

# TN-1103

# APPLICATIONS

## Analyzing Testosterone in Human Serum by UHPLC using High Efficiency Kinetex<sup>®</sup> 1.7 $\mu$ m C18 Core-Shell Columns

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Testosterone was extracted from human serum by strong anion exchange polymeric SPE (solid phase extraction) and analyzed using a Kinetex C18, 30 x 2.1 mm, 1.7  $\mu$ m column and positive polarity ESI LC/MS/MS system. Kinetex sub-2  $\mu$ m core-shell technology offers higher efficiencies than traditional sub-2  $\mu$ 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. Monitoring body concentration of testosterone is an aid in diagnosing and treating disease state related to the hormonal imbalance.

### Materials and Methods

#### Sample Preparation

The sample preparation is based on a simple solid phase extraction method using strong anion exchange SPE (Strata<sup>™</sup>-X-A 30 mg/3 mL tubes) to produce a clean extract from human serum.

1	<b>Condition:</b> 1. Methanol 2 mL 2. Water 2 mL
2	<b>Load:</b> Into individually labeled test tube 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).
3	<b>Wash:</b> 50% Methanol in Water 0.6 mL
4	<b>Dry:</b> 5 minutes under high vacuum
5	<b>Elute:</b> Methanol 0.3 mL (x2)

#### Final Prep and Analysis:

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

#### HPLC Conditions:

Following the solid phase extraction, testosterone is derivatized to form an oxime which is then analyzed, using a short-length 30mm x 2.1 mm ID, 1.7  $\mu$ m Kinetex C18 UHPLC column, in positive mode ESI LC/MS/MS under multiple-reactions-monitoring function<sup>1</sup>.

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.

Table 1.

LC Gradient Program

Step	Total Time (min)	Flow Rate ( $\mu$ 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

An AB Sciex API 5000<sup>™</sup> 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

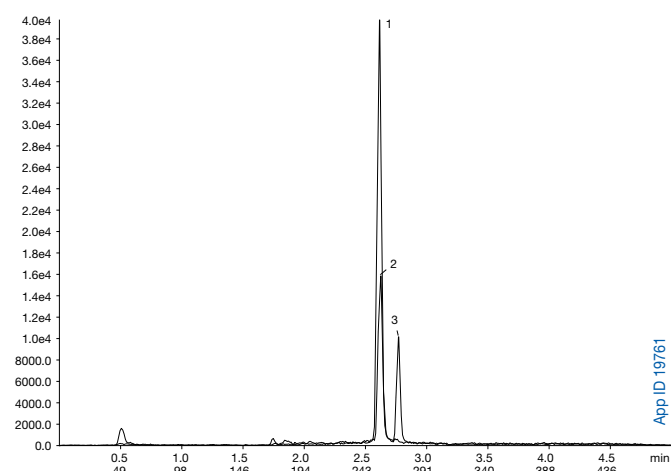
Peak Name	MRM Channel
Testo (1)	304.3 $\rightarrow$ 124.0
Testo (2)	304.3 $\rightarrow$ 112.0
IS (Testo-D3 1)	307.3 $\rightarrow$ 124.0
IS (Testo-D3 2)	307.3 $\rightarrow$ 112.0

### Results and Discussion

As demonstrated in Figure 1, the Kinetex 1.7  $\mu$ 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



<b>Column:</b>	Kinetex 1.7 $\mu$ m C18 100 Å		
<b>Dimensions:</b>	30 x 2.1 mm		
<b>Part No.:</b>	00A-4475-AN		
<b>Mobile Phase:</b>	A: 0.1% Formic Acid +1 mM Ammonium Formate in Water		
	B: 0.1% Formic Acid +1 mM Ammonium Formate in Acetonitrile		
<b>Gradient:</b>	<b>Time (min)</b>	<b>B (%)</b>	<b>Flow Rate:</b> 0.4 mL/min
	0	10	<b>Temperature:</b> 55 °C
	2.5	90	<b>Detection:</b> LC/MS/MS ESI+
	3.5	90	<b>Analyte:</b> 1. Testosterone
	3.6	10	2. Testosterone – D3 (IS)
			3. Epitestosterone

# TN-1103 APPLICATIONS

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## References

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

## Kinetex Ordering Information

### 2.6 µm Analytical Columns (mm)

	30 x 4.6	50 x 4.6	75 x 4.6	100 x 4.6	150 x 4.6
<b>XB-C18</b>	—	00B-4496-E0	00C-4496-E0	00D-4496-E0	00F-4496-E0
<b>C18</b>	00A-4462-E0	00B-4462-E0	00C-4462-E0	00D-4462-E0	00F-4462-E0
<b>C8</b>	—	00B-4497-E0	00C-4497-E0	00D-4497-E0	00F-4497-E0
<b>PFP</b>	00A-4477-E0	00B-4477-E0	00C-4477-E0	00D-4477-E0	00F-4477-E0
<b>HILIC</b>	—	00B-4461-E0	00C-4461-E0	00D-4461-E0	00F-4461-E0

### 2.6 µm Solvent Saver MidBore™ Columns (mm)

	30 x 3.0	50 x 3.0	75 x 3.0	100 x 3.0	150 x 3.0
<b>XB-C18</b>	00A-4496-Y0	00B-4496-Y0	00C-4496-Y0	00D-4496-Y0	—
<b>C18</b>	00A-4462-Y0	00B-4462-Y0	00C-4462-Y0	00D-4462-Y0	00F-4462-Y0
<b>C8</b>	00A-4497-Y0	00B-4497-Y0	00C-4497-Y0	00D-4497-Y0	—
<b>PFP</b>	00A-4477-Y0	00B-4477-Y0	00C-4477-Y0	00D-4477-Y0	00F-4477-Y0
<b>HILIC</b>	00A-4461-Y0	—	—	—	00F-4461-Y0

### 2.6 µm Minibore Columns (mm)

	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1
<b>XB-C18</b>	00A-4496-AN	00B-4496-AN	00D-4496-AN	00F-4496-AN
<b>C18</b>	00A-4462-AN	00B-4462-AN	00D-4462-AN	00F-4462-AN
<b>C8</b>	00A-4497-AN	00B-4497-AN	00D-4497-AN	00F-4497-AN
<b>PFP</b>	00A-4477-AN	00B-4477-AN	00D-4477-AN	00F-4477-AN
<b>HILIC</b>	—	00B-4461-AN	00D-4461-AN	00F-4461-AN

### 1.7 µm Minibore Columns (mm)

	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1
<b>XB-C18</b>	00A-4498-AN	00B-4498-AN	00D-4498-AN	00F-4498-AN
<b>C18</b>	00A-4475-AN	00B-4475-AN	00D-4475-AN	00F-4475-AN
<b>C8</b>	00A-4499-AN	00B-4499-AN	00D-4499-AN	00F-4499-AN
<b>PFP</b>	00A-4476-AN	00B-4476-AN	00D-4476-AN	00F-4476-AN
<b>HILIC</b>	00A-4474-AN	00B-4474-AN	00D-4474-AN	00F-4474-AN

## KrudKatcher™ Ultra In-Line Filter Ordering Information

Part No.	Description	Unit
AF0-8497	HPLC KrudKatcher Ultra Column In-Line Filter, 0.5 µm Depth Filter x 0.004 in. ID	3/pk

KrudKatcher Ultra requires 5/16 in. wrench. Wrench not provided.



If Phenomenex products in this technical note do not provide at least an equivalent separation as compared to a 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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