

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

**Column:** Kinetex® 2.6 µm 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

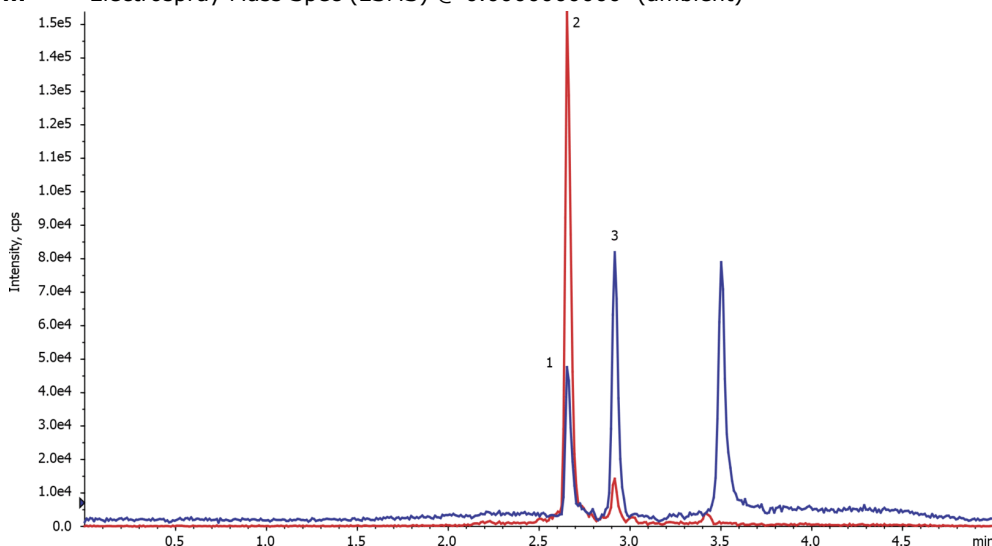
**Eluent B:** 10 mm ammonium acetate in methanol

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



### 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



Products used in this application:



# Sample Preparation Details

for HPLC Application ID No.: 22785

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

### 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:

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#### Load:

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Sample pre-treatment:

Dilute 150  $\mu$ L of human plasma (spiked with 25 ng/mL and 125 ng/mL of cortisone and prednisolone respectively) with 150  $\mu$ 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:

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#### Dry:

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#### Elute:

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#### Final Prep and Analysis:

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Sample pre-treatment

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

Inject: 10  $\mu$ L on HPLC Electrospray Mass Spec (ESMS) @ 0.0000000000 (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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For more information contact your Phenomenex Representative at [international@phenomenex.com](mailto:international@phenomenex.com).



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[international@phenomenex.com](mailto:international@phenomenex.com)