Extraction of Acidic Catecholamine Metabolites in Plasma Using EVOLUTE® EXPRESS ABN Prior to LC-MS/MS Analysis

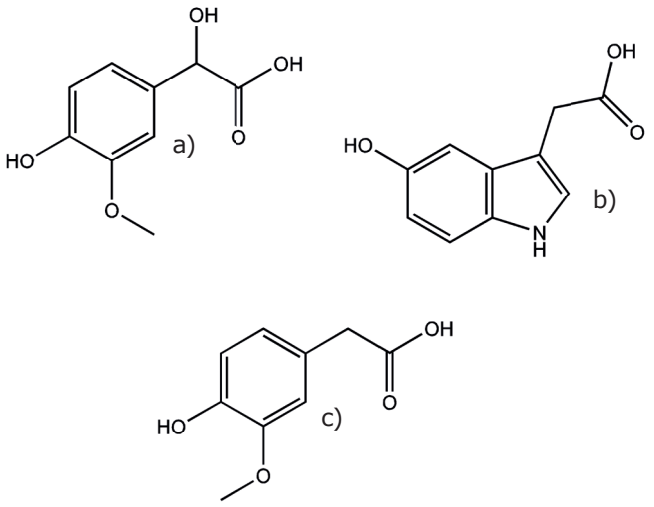

Figure 1. Analyte structures: a) vanillylmandelic acid, b) 5-hydroxyindoleacetic acid, c) homovanillic acid.

Introduction

This application note describes a solid phase extraction protocol for the extraction of three catecholamine metabolites (vanillylmandelic acid, homovanillic acid and 5-hydroxyindoleacetic acid) from plasma prior to LC-MS/MS detection. The method described in this application achieves high reproducible recoveries for a number of common catecholamine analytes in plasma.

EVOLUTE® EXPRESS SPE products dramatically improve flow characteristics, and enhance sample preparation productivity.

Analytes

Vanillylmandelic acid (VMA), 5-hydroxyindoleacetic acid (5-HIAA), homovanillic acid (HVA) and internal standards (D_3-vanillylmandelic acid, D_5-5-hydroxyindoleacetic acid, D_5-homovanillic acid) obtained from Sigma-Aldrich Company Ltd., Gillingham UK).

Sample Preparation Procedure

Format

EVOLUTE® EXPRESS ABN 10 mg plate, part number 600-0010-PX01.

Sample Pre-treatment

Add internal standard solution to plasma (100 µL) and 1% formic acid (300 µL). Mix.

Condition

Condition wells with methanol (500 µL).

Equilibration

Condition wells with 18.2MΩ-cm water (500 µL).

Sample Loading

Load 400 µL of the pre-treated plasma into each well of EVOLUTE EXPRESS ABN plate (equivalent to 100 µL plasma).

Wash

Elute interferences with 0.1% formic acid (aq) (500 µL). Processing conditions: 1–3 psi when using a Biotage® PRESSURE+ 96 manifold.

Elution

Elute analytes with 60% methanol (aq) (200 µL). ensure complete elution by applying 40–50 psi for 30 s (Biotage® PRESSURE+ 96).

Post Elution and Reconstitution

Dry the extract in a stream of air or nitrogen using a Biotage® SPE Dry 96 (30 °C at 60 L min^{-1}) or TurboVap® 96 (30 °C at 1.0 bar). Reconstitute the extracts with 0.1% acetic acid in 10% acetonitrile (aq) (200 µL). Mix thoroughly.

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UHPLC Conditions

Instrument
Shimadzu Nexera UHPLC

Column
Restek Raptor Biphenyl 2.1 x 100 mm, 2.7 µm analytical column and Biphenyl 2.1 x 5 mm, 2.7 µm EXP® guard cartridge.

Mobile Phase
A: 1 mM ammonium fluoride (aq)

B: Methanol

Flow Rate
0.5 mL min^{-1}

Table 1. Gradient and Divert Valve Settings.

Time (min)	%A	%B	Divert Valve
0.00	90	10	waste
0.50	90	10	MS
0.68	85	15	
1.40	85	15	
3.10	50	50	waste
3.40	10	90	
4.40	10	90	
4.90	90	10	
6.50	90	10	

Column Temperature
40 °C

Injection Volume
10 µL (flow-through needle)

Sample Temperature
15 °C

Table 2. Typical Retention Times.

Analyte	Retention Time (min)
VMA	0.8
5-HIAA	1.8
HVA	2.1

Mass Spectrometry Conditions

Dual polarity ions were acquired using electrospray ionization in multiple reaction monitoring mode and two periods.

Instrument
Sciex Triple Quad 5500 mass spectrometer

Source Temperature (TEM)
600 °C

Curtain Gas (CUR)
40

Source Gas 1 (GS1)
50

Source Gas 2 (GS2)
60

Table 3. MRM Parameters.

Analyte	Transition (Da)	IS (V)	DP (V)	EP (V)	CE (V)	CXP (V)
VMA	197.1 > 137.1	-1500	-50	-10	-28	-21
D_3-VMA	199.9 > 139.9	-1500	-50	-10	-28	-16
5-HIAA	192.0 > 145.7	2000	80	13	45	22
D_5-5-HIAA	197.0 > 150.1	2000	70	12	45	19
HVA	181.0 > 136.4	-1500	-50	-10	-11	-18
D_5-HVA	186.1 > 142.0	-1500	-60	-12	-10	-19

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Results

Extraction Recovery

Extraction recoveries were determined at 20 ng mL^{-1}, equivalent to 2 ng when extracting 400 µL of pre-treated plasma.

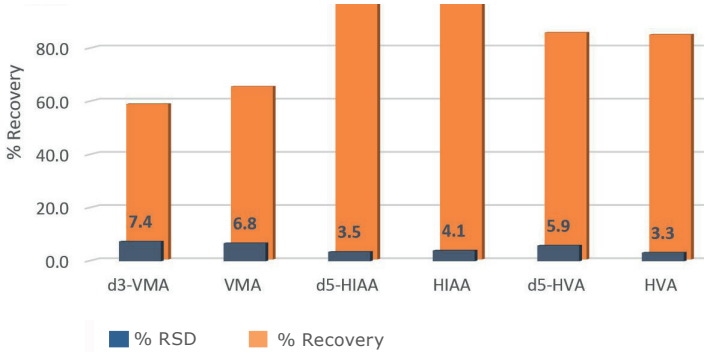

Figure 2. Extraction recovery and RSD (n=6) of acidic catecholamine metabolites (20 ng mL^{-1} spike).

Linearity

Extraction linearity was determined between 2 and 200 ng mL^{-1} from a mixed stock spiked into pooled plasma at 200 ng mL^{-1} then serially diluting in pooled plasma. Each calibration level was extracted in duplicate with a fixed concentration of internal standards. Figure 3 demonstrates representative calibration curves.

Table 4. Linearity data for Catecholamine Acid Metabolites extracted using EVOLUTE® EXPRESS ABN 10 mg.

Analyte	Linear Range (ng mL^{-1})	Coefficient (r^2)	LOQ (ng mL^{-1})	LOD (ng mL^{-1})
VMA	10 to 200	0.996	10	2
5-HIAA	10 to 200	0.991	10	2
HVA	10 to 200	0.995	10	2

Phospholipid Removal

Removal of phospholipids was demonstrated by monitoring MRM transitions for the common product ion at m/z 184. Unspiked plasma extracted using the optimized SPE protocol was compared to plasma extracted using a solvent crash 1:4 in acetonitrile. Figure 4 demonstrates a reduction in lysophospholipids (a: 0 to 4.00 min) and a significant reduction in phospholipids (b: 4.00 to 8.00 min) when using the optimized SPE protocol compared to a 1:3 solvent crash. A range expansion of x100 was used to display the EVOLUTE® Express ABN 10 mg extracted plasma phospholipids total ion chromatogram.

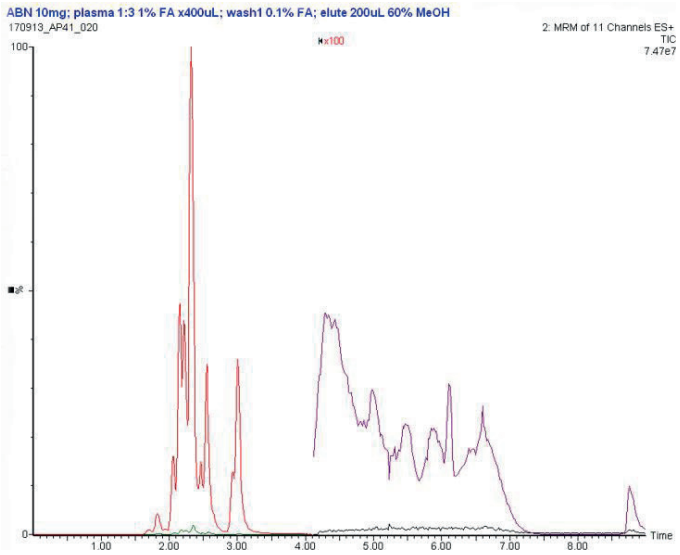

Figure 4. Overlaid TIC for the 184.0 m/z product ion comparing plasma 1:4 (v/v) acetonitrile (red/purple) to plasma extracted using the optimized EVOLUTE® Express 10 mg protocol (green/black).

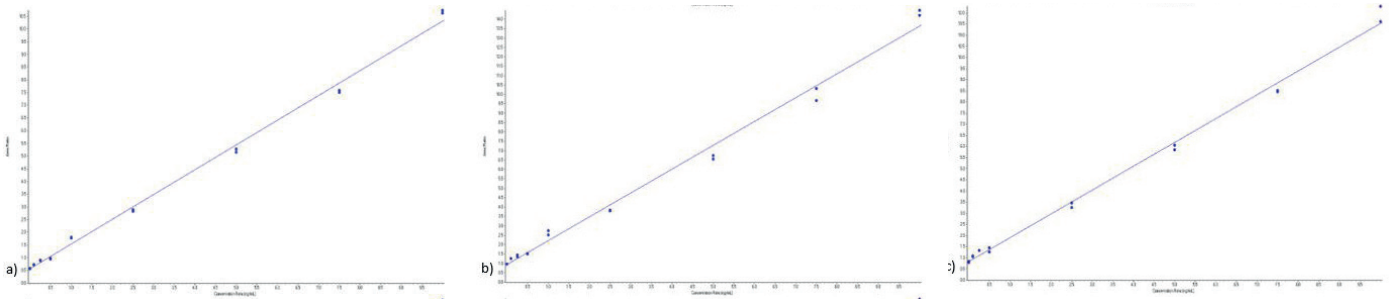

Figure 3. Representative Extracted Catecholamine Acid Metabolite Calibration Curves, 2 to 200 ng mL^{-1} relative to 50 ng mL^{-1} IS: a) VMA, b) 5-HIAA, c) HVA.

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Processing Conditions

Unless otherwise stated, all SPE processing steps were performed using a Biotage Pressure+ 96 positive pressure manifold at a pressure of 1–3 psi.

Other Chemicals and Reagents

- 18.2 MΩ.cm water was drawn fresh daily from a Direct-Q 5 water purifier (Merck Millipore, Watford, UK).
- Solid reagents and chemicals were obtained from Sigma-Aldrich Chemical Co. (Gillingham, UK), HPLC grade or higher.
- Liquid reagents and solvents were obtained from Honeywell Research Chemicals (Bucharest, Romania), HPLC grade or higher.
- Pooled human plasma was obtained from the Welsh Blood Service (Pontyclun, UK).

Ordering Information

Part Number	Description	Quantity
600-0010-PX01	EVOLUTE® EXPRESS ABN 10 mg Plate	1
PPM-96	Biotage® PRESSURE+ 96 Positive Pressure Manifold	1
SD-9600-DHS-EU	Biotage® SPE Dry 96 Sample Evaporator 220/240 V	1
SD-9600-DHS-NA	Biotage® SPE Dry 96 Sample Evaporator 100/120 V	1
C103264	Turbovap® 96 Evaporator 220/240 V	1
C103263	Turbovap® 96 Evaporator 100/120 V	1

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