

APPLICATION

Extraction and LC/MS/MS Analysis of Amphetamines from Human Urine Using Strata®-X-C Microelution 96-Well Solid Phase Extraction (SPE) Plates and a Kinetex® 2.6 µm Biphenyl Core-Shell HPLC Column

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Overview

This application note describes an ultra-sensitive extraction method to isolate five amphetamines from urine using Strata-X-C Microelution 96-well SPE plates followed by LC/MS/MS analysis using a Kinetex 2.6µm Biphenyl Core-Shell HPLC column. By using the Microelution SPE format, we were able to skip the dry down step saving at least 30 minutes without negatively impacting the sensitivity of our analysis. The five amphetamines were accurately quantified at detection levels down to 25% below the cutoff levels specified by the Substance Abuse and Mental Health Services Administration (SAMHSA).

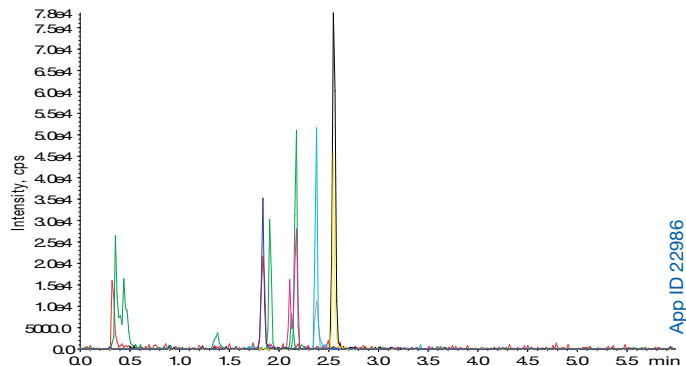
Analytes

1. Amphetamine
2. Methamphetamine
3. MDA
4. MDMA
5. MDEA

Extraction Procedure

Microelution 96-Well Plate: Strata-X-C Microelution 96-Well Plate, 2 mg/well
Part No.: 8M-S029-4GA
Condition: 200 µL Methanol
Equilibrate: 200 µL Water
Load: 400 µL diluted urine (200 µL urine diluted 1:1 with water)
Wash 1: 200 µL 2% Formic acid in water
Wash 2: 200 µL Methanol
Elute: 2x 25 µL 5% Ammonium hydroxide in acetonitrile/methanol (60:40)
Injection: 2 µL

Representative Chromatogram of Amphetamines Extracted from Human Urine



Column: Kinetex 2.6 µm Biphenyl
Dimensions: 50 x 2.1 mm
Part No.: 00B-4622-AN
Mobile Phase: A: 0.1 % Formic acid in Water
 B: 0.1 % Formic acid in Methanol
Gradient:

Time (min)	% B
0.00	10
3.00	95
4.00	95
4.01	10
6.00	10

Flow Rate: 500 µL/min
Temperature: Ambient
Detection: MS/MS, Triple Quad™ 4500 (SCIEX), ESI+

Absolute Recovery of Amphetamines Extracted from Human Urine

Amphetamines	Concentration (ng/mL) (25% below SAMHSA cut off)	RT (min)	% Absolute Recovery	% CV (N=8)
Amphetamine	125	1.83	82	13.1
Methamphetamine	125	2.12	107	15.1
MDA	62.25	2.15	106	4.2
MDMA	62.25	2.36	99	15.7
MDEA	62.25	2.53	108	10.5

Ordering Information

Strata-X-C Solid Phase Extraction (SPE)

Format	Sorbent Mass	Part Number	Unit
Microelution 96-Well Plate			
	2 mg	8M-S029-4GA	1 Plate/Box
96-Well Plate			
	10 mg	8E-S029-AGB	2 Plates/Box
	30 mg	8E-S029-TGB	2 Plates/Box
	60 mg	8E-S029-UGB	2 Plates/Box
Tube			
	30 mg	8B-S029-TAK*	1 mL(100/box)
	30 mg	8B-S029-TBJ	3 mL (50/box)
	60 mg	8B-S029-UBJ*	3 mL (50/box)
	100 mg	8B-S029-EBJ	3 mL (50/box)
	100 mg	8B-S029-ECH	6 mL (30/box)
	200 mg	8B-S029-FBJ	3 mL (50/box)
	200 mg	8B-S029-FCH	6 mL (30/box)
	500 mg	8B-S029-HBJ	3 mL (50/box)
	500 mg	8B-S029-HCH	6 mL (30/box)

*tab-less tubes available

Ordering Information (cont'd)



APPLICATION

Ordering Information (cont'd)

Kinetex[®] Core-Shell HPLC/UHPLC Columns

Phase	5 µm Minibore Columns (mm)			SecurityGuard [™] ULTRA Cartridges [†]
	50 x 2.1	100 x 2.1	3/pk	
Biphenyl	00B-4627-AN	00D-4627-AN	AJ0-9209	for 2.1 mm ID

Phase	5 µm MidBore [™] Columns (mm)			SecurityGuard [™] ULTRA Cartridges [†]
	50 x 3.0	100 x 3.0	3/pk	
Biphenyl	00B-4627-Y0	00D-4627-Y0	AJ0-9208	for 3.0 mm ID

Phase	5 µm Analytical Columns (mm)				SecurityGuard [™] ULTRA Cartridges [†]
	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	3/pk
Biphenyl	00B-4627-E0	00D-4627-E0	00F-4627-E0	00G-4627-E0	AJ0-9207 for 4.6 mm ID

Phase	2.6 µm Minibore Columns (mm)				SecurityGuard [™] ULTRA Cartridges [†]
	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
Biphenyl	00A-4622-AN	00B-4622-AN	00D-4622-AN	00F-4622-AN	AJ0-9209 for 2.1 mm ID

Phase	2.6 µm MidBore Columns (mm)			SecurityGuard [™] ULTRA Cartridges [†]
	50 x 3.0	100 x 3.0	150 x 3.0	3/pk
Biphenyl	00B-4622-Y0	00D-4622-Y0	00F-4622-Y0	AJ0-9208 for 3.0 mm ID

Phase	2.6 µm Analytical Columns (mm)				SecurityGuard [™] ULTRA Cartridges [†]
	50 x 4.6	100 x 4.6	150 x 4.6	3/pk	
Biphenyl	00B-4622-E0	00D-4622-E0	00F-4622-E0	AJ0-9207	for 4.6 mm ID

Phase	1.7 µm Minibore Columns (mm)				SecurityGuard [™] ULTRA Cartridges [†]
	50 x 2.1	100 x 2.1	150 x 2.1	3/pk	
Biphenyl	00B-4628-AN	00D-4628-AN	00F-4628-AN	AJ0-9209	for 2.1 mm ID

[†] SecurityGuard ULTRA Cartridges require holder, Part No.: AJ0-9000



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Strata-X is patented by Phenomenex. U.S. Patent No. 7,119,145

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