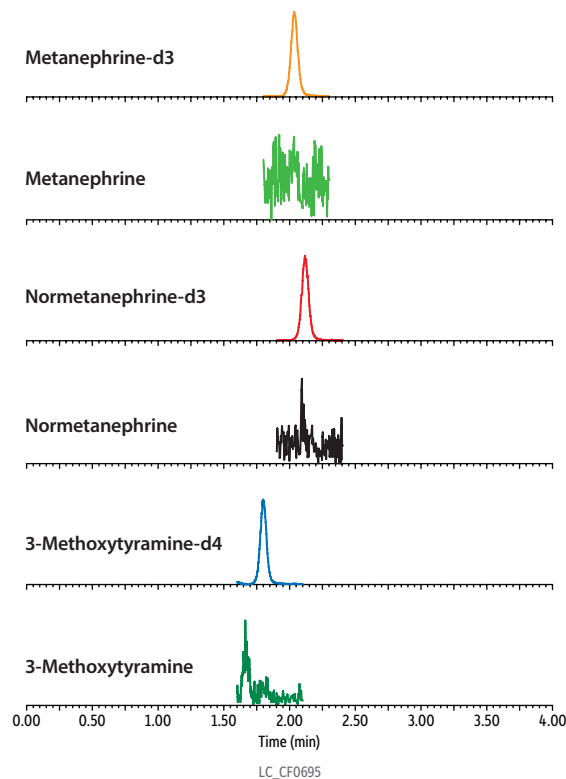


Analysis of Blank Human Plasma for Metanephrine, Normetanephrine, and 3-Methoxytyramine on Raptor HILIC-Si



Peaks	Retention Time (min)	Concentration (pg/mL)	Precursor Ion	Product Ion
1. 3-Methoxytyramine-d4 (IS)	1.80	400	155.07	122.93
2. 3-Methoxytyramine	-	-	151.00	119.00
3. Metanephrine-d3 (IS)	2.04	200	183.00	151.15
4. Metanephrine	-	-	179.94	148.22
5. Normetanephrine-d3 (IS)	2.12	400	169.00	136.96
6. Normetanephrine	-	-	166.00	134.02

Column Raptor HILIC-Si (cat.# 9310A52)
Dimensions: 50 mm x 2.1 mm ID
Particle Size: 2.7 µm
Pore Size: 90 Å
Temp.: 30 °C
Standard/Sample Diluent: Mobile phase A:mobile phase B (10:90)
Inj. Vol.: 10 µL
Mobile Phase
A: Water, 100 mM ammonium formate, pH 3.0
B: Acetonitrile

Time (min)	Flow (mL/min)	%A	%B
0.00	0.3	10	90
5.00	0.3	10	90

Detector MS/MS
Ion Mode: ESI+
Mode: MRM
Instrument UHPLC

Sample Preparation Internal standard solution (IS) was prepared at 4 ng/mL for metanephrine-d3 and at 8 ng/mL for normetanephrine-d3 and 3-methoxytyramine-d4 in methanol. Charcoal stripped plasma (200 µL) was mixed with 10 µL of IS solution and 600 µL of 50 mM ammonium acetate solution. The mixture was loaded to the EVOLUTION EXPRESS WCX 96-well plate (30 mg) and washed with 1 mL water and 1 mL methanol:acetonitrile (50:50). The elution was performed twice with 0.9 mL of 5% formic acid in methanol:acetonitrile (50:50) and evaporated to dryness at 55 °C under a gentle stream of nitrogen. Dried extract was reconstituted with 100 µL of diluent and injected (10 µL) for analysis.