

HPLC Application

ID No.: 22787

Extraction of Total Cortisol from Human Plasma Using Novum SLE & Kinetex Biphenyl

Column: Kinetex[®] 2.6 µm Biphenyl 100 Å, LC Column 50 x 2.1 mm, Ea

Dimensions: 50 x 2.1 mm ID

Order No: 00B-4622-AN

Elution Type: Gradient

Eluent A: 10 mM ammonium acetate in water

Eluent B: 10 mM ammonium acetate in methanol

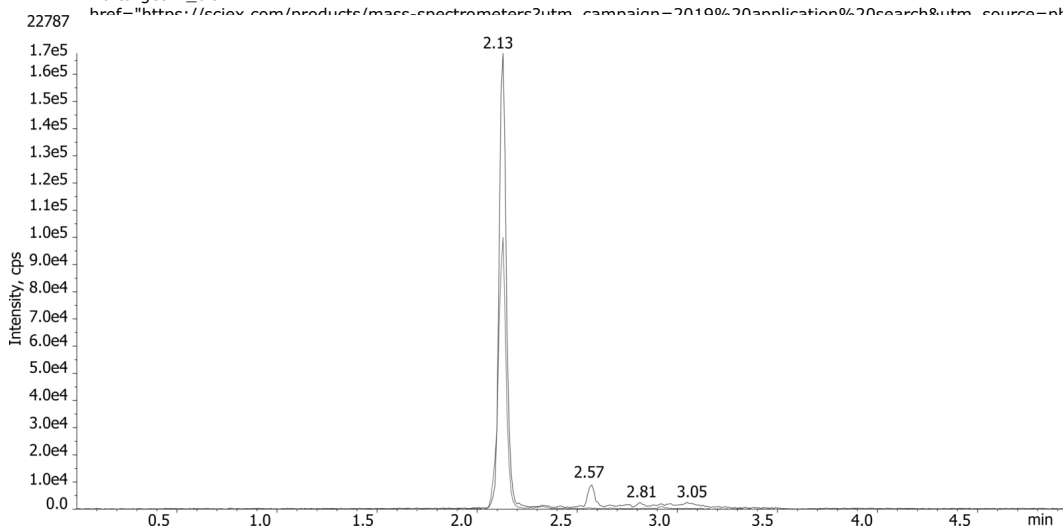
Gradient Profile:	Step No.	Time (min)	Pct A	Pct B
	1	0	50	50
	2	2	5	95
	3	3.1	5	95
	4	3.11	50	50
	5	5	50	50

Flow Rate: 0.45 mL/min

Col. Temp.: 25 °C

Detection: Tandem Mass Spec (MS-MS) @ (ambient)

Detector Info: SCIEX<



Products used in this application:



ANALYTES:

1 Cortisol

Retention Time: 2.13 min

2 Cortisol-D4 (Internal standard)

Retention Time: 2.13 min



Sample Preparation Details

for HPLC Application ID No.: 22787

Extraction of Total Cortisol from Human Plasma Using Novum SLE & Kinetex Biphenyl

PRODUCT DESCRIPTION:

Novum SLE MAX 96-Well Plate, 1/Pk

Order No.: 8E-S138-5GA

SOLID PHASE EXTRACTION (SPE) PROCEDURE:

Note: The solvent volumes shown below are for a 400 mg bed mass.

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

Condition:

Load:

Sample pre-treatment: Dilute 200 μ L of human plasma with 200 μ L of 50 mM sodium phosphate dibasic heptahydrate, pH unadjusted. Vortex briefly (3-5 sec). Sample loading: Load the sample from pretreatment step above onto the Novum plate and apply a short and gentle pulse of vacuum (\sim 5" of Hg for 5-10 sec) or until the sample has completely entered the media. Wait for 5 minutes.

Wash:

Dry:

Elute:

Final Prep and Analysis:

Extraction Procedure

Sample pre-treatment: Dilute 200 μ L of human plasma with 200 μ L of 50 mM sodium phosphate

Inject: 10 μ L on HPLC Tandem Mass Spec (MS-MS) @ (ambient)

ANALYTES:	Spiked Conc. (ng/mL)	Log P	pKa	% Rec	%RSC (n=0)
1 Cortisol	25			79	
2 Cortisol-D4 (Internal standard)	50				

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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