Extraction of Epinephrine, Norepinephrine and Dopamine from Human Plasma Using EVOLUTE® EXPRESS WCX Prior to LC-MS/MS Analysis

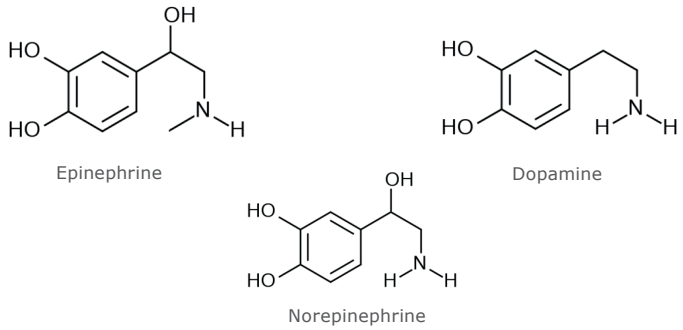

Figure 1. Structures of catecholamines.

Introduction

Catecholamines are biomarkers used for the detection of diseases such as hypertension, pheochromocytoma and neuroblastoma. The main target analytes are dopamine, epinephrine and norepinephrine (see Figure 1. for details), which are traditionally analyzed using liquid chromatography with electrochemical detection.

This application note describes a mixed-mode weak cation exchange solid phase extraction protocol using EVOLUTE® EXPRESS WCX 96-well plates for the extraction of three catecholamines (epinephrine, norepinephrine and dopamine) from human plasma prior to LC-MS/MS detection, which allows low level quantification of the analytes.

EVOLUTE EXPRESS solid phase extraction products combine powerful EVOLUTE sorbent chemistry with enhanced 'EXPRESS' column components. EVOLUTE EXPRESS 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 and Dopamine

Internal Standards

D_6 Epinephrine, D_6 Norepinephrine, D_4 Dopamine (LGC, Teddington, UK).

See Notes for further details on sample and standard preparation.

Sample Preparation Procedure

Format:

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

Sample Pre-treatment

Mix plasma sample (250 µL) containing internal standard with 0.05% (v/v) formic acid (250 µL).

Condition

Optional. Not required in load-wash-elute procedure.
If desired condition wells with methanol (0.5 mL).

Equilibration

Optional. Not required in load-wash-elute procedure.
If desired equilibrate wells with 10mM ammonium acetate (0.5 mL).

Sample Loading

Load 500 µL of pre-treated sample.

Wash 1

Elute interferences with ammonium acetate (10 mM, 0.5 mL)

Wash 2

Elute interferences with propan-2-ol (0.5 mL). Dry thoroughly (e.g. 50 psi, 5 mins, using Biotage® PRESSURE+ 96).

Elution

Elute analytes with 0.1% formic acid in water : propan-2-ol (85:15) (0.2mL), dry thoroughly as above.

Note

To ensure high analyte recoveries and low RSDs, the SPE plate is dried thoroughly immediately before and after elution.

Post Elution

Due to the low elution volume, no evaporation of the extract is required prior to analysis.

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HPLC Conditions

Instrument
Shimadzu Nexera UHPLC system

Column
ACE Excel 1.7 C18 PFP 100 x 2.1 mm

Mobile Phase
A: Water containing 0.25mM ammonium acetate and formic acid

B: Methanol containing 0.25mM ammonium acetate and formic acid
(See 'Additional Notes' for mobile phase preparation details)

Flow Rate
0.4 mL min^{-1}

Injection
20 µL

Gradient
Starting conditions: 95% mobile phase A/5% mobile phase B; hold until 1.2 minutes; Step gradient to 95% and hold until 3.2 minutes.

Resume initial starting conditions at 3.2 minutes and equilibrate for 4 minutes.

Column Temperature
40 °C

Sample Temperature
20 °C

MS Conditions

Positive ions were selected in order to achieve maximum sensitivity using multiple reaction monitoring. Dehydrated precursor ions were selected for epinephrine and norepinephrine to improve sensitivity.

Instrument
AB Sciex 5500

Curtain Gas
35

Collision Gas
7

IonSpray Voltage
5500

Temperature
700 °C

Ion Source Gas 1 (GS1)
50

Ion Source Gas (GS2)
50

Setting Time
40 ms

Pause Between Mass Ranges
5.007 ms

Table 1. Typical retention times for catecholamines

Compound	Retention Time (min)
Epinephrine	0.92
Norepinephrine	0.79
Dopamine	1.04

Switching Valve Settings

Initial Setting	Switch to Waste
Switch to MS	0.4 min
Switch to waste	1.5 min

Table 2. Mass Spectrometer conditions for catecholamines and internal standards

Analyte	Transition	DP (V)	EP (V)	CE (V)	CE(V)
Epinephrine	166.1 to 107.1	148	8	24	16
Norepinephrine	152.1 to 107.1	25	2	22	25
Dopamine	154.1 to 91.1	50	9	29	13
D_6 Epinephrine	172.1 to 112.1	148	8	24	16
D_6 Norepinephrine	158.1 to 111.1	25	2	22	25
D_4 Dopamine	158.1 to 95.1	50	9	29	13

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Results

Table 3. Extraction recoveries (analytes spiked into human plasma at a concentration of 1.28 ng/mL)

Analyte	Standard SPE		EXPRESS Load-Wash-Elute SPE	
	Extraction Recovery	% RSD	Extraction Recovery	% RSD
Epinephrine	88.2	5.0	81.1	2.6
Norepinephrine	81.6	3.7	70.8	4.2
Dopamine	84.3	1.0	77.4	0.8

Linearity was checked from the concentration used to assess recovery (1.28 ng/mL) down to a common lower spiked level of 20 pg/mL. Calibration lines for each analyte are displayed using the full extraction method (left) and a simplified load-wash-elute procedure (right).

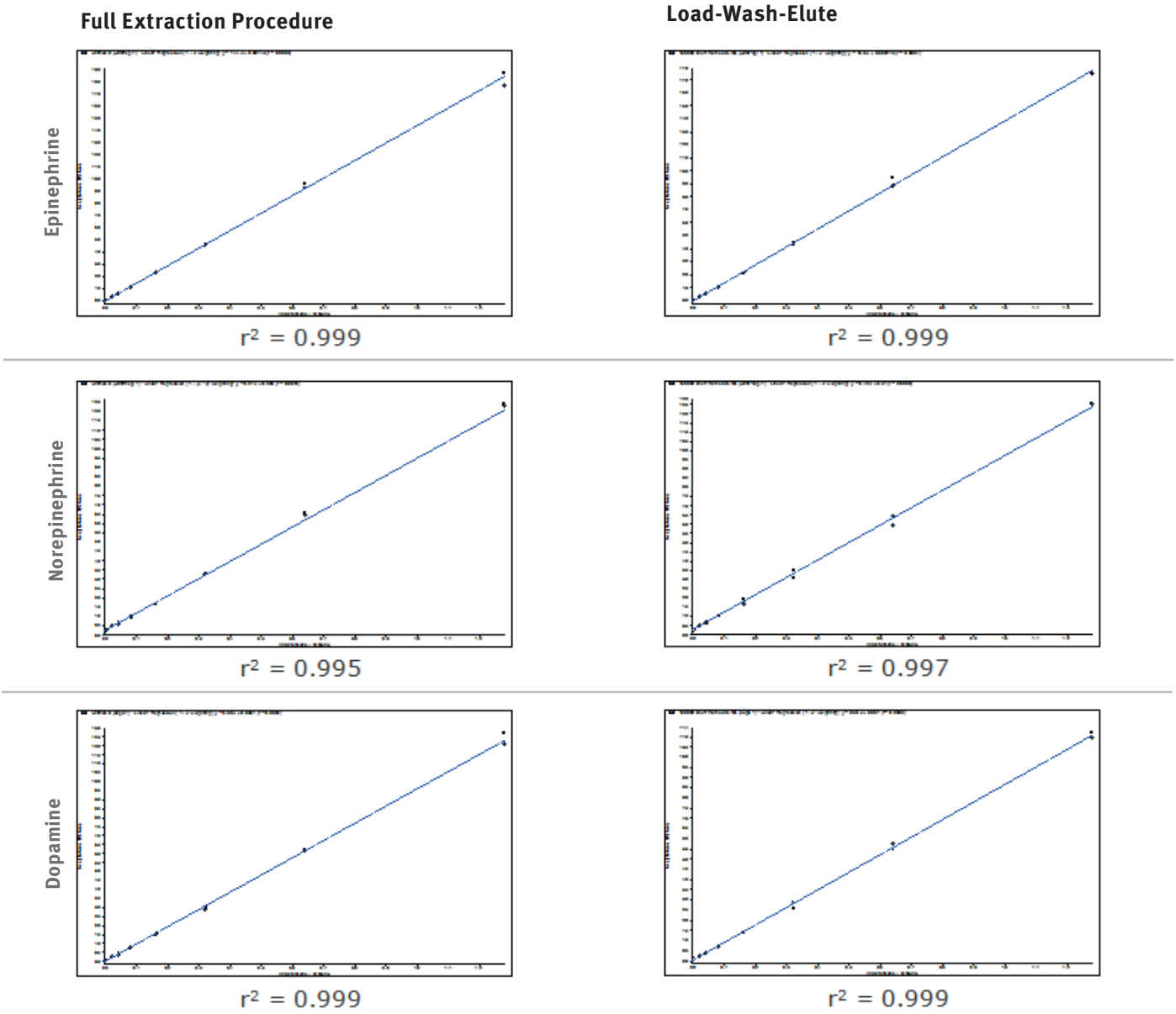

Figure 2. Representative calibration plots of catecholamines.

As no catecholamine free plasma was available for testing, sensitivity was demonstrated by comparing the chromatograms of endogenous levels and those over-spiked at a concentration of 20 pg/mL (displayed on the same scale).

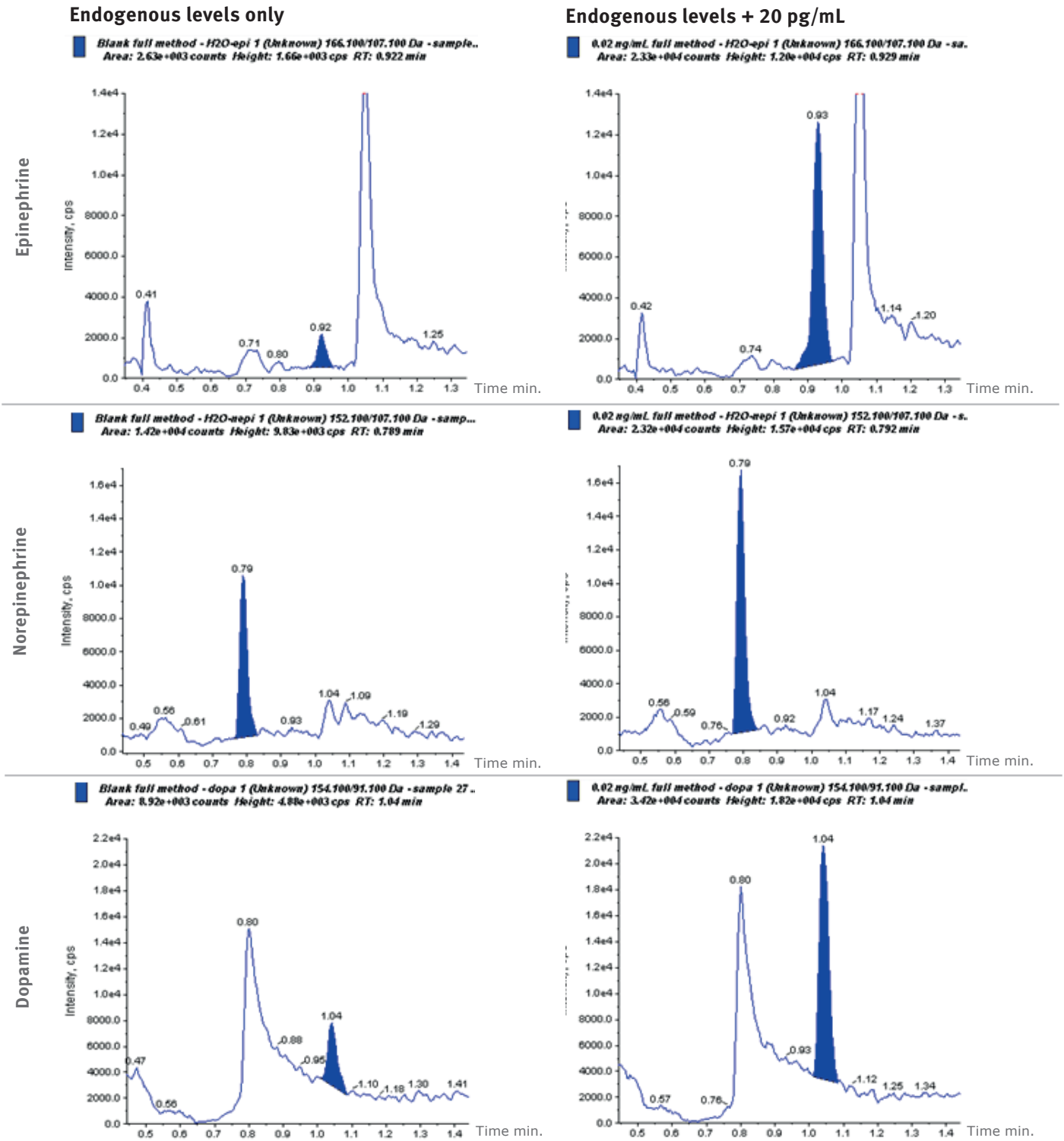

Figure 3. Representative chromatograms of catecholamines spiked into plasma.

Additional Notes

- This method was tested using both standard SPE and the EXPRESS load-wash-elute SPE procedure. EVOLUTE® EXPRESS load-wash-elute procedures eliminate 2 processing steps, saving time and reducing solvent usage.
- In our tests instrument sensitivity of the internal standards was significantly lower than that for the analytes. Therefore, instead of setting the internal standard concentration near the middle of the calibration range as is often recommended they were set at the upper limit of quantitation (1.28 ng/mL) .
- As the samples are not reconstituted in an organic solvent we recommend that an in-line filter is used before the HPLC column and that this is regularly replaced.
- It may be possible to further reduce the HPLC run time to increase throughput. This should be evaluated during method optimization.
- **Reagent Preparation.**
  1. Water used throughout: 18.2 MΩ.cm drawn from a Direct-Q 5 water purifier (Merck Millipore, Watford, UK).
  2. 10 mM ammonium acetate (SPE equilibration (optional) and wash) 1: 77.1 mg ammonium acetate is dissolved in 100 mL of deionized water.
  3. 0.1% formic acid in water : propan-2-ol (85:15) (SPE elution solvent): 0.5mL of Formic acid added to a mixture of 425 mL deionized water and 75 mL propan-2-ol
  4. Ammonium formate/formic acid for HPLC mobile phase. Prepare a 500 mM solution by combining 38.75 mg of ammonium formate, 71 µL of formic acid and water to a total volume of 5 mL. Add to both mobile phases as 500 µL of the ammonium formate / formic acid solution per litre of mobile phase.
- **Preparation of samples and standards.**
  1. Dilute analytes to a concentration of 2 µg/mL in water and stored at approximately 4 °C.
  2. Prepare a single 32 ng/mL solution of all three catecholamines in water from these as a fortification solution.
  3. Dilute the fortification solution 1:25 (v/v) with plasma to prepare the top calibration solution at a concentration of 1.28 ng/mL, dilute further to give additional calibration standards at 0.64, 0.32, 0.16, 0.08, 0.04 and 0.02 ng/mL.
  4. Prepare a 38.4ng/mL solution of the deuterated internal standards in water. Combine a 10 µL volume of internal standard with 0.3mL of standard, quality control or sample to give an internal standard concentration ng/mL concentration equivalent to the top standard. Then combine this with a 0.3 mL volume of 0.05% formic acid (v/v) solution from which a 0.5 mL sample volume can easily be removed.

Ordering Information

Part Number	Description	Quantity
602-0010-PX01	EVOLUTE® EXPRESS WCX 10 mg Fixed Well Plate	1
PPM-96	PRESSURE+ 96 Positive Pressure Manifold 96 well	1
121-5203	Collection Plate, 2 mL, Square	50
121-5204	Piercable Sealing Cap	50

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