compared to a C18 phase as illustrated by the retention order reversal for the two later eluting compounds.

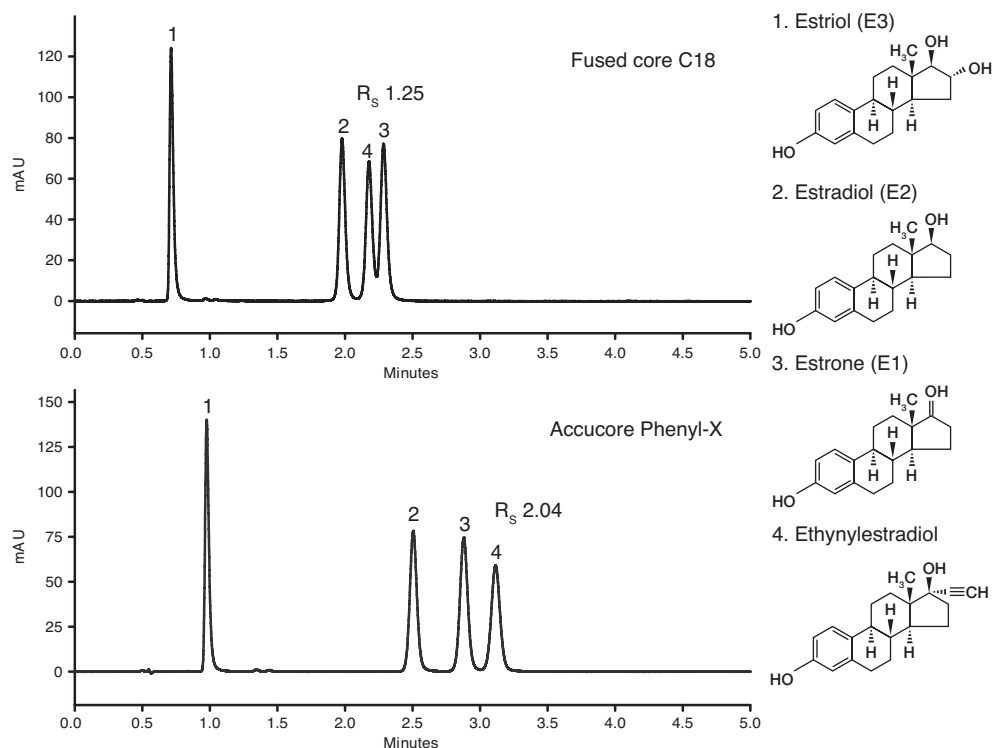


Figure 1: Separation of Estrogens

## Conclusion

The Accucore Phenyl-X HPLC column successfully separated all 4 estrogens with a short retention time. This application demonstrates that Accucore Phenyl-X HPLC columns

- retain and resolve aromatic compounds better than a C18 HPLC column
- provide a high aromatic selectivity
- provide a unique and complementary selectivity for aromatic compounds when compared with a C18 phase

## References

1. [www.women-health-info.com](http://www.women-health-info.com)
2. Dionex.com

[thermoscientific.com/chromatography](http://thermoscientific.com/chromatography)

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