HPLC Application

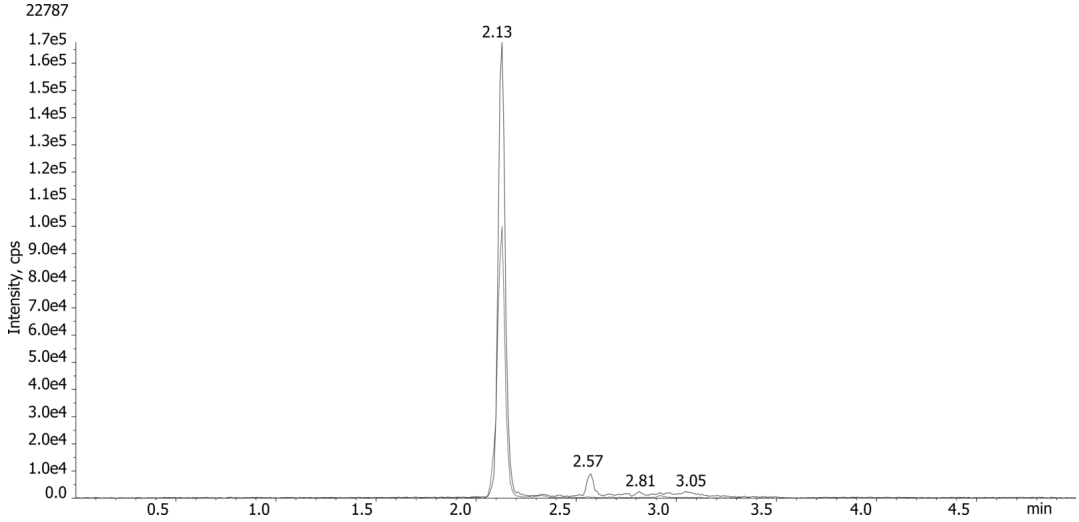
ID No.: **22787**

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: Electrospray Mass Spec (ESMS) @ 0.0000000000 (ambient)



Products used in this application:

KINETEX®

ANALYTES:

1 Cortisol
Retention Time: 2.13 min

2 Cortisol-D4 (Internal standard)
Retention Time: 2.13 min

For more information contact your Phenomenex Representative at support@phenomenex.com

Phenomenex products are available worldwide.

Sample Preparation Details

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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) PRODCEDURE:

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 uL of human plasma with 200 ul of 50 mM sodium phosphate dibasic heptahydrate, pH unadjusted. Vortex briefly (3-5 sec). Sample loading: Load the sample from pretreatment step abive onto the Novum plate and apply a short and gentle pulse of vacuum (~ 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 uL of human plasma with 200 ul of 50 mM sodium phosphate

Inject: 10 µL on HPLC Electrospray Mass Spec (ESMS) @ 0.0000000000 (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.*