Fast Extraction of Metanephrine and Normetanephrine from Pooled Plasma Using EVOLUTE® EXPRESS WCX in a 96-Well Plate Format Prior to LC-ESI-MS/MS

This application note describes a method of extraction for metanephrine and normetanephrine from pooled plasma using EVOLUTE® EXPRESS WCX 96-well SPE plates.

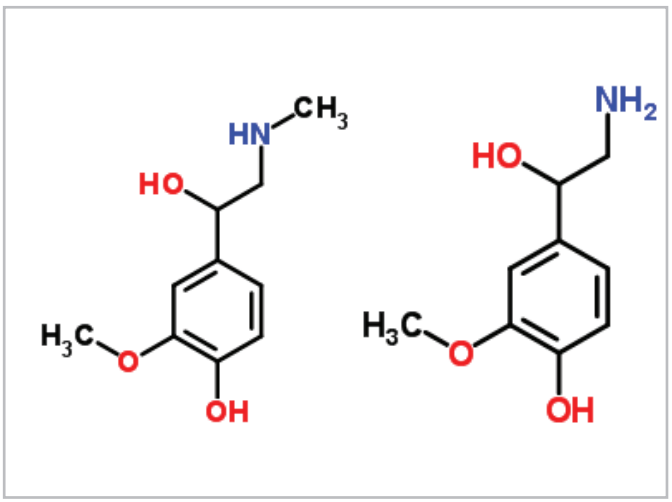

Figure 1. Structures metanephrine and normetanephrine

Introduction

The normalized metabolite concentrations of epinephrine and norepinephrine precursors have common utility in clinical diagnostics related to neuroendocrine tumors. Of particular interest are the selected metabolites metanephrine and normetanephrine. The challenges in population screening for these metabolites are time, cost and efficacy of sample preparation strategies required for trace level determinations (pg/mL-ng/mL levels) by liquid chromatography-tandem mass spectrometry. A new solid phase extraction technology has been developed to reduce the time associated with sample preparation methods by eliminating traditional steps in the extraction workflow. Leveraging this technology, a method protocol was developed using a load, wash and elute approach. This method was optimized for pooled plasma.

EVOLUTE EXPRESS WCX ion exchange SPE materials offer an efficient alternative to traditional ion exchange SPE plates for bioanalytical sample preparation, providing high analyte recoveries and significantly reduced sample preparation time.

Analytes

Metanephrine, normetanephrine

Sample Preparation Procedure

Format:	EVOLUTE EXPRESS WCX 30 mg 96-well plate, part number 602-0030-PXO1
Matrix	Pooled plasma
Sample Pre-treatment	Dilute plasma sample with ammonium acetate (3:1 v/v, 0.05 M, pH = 7)
Sample Loading:	Load 0.4 mL of the diluted sample into each well. Apply positive pressure (PRESSURE+ 96 Positive Pressure Manifold, PPM-96) to maintain a steady flow rate of 1 mL/min (10-12 drops)
Wash 1*:	Remove interferences with water (1 mL)
Wash 2*:	Remove interferences with methanol/acetonitrile (1:1 v/v, 1 mL)
Elution:	Elute analytes with methanol/acetonitrile (1:1 v/v):formic acid (95:5, v/v)
Blow down/recon:	Evaporate eluate to dryness and reconstitute in 0.4 mL of mobile phase

*Suggested variable to try on implementation: evaluate 2x wash steps for additional extract cleanliness.

HPLC Conditions

Instrument: Agilent 1200 Liquid Handling System (Agilent Technologies, Berkshire, UK)

Column: Restek Organic Acids 150 mm x 4.6 mm (5 µm) (catalog # 9165565)

Mobile Phase: Solvent A: 1% Formic acid in water
Solvent B: Acetonitrile

Flow rate: 1 mL /min

Injection Volume: 100 µL

Temperature: 35 °C

Table 1. Liquid Chromatography parameters

Step	Time (min)	Flow Rate (mL/min)	%A	%B
1	0.0	1.0	90	10
2	3.0	1.0	90	10

Mass Spectrometry Conditions

Instrument: Applied Biosystems/MDS Sciex 4000 Q-Trap triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA.) equipped with a Turbo Ionspray® interface for mass analysis.

Ion Source Temperature: 600 °C

Table 2. MRM transitions in positive mode Turbo Ionspray

Scan Function	Analyte	MRM Transition	Declustering Potential (DP)	Collision Energy (CE)	Cell Exit Potential (CXP)
1	Metanephrine	180 → 148	25	20	16
2	Normetanephrine	166 → 134	25	20	16

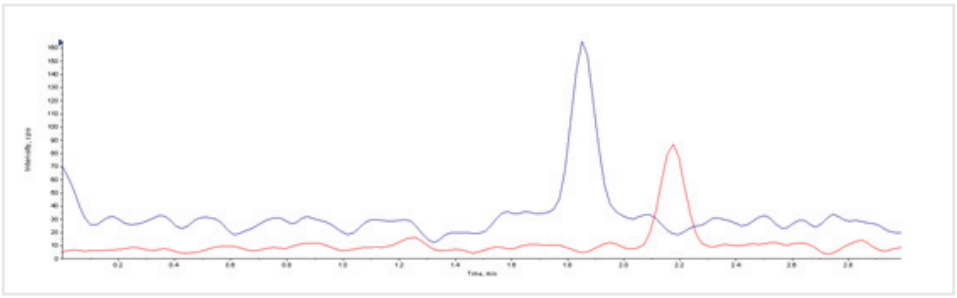

Figure 2. A typical ion chromatogram for plasma fortified at 150 ng/mL

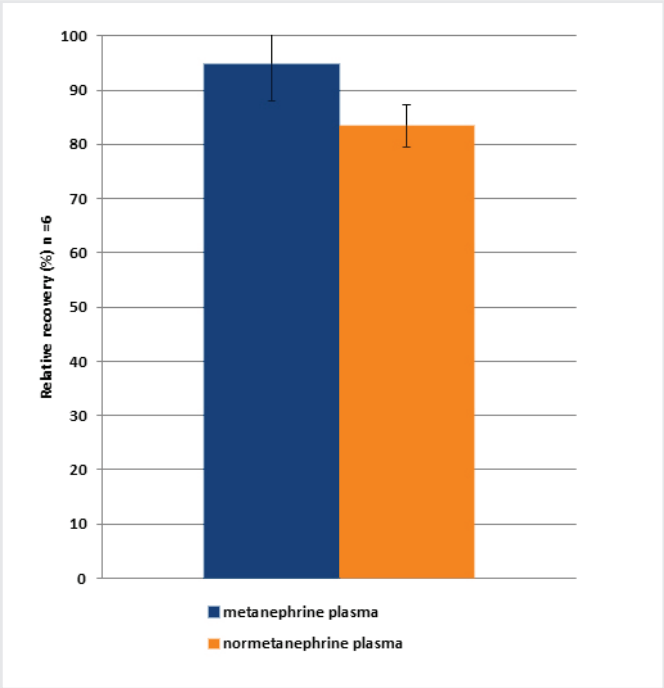

Figure 3. Relative recovery of metanephrine and normetanephrine from fortified plasma using an EVOLUTE® EXPRESS WCX 30 mg 96-well plate. Repeatability as %RSD was <15% for both analytes.

Ordering Information

Part Number	Description	Quantity
602-0030-PX01	EVOLUTE® EXPRESS WCX 30 mg Fixed Well Plate	1
PPM-96	Biotage® PRESSURE+ 96 Positive pressure Manifold 96 well	1
SD-9600-DHS-EU	Biotage® SPE Dry Sample Concentrator System 220 V	1
SD-9600-DHS-NA	Biotage® SPE Dry Sample Concentrator System 110 V	1

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