

Analysis of Testosterones Using a Core Enhanced Technology Accucore HPLC Column

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

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 | |

Thermo Scientific Accela

| | |
|--------------------|------------|
| 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)

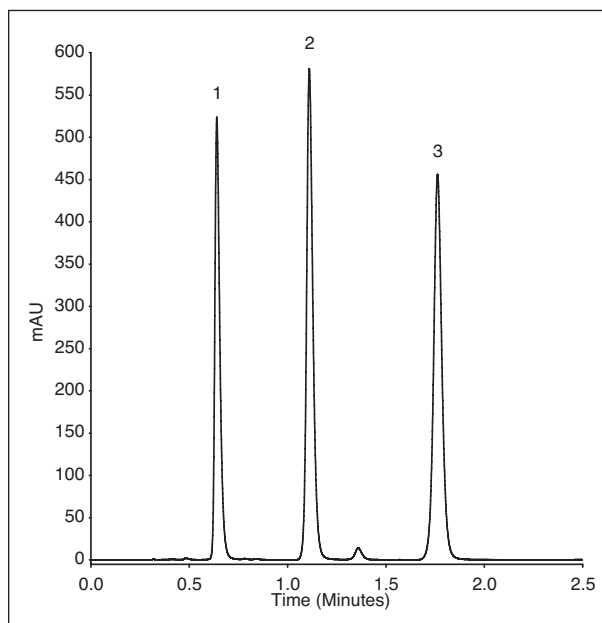


Figure 1: Chromatogram for 11-ketotestosterone (1), 19-nortestosterone (2), and epitestosterone (3) separated on an Accucore RP-MS 2.6 µm 100 x 2.1 mm column

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.

In addition to these offices, Thermo Fisher Scientific maintains a network of representative organizations throughout the world.

**North America
USA and Canada**
+1 800 332 3331

**Europe
France**
+33 (0)1 60 92 48 34

Germany
+49 (0) 2423 9431 -20
or -21

Switzerland
+41 56 618 41 11

United Kingdom
+44 1928 534110

**Asia
Japan**
+81 3 5826 1615

China
+86-21-68654588
or +86-10-84193588
800-810-5118

India
1800 22 8374 (toll-free)
+91 22 6716 2200

**Thermo Fisher
Scientific Australia
Pty Ltd**
1300 735 292 (free call
domestic)

**Thermo Fisher
Scientific New
Zealand Ltd**
0800 933 966 (free call
domestic)

All Other Enquiries
+44 (0) 1928 534 050

Technical Support

North America
800 332 3331

**Outside North
America**
+44 (0) 1928 534 440

www.thermoscientific.com/chromatography

© 2011 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries. Specifications, terms and pricing are subject to change. Not all products are available in all countries. Please consult your local sales representative for details.

ANCCSCETTESTOST 0811