

Analysis of Steroids Using a Core Enhanced Technology Accucore HPLC Column

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Key Words

- Progestogens
- 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 six steroids.

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 2.6µm diameter of Accucore particles provides much lower backpressures than typically seen with sub-2 µm materials.

Hydrocortisone, cortisone, corticosterone, 11-alpha hydroxyprogesterone, 17-alpha hydroxyprogesterone and progesterone belong to the progestogens class of steroid hormones. Progestogens are characterized by the 21 carbon backbone and are primarily used to maintain pregnancy.

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



Sample Preparation

Working standard contained 20 µg/mL of each steroid in acetonitrile

Thermo Scientific Column	Part Number
Accucore RP-MS 2.6 µm 100 x 2.1 mm	17626-102130
Measured pressure: 400 bar	

Thermo Scientific Accela

Column temperature	25 °C
Injection volume	1.0 µL
Flow rate	0.6 mL/min
UV detection	254 nm

Mobile Phase

Mobile phase A: water
Mobile phase B: acetonitrile
Gradient: 25-75%B in 4 minutes

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 μm 100 x 2.1 mm column. The six steroids eluted in approximately 4 minutes (Figure 1.)

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

Analyte	Analyte name	t_r (min)	%RSD on t_r
1	Hydrocortisone	1.41	0.71
2	Cortisone	1.48	0.70
3	Corticosterone	1.96	0.57
4	11-alpha Hydroxyprogesterone	2.26	0.53
5	17-alpha Hydroxyprogesterone	2.99	0.42
6	Progesterone	4.01	0.32

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

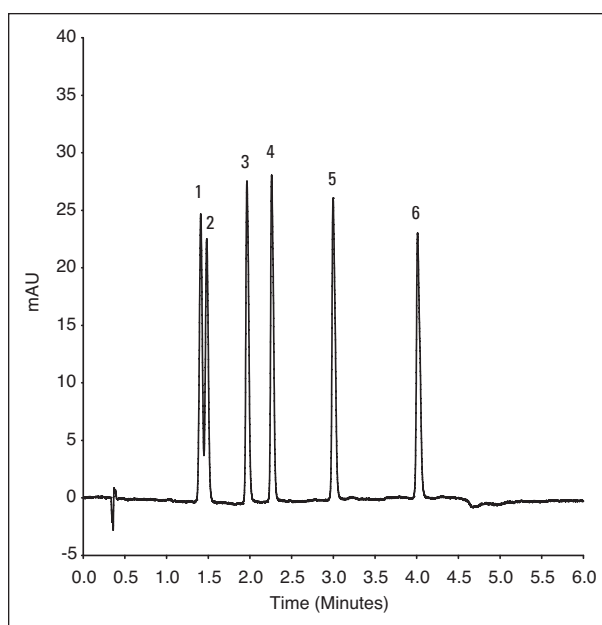


Figure 1: Separation of hydrocortisone (1), cortisone (2), corticosterone (3), 11-alpha hydroxyprogesterone (4), 17-alpha hydroxyprogesterone (5) and progesterone (6) on an Accucore RP-MS 2.6 μm 100 x 2.1 mm column

Conclusions

Accucore RP-MS column successfully separated six steroids in approximately 4 minutes. Accucore RP-MS columns are therefore an excellent choice for the fast analysis of steroids, allowing high sample throughput.

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