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Extraction of Urinary Catecholamines and Metanephrines Using EVOLUTE® EXPRESS WCX SPE Prior to LC-MS/MS Analysis

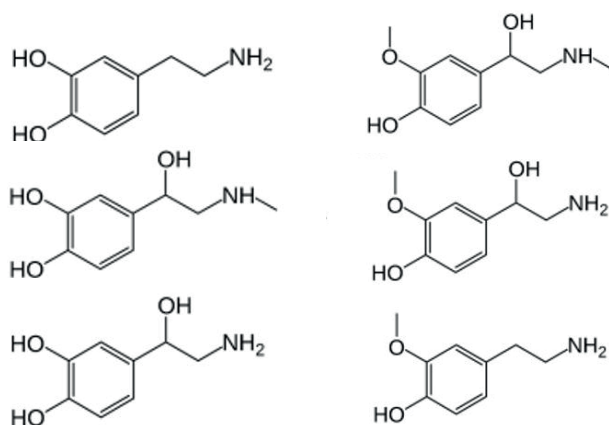


Figure 1. Structures of dopamine, epinephrine and norepinephrine (left), metanephrine, normetanephrine and 3-methoxytyramine (right).

Introduction

This application note describes a mixed-mode weak cation exchange SPE protocol for the extraction of three catecholamines (epinephrine, norepinephrine and dopamine) and three metanephrine metabolites (metanephrine, normetanephrine and 3-methoxytyramine) from urine prior to LC-MS/MS detection.

The method described in this application achieves high reproducible recoveries for a number of common catecholamine analytes in urine.

EVOLUTE® EXPRESS SPE products dramatically improve flow characteristics, and enhance sample preparation productivity. By truly eliminating the need for column conditioning and equilibration, samples can be prepared using a simple, fast load-wash-elute procedure.

Analytes

Epinephrine, Norepinephrine, Dopamine, Metanephrine, Normetanephrine and 3-Methoxytyramine (Sigma Aldrich Chemical Co, Poole, UK).

Internal Standards

D₆-Epinephrine, D₆-Norepinephrine, D₄-Dopamine, D₃-Metanephrine, D₃-Normetanephrine (Sigma Aldrich Chemical Co, Poole, UK).

Solid Phase Extraction Procedure

Format:

EVOLUTE® EXPRESS WCX 10 mg fixed well plate, part number 602-0010-PX01.

Sample Pretreatment:

Mix urine (75 µL) with 10 µL of internal standard solution and 250 mM ammonium acetate solution (150 µL). Mix.

Condition:

OPTIONAL. NOT REQUIRED in load-wash-elute procedure. Condition wells with methanol (500 µL). Processing conditions: load at 1–2 psi when using a Biotage® PRESSURE+ 96 manifold.

Equilibration:

OPTIONAL. NOT REQUIRED in load-wash-elute procedure. Condition wells with 10 mM ammonium acetate (500 µL). Processing conditions: load at 1–2 psi when using a Biotage® PRESSURE+ 96 manifold.

Sample Loading:

Load pre-treated urine (150 µL). Processing conditions: load at 1–2 psi when using a Biotage® PRESSURE+ 96 manifold.

Wash 1:

Elute interferences with 10mM ammonium acetate (500 µL). Processing conditions: load at 1–2 psi, followed by 50 psi for 2s when using a Biotage® PRESSURE+ 96 manifold.

Wash 2:

Elute interferences with propan-2-ol (500 µL). Processing conditions: load at 1–2 psi when using a Biotage® PRESSURE+ 96 manifold. Dry thoroughly at 50 psi for 1 min.

Elution:

Elute analytes with 125 µL of water: propan-2-ol (85:15, v/v) containing formic acid (0.1% v/v). Processing conditions: load at 1–2 psi when using a Biotage® PRESSURE+ 96 manifold. Dry thoroughly at 50 psi for 1 min.

Post Elution:

Cap the SPE plate. No evaporation step is required.

UPLC Conditions

Instrument

Shimadzu Nexera UHPLC system

Column

ACE Excel 1.7 C18 PFP 100 x 2.1 mm

Guard

Securityguard C18

Mobile Phase

A: Water containing 0.25 mM ammonium formate and formic acid

B: Methanol containing 0.25 mM ammonium formate and formic acid

(See “Reagent Preparation” for preparation procedure)

Flow Rate:

0.4 mL min⁻¹

Injection

7.5 µL

Gradient

Initial to 1.3 min hold at 5 % B

1.4 to 4.3 min hold at 95 % B

4.4 to 7.5 min hold at 5 % B

Column Temperature

40 °C

Sample Temperature

5 °C

Table 1. Typical retention times for catecholamines and metanephrines.

Compound	Retention time (min)
Epinephrine	0.76
Norepinephrine	1.02
Dopamine	1.24
Normetanephrine	1.22
Metanephrine	1.24
3-Methoxytyramine	2.9

Refer also to additional notes for more information.

Switching Valve Settings

Initial setting: Waste

Switch to MS: 0.4 min

Switch to waste: 3.4 min

NOTE: Other U/HPLC methods may be appropriate.

MS Conditions

Ions were selected in order to achieve maximum sensitivity using multiple reaction monitoring.

Instrument

AB Sciex 5500

Curtain Gas

35

Collision Gas

7

Ion Spray Voltage

5500

Temperature

700 °C

Ion Source Gas 1 (GS1)

50

Ion Source Gas (GS2)

50

MRM Parameters

Table 2. MRM Parameters.

Analyte	Transition	DP, V	EP, V	CE, V	CXP, V
Epinephrine	166.1 > 107.1	148	8	24	16
D ₆ -Epinephrine	172.1 > 112.1	148	8	24	16
Norepinephrine	152.1 > 107.1	25	2	22	25
D ₆ -Norepinephrine	158.1 > 111.1	25	2	22	25
Dopamine	154.1 > 91.1	50	9	29	13
D ₄ -Dopamine	158.1 > 95.1	50	9	29	13
Metanephrine	180.1 > 148.0	25	8	22	16
D ₃ -Metanephrine	183.1 > 151.0	25	8	22	16
Normetanephrine	166.1 > 134.0	25	9	20	16
D ₃ -Normetanephrine	169.1 > 137.0	25	9	20	16
3-Methoxytyramine	151.2 > 90.9	110	9	26	11

Results

Extraction recoveries were calculated at the top calibration concentration as follows:

Table 3. Top calibration concentrations used for recovery calculation.

Analytes	Top Standard Concentration
Norepinephrine	250 ng/mL
Epinephrine	25 ng/mL
Dopamine	625 ng/mL
Normetanephrine	125 ng/mL
Metanephrine	125 ng/mL
3-Methoxytyramine	250 ng/mL

Data is summarized from the results of n=8 extracted samples compared to n=5 blank samples fortified with an amount of analytes equivalent to 100% after extraction.

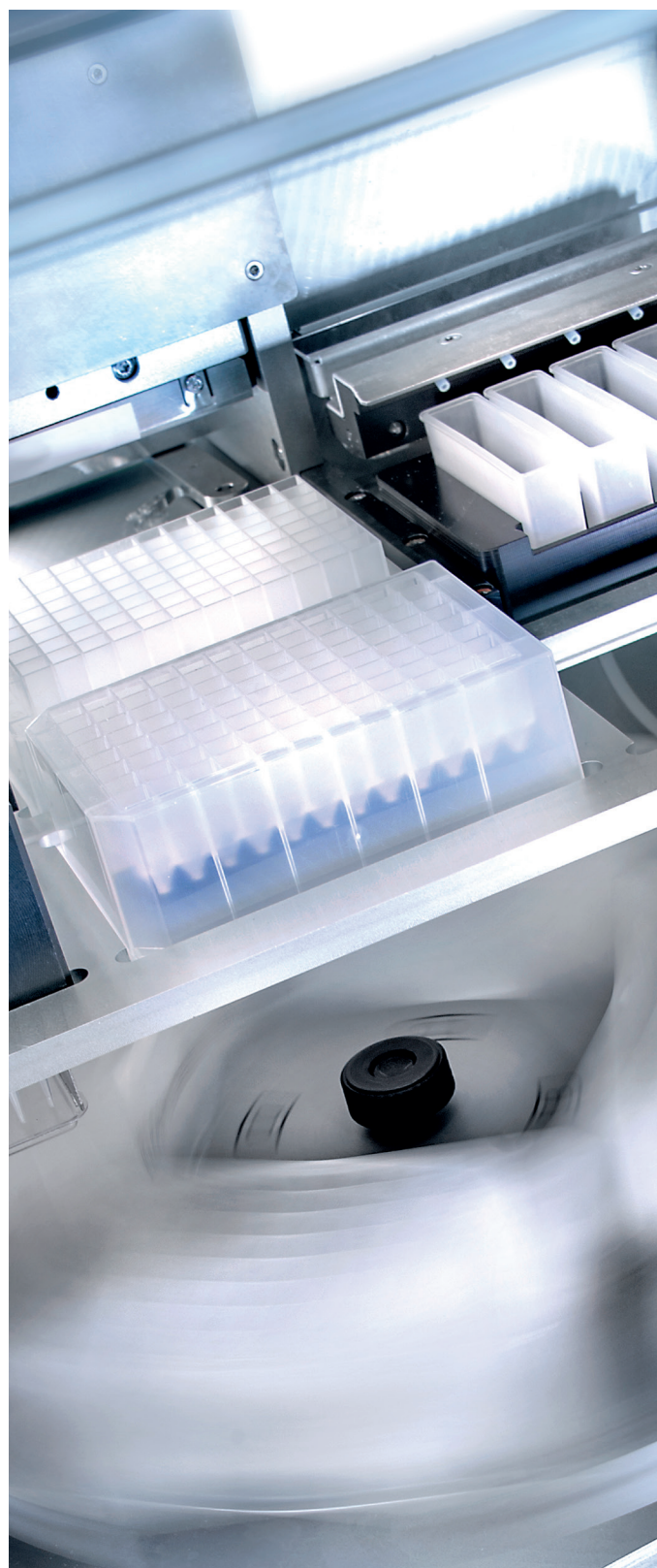
Table 4. Extraction recoveries (Manual procedure)

	Traditional Method		Load-Wash-Elute Method	
	Extraction Recovery	% RSD	Extraction Recovery	% RSD
Norepinephrine	88.8	3.5	76.1	7.0
Epinephrine	109.3	7.0	108.3	8.5
Dopamine	91.8	3.1	88.2	3.3
Normetanephrine	87.9	6.6	83.6	8.6
Metanephrine	83.3	5.0	88.4	6.8
3-Methoxytyramine	84.2	4.2	83.6	2.1

Table 5. Extraction recoveries (Biotage® Extrahera™ procedure)

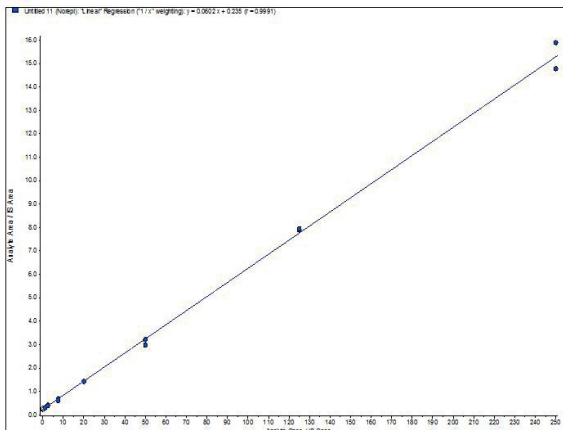
	Traditional Method		Load-Wash-Elute Method	
	Extraction Recovery	% RSD	Extraction Recovery	% RSD
Norepinephrine	77.4	4.9	69.5	5.8
Epinephrine	110.4	7.4	93.7	6.0
Dopamine	87.4	4.2	88.4	2.0
Normetanephrine	80.7	7.4	77.2	7.2
Metanephrine	83.5	5.8	82.4	5.7
3-Methoxytyramine	78.0	5.8	80.1	4.8

Linearity was determined between 0.1 and 25 ng/mL for epinephrine, between 0.5 and 125 ng/mL for metanephrine and normetanephrine, between 1 and 250 ng/mL for norepinephrine and 3-methoxytyramine and between 2.5 and 625 ng/mL for dopamine. Calibration lines for each analyte are displayed on page 4. Similar performance was observed for both standard and Load-Wash-Elute methods, whether processed manually (using the Biotage® Pressure +96 positive pressure manifold), or Biotage® Extrahera™.



Typical Calibration Lines

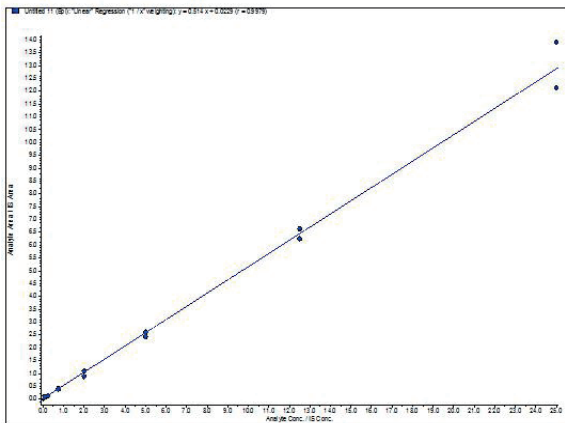
Norepinephrine



Precision, r^2

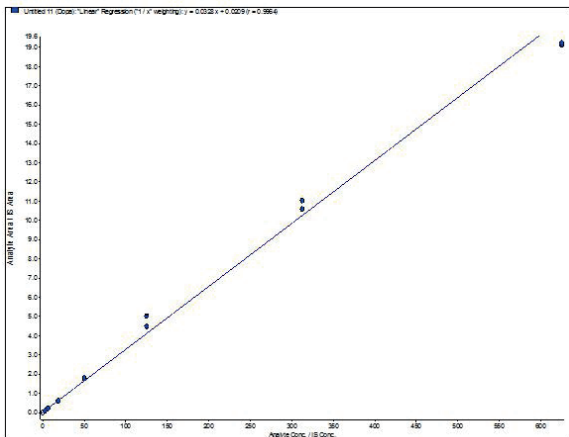
Manual	Biotage® Extrahera™
0.996	0.997
Manual Load-Wash-Elute	Biotage® Extrahera™ Load-Wash-Elute
0.998	0.996

Epinephrine



Manual	Biotage® Extrahera™
0.994	0.995
Manual Load-Wash-Elute	Biotage® Extrahera™ Load-Wash-Elute
0.996	0.991

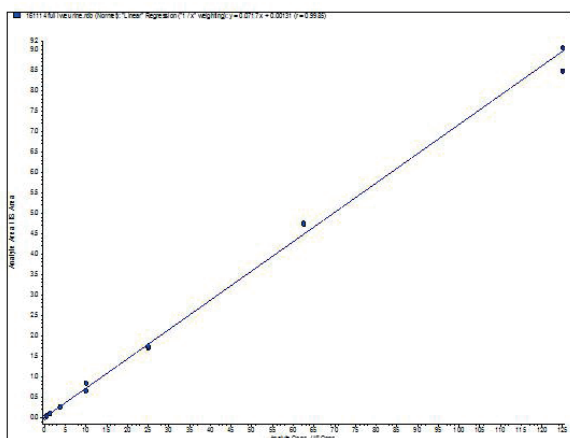
Dopamine



Manual	Biotage® Extrahera™
0.991	0.995
Manual Load-Wash-Elute	Biotage® Extrahera™ Load-Wash-Elute
0.993	0.993

Typical Calibration Lines

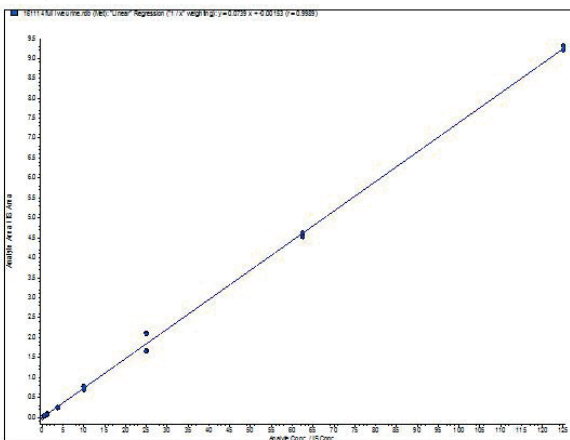
Normetanephrine



Precision, r^2

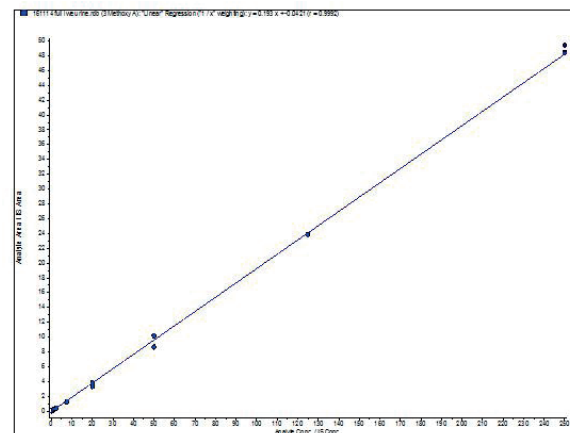
Manual	Biotage® Extrahera™
0.993	0.995
Manual Load-Wash-Elute	Biotage® Extrahera™ Load-Wash-Elute
0.997	0.998

Metanephrine



Manual	Biotage® Extrahera™
0.996	0.990
Manual Load-Wash-Elute	Biotage® Extrahera™ Load-Wash-Elute
0.998	0.996

3 Methoxytyramine



Manual	Biotage® Extrahera™
0.998	0.997
Manual Load-Wash-Elute	Biotage® Extrahera™ Load-Wash-Elute
0.998	1.000

Figure 2. Representative calibration plots of catecholamines.

Preparation of Standards

The calculations below assume all analyte stocks solutions are at a concentration of 1 mg/mL.

- I. An epinephrine substock solution A was prepared by diluting 5 µL of epinephrine stock to 1 mL with water.
- II. A combined substock solution B was prepared by diluting norepinephrine stock (10 µL), 3-methoxytyramine stock (10 µL), metanephrine stock (5 µL), normetanephrine stock (5 µL), and 200 µL of substock A (200 µL), to 1 mL with water.
- III. A final catecholamine and metanephrines substock solution C was produced by diluting 10 µL of dopamine stock and 400 µL of substock B to a total volume of 1 mL with water. The catecholamine and metanephrines substock solution C contained dopamine at 10 µg/mL, norepinephrine and 3-methoxytyramine at 4 µg/mL, metanephrine and normetanephrine at 2 µg/mL and epinephrine at 0.4 µg/mL.
- IV. A top concentration urine solution was then prepared by diluting the final catecholamine and metanephrines substock C by a factor of 1 in 16 with urine giving concentrations of dopamine of 625 ng/mL down to epinephrine at 25 ng/mL. This solution was then further diluted in urine to generate the standards as listed below.

A combined deuterated internal standard (IS) solution was also prepared in water using separate 100 µg/mL stock solutions:

- I. An epinephrine IS substock D solution was prepared by diluting a 5 µL volume of epinephrine IS stock to 1 mL using water.
- II. A combined IS substock E was prepared by diluting normetanephrine IS stock (5 µL), metanephrine IS stock (5 µL) and of epinephrine IS substock D (200 µL) to 1 mL with water.
- III. A catecholamine and metanephrines IS substock F was prepared by diluting norepinephrine IS stock (5 µL) and 500 µL of the combined IS substock E to 1 mL with water.
- IV. A final catecholamine and metanephrines IS substock G was combined by diluting 5 µL dopamine IS stock and 400 µL catecholamine and metanephrines IS stock F to 1 mL with 250 mM ammonium acetate. The final catecholamine and metanephrines IS substock solution G contained dopamine internal standard at 500 ng/mL, norepinephrine IS at 200 ng/mL, metanephrine and normetanephrine IS at 100 ng/mL and epinephrine IS at 20 ng/mL.

All substock and internal standard solutions were prepared daily to prevent degradation.

Table 6. Concentrations of standard solutions used.

Analyte	Std 7	Std 6	Std 5	Std 4	Std 3	Std 2	Std 1
Norepinephrine	250	125	50	20	7.5	2.5	1
Epinephrine	25	12.5	5	2	0.75	0.25	0.1
Dopamine	625	312.5	125	50	18.75	6.25	2.5
Normetanephrine	125	62.5	25	10	3.75	1.25	0.5
Metanephrine	125	62.5	25	10	3.75	1.25	0.5
3-Methoxytyramine	250	125	50	20	7.5	2.5	1

Other Chemicals & Reagents

- » Water (18.2 MΩ.cm) drawn from a Direct-Q 5 water purifier (Merck Millipore, Watford, UK).
- » Ammonium Acetate & Ammonium Formate- Sigma-Aldrich Chemical Co. (Poole, UK).
- » Propan-2-ol Sigma-Aldrich Chemical Co. (Poole, UK).
- » Methanol, MS grade Sigma-Aldrich Chemical Co. (Poole, UK).
- » Urine (MSG5000, ultra low hormone, steroid, drug and alcohol free) was sourced from Golden West Biologicals Inc, Temecula, CA, USA



Reagent Preparation

- » SPE elution solvent, prepared daily
 - » 15 mL of IPA was transferred into a bottle, to this was added 85 mL of water and then 100µL of formic acid.
- » 0.05% formic acid
 - » 250 mL water was transferred to a bottle and to this was added 125 µL of formic acid
- » 10 mM Ammonium acetate
 - » Approximately 350 mg of ammonium acetate was accurately weighed and emptied into a bottle, to this was added water such that 500 mL water was added for every 385.4 mg of ammonium acetate weighed.
- » HPLC mobile phase A and B
 - » Ammonium formate/formic acid. A concentrated solution was prepared by combining 38.75 mg of ammonium formate, with 71 µL of formic acid and diluting with water to a total volume of 5 mL. This was then added to both mobile phases (A: water and B: methanol) as 500 µL of the ammonium formate / formic acid solution per litre of mobile phase.

All other reagents used were pure solvents. The catecholamine and metanephrine sub stocks used were prepared in water.

Additional notes

1. The method described in this application note covers different calibration ranges because of wide native endogenous levels expected from one analyte to another in urine.
2. The method can either be performed using either a traditional SPE procedure or the EVOLUTE® EXPRESS Load-Wash-Elute method (with no conditioning or equilibration steps).
3. Stripped urine (from Golden West) was used to construct calibration lines as control urine contained significant values of all analytes. Comparison plots of blank and lowest concentration standards indicate however that if ultra low measurement of epinephrine or norepinephrine is required then some form of proxy matrix or synthetic urine should be used instead for calibration line and low concentration QC preparation.
4. Catecholamines are relatively unstable so a number of handling procedures were employed for these analytes. Analytes were purchased as solids, stored according to their label and regularly re-prepared. Internal standards were regularly compared for sensitivity alongside analytes increasing their concentrations if necessary. A low autosampler temperature of approximately 5 °C is also recommended to minimize any potential autosampler instability. Working solutions were prepared from stocks on a daily basis.
5. The method described here is suitable for urine only. For extraction from plasma matrix, see Biotage application note AN874.
6. It is essential to the method performance that all traces of wash 2 solvent is removed prior to analyte elution. If using The Biotage® Extrahera™ (with a dual flow head) or Biotage® Pressure +96 manifold, use of a maximum flow setting for at least 10 minutes is recommended. If using other equipment optimize drying conditions to ensure wash 2 solvent is fully removed.
7. With the chromatographic system documented in this application note, three of the components: dopamine, normetanephrine and metanephrine along with their deuterated internal standards elute very close to each other. Minor changes to gradient conditions did not further resolve these peaks significantly. However, analysis of individual components (both analytes and internal standards) confirmed that there was no contribution of one of these analytes or internal standards to the MS transition of another.
8. The chromatography system relies on the combination of a C18 guard followed by a PFP analytical column. The retention times were found to vary slightly depending upon the ages of the columns and so the timings quoted in the application note should only be used as an approximate guide.
9. As the method does not involve an evaporation step extraction recoveries were calculated by comparing extracts of spiked samples + 10 µL of water compared to extracts of blank samples + 10 µL of fortified analytes in water.
10. Due to the high levels of dopamine in urine the calibration line for this analyte could show some non-linearity at higher concentrations due to saturation. If this is significant and high concentrations are expected then the dopamine can be 'detuned' by for example reducing its collision energy from 29 V to 18 V. (see fig 4a and 4b below) The calibration details quoted in this application note were for correctly tuned dopamine with a linear fit.

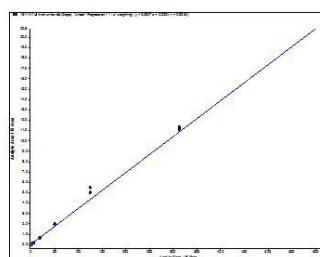


Figure 4a. Dopamine plot showing some non-linearity.

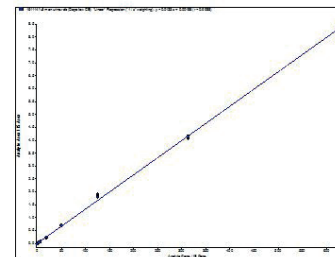


Figure 4b. Detuned Dopamine giving linear plot.

Ordering Information

Part Number	Description	Quantity
602-0010-PX01	EVOLUTE® EXPRESS WCX 10 mg Fixed Well Plate	1
414001	Biotage® Extrahera™ Automation System	1
415040	Configuration Kit 96 Positions Dual Flow	1
414141	Extrahera clear tips	960
PPM-96	Biotage® PRESSURE+ 96 Positive Pressure Manifold (96 well)	1
121-5203	Collection plate, 2 mL, square	50
121-5204	Piercable sealing cap	50

Appendix

Biotage® Extrahera™ Settings

The Load-Wash-Elute SPE method described in this application note was automated on the Biotage® Extrahera™ using EVOLUTE® EXPRESS WCX 10 mg plates. This appendix contains the software settings required to configure Extrahera to run this method. An importable electronic copy of this method for Extrahera can be downloaded from www.biotage.com.

Using this automated procedure, 96 samples can be processed in a total of 51.52 mins.

Biotage® Extrahera™ Data

Analyte	Recovery (n=8)*	% RSD	Linearity (r ²)	LLOQ ng/mL
Norepinephrine	69.5	5.8	0.996	1
Epinephrine	93.7	6.0	0.991	0.1
Dopamine	88.4	2.0	0.993	2.5
Normetanephrine	77.2	7.2	0.998	0.5
Metanephrine	82.4	5.7	0.996	0.5
3-methoxytyramine	80.1	4.8	1.000	1

*Recovery measured at top calibration concentration

Method Name:	Metanephrines and Catecholamines in urine
Sample Plate/Rack:	2 mL sample plate, 96
Extraction Media:	EVOLUTE® EXPRESS WCX



< Cancel Edit SPE Method - Metanephrines & Catechola... Save >

Method name: **Metanephrines & Catecholamines in urine** Sample plate/rack: **2 mL sample plate, 96** Extraction media: **EVOLUTE WCX EXPRESS**

Pre-treatment: On

Conditioning: Off

Equilibration: Off

Load: On

Wash: On

Elution: On

Sample type: **Aqueous sample**

Starting sample volume in plate/rack (µL): **75**

Method comment: **Catecholamines and Metanephrines in human urine using 10mg WCX evolute express. 75 µL urine is added to the block before the start combined with 10 µL of internal standard. The method can be run as a full SPE procedure or as a load wash elute format. If performing as a load wash elute procedure make sure that the conditioning and equilibration steps in this method are left unselected. This method is only recommended for an Extrahera containing a high flow pressure head and for urine analysis.**

Settings

"Sample" Tab

Sample Type:

Aqueous Sample

Starting Sample Volume (µL):

75

Method Comment:

Catecholamines and Metanephrines in human urine using 10 mg EVOLUTE® EXPRESS WCX. 75 µL urine is added to the block before the start combined with 10 µL of internal standard. The method can be run as a full SPE procedure or as a load wash elute format. If performing as a load wash elute procedure make sure that the conditioning and equilibration steps in this method are left unselected. This method is only recommended for an Biotage® Extrahera™ containing a high flow pressure head and for urine analysis.

Screenshot

Method name: Metanephrines & Catecholamines in urine

Sample plate/rack: 2 mL sample plate, 96

Extraction media: EVOLUTE WCX EXPRESS

Number of steps: 1

Solvent: Ammonium acetate 250mM

Volume (µL): 150

Wait time (min): 0

Buttons: Pretreatment (On), Conditioning (Off), Equilibration (Off), Load (On), Wash (On), Elution (On)

Settings

Pre-treatment	Activated
No. of steps	1
Pause after last step	No
Dispose tips after last step	No

Solvent	
1	Ammonium acetate 250 mM
2	
3	
4	

	1	2	3	4
Volume (µL)	150			
Wait Time (min)	0			

Conditioning	Not Activated
No. of steps	
Pressure (Bar)	
Dispose tips after last step	

Solvent	
1	
2	
3	
4	

	1	2	3	4
Volume (µL)				
Position				
Pressure time (s)				
Repeat				
Pause after this step	No			

'Advanced Settings'

Equilibration	Not Activated
No. of steps	
Pressure (Bar)	
Dispose tips after last step	

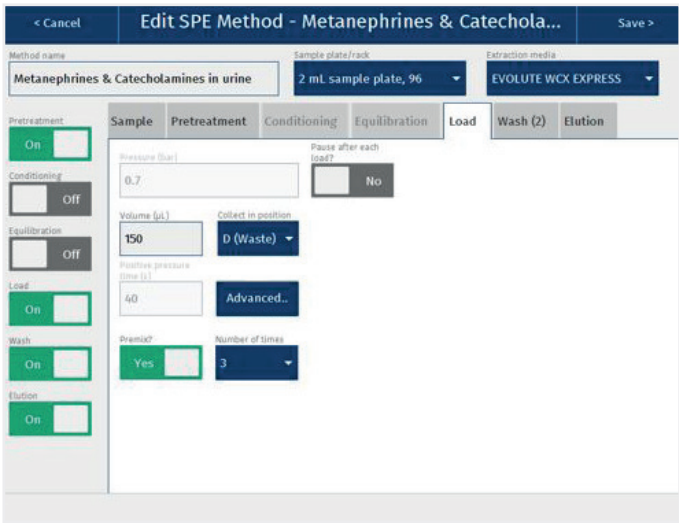
Solvent
1
2
3
4

	1	2	3	4
Volume (µL)				
Position				
Pressure time (s)				
Repeat				
Pause after this step				

'Advanced Settings'

Load	Activated
Pressure (Bar)	Advanced
Pause after each load	No
Volume (µL)	150
Collect in position	D
Positive pressure time (s)	Advanced
Premix	Yes
Number of times	3

'Advanced Settings'
0.0 bar for 300 s then 0.7 bar for 90 s. Plate Dry OFF



Edit SPE Method - Metanephrines & Catechola...

Method name: **Metanephrines & Catecholamines in urine** | Sample plate/rack: **2 mL sample plate, 96** | Extraction media: **EVOLUTE WCX EXPRESS**

Wash (2)

Number of steps	Pressure (bar)	Plate dry after last wash?	Plate dry time (s)	Dispose tips after each step?
2	1.0	No	0	No

1

Solvent: **10mM NH₄OAc pH7**

Volume (µL): **500** | Collect in position: **D (Waste)**

Positive pressure time (s): **40** | **Advanced...**

Repeat (number of times): **1** | Pause after this step? **No**

2

Solvent: **IPA**

Volume (µL): **500** | Collect in position: **D (Waste)**

Positive pressure time (s): **60** | **Advanced...**

Repeat (number of times): **1** | Pause after this step? **No**

Wash	Activated
No. of steps	2
Pressure (Bar)	Advanced
Plate dry after last wash	No
Plate dry time (s)	N/A
Dispose tips after each step	No

Solvent	
1	10 mM NH ₄ OAc pH7
2	IPA
3	
4	

	1	2	3	4
Volume (µL)	500	500		
Collect in position	D	D		
Pressure time (s)	Adv.	Adv.		
Repeat	1	1		
Pause after this step	No	No		

'Advanced Settings'

For 10 mM NH₄OAc pH7 0.0 bar for 180 s then 0.5 bar for 90 s then 5.0 bar for 15 s. Plate Dry ON for 5 s

For IPA 0.5 bar for 90 s then 1.5 bar for 60 s the 5.0 bar for 30 s. Plate Dry ON for 600 s

Edit SPE Method - Metanephrines & Catechola...

Method name: **Metanephrines & Catecholamines in urine** | Sample plate/rack: **2 mL sample plate, 96** | Extraction media: **EVOLUTE WCX EXPRESS**

Elution

Number of steps	Pressure (bar)	Plate dry after last elution?	Plate dry time (s)	Dispose tips after each step?
1	0.5	No	0	No

Solvent: **15% IPA 0.1% Formic acid**

Volume (µL): **125** | Collect in position: **A**

Positive pressure time (s): **60** | **Advanced...**

Repeat (number of times): **1** | Pause after this step? **No**

Elution	Activated
No. of steps	1
Pressure (Bar)	Advanced
Plate dry after last elution	No
Plate dry time (s)	N/A
Dispose tips after each step	No

Solvent	
1	15% IPA 0.1% Formic acid
2	
3	
4	

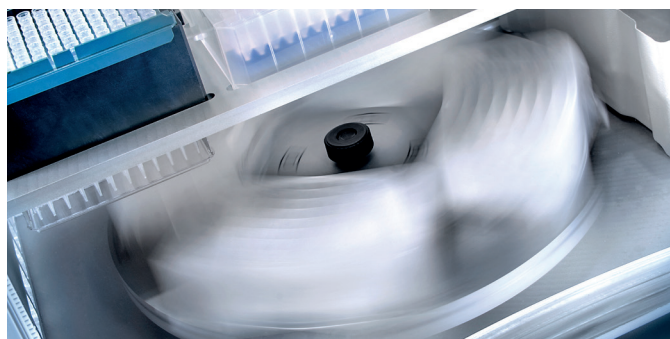
	1	2	3	4
Volume (µL)	125			
Position	A			
Pressure time (s)	Adv.			
Repeat	1			
Pause after this step	No			

'Advanced Settings'

0.0 bar for 120 s then 0.7 bar for 30 s then 2.0 bar for 30 s. Plate Dry ON for 60 s

Solvent Properties

Solvent Description	
1	Solvent description
2	Ammonium acetate 250mM
3	10 mM NH ₄ OAc pH7
4	IPA
5	15% IPA 0.1% Formic acid
6	
7	
8	
9	
10	



Solvent	1	2	3	4	5	6	7	8	9	10
Reservoir Type	Refillable				Non Refillable					
Capacity	N/A	N/A	N/A	N/A	N/A					
Aspiration flow rate (mL/min)	10	10	10	10						
Dispense flow rate (mL/min)	20	20	5	5						
Lower air gap flow rate (mL/min)	20	20	5	5						
Lower air gap volume (µL)	5	5	5	5						
Upper air gap flow rate (mL/min)	20	120	120	120						
Upper air gap volume (µL)	100	100	100	100						
Upper air gap dispense pause	300	300	300	300						
Conditioning?	Yes	Yes	Yes	Yes						
Conditioning number of times	2	2	3	3						
Conditioning flow rate (mL/min)	20	20	5	5						
Chlorinated	No	No	No	No						
Serial dispense	No	No	No	No						

< Cancel Edit Sample - Aqueous sample Save >

Sample	Air Gap
Sample name Aqueous sample	Lower air gap flow rate (mL/min) 20
Sample description Default settings for aqueous	Lower air gap volume (µL) 5
Aspiration flow rate (mL/min) 10	Upper air gap flow rate (mL/min) 120
Dispense flow rate (mL/min) 20	Upper air gap volume (µL) 100
	Upper air gap dispense pause (ms) 300

"Sample" Screen

Sample name	Aqueous sample
Sample description	Aqueous sample
Aspiration flow rate (mL/min)	10
Dispense flow rate (mL/min)	20
Lower air gap flow rate (mL/min)	20
Lower air gap volume (µL)	5
Upper air gap flow rate (mL/min)	120
Upper air gap volume (µL)	100
Upper air gap dispense pause	300

< Cancel Edit Extraction Media - EVOLUTE WCX EXPRESS Save >

Extraction Media	Pipetting Height
Name EVOLUTE WCX EXPRESS	Solvent dispensation height (mm) -125.0
Manufacturer Biotage	Sample dispensation height (mm) -135.0
Part number 602-0010-PX01	Aspiration height (mm) -135.0
Sorbent load (mg) 10	Tune Pipetting Heights...
Capacity volume (µL) 1000	
Format 96	
Comment	

"Extraction Media" Screen

Name	EVOLUTE® EXPRESS WCX
Manufacturer	Biotage
Part number	602-0010-PX01
Sorbent load (mg)	10
Capacity volume (µL)	1000
Format	96
Comment	
Solvent dispensation height (mm)	-125.0
Sample dispensation height (mm)	-135.0
Aspiration height (mm)	-135.0

< Cancel Edit Sample Plate/Rack - 2 mL sample plate, 96 Save >

Sample Plate/Rack	Pipetting Height
Name 2 mL sample plate, 96	Aspiration height (mm) -162.0
Capacity volume (µL) 1800	Pre-treatment dispensation height (mm) -128.0
Format 96	Tune Pipetting Heights...

"Sample Plate/Rack" Screen

Name	2 mL Sample plate, 96
Capacity volume (µL)	1800
Format	96
Aspiration height (mm)	-162.0
Pre-treatment dispensation height (mm)	-128.0

< Cancel	Edit Pipette Tip - 1000 µL Biotage tip	Save >
<div style="border: 1px solid gray; padding: 5px; width: fit-content; margin: auto;"> <p>Pipette Tip</p> <p>Name 1000 µL Biotage tip</p> <p>Manufacturer Biotage</p> <p>Part number 414141</p> <p>Capacity (µL) 1000</p> <p>Length (mm) 95</p> </div>		

"Pipette tip" Screen

Name	1000 µL Biotage Tip
Manufacturer	Biotage
Part number	414141
Capacity (µL)	1000
Length (mm)	95

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