HPLC Application

ID No.: **22985**

Extraction of Procaine from Serum using Strata-X-C 10mg Plate & Kinetex 2.6u Biphenyl, 50x2.1 Column

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: 0.1% formic acid in water
Eluent B: 0.1% formic acid in acetonitrile

Gradient Profile:

Step No.	Time (min)	Pct A	Pct B
1	0	95	5
2	0.5	95	5
3	3	5	95
4	3.5	5	95
5	3.51	95	5
6	5.5	95	5

Flow Rate: 0.5 mL/min
Col. Temp.: 25 °C
Detection: Electrospray Mass Spec (ESMS) @ 0.0000000000 (ambient)
Analyst Note: Strata X-C 10 mg Plate Protocol

Condition

500 µL methanol

Equilibrate

500uL water

Load

750 µL diluted serum

(375 µL plasma diluted 1:1 with 4% phosphoric acid in water)

Wash1

500 µL 2% formic acid in water

Wash 2

500 µL methanol

Elute

375 µL(3x125 µL) 5% ammonium hydroxide in acetonitrile:methanol(60:40)

Inject

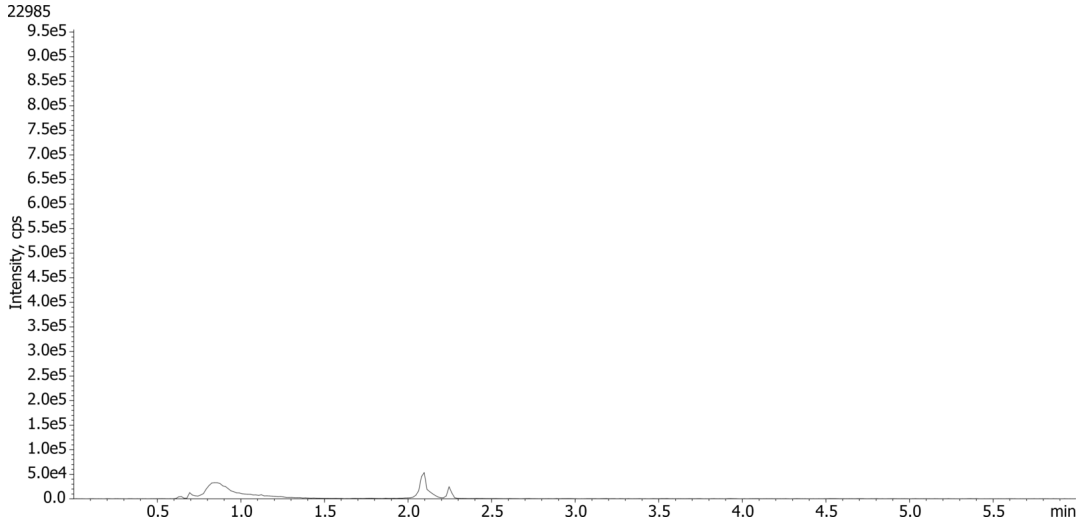
1 µL

Note: Spiked conc. of analyte was 1 ng/mL serum

Products used in this application:

KINETEX®

Extraction of Procaine from Serum using Strata-X-C 10mg Plate & Kinetex 2.6u Biphenyl, 50x2.1 Column



ANALYTES:

1 Procaine

Retention Time: 2.09 min

Sample Preparation Details

for **HPLC** Application ID No.: **22985**

Extraction of Procaine from Serum using Strata-X-C 10mg Plate & Kinetex 2.6u Biphenyl, 50x2.1 Column

PRODUCT DESCRIPTION:

Strata™-X-C 33 µm Polymeric Strong Cation, 10 mg / well, 96-Well Plates , 2/Pk

Order No.: 8E-S029-AGB

SOLID PHASE EXTRACTION (SPE) PRODCEDURE:

Note: The solvent volumes shown below are for a 10 mg bed mass.
The solvent volumes will need to be adjusted for a smaller or larger bed mass.

Condition:

Load:

Dilute 375µL serum with 375µL of 4% Phosphoric acid in water.
Dilute 150 uL of human plasma (spiked with 25 ng/mL and 125 ng/mL of cortisone and prednisolone respectively) with 150 uL 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 (~ 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:

No dry down step was performed. 1µL of final eluent was direct injected.
Inject: 1 µL on HPLC Electrospray Mass Spec (ESMS) @ 0.0000000000 (ambient)

ANALYTES:	Spiked Conc. (ng/mL)	Log P	pKa	% Rec	%RSC (n=0)
1 Procaine	1				

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.*