

# Analysis of Estradiol and Estrone Using a Core Enhanced Technology Accucore HPLC Column

Joanne Gartland, Thermo Fisher Scientific, Runcorn, Cheshire, UK

## Key Words

- Steroids
- Accucore RP-MS
- Fused core
- Superficially porous
- Core Enhanced Technology

## Abstract

This application note demonstrates the use of the Thermo Scientific Accucore RP-MS HPLC column for the fast analysis of estradiol and estrone.

## 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 optimised phase bonding creates a series of high coverage, robust phases. Accucore RP-MS uses an optimized alkyl chain length for more effective coverage of the silica surface. This coverage results in a significant reduction in secondary interactions and thus highly efficient peaks with very low tailing. The tightly controlled 2.6 µm diameter of Accucore particles provides much lower backpressures than typically seen with sub-2 µm materials.

Estradiol and estrone are naturally occurring estrogens. Estradiol is the predominate hormone produced by pubescent females and is involved in numerous bodily functions. Estradiol levels are tested to verify the functionality of the ovaries and can be used for confirmation of ovarian tumors. In addition to this, estradiol is monitored in women undergoing fertility treatment. Estradiol is also readily found in males as it is a synthesized from testosterone.

Estradiol can be used:

- To treat symptoms of menopause
- In the prevention of osteoporosis in postmenopausal women
- As part of the treatment of cancer in both men and women

Estrone is less active than estradiol. It is primarily found in postmenopausal women and is secreted by the ovaries.

The separation of these two compounds and more significantly the speed of analysis are demonstrated in this application.



## Sample Preparation

Working standard contained 50 µg/mL of estradiol and 50 µg/mL of estrone in mobile phase.

Thermo Scientific Column	Part Number
Accucore RP-MS 2.6 µm 100 x 2.1 mm, Measured pressure: 280 bar	17626-102130

## Thermo Scientific Accela

Column temperature	40 °C
Injection volume	1 µL
Flow rate	0.6 mL/min
UV detection	220 nm

## Mobile Phase

Mobile phase: 1:1 (v/v) Water / Acetonitrile

Consumables	Part Number
Fisher Scientific HPLC grade water	W/0106/17
Fisher Scientific HPLC grade acetonitrile	A/0626/17
NSC Mass Spec Certified 2 mL clear vial with blue bonded PTFE silicone cap	MSCERT4000-34W

## Results

The analysis was carried out on an Accucore RP-MS 2.6  $\mu\text{m}$  100 x 2.1 mm column. As shown on Figure 1, estradiol and estrone eluted in under 1 minute.

Replicate injections of the estrogen mix showed that Accucore RP-MS produced stable and reproducible results (Table 1).

	Estradiol	Estrone
Asymmetry	1.56	1.43
%RSD $t_r$	0.08	0.09
%RSD Peak area	0.68	0.84

Table 1: Method precision (%RSD) for estradiol and estrone (data calculated from six replicate injections)

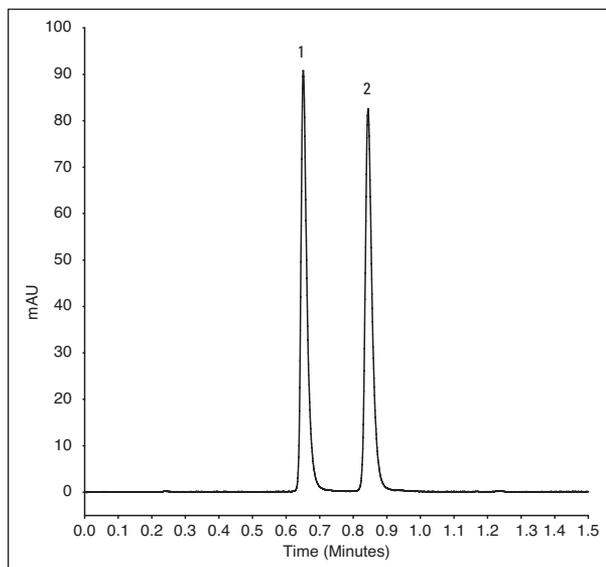


Figure 1: Chromatogram for 1. estradiol and 2. estrone separated on an Accucore RP-MS 2.6  $\mu\text{m}$  100 x 2.1 mm column

## Conclusions

The use of Accucore RP-MS column allowed to successfully separate estradiol and estrone in less than 1 minute. Accucore RP-MS columns are therefore an excellent choice for the fast analysis of steroids, allowing high sample throughput.

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