Fast Extraction of VMA, 5-HIAA, and HVA from Synthetic Urine using EVOLUTE® EXPRESS AX 96-well SPE Plates Prior to LC-ESI-MS/MS

This application note describes the extraction of the acidic catecholamine metabolites VMA, 5-HIAA, and HVA from synthetic urine using EVOLUTE® EXPRESS AX 96-well SPE plates.

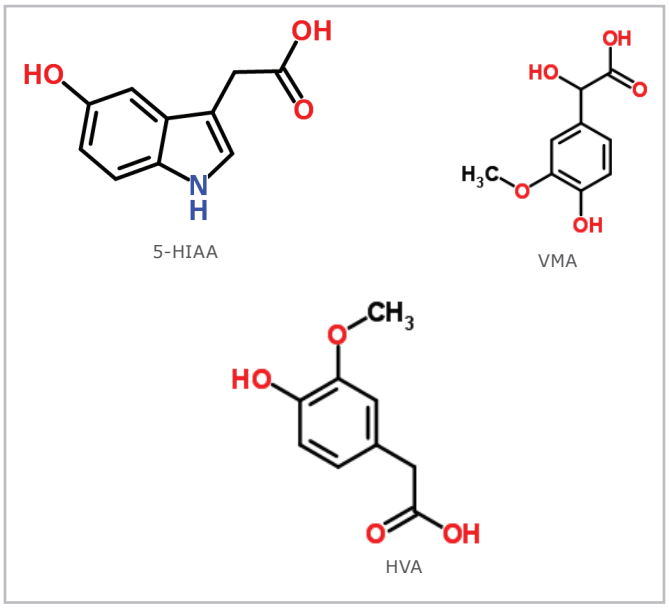

Figure 1. Structures of 5-HIAA, HVA and VMA

Introduction

Analytical measurements that target the metabolites of serotonin probe defects in the biochemical systems related to the progressive and degenerative conditions of the gastrointestinal system as well as cognitive disorders are required in clinical laboratories. The selected metabolites homovanillic acid (HVA), 5-hydroxyindoleacetic acid (5-HIAA) and vanillylmandelic acid (VMA) are endogenous metabolites found in urine. This application note describes a method developed to extract the targeted metabolites from urine using a mixed-mode anion exchange retention mechanism, prior to analysis via LC-MS/MS. In the absence of a true blank with real clinical specimens, the method described in this report was developed using synthetic urine.

EVOLUTE EXPRESS 96-well SPE plates are optimised for quicker and easier SPE processing. Using a load-wash-elute procedure, traditional conditioning and equilibration steps are eliminated, reducing solvent costs and increasing sample throughput.

Analytes

5-hydroxyindoleacetic acid (5-HIAA); homovanillic acid (HVA); vanillylmandelic acid (VMA)

Sample Preparation Procedure

Format:	EVOLUTE EXPRESS AX 96-well plate, part number 603-0030-PX01.
Sample Pre-Treatment:	Dilute synthetic urine 1:1 (v/v) with HPLC grade water.
Sample Loading:	Load pre-treated sample (1 mL) into wells. Apply positive pressure (PRESSURE+ 96 Positive Pressure Manifold PPM-96) to maintain a flow rate of 1 mL/min (10-12 drops/min). Note: no conditioning or equilibration steps are required.
Wash:	Wash with HPLC grade water/methanol (75:25, v/v, 1 mL).
Analyte Elution:	Elute analytes of interest with methanol/formic acid (98:2, v/v, 1 mL).
Post Extraction:	Evaporate sample to dryness (SPE Dry Dual) and reconstitute in mobile phase (1 mL).
Sample Throughput:	Sample plates can be loaded onto a Positive Pressure+ 96 manifold and processed in 1 hour or less

Biotage®

HPLC Conditions

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

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

Mobile Phase: A: 1% Formic acid in Water
B: Acetonitrile

Injection Volume: 20 µL

Temperature: 35 °C

Table 1: Liquid Chromatography parameters

Step	Time (min)	Flow Rate (mL/min)	% A	% B
1	0.0	1.0	80	20
2	9.0	1.0	80	20

MS 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	5-hydroxyindoleacetic acid (5-HIAA) (5-HIAA)	192>146	30	25	16
2	homovanillic acid (HVA)	183>137	30	20	16
3	vanillylmandelic acid (VMA)	181>149	30	25	16
4	5-hydroxyindole-4,6,7-D3-acetic-D2-acid (5-HIAA-D5)	180>105	30	20	16

Results

Fortified synthetic urine samples were processed using the protocol outlined in sample preparation section. The plate performance was evaluated as a function of relative recovery and repeatability of replicate preparations. A mixed solution standard for both analytes was obtained from Cerilliant Corp (Round Rock, TX). A working standard solution was prepared in methanol at a concentration of 1 µg/mL. Seven replicates of a 40 ng/mL fortified synthetic urine sample were then prepared. The synthetic urine stock solution was obtained from Carolina Biological (Burlington, NC).

The relative recovery was determined for each replicate sample after each was processed with the SPE method detailed in sample preparation section. The results are shown in Figure 3. Error bars represent +/- 1 standard deviation of the mean. The method precision was determined as %RSD of 3.5%, 4.8% and 3.2% for HVA, 5-HIAA and VMA, respectively. The method was applied to a real urine specimen fortified with deuterated 5-HIAA. Seven replicate extractions were prepared and anayzed. The final spike concentration of the deuterated 5-HIAA in the urine was 200 ng/mL. The results of the percent recovery study are given in Figure 4. The %RSD (n =7) was determined to be < 5.

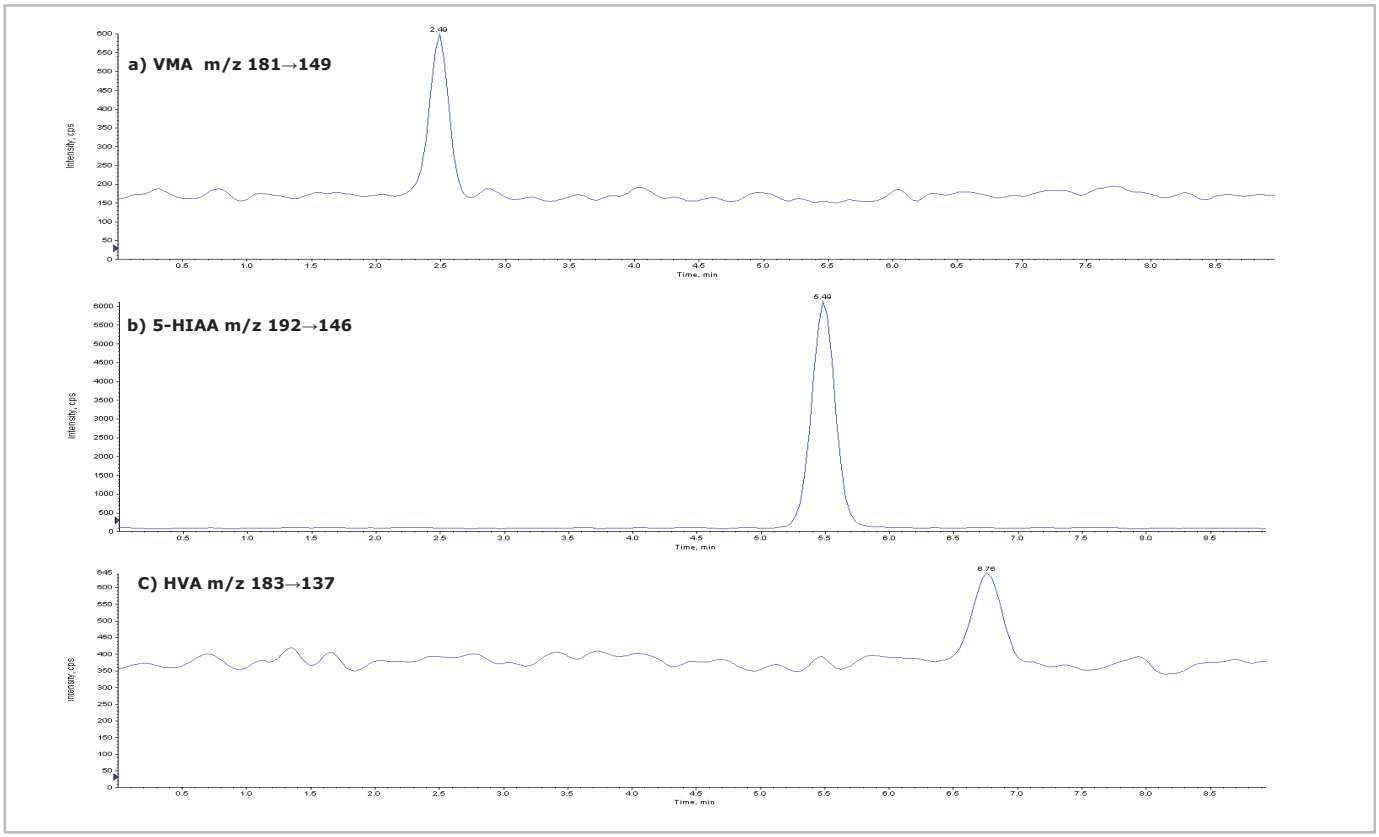

Figure 2: Typical extracted ion chromatograms for a : a) VMA b) 5-HIAA and c) HVA extracted from a 40 ng/mL fortified synthetic urine specimen using EVOLUTE® EXPRESS AX 96 fixed well plates.

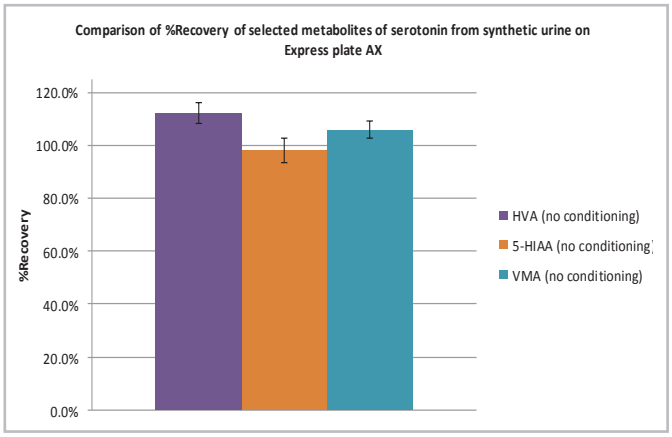

Figure 3: Recovery (n =7) of the selected 40 ng/mL metabolite mixed standard extracted from the synthetic urine matrix with the EVOLUTE® EXPRESS AX 96-well plate.

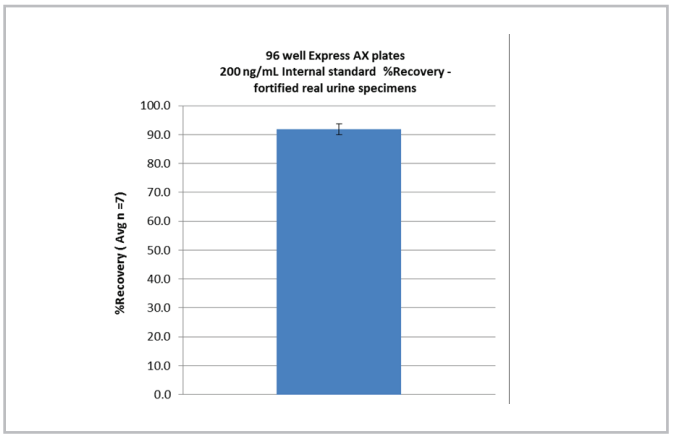

Figure 4: Average recovery (n =7) for a real urine specimen fortified (200 ng/mL) with 5-HIAA-D5

Ordering Information

Part Number	Description	Quantity
603-0030-PXO1	EVOLUTE® EXPRESS AX 30 mg 96-well plate	1
PPM-96	Biotage® PRESSURE+ 96 Positive Pressure Manifold	1
SD2-9600-DHS-NA	Biotage® SPE Dry Dual Sample Concentrator System, 110V	1
SD2-9600-DHS-EU	Biotage® SPE Dry Dual Sample Concentrator System, 220V	1

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