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 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 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)

Products used in this application:

KINETEX®

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 uL methanol

Equilibrate: 200 uL water

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

Wash 1: 200 uL 2% formic acid/water

Wash 2: 200 uL methanol

Elute: 2x25uL 5% ammonium hydroxide/acetonitrile: methanol (60:40)

Direct injection: injection of 2 uL sample directly

HPLC:

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

Flow rate=0.5 mL/min; Inj. vol=2uL.

Step	Total Time(min)	Flow Rate (µl/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

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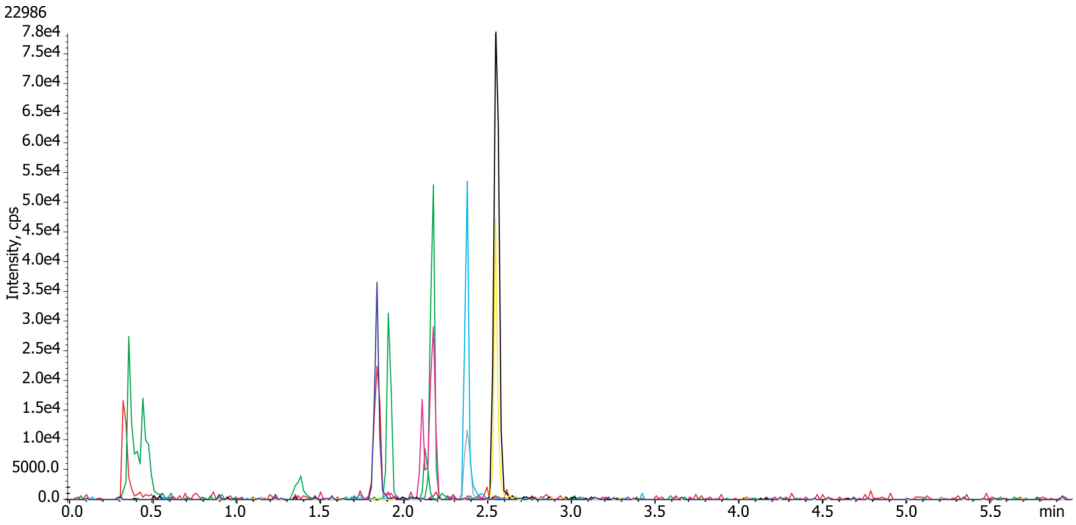

ANALYTES:

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) PRODCEDURE:

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 µL 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.*