

Extraction of Catecholamines from Human Plasma Using EVOLUTE® WCX and RapidTrace® Workstation

Introduction

This application note describes the extraction of Catecholamines from human plasma using EVOLUTE WCX.

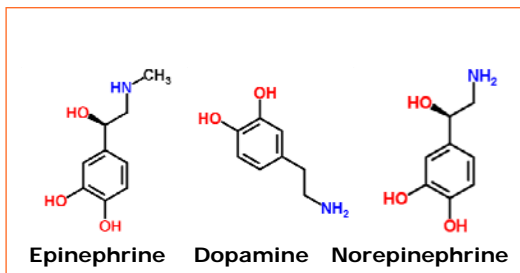


Figure 1. Structures of Catecholamines

Catecholamines are a class of compounds defined by the presence of a dihydroxy phenyl group or “catechol” attached in the para position by an aliphatic amine side chain. The three most commonly studied catecholamines are epinephrine, dopamine and norepinephrine. Catecholamines are classic biomarkers for the detection of diseases like hypertension, pheochromocytoma and neuroblastoma. The detection of these analytes in biological matrices like serum, plasma and urine at low nanogram per milliliter levels is critical in the clinical diagnosis of these and other diseases.

EVOLUTE WCX is a mixed-mode non-polar/weak cation exchange resin based SPE sorbent that extracts a wide range of basic analytes from biological fluids (e.g. plasma, serum, urine) and other aqueous matrices using allowing elution under acidic conditions. EVOLUTE WCX removes matrix interferences like proteins, salts, non-ionisable molecules and phospholipids, delivering cleaner extracts with reproducible recoveries for accurate quantitation.

Analytes

Epinephrine, Dopamine, Norepinephrine.

EVOLUTE WCX Configuration

EVOLUTE WCX 50mg/3mL part number 612-0005-B.

RapidTrace Procedure

The SPE steps were configured schematically using the RapidTrace® software as follows:

No.	Step	Source	Destination	Volume (mL)	Flow (mL/min)
1	Condition	Methanol	Organic Waste	1.0	15.0
2	Condition	50mM Amm. acetate	Aqueous Waste	1.0	15.0
3	Load	Pre-treated Plasma	Biological Waste	2.0	2.0
4	Rinse	10% Methanol	Aqueous Waste	1.0	10.0
5	Rinse	Isopropanol	Organic Waste	1.0	10.0
6	Collect	Methanol:Formic acid	Fraction Collection 1	1.0	1.0
7	Collect	Methanol:Formic acid	Fraction Collection 1	0.5	1.0
8	Purge-Cannula	Water	Cannula Waste	5.0	30.0

Reagent Lines:

Line 1: Water

Line 2: Methanol

Line 3: 50mM Ammonium acetate, pH 7

Line 4: Methanol:water (10:90, v/v)

Line 5: Isopropanol

Line 7: Methanol:Formic acid (95:5, v/v)

**Biotage recommends TurboVap® Workstation post elution to blow down your samples.

TurboVap Drying Conditions

Water bath temperature:	40°C.
Run time:	10 minutes.
Pressure:	20psi.
Gas:	Nitrogen.

HPLC Conditions

Instrument:	Agilent 1200 Liquid Handling System (Agilent Technologies, Berkshire, UK).
Column:	Agilent Poroshell 120 C18-EC 2.7 µm analytical column (50 x 3.0 mm id) (Agilent Technologies, Berkshire, UK).
Mobile Phase:	0.1% formic acid aq (A) and methanol (B) .
Isocratic:	Initial solvent flow of 98% A and 2% B at a flow rate of 0.300mL/ min for 2 minutes.
Injection Volume:	10 µL.

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:	700 °C

Scan Function	Analyte	MRM Transition	Cone Voltage (V)	Collision Energy
1	Norepinephrine	170.2>152.2	40	10
2	Epinephrine	184.1>166.1	40	10
3	Dopamine	154.3>137.3	40	10

Table 1. MRM Transitions for Catecholamines

Results

All results show averaged inter-run recoveries above 75% with intra-run RSDs below 10% for epinephrine and dopamine at 20 ng/mL and norepinephrine at 60 ng/mL. **Figure 2** shows charted averaged recoveries for the catecholamines at low nanogram levels in plasma.

LC-MS/MS analysis of reconstituted sample solutions was conducted at varied spiked concentration levels in plasma. **Figure 3** shows a typical total ion chromatogram obtained for concentrations of epinephrine, dopamine and norepinephrine at low nanogram detection levels.

Note: Complete sample preparation and analytical methods for the lower limit of detection of catecholamines using EVOLUTE WCX, 50mg/3mL cartridges can be found in Application Note AN739.

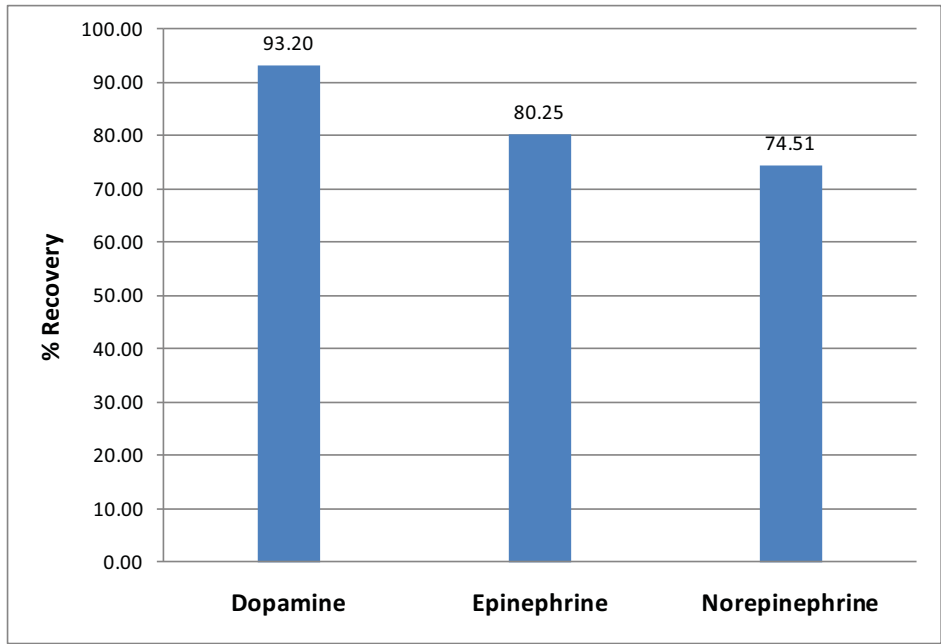


Figure 2. Averaged inter-run percent recovery (n=27) for Epinephrine and Dopamine (20 ng/mL) and Norepinephrine (60 ng/mL) run on RapidTrace Workstation.

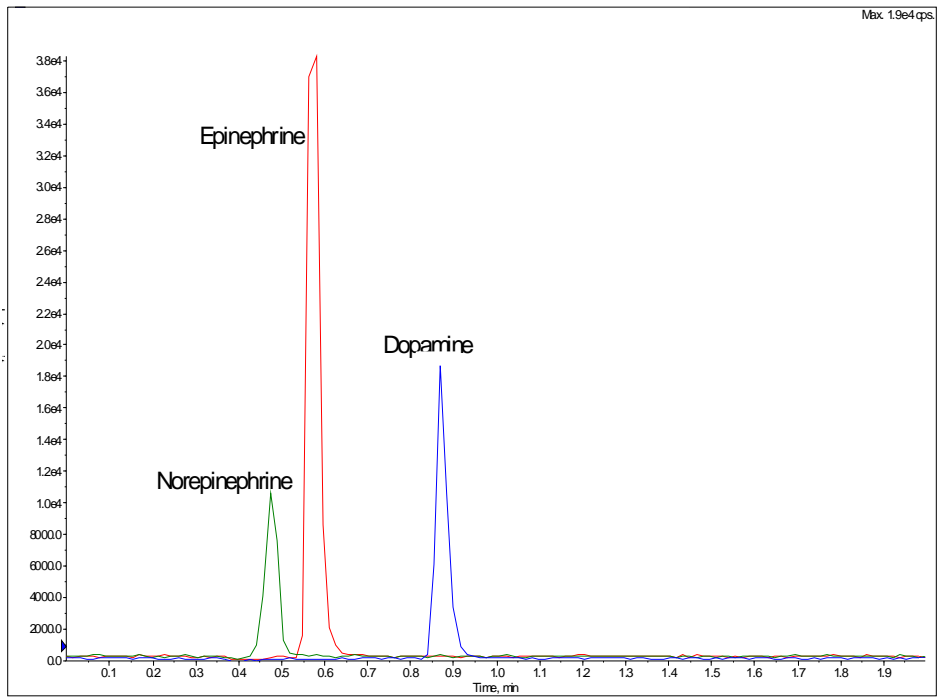


Figure 3. Total Ion Chromatogram for Epinephrine and Dopamine (20 ng/mL) and Norepinephrine (60.0 ng/mL) extracted from spiked plasma with EVOLUTE WCX. HPLC conditions are isocratic flow of 1% Formic acid and methanol with 1% Formic acid (98:2) at 500 μ L/min flow rate.

Ordering information

Part number	Description	Quantity
612-0005-B	EVOLUTE WCX 50 mg/3 mL	50
C103198	TurboVap LV	1
C103198	1 mL and 3 mL RapidTrace Workstation	1

Additional assistance

The TurboVap LV Concentration Evaporator Workstation provides simultaneous evaporation of up to 50 samples. This evaporation system offers many interchangeable tube racks giving you the flexibility for automated low volume sample preparation ranging in volumes size from 1.5 mL to 30 mL. The micro-processor-control provides monitoring of the timed operation and water bath temperature. It also provides automatic gas shutoff and operational diagnostics.



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