

APPLICATION

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

Shahana Huq and Matthew Brusius
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

Overview

This application note describes an ultra-sensitive extraction method to isolate six benzodiazepines from urine using Strata-X-C Microelution 96-well SPE plates followed by LC/MS/MS analysis using a Kinetex 2.6 µm Biphenyl 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 six benzodiazepines were accurately quantified at detection levels down to 25 % below the cutoff levels specified by the Substance Abuse and Mental Health Services Administration (SAMHSA).

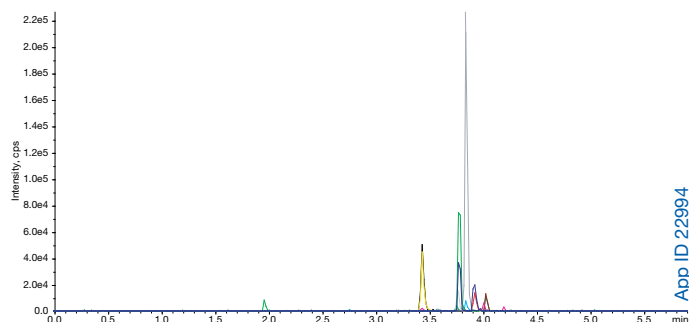
Analytes

1. Midazolam
2. α-OH-Alprazolam
3. Nordiazepam
4. Lorazepam
5. Alprazolam
6. Diazepam

Extraction Procedure

Microelution 96-Well Strata-X-C Microelution 96-Well Plate, 2 mg/well
Plate:
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 Benzodiazepines 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 Benzodiazepines Extracted from Human Urine

Benzodiazepines	Concentration (ng/mL) (25 % below SAMHSA cut off)	RT (min)	% Absolute Recovery	% CV (N=4)
Midazolam	25	3.40	94	10.4
α-OH-Alprazolam	25	3.72	115	4.2
Nordiazepam	25	3.74	101	10.6
Lorazepam	25	3.81	111	14.3
Alprazolam	25	3.88	88	10.3
Diazepam	25	4.00	77	9.5

Ordering Information

Strata-X-C Solid Phase Extraction (SPE)

Format	Sorbent Mass	Part Number	Unit
Microelution 96-Well	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)

*Tabless 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

Australia
t: +61 (0)2-9428-6444
f: +61 (0)2-9428-6445
auinfo@phenomenex.com

Austria
t: +43 (0)1-319-1301
f: +43 (0)1-319-1300
anfrage@phenomenex.com

Belgium
t: +32 (0)2 503 4015 (French)
t: +32 (0)2 511 8666 (Dutch)
f: +31 (0)30-2383749
beinfo@phenomenex.com

Canada
t: +1 (800) 543-3681
f: +1 (310) 328-7768
info@phenomenex.com

China
t: +86 (0)20 2282-6668
f: +86 (0)20 2809-8130
chinainfo@phenomenex.com

Denmark
t: +45 4824 8048
f: +45 4810 6265
nordicinfo@phenomenex.com

Finland
t: +358 (0)9 4789 0063
f: +45 4810 6265
nordicinfo@phenomenex.com

France
t: +33 (0)1 30 09 21 10
f: +33 (0)1 30 09 21 11
franceinfo@phenomenex.com

Germany
t: +49 (0)6021-58830-0
f: +49 (0)6021-58830-11
anfrage@phenomenex.com

India
t: +91 (0)40-3012 2400
f: +91 (0)40-3012 2411
indiainfo@phenomenex.com

Ireland
t: +353 (0)1 247 5405
f: +44 1625-501796
eireinfo@phenomenex.com

Italy
t: +39 051 6327511
f: +39 051 6327555
italiainfo@phenomenex.com

Luxembourg
t: +31 (0)30-2418700
f: +31 (0)30-2383749
nlinfo@phenomenex.com

Mexico
t: 001-800-844-5226
f: 001-310-328-7768
tecnicomx@phenomenex.com

The Netherlands
t: +31 (0)30-2418700
f: +31 (0)30-2383749
nlinfo@phenomenex.com

New Zealand
t: +64 (0)9-4780951
f: +64 (0)9-4780952
nzinfo@phenomenex.com

Norway
t: +47 810 02 005
f: +45 4810 6265
nordicinfo@phenomenex.com

Puerto Rico
t: +1 (800) 541-HPLC
f: +1 (310) 328-7768
info@phenomenex.com

Spain
t: +34 91-413-8613
f: +34 91-413-2290
espinfo@phenomenex.com

Sweden
t: +46 (0)8 611 6950
f: +45 4810 6265
nordicinfo@phenomenex.com

United Kingdom
t: +44 (0)1625-501367
f: +44 (0)1625-501796
ukinfo@phenomenex.com

USA
t: +1 (310) 212-0555
f: +1 (310) 328-7768
info@phenomenex.com

**All other countries
Corporate Office USA** 
t: +1 (310) 212-0555
f: +1 (310) 328-7768
info@phenomenex.com



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