

# HPLC Application

ID No.: 22986

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

**Column:** Kinetex<sup>®</sup> 2.6  $\mu$ m 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 methanol

<b>Gradient Profile:</b>	<b>Step No.</b>	<b>Time (min)</b>	<b>Pct A</b>	<b>Pct B</b>
	<b>1</b>	0	90	10
	<b>2</b>	3	5	95
	<b>3</b>	4	5	95
	<b>4</b>	4.01	90	10
	<b>5</b>	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  $\mu$  Elution 96-well Plate

Condition: 200  $\mu$ L methanol

Equilibrate: 200  $\mu$ L water

Load: 400  $\mu$ L diluted human urine (200  $\mu$ L sample diluted 1:1 with water)

Wash 1: 200  $\mu$ L 2% formic acid/water

Wash 2: 200  $\mu$ L methanol

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

Direct injection: injection of 2  $\mu$ L sample directly

HPLC:

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

Flow rate=0.5 mL/min; Inj. vol=2 $\mu$ L.

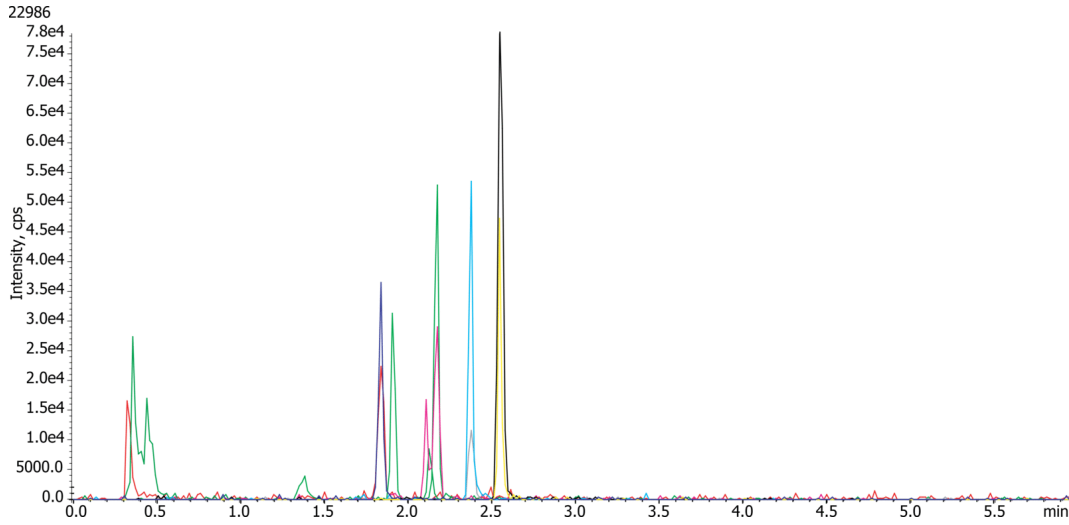
Step	Total Time(min)	Flow Rate ( $\mu$ L/min)	A (%)	B (%)
0	0.00	500	90.0	10.0
1	3.00	500	5.0	95.0
2	4.0	500	5.0	95.0
3	4.01	500	90.0	10.0
4	6.0	500	90.0	10.0



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:

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#### Load:

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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:

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#### Dry:

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#### Elute:

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### Final Prep and Analysis:

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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)

<b>ANALYTES:</b>	<b>Spiked Conc. (ng/mL)</b>	<b>Log P</b>	<b>pKa</b>	<b>% Rec</b>	<b>%RSC (n=0)</b>
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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