

HPLC Application

ID No.: 19761

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)

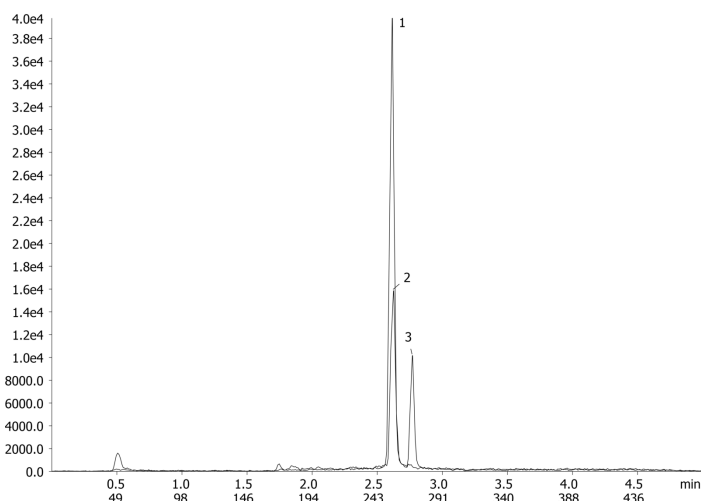
Detector Info: <a target="_blank"

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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 μ m C18

PRODUCT DESCRIPTION:

Strata[™]-X-A 33 μ m 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 μ L 25% hydroxylamine solution and heat at 60-65 C for 5-10 min, then add 200 μ L 5%

Inject: 0 μ L 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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