

Fast Extraction of Metanephrine and Normetanephrine from Synthetic Urine using EVOLUTE® EXPRESS CX in a 96 Fixed Well Plate Prior to LC-ESI-MS/MS

This application note describes a method of extraction for metanephrine and normetanephrine from synthetic urine using EVOLUTE EXPRESS CX 96 fixed well plates.

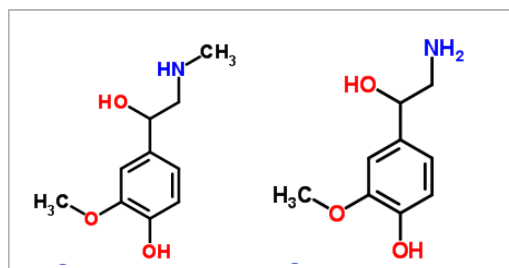


Figure 1. Structure of metanephrine and normetanephrine

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 steps in the extraction workflow. Leveraging this technology, a method protocol was developed using a load, wash and elute approach. The method was evaluated for relative recovery, repeatability and linearity of analyte response with concentration. Recoveries exceeding 80% were achieved for both metanephrine and normetanephrine.

EVOLUTE EXPRESS CX 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

| | |
|------------------------------|--|
| Plate configuration: | EVOLUTE EXPRESS CX 96 30 mg fixed well plate, part number 601-0030-PX01 |
| Sample pre-treatment: | Dilute 1:1 with HPLC grade water. |
| Sample loading: | Load pre-treated sample (1 mL). Apply positive pressure (PRESSURE+ 96 Positive Pressure Manifold PPM-96) to maintain a steady flow rate of 1 mL/min (10-12 drops). |
| Interference wash 1: | Remove polar and ionic interferences with water (1 mL). |
| Interference wash 2: | Remove organic material with methanol (1 mL). |
| Extraction: | Elute analytes of interest methanol/ concentrated ammonium hydroxide (95:5, v/v) (1 mL) . |
| Post extraction: | Evaporate to dryness and reconstitute in 80/20 (v/v, mobile phase A/mobile phase B) (0.5 mL). |

HPLC Conditions

| | |
|--------------------------|---|
| Instrument: | Agilent 1200 Liquid Handling System (Agilent Technologies, Berkshire, UK) |
| Column: | Restek Organic Acids 150 mm x 4.6 mm (5 µm) (catalogue # 9165565) |
| Mobile Phase: | Solvent A: 1% Formic acid in Water Solvent B: Acetonitrile |
| Injection Volume: | 20 µL |
| Temperature: | 35° C |

Gradient:

| Step | Time (min) | Flow Rate (mL/min) | %A | %B |
|------|------------|--------------------|----|----|
| 1 | 0.0 | 1.0 | 80 | 20 |
| 2 | 3.0 | 1.0 | 80 | 20 |

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. |
|--------------------|--|

Table 1. MRM transitions in positive mode Turbo Ionspray.

| Scan Function | Analyte | MRM Transition | Declustering Potential (DP) | Collision Energy (CE) | Cell Exit Potential (CXP) |
|---------------|-----------------|----------------|-----------------------------|-----------------------|---------------------------|
| 1 | Metanephrine | 198 → 165 | 30 | 15 | 16 |
| 2 | Normetanephrine | 184 → 166 | 30 | 30 | 16 |

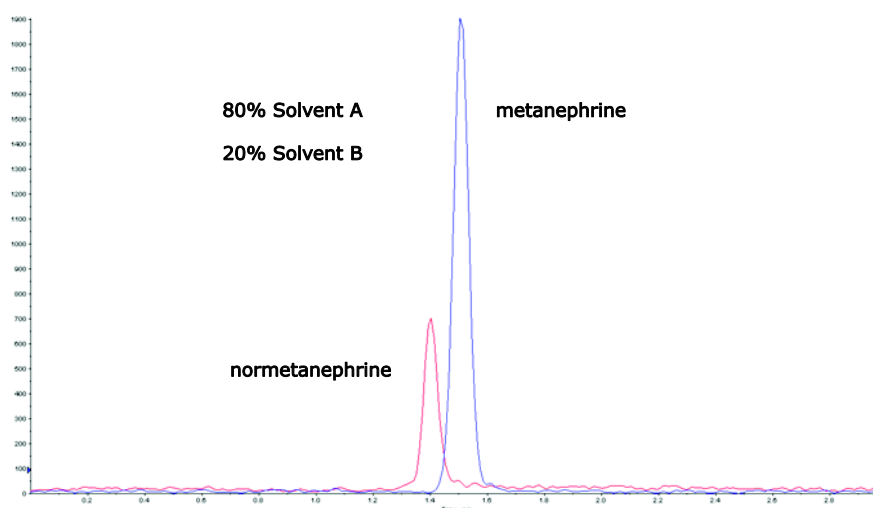


Figure 2. The analytes of interest were eluted from an Organic Acids HPLC column post extraction from the EVOLUTE EXPRESS CX sorbent. A typical ion chromatogram for a 20 ng/mL fortified synthetic urine standard is shown above.

RESULTS

Calibration standards were prepared in synthetic urine matrix. The selected metabolites were ordered as a mixed solution standard from Cerilliant (Round Rock, Texas) at a 1 mg/mL concentration in methanol. A working stock solution (1 µg/mL in methanol) was prepared. Synthetic urine was obtained from Carolina Biological Supply (Burlington, NC). The fortified synthetic urine calibration standards prepared were 10 ng/mL, 30 ng/mL, 40 ng/mL, 60 ng/mL, 70 ng/mL. Recoveries exceeding 80% were achieved for each analyte as demonstrated in figure 5.

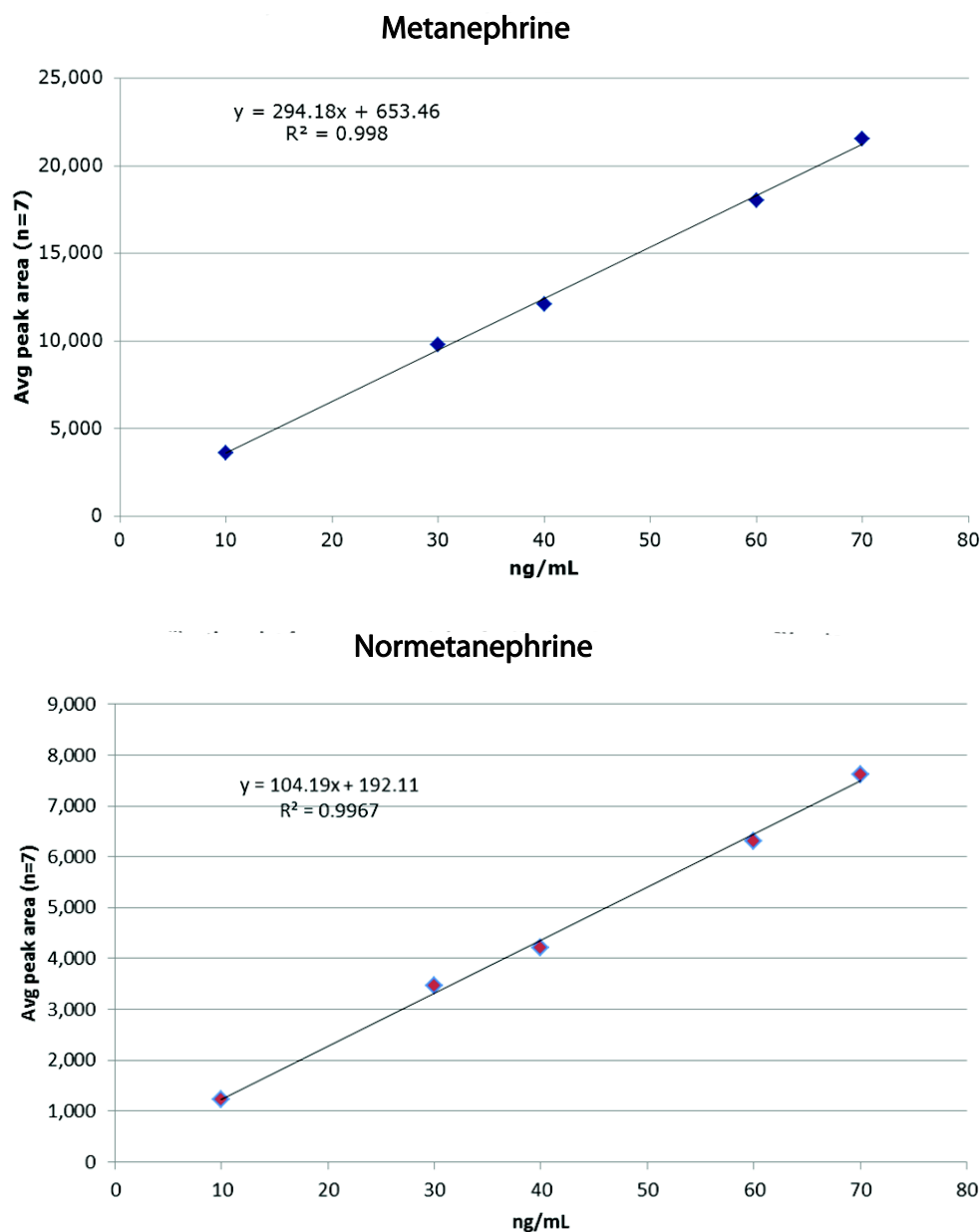


Figure 4. Calibration curves for Metanephrine and normetanephrine showing linearity over the range from 10–70 ng/mL

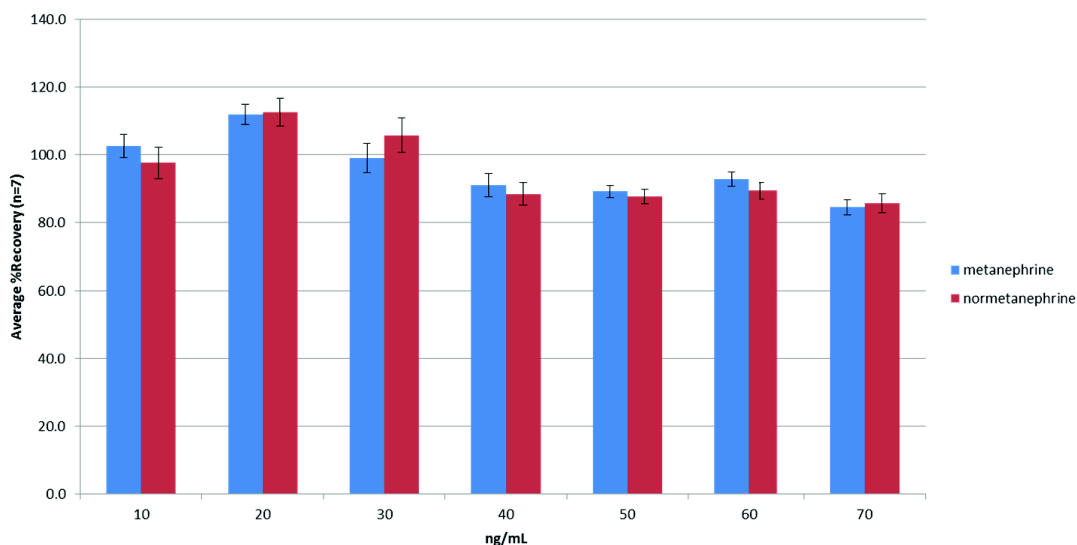


Figure 5. Analyte %recoveries for metanephrine and normetanephrine using the EVOLUTE EXPRESS CX protocol

Ordering information

| Part Number | Description | Quantity |
|----------------|--|----------|
| 601-0030-PX01 | EVOLUTE EXPRESS CX 30 mg 96 fixed well plate | 1 |
| PPM-96 | PRESSURE+48 Positive Pressure Manifold | 1 |
| SD-9600-DHS-NA | SPE Dry Dual Sample Concentrator 110 V | 1 |

To search and download more of Biotage's extensive database of sample preparation applications please visit <http://www.biotage.com/applications> or scan the QR code with your smart phone to go direct.



EUROPE
Main Office: +46 18 56 5900
Fax: +46 18 59 1922
Order Tel: +46 18 56 57 10
Order Fax: +46 18 56 57 05
order@biotage.com
EU-1pointsupport@biotage.com

NORTH AMERICA
Main Office: +1 704 654 4900
Toll Free: +1 800 446 4752
Fax: +1 704 654 4917
Order Tel: +1 704 654 4900
Order Fax: +1 434 296 8217
ordermailbox@biotage.com
US-1pointsupport@biotage.com

JAPAN
Tel: +81 3 5627 3123
Fax: +81 3 5627 3121
jp_order@biotage.com
JP-1pointsupport@biotage.com

CHINA
Tel: +86 21 2898 6655
Fax: +86 21 2898 6153
cn_order@biotage.com
CN-1pointsupport@biotage.com
www.biotage.com

For regional distributors, products and offers, please visit www.biotage.com