

## Separation of Testosterone and Epitestosterone by LC/MS/MS using Strata-X-A and Kinetex 1.7 µm C18

**Column:** Kinetex® 1.7 µm C18 100 Å, LC Column 30 x 2.1 mm, Ea

**Dimensions:** 30 x 2.1 mm ID

**Order No:** 00A-4475-AN

**Elution Type:** Gradient

**Eluent A:** 0.1% Formic Acid +1 mM Amm Formate in Water

**Eluent B:** 0.1% Formic Acid +1 mM Amm Formate in ACN

Gradient Profile:	Step No.	Time (min)	Pct A	Pct B
	1	0	90	10
	2	2.5	10	90
	3	3.5	10	90
	4	3.6	90	10

**Flow Rate:** 0.4 mL/min

**Col. Temp.:** ambient

**Detection:** Mass Spectrometer (MS) @ amu (ambient)

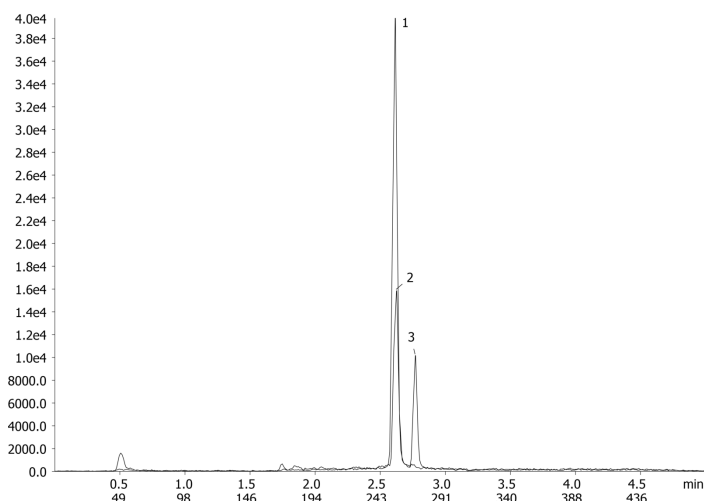
**Detector Info:** <a target="\_blank"

19761

href="https://sciex.com/products/mass-spectrometers?utm\_campaign=2019%20application%20search&utm\_source=phenomenex&utm\_medium=referral">SCIEX<



Products used in this application:



### ANALYTES:

- 1 Testosterone  
Retention Time: 2.62 min
- 2 Testosterone-d3  
Retention Time: 2.61 min
- 3 Epitestosterone  
Retention Time: 2.77 min



# Sample Preparation Details

for HPLC Application ID No.: 19761

## Separation of Testosterone and Epitestosterone by LC/MS/MS using Strata-X-A and Kinetex 1.7 µm C18

### PRODUCT DESCRIPTION:

Strata<sup>TM</sup>-X-A 33 µm Polymeric Strong Anion, 30 mg / 3 mL, Tubes , 50/Pk

Order No.: 8B-S123-TBJ

### SOLID PHASE EXTRACTION (SPE) PROCEDURE:

**Note:** The solvent volumes shown below are for a 30 mg bed mass.

The solvent volumes will need to be adjusted for a smaller or larger bed mass.

#### Condition:

#### Load:

#### Wash:

#### Dry:

Dry for 5 min under high vacuum

#### Elute:

#### Final Prep and Analysis:

Following evaporation of elution solvent @ 50-55 C under gentle nitrogen stream;  
Add 50 µL 25% hydroxylamine solution and heat at 60-65 C for 5-10 min, then add 200 µL 5%  
Inject: 0 µL on HPLC Mass Spectrometer (MS) @ amu (ambient)

ANALYTES:	Spiked Conc. (ng/mL)	Log P	pKa	% Rec	%RSC (n=0)
1 Testosterone	100				
2 Testosterone-d3	0.5				
3 Epitestosterone	0				

**Note:** This method is designed as a convenient starting point for further investigation and can be tailored to meet your extraction goals.  
Call your local Phenomenex Representative for assistance in method development and optimization techniques.

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For more information contact your Phenomenex Representative at [info@phenomenex.com](mailto:info@phenomenex.com)



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