

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

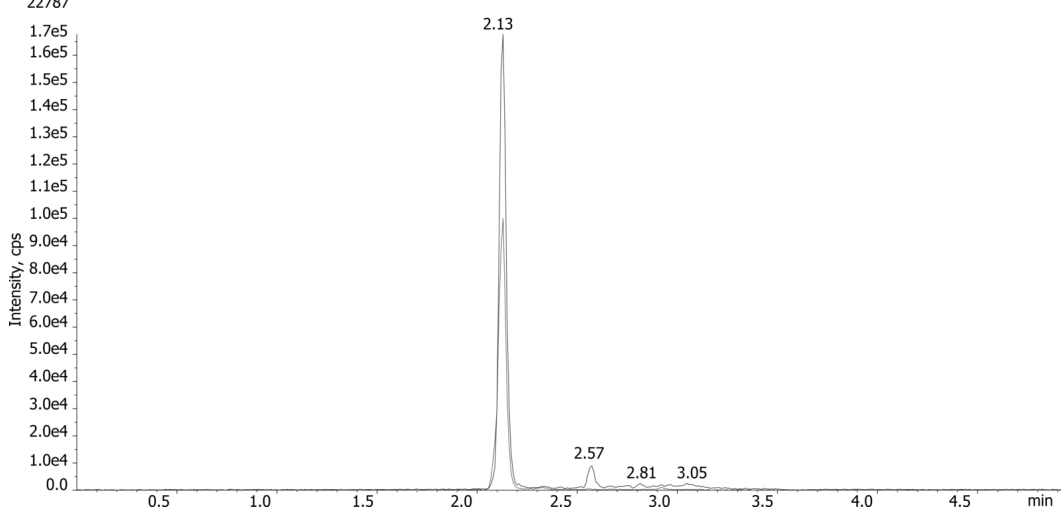
Col. Temp.: 25 °C

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

Detector Info:

<a target="_blank"

href="https://sciex.com/products/mass-spectrometers?utm_campaign=2019%20application%20search&utm_source=phenomenex&utm_medium=referral">SCIEX<



ANALYTES:

1 Cortisol

Retention Time: 2.13 min

2 Cortisol-D4 (Internal standard)

Retention Time: 2.13 min



Products used in this application:



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.

©2024 Phenomenex Inc. All rights reserved.

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



Phenomenex products are available worldwide.

www.phenomenex.com

support@phenomenex.com