

Analysis of Testosterones Using a Core Enhanced Technology Accucore HPLC Column

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Key Words

- 11-ketotestosterone
- Epitestosterone
- 19-nortestosterone
- Nandrolone
- Accucore RP-MS
- Fused core
- Superficially porous
- Core Enhanced Technology
- Testosterones

Abstract

This application note demonstrates the use of the Thermo Scientific Accucore RP-MS HPLC column for the fast analysis of three testosterones.

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.

11-ketotestosterone, 19-nortestosterone (nandrolone) and epitestosterone are all related to testosterone. 11-Ketotestosterone is an oxidized form of testosterone. Nandrolone contains an additional methyl group in comparison to testosterone and epitestosterone and is an inactive epimer of testosterone. All three compounds are anabolic steroids, which promote protein synthesis within cells, particularly in muscles. Nandrolone occurs naturally at low levels (0.4 ng/mL) in the body and is routinely tested for in athletes.

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



Sample Preparation

Working standard contained 50 µg/mL of each testosterone in mobile phase

Thermo Scientific Column	Part Number
Accucore RP-MS 2.6 µm 100 x 2.1 mm	17626-102130
Measured pressure: 300 bar	

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Column temperature	40 °C
Injection volume	1 µL
Flow rate	0.6 mL/min
UV detection	254 nm

Mobile Phase

60:40 (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 µm 100 x 2.1 mm column. 11-ketotestosterone, 19-nortestosterone and epitestosterone eluted in under 2 minutes (Figure 1.)

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

	Peak 1 11-ketotestosterone	Peak 2 19-nortestosterone	Peak 3 epitestosterone
Asymmetry	1.48	1.28	1.11
%RSD t_r	0.00	0.05	0.06
%RSD Peak area	0.31	0.26	0.44

Table 1: Method precision (%RSD) for 11-ketotestosterone, 19-nortestosterone and epitestosterone (data calculated from six replicate injections)

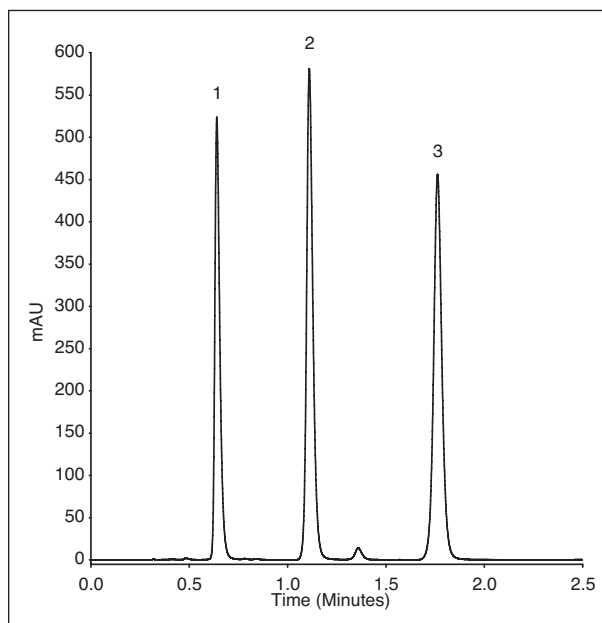


Figure 1: Chromatogram for 11-ketotestosterone (1), 19-nortestosterone (2), and epitestosterone (3) separated on an Accucore RP-MS 2.6 µm 100 x 2.1 mm column

Conclusions

Accucore RP-MS column successfully separated 11-ketotestosterone, 19-nortestosterone and epitestosterone in less than 2 minutes, providing an excellent choice for the fast analysis of testosterone, allowing high sample throughput.

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