

APPLICATIONS

A Sensitive Extraction and Analysis of Urinary Metanephries Using Strata[®]-X-CW Microelution Solid Phase Extraction (SPE) and Kinetex[®] Biphenyl HPLC Column

Matthew Brusius and Jeff Layne
 Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA



Matt Brusius
Product Manager,
Sample Preparation
Matt Brusius is an avid ice hockey player. He likes skating backwards and taking slapshots from the point.



Introduction

Metanephrine and normetanephrine are both metabolites of epinephrine and norepinephrine. In this technical note we will explore how to use Strata-X-CW Microelution SPE 96-Well Plates in conjunction with a Kinetex Biphenyl HPLC column in order to resolve an interference that coelutes with 3-Methoxytyramine on a standard C18 HPLC column, while reaching low limits of quantification for metanephrine and normetanephrine.

Materials and Methods

Sample Preparation

Urine Pretreatment: 500 μ L of urine was diluted with 500 μ L of 50 mM Ammonium acetate buffer, (pH 7). Urine was pre-spiked from 10 ng/mL to 63 pg/mL with metanephrine, normetanephrine, and 3-methoxytyramine (standards provided by Cerilliant[®]).

Solid Phase Extraction Method

Microelution 96-Well Plate: Strata-X-CW Microelution 96-Well Plate, 2 mg/well
Part No.: 8M-S035-4GA
Condition: 200 μ L Methanol
Equilibrate: 200 μ L 50 mM Ammonium acetate buffer, pH 7
Load: 1 mL of pretreated sample
Wash 1: 200 μ L of 50 mM Ammonium acetate buffer, pH 7
Wash 2: 200 μ L Acetonitrile/IPA (1:1)
Elute: 2 x 25 μ L of Water/Acetonitrile/Formic acid (85:10:5)*
Injection: Dilute eluent with 100 μ L of 0.1% Formic acid in water**

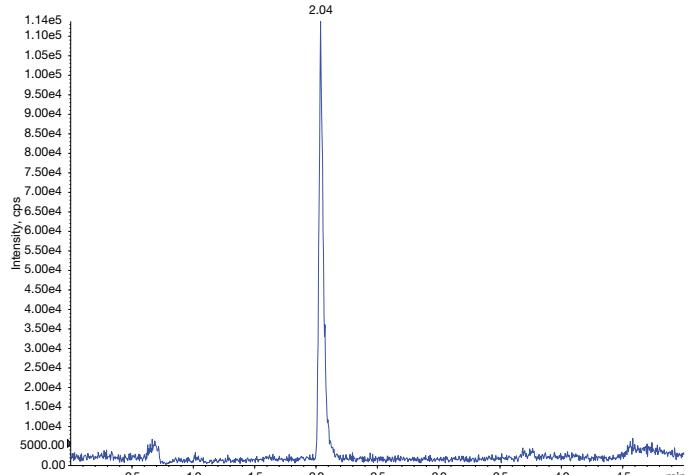
HPLC Conditions

Column: Kinetex 5 μ m Biphenyl
Dimensions: 50 x 4.6 mm
Part No.: 00B-4627-E0
SecurityGuard Cartridge: AJ0-9207
Mobile Phase: A: 0.1% Formic acid in Water
 B: 0.1% Formic acid in Methanol
Gradient: Time (min) B (%)
 0 5
 3 90
 3.1 5
 5 5
Flow Rate: 0.7 mL/min
Injection Volume: 30 μ L
Temperature: Ambient
Detection: API 4000TM MS/MS (SCIEX[®])

Table 1. Recovery Values from 10 ng/mL to 63 pg/mL

Analyte Concentration (ng/mL)	Average % Recovery	%CV (n=6)
Metanephrine		
10	102	5
1	102	3
0.5	99	2
0.25	99	3
0.125	97	3
0.063	94	6
Normetanephrine		
10	100	10
1	87	12
0.5	110	10
0.25	89	9
0.125	110	13
0.063	108	15
3-Methoxytyramine		
10	91	3
1	89	6
0.5	95	4
0.25	86	5
0.125	87	6
0.063	92	7

Figure 1. Chromatogram of unresolved interference for 3-methoxytyramine using C18 1ng/mL



App ID 23845

*Elution solvent optimized to minimize amount of organic to allow for minimal post elution dilution

**Internal standard Metanephrine-D3 was included at 1 ng/mL in this portion of 100 μ L diluent

Figure 2. Chromatogram of resolved interference for 3-methoxytyramine using Kinetex[®] Biphenyl at 1 ng/mL

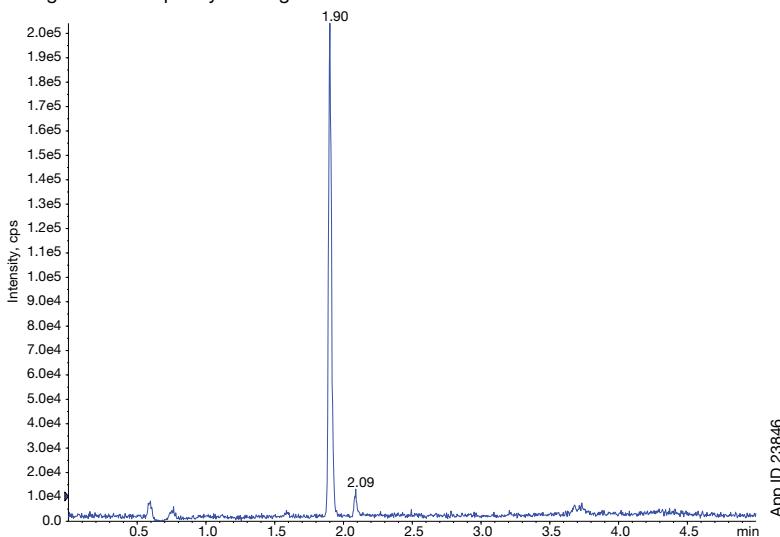


Figure 3. 3-Methoxytyramine separated interference at 63 pg/mL on Kinetex Biphenyl

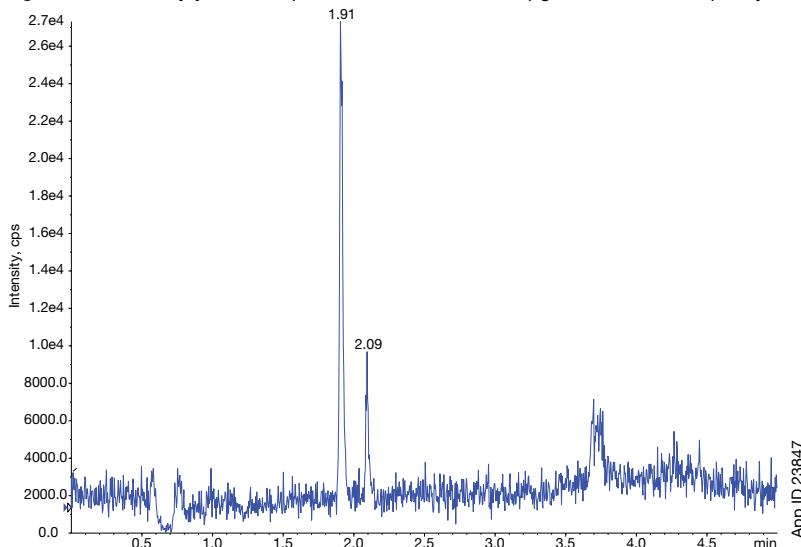


Figure 4. Representative Chromatogram of Urinary Catecholamines

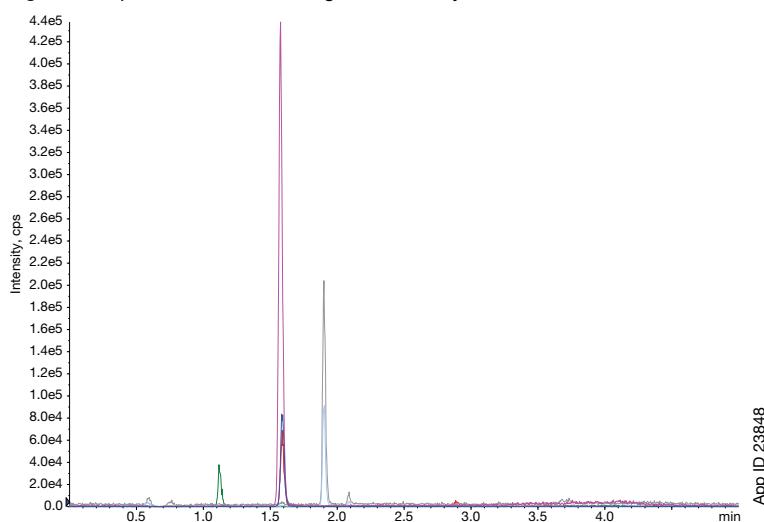


Figure 5. Representative Calibration Curve for Metanephrine

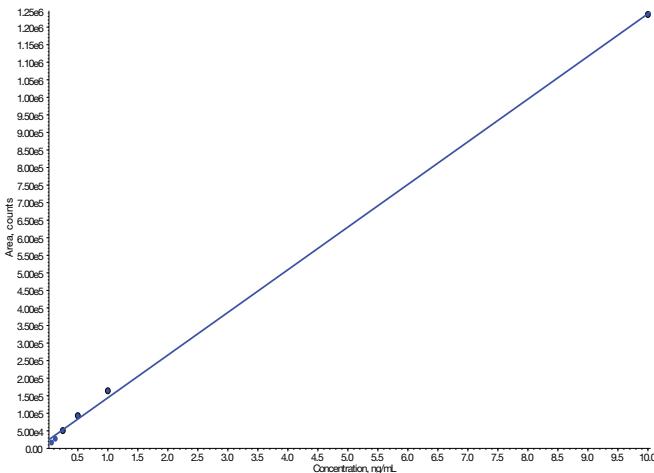


Figure 7. Representative Calibration Curve for 3-Methoxytyramine

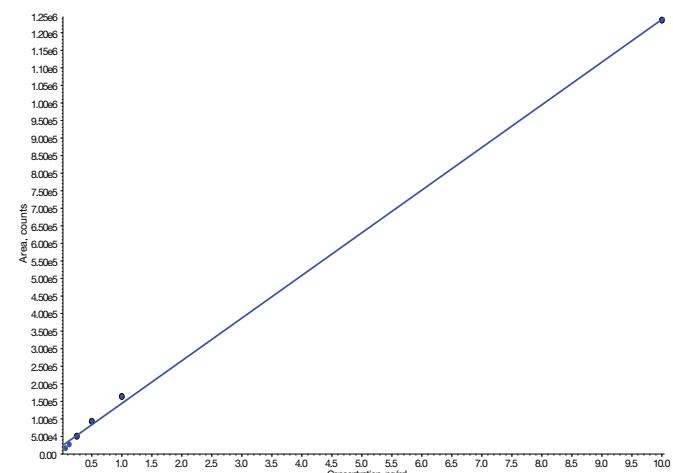
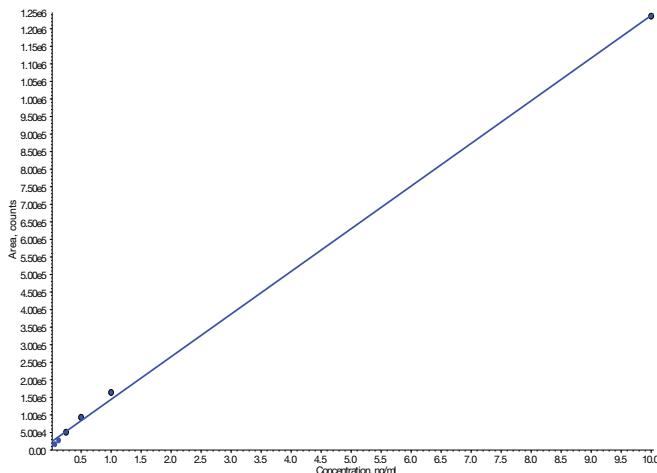


Figure 6. Representative Calibration Curve for Normetanephrine



Results and Discussion

HPLC: **Figure 1** shows the Extracted-Ion Chromatography (XIC) for 3-Methoxytyramine using a standard C18 50 x 4.6 HPLC column. This figure shows only one, albeit tailing, peak. By contrast, **Figure 2** is the XIC for 3-Methoxytyramine using a Kinetex[®] Biphenyl HPLC column which clearly shows two distinct peaks, one at 1.9 minutes as well as one at 2.09 minutes. This indicates that the biphenyl selectivity is able to resolve an interference in the sample that is not resolved with the standard C18 column.

Figure 3 shows the same separation as **Figure 1**, however it is at a much lower level (63 pg/mL), making it even more important that the interference is completely resolved. **Figure 4** exhibits a chromatogram for all analytes in the suite including Metanephrine-D3 at 1 ng/mL.

SPE: **Table 1** provides absolute recovery values for all three of the compounds analyzed, and **Figures 5, 6 and 7** show representative calibration curves for each analyte from 10 ng down to 63 pg where $R > 0.9995$. All absolute recovery values are greater than

86 % and the highest % CV is for normetanephrine at 63 pg/mL (15 %).

The 10 % acetonitrile elution is chosen purposefully. By minimizing the amount of organic in the elution step, the need for post elution dilution (to minimize peak distortion/splitting) is also decreased and therefore only a 2:1 dilution is required. By starting at 500 μ L (urine sample) and ending in 150 μ L (elution volume plus diluent), the solid phase extraction process concentrated the samples 3x without the need for dry down and reconstitution.

Conclusion

In conclusion, we have shown that by implementing an SPE step using Strata[®]-X-CW microelution in conjunction with a Kinetex[®] Biphenyl HPLC column we were able to maximize sensitivity and accuracy and display the utility of a method for detecting catecholamines that can reach at least 63 pg/mL on an API 4000[™] LC-MS/MS (SCIEX[®]).

APPLICATIONS

Ordering Information

Strata[®]-X Microelution 96-Well SPE Plates

Part No.	Description	Unit
8M-S035-4GA	Strata-X-CW 33 µm Polymeric Weak Cation-Exchange Microelution 96-Well Plate, 2 mg/well	1/pk
8M-S029-4GA	Strata-X-C 33 µm Polymeric Strong Cation-Exchange Microelution 96-Well Plate, 2 mg/well	1/pk
8M-S100-4GA	Strata-X 33 µm Polymeric Reversed Phase Microelution 96-Well Plate, 2 mg/well	1/pk
8M-S123-4GA	Strata-X-A 33 µm Polymeric Strong Anion-Exchange Microelution 96-Well Plate, 2 mg/well	1/pk
8M-S038-4GA	Strata-X-AW 33 µm Polymeric Weak Anion-Exchange Microelution 96-Well Plate, 2 mg/well	1/pk

Kinetex [®] 2.6 µm Analytical Columns (mm)				SecurityGuard ULTRA Cartridges [†]
Phase	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

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

Presston[™] 1000 Positive Pressure Manifold

Part No.	Description
AH1-7033	Presston 1000 Positive Pressure Manifold, 96-Well Plate

Phenomenex warrants the Presston 1000 will be free of defects in materials and workmanship under normal installation, use, and maintenance for a period of 12 months following delivery. Please visit www.phenomenex.com/Presstonwarranty for complete warranty information.



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

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

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

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

China
t: +86 400-606-8099
cninfo@phenomenex.com

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

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

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

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

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

Ireland
t: +353 (0)1 247 5405
ireinfo@phenomenex.com

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

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

Mexico
t: 01-800-844-5226
tecnicomx@phenomenex.com

www.phenomenex.com

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

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