

# APPLICATION

## Extraction and LC/MS/MS Analysis of Benzodiazepines from Human Urine Using Strata<sup>®</sup>-X-C Microelution 96-Well Solid Phase Extraction (SPE) Plates and a Kinetex<sup>®</sup> 2.6 $\mu$ m Biphenyl HPLC Column

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### 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  $\mu$ m 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).

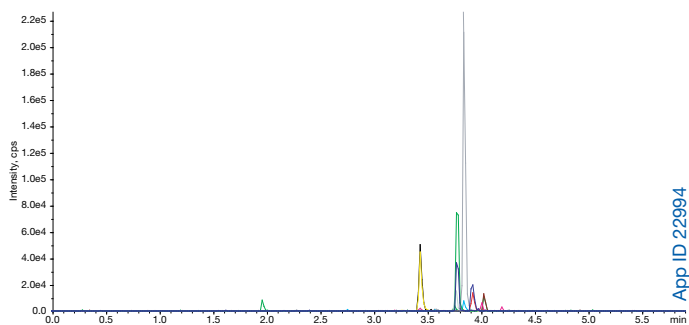
### Analytes

1. Midazolam
2.  $\alpha$ -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  $\mu$ L Methanol  
**Equilibrate:** 200  $\mu$ L Water  
**Load:** 400  $\mu$ L diluted urine (200  $\mu$ L urine diluted 1:1 with water)  
**Wash 1:** 200  $\mu$ L 2% Formic acid in water  
**Wash 2:** 200  $\mu$ L Methanol  
**Elute:** 2x 25  $\mu$ L 5% Ammonium hydroxide in acetonitrile/methanol (60:40)  
**Injection:** 2  $\mu$ L

### Representative Chromatogram of Benzodiazepines Extracted from Human Urine



**Column:** Kinetex 2.6  $\mu$ m 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  $\mu$ L/min  
**Temperature:** Ambient  
**Detection:** MS/MS, Triple Quad<sup>™</sup> 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
$\alpha$ -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<sup>®</sup> Core-Shell HPLC/UHPLC Columns

Phase	5 µm Minibore Columns (mm)		SecurityGuard <sup>™</sup> ULTRA Cartridges <sup>†</sup>
	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 <sup>™</sup> Columns (mm)			SecurityGuard <sup>™</sup> ULTRA Cartridges <sup>†</sup>
	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 <sup>™</sup> ULTRA Cartridges <sup>†</sup>
	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 <sup>™</sup> ULTRA Cartridges <sup>†</sup>
	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 <sup>™</sup> ULTRA Cartridges <sup>†</sup>
	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 <sup>™</sup> ULTRA Cartridges <sup>†</sup>
	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 <sup>™</sup> ULTRA Cartridges <sup>†</sup>
	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	

<sup>†</sup> 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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