

HPLC Application

ID No.: 22785

Automated Extraction of Prednisolone & Cortisone from Plasma Using Novum SLE & Kinetex 2.6u Biphenyl

Column: Kinetex[®] 2.6 μ m Biphenyl 100 Å, LC Column 50 x 3.0 mm, Ea

Dimensions: 50 x 3 mm ID

Order No: 00B-4622-Y0

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

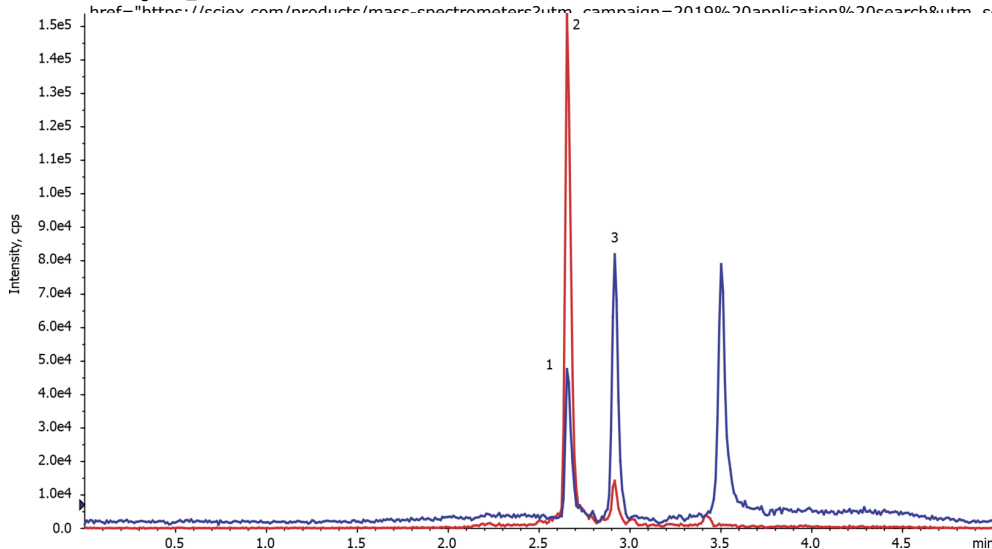
Col. Temp.: 25 °C

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

Detector Info: SCIEX



Products used in this application:



ANALYTES:

- 1** Cortisone
Retention Time: 2.66 min
- 2** Cortisol-D4 (Internal standard)
Retention Time: 2.69 min
- 3** Prednisolone
Retention Time: 2.92 min

Sample Preparation Details

for HPLC Application ID No.: 22785

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PRODUCT DESCRIPTION:

Novum SLE MINI 96-Well Plate, 1/Pk

Order No.: 8E-S138-FGA

SOLID PHASE EXTRACTION (SPE) PROCEDURE:

Note: The solvent volumes shown below are for a 200 mg bed mass.

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

Condition:

Load:

Sample pre-treatment:

Dilute 150 μ L of human plasma (spiked with 25 ng/mL and 125 ng/mL of cortisone and prednisolone respectively) with 150 μ L of 50 mM sodium phosphate dibasic heptahydrate, pH unadjusted.

Mix briefly (3-5 sec).

Sample loading:

Load the sample from pre-treatment step above onto the Novum plate and apply a short and gentle pulse of vacuum ($\sim 10''$ of Hg for 20 secs) until the sample has completely entered the media.

Wait for 5 minutes

Wash:

Dry:

Elute:

Final Prep and Analysis:

Sample pre-treatment

Dilute 150 μ L of human plasma (spiked with 25 ng/mL and 125 ng/mL of cortisone and

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 Cortisone	25			99	
2 Cortisol-D4 (Internal standard)	50				
3 Prednisolone	125			95	

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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