

APPLICATIONS

Analyzing Testosterone in Human Serum by UHPLC using High Efficiency Kinetex[®] 1.7 μ m C18 Core-Shell Columns

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Testosterone was extracted from human serum by Strata[®]-X-A strong anion-exchange polymeric Solid Phase Extraction (SPE) and analyzed using a Kinetex C18, 30 x 2.1 mm, 1.7 μ m column and positive polarity ESI LC/MS/MS system. Kinetex sub-2 μ m core-shell technology offers higher efficiencies than traditional sub-2 μ m columns, producing greater chromatographic resolution, sensitivity, and higher peak capacities.

Introduction

Testosterone is an androgenic steroid responsible for the development of male reproductive organs, maintaining (or increasing) muscle mass, and bone density. As anabolic steroids, testosterone has been used (or abused) to increase muscle mass and enhance the athletic performance. The concentration of testosterone is lower in the female population than men and in general diminishes with advancing age.

Materials and Methods

Sample Preparation

The sample preparation is based on a simple solid phase extraction method using strong anion-exchange to produce a clean extract from human serum.

Solid Phase Extraction Method

Cartridge:	Strata-X-A, 30 mg/3 mL
Part No.:	8B-S123-TBJ
Condition:	2 mL Methanol
Equilibrate:	2 mL Water
Load:	Into individual labeled test tubes, combine 0.25 mL serum sample (or calibrator or QC sample), 1 mL DI water and 0.1 mL working internal standard solution (2 ng/mL).
Wash:	0.6 mL Methanol/Water (1:1)
Dry:	5 minutes under high vacuum
Elute:	2 x 0.3 mL Methanol

Final Prep and Analysis:

Following evaporation of elution solvent @ 50-55 °C under gentle nitrogen stream; Add 50 μ L 25% hydroxylamine solution and heat at 60-65 °C for 5-10 min, then add 200 μ L 5% aqueous formic acid and vortex the tubes. Transfer the solution to autosampler vials and inject 25 μ L on column. Inject 20 μ L on HPLC / Mass Spectrometer (MS) @ amu (ambient).

HPLC Conditions:

Following the SPE, testosterone is derivatized to form an oxime which is then analyzed, using a short-length 30 x 2.1 mm ID, 1.7 μ m Kinetex C18 UHPLC column, in positive mode ESI LC/MS/MS under multiple-reactions-monitoring function¹.

The mobile phase consisted of 0.1% formic acid with 1 mM ammonium formate with no pH adjustment, in water (mobile phase A) and acetonitrile (mobile phase B). A typical LC gradient (Table 1) is used for the separation.



Sean Orlowicz
Manager, PhenoLogix
When not in the lab, Sean enjoys just about anything involving the outdoors: hiking, climbing, surfing, etc. He is especially at home in the mountains, being an avid skier and motorcyclist.



Table 1.
LC Gradient Program

Step	Total Time (min)	Flow Rate (μ L/min)	B (%)
0	0	400	10
1	2.5	400	90
2	3.5	400	90
3	3.6	400	10
4	5	400	10

SCIEX API 5000[™] triple-quadrupole tandem mass spectrometer is used for analysis equipped with an ESI probe operating in positive polarity mode. Under an MRM mode, two channels were monitored for Testosterone and Testosterone-D3 (Table 2).

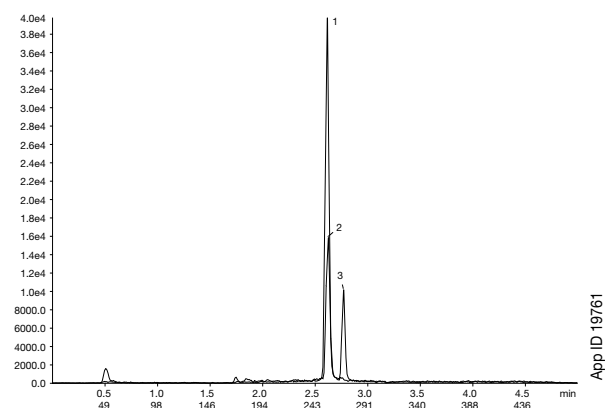
Table 2.
MRM Transitions Used for Data Analysis

Peak Name	MRM Channel
Testo (1)	304.3 \rightarrow 124.0
Testo (2)	304.3 \rightarrow 112.0
IS (Testo-D3 1)	307.3 \rightarrow 124.0
IS (Testo-D3 2)	307.3 \rightarrow 112.0

Results and Discussion

As demonstrated in Figure 1, the Kinetex 1.7 μ m 30 x 2.1 mm UHPLC column efficiently separates testosterone from its isomeric form epitestosterone. This column provides a very high degree of selectivity, even in a short dimension, resulting in superior chromatographic separation in a short run time.

Figure 1.
Separation of Testosterone and Epitestosterone by LC/MS/MS



Column:	Kinetex 1.7 μ m C18 100 \AA										
Dimensions:	30 x 2.1 mm										
Part No.:	00A-4475-AN										
Mobile Phase:	A: 0.1% Formic acid +1 mM Ammonium formate in Water B: 0.1% Formic acid +1 mM Ammonium formate in Acetonitrile										
Gradient:	<table border="0"> <thead> <tr> <th>Time (min)</th> <th>B (%)</th> </tr> </thead> <tbody> <tr> <td>0</td> <td>10</td> </tr> <tr> <td>2.5</td> <td>90</td> </tr> <tr> <td>3.5</td> <td>90</td> </tr> <tr> <td>3.6</td> <td>10</td> </tr> </tbody> </table>	Time (min)	B (%)	0	10	2.5	90	3.5	90	3.6	10
Time (min)	B (%)										
0	10										
2.5	90										
3.5	90										
3.6	10										
Flow Rate:	0.4 mL/min										
Temperature:	55 °C										
Detection:	LC/MS/MS ESI+										
Analyte:	1. Testosterone 2. Testosterone – D3 (IS) 3. Epitestosterone										

App ID 19761



APPLICATIONS

References:

- M. M. Kushnir et al., Clinical Chemistry 52:1, 120-128 (2006)





Kinetex Ordering Information

1.7 μ m Minibore Columns (mm)					SecurityGuard TM ULTRA Cartridges [†]
Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
EVO C18	—	00B-4726-AN	00D-4726-AN	00F-4726-AN	AJO-9298
F5	—	00B-4722-AN	00D-4722-AN	00F-4722-AN	AJO-9322
Biphenyl	—	00B-4628-AN	00D-4628-AN	00F-4628-AN	AJO-9209
XB-C18	00A-4498-AN	00B-4498-AN	00D-4498-AN	00F-4498-AN	AJO-8782
C18	00A-4475-AN	00B-4475-AN	00D-4475-AN	00F-4475-AN	AJO-8782
C8	00A-4499-AN	00B-4499-AN	00D-4499-AN	00F-4499-AN	AJO-8784
HILIC	00A-4474-AN	00B-4474-AN	00D-4474-AN	—	AJO-8786
Phenyl-Hexyl	—	00B-4500-AN	00D-4500-AN	00F-4500-AN	AJO-8788

[†] SecurityGuard ULTRA Cartridges require holder, Part No.: AJO-9000.

for 2.1 mm ID

Strata[®]-X-A Ordering Information

Format	Sorbent Mass	Part Number	Unit
Tube			
	30 mg	8B-S123-TAK**	1 mL (100/box)
	30 mg	8B-S123-TBJ	3 mL (50/box)
	60 mg	8B-S123-UBJ	3 mL (50/box)
	100 mg	8B-S123-EBJ	3 mL (50/box)
	100 mg	8B-S123-ECH	6 mL (30/box)
	200 mg	8B-S123-FBJ	3 mL (50/box)
	200 mg	8B-S123-FCH	6 mL (30/box)
	500 mg	8B-S123-HBJ	3 mL (50/box)
	500 mg	8B-S123-HCH	6 mL (30/box)
Giga[™] Tube			
	500 mg	8B-S123-HDG	12 mL (20/box)
	1 g	8B-S123-JDG	12 mL (20/box)
	1 g	8B-S123-JEG	20 mL (20/box)
	2 g	8B-S123-KEG	20 mL (20/box)
	5 g	8B-S123-LFF	60 mL (16/box)
96-Well Plate			
	10 mg	8E-S123-AGB	2 Plates/Box
	30 mg	8E-S123-TGB	2 Plates/Box
	60 mg	8E-S123-UGB	2 Plates/Box
96-Well Microelution Plate			
	2 mg	8M-S123-4GA	ea

** Tab-less tubes available. Contact Phenomenex for details.



If Phenomenex products in this technical note do not provide at least an equivalent separation as compared to other products of the same phase and comparable dimensions, return the product with comparative data within 45 days for a FULL REFUND.

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Strata-X is patented by Phenomenex. U.S. Patent No. 7, 119, 145

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