HPLC Application

ID No.: 22984



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

Kinetex® 2.6 μ m Biphenyl 100 Å, LC Column 50 x 2.1 mm, Ea

50 x 2.1 mm ID **Dimensions:** 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	Step No.	Time (min)	Pct A	Pct B
Profile:	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)

Strata X-C Elution Plate Protocol Analyst Note:

> 200 µL methanol Equilibrate 200uL water Load

750 μL diluted serum

(375 μ L 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:

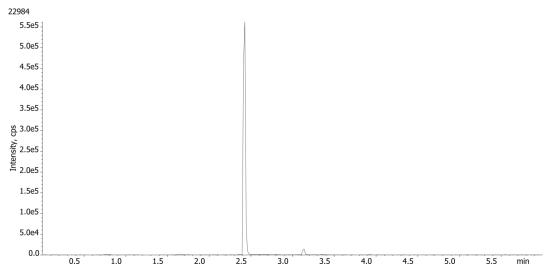




HPLC Application ID No.: **22984**



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



ANALYTES:

1 Timolol

Retention Time: 2.42 min

Sample Preparation Details for HPLC Application ID No.: 22984



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

PRODUCT DESCRIPTION:

Novum SLE MINI 96-Well Plate, 1/Pk

Order No.: 8E-S138-FGA

SOLID PHASE EXTRACTION (SPE) PRODCEDU	JRE
---------------------------------------	-----

	s shown below are for a 200 m	•				
The solvent volume	s will need to be adjusted for a	a smaller or	larger bed r	nass.		
Condition:						
Load:						
Sample pre-treatment:						
Dilute 150 uL of human plasma (s	piked with 25 ng/mL and 125 ng/mL of cortisone	and prednisolone	respectively) with	150 uL of 50 mM sodium	phosphate dibasic heptahy	drate, pH unadjusted.
Mix briefly (3-5 sec).						
Sample loading:						
Load the sample from pre-treatme	ent step above onto the Novum plate and apply a	short and gentle p	oulse of vacuum (~	10" of Hg for 20 secs) u	ntil the sample has complet	ely entered the media.
Wait for 5 minutes						
Wash:						
Dry:						
Eluto						
Elute:						
Final Prep and Analysis	<u>:</u>					
Sample pre-treatmer						
Dilute 150 uL of hum	an plasma (spiked with 25 ng/	mL and 125	ng/mL of c	ortisone and		
Inject: 1 µL on HPLC	Tandem Mass Spec (MS-MS) @	ambien	t)			
	, , ,					
ANALYTES:	Spiked Conc.	Log P	pKa	% Rec	%RSC	
ANALI ILOI	(ng/mL)	5 -	•		(n=0)	
1 Timolol	1			97	(•)	
1 11110101	1			31		

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.

©2025 Phenomenex Inc. All rights reserved.

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

