

HPLC Application

ID No.: 22984

Extraction of Timolol from Serum using Strata-X-C Microelution & a Kinetex 2.6u Biphenyl HPLC 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 Elution Plate Protocol

Condition

200 µL methanol

Equilibrate

200µL water

Load

750 µL diluted serum

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

Wash1

200 µL 2% formic acid in water

Wash 2

200 µL methanol

Elute

25 µL 5% ammonium hydroxide in acetonitrile:methanol(60:40)

Inject

1 µL

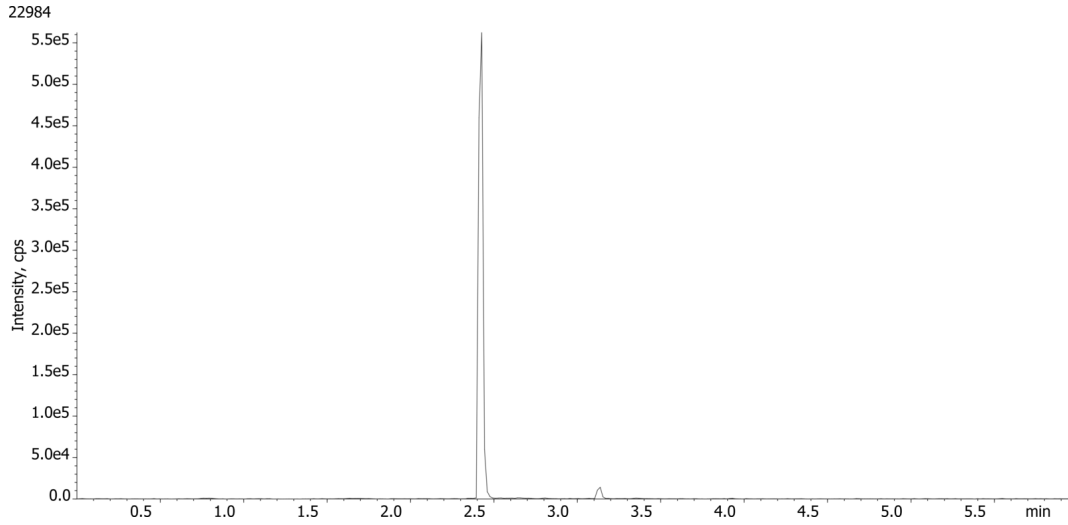
Note: Spiked conc. of analyte was 1 ng/mL serum



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.: 22984

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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: 1 μ L on HPLC Tandem Mass Spec (MS-MS) @ (ambient)

ANALYTES:	Spiked Conc. (ng/mL)	Log P	pKa	% Rec	%RSC (n=0)
1 Timolol	1			97	

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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