

HPLC Application

ID No.: 22982

Extraction of Timolol from Serum using Strata-X-C 10 mg 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: Tandem Mass Spec (MS-MS) @ (ambient)

Analyst Note: Strata X-C 10 mg Plate Protocol

Condition

500 µL methanol

Equilibrate

500µL 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

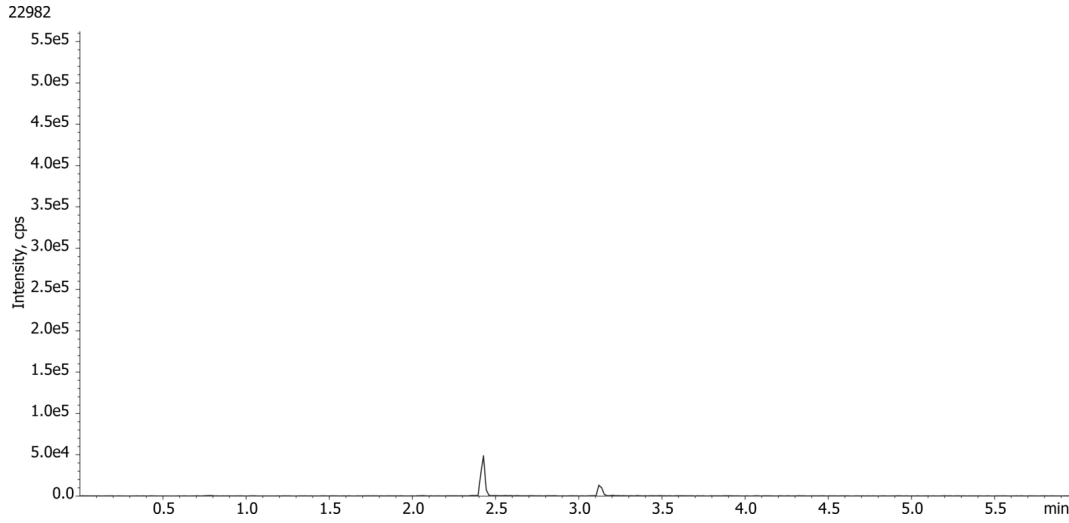
Note: Spiked conc. of analyte was 1 ng/mL serum



Products used in this application:



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ANALYTES:

1 Timolol

Retention Time: 2.42 min



Sample Preparation Details

for HPLC Application ID No.: 22982

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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) PROCEDURE:

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 150 µL of human plasma (spiked with 25 ng/mL and 125 ng/mL of cortisone and prednisolone respectively) with 150 µL 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. 1 µL of final eluent was injected.

Inject: 1 µL on HPLC Tandem Mass Spec (MS-MS) @ (ambient)

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

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



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