HPLC Application

ID No.: **19762**

Testosterone from Female Human Plasma 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:

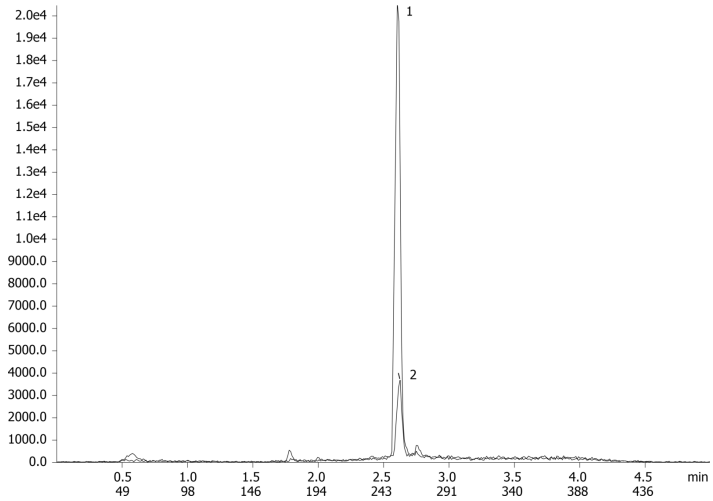
Step No.	Time (min)	Pct A	Pct B
1	0	90	10
2	2.5	10	90
3	3.5	10	90
4	3.6	90	10

Flow Rate: 0.4 mL/min
Col. Temp.: ambient
Detection: Mass Spectrometer (MS) @ amu (ambient)
Detector Info: <a target="_blank" href="https://sciex.com/products/mass-spectrometers?utm_campaign=2019%20application%20search&utm_source=phenomenex&utm_medium=referral">SCIEX<,

Products used in this application:

KINETEX®

19762



ANALYTES:

1 Testosterone
Retention Time: 2.62 min

2 Testosterone-d3
Retention Time: 2.61 min

Sample Preparation Details

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PRODUCT DESCRIPTION:

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

Order No.: 8B-S123-TBJ

SOLID PHASE EXTRACTION (SPE) PRODCEDURE:

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 uL 25% hydroxylamine solution and heat at 60-65 C for 5-10 min, then add 200 uL 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	0				
2	Testosterone-d3	0.5				

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.*