

HPLC Application

ID No.: 22986

Extraction of Amphetamines from Urine using Strata-X-C Microelution & Kinetex 2.6u Biphenyl, 50x2.1

Column: Kinetex[®] 2.6 μ m Biphenyl 100 \AA , 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 methanol

Gradient Profile:	Step No.	Time (min)	Pct A	Pct B
	1	0	90	10
	2	3	5	95
	3	4	5	95
	4	4.01	90	10
	5	6	90	10

Flow Rate: 0.5 mL/min

Col. Temp.: 25 °C

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

Analyst Note: Extraction of Amphetamines from Human Urine Using Strata-X-C u-Elution Plate

Sorbent: Strata-X-C μ Elution 96-well Plate

Condition: 200 μ L methanol

Equilibrate: 200 μ L water

Load: 400 μ L diluted human urine (200 μ L sample diluted 1:1 with water)

Wash 1: 200 μ L 2% formic acid/water

Wash 2: 200 μ L methanol

Elute: 2x25 μ L 5% ammonium hydroxide/acetonitrile: methanol (60:40)

Direct injection: injection of 2 μ L sample directly

HPLC:

Kinetex Biphenyl, 2.6 μ , 50x2.1; A=0.1% FA/water, B=0.1% FA/methanol;

Flow rate=0.5 mL/min; Inj. vol=2 μ L.

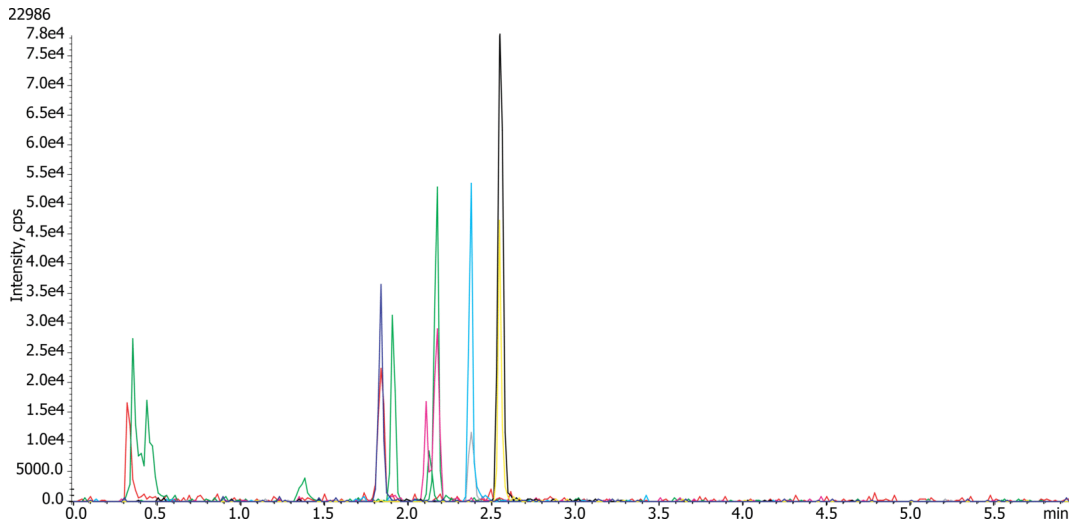
Step	Total Time(min)	Flow Rate (μ L/min)	A (%)	B (%)
0	0.00	500	90.0	10.0
1	3.00	500	5.0	95.0
2	4.0	500	5.0	95.0
3	4.01	500	90.0	10.0
4	6.0	500	90.0	10.0



Products used in this application:



Extraction of Amphetamines from Urine using Strata-X-C Microelution & Kinetex 2.6u Biphenyl, 50x2.1



ANALYTES:

- 1** Amphetamine
Retention Time: 1.83 min
- 2** Methamphetamine
Retention Time: 2.12 min
- 3** MDMA
Retention Time: 2.36 min
- 4** MDA
Retention Time: 2.15 min
- 5** MDEA
Retention Time: 2.53 min



Sample Preparation Details

for HPLC Application ID No.: 22986

Extraction of Amphetamines from Urine using Strata-X-C Microelution & Kinetex 2.6u Biphenyl, 50x2.1

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 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:

Sample pre-treatment

Dilute 150 uL of human plasma (spiked with 25 ng/mL and 125 ng/mL of cortisone and

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

ANALYTES:	Spiked Conc. (ng/mL)	Log P	pKa	% Rec	%RSC (n=0)
1 Amphetamine	125			82	
2 Methamphetamine	125			107	
3 MDMA	62.25			99	
4 MDA	62.25			106	
5 MDEA	62.25			108	

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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