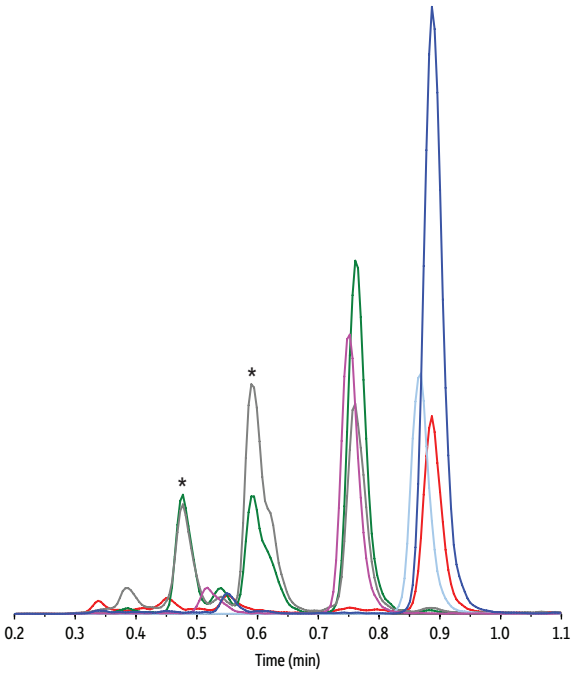
Cortisol and Cortisone in Extracted Female Human Urine on Raptor Biphenyl by LC-MS/MS



Peaks	t_R (min)	Precursor Ion	Product Ion	Product Ion
1. Cortisol-d4	0.75	367.4	121.0	-
2. Endogenous cortisol	0.76	363.3	120.9	91.1
3. Cortisone-d8	0.88	369.4	167.9	-
4. Endogenous cortisone	0.89	361.3	163.2	91.0
*Isobaric matrix peaks				

Column Raptor Biphenyl (cat.# 9309A52)
Dimensions: 50 mm x 2.1 mm ID
Particle Size: 2.7 µm
Pore Size: 90 Å
Guard Column: Raptor Biphenyl EXP guard column cartridge 5 mm, 2.1 mm ID, 2.7 µm (cat.# 9309A0252)
Temp.: 40 °C
Standard/Sample
Diluent: Mobile phase A
Conc.: Calculated concentration is 105.1 ng/mL and 135.9 ng/mL for cortisol and cortisone, respectively
Inj. Vol.: 10 µL
Mobile Phase
A: Water, 0.1% formic acid
B: Acetonitrile, 0.1% formic acid

Time (min)	Flow (mL/min)	%A	%B
0.00	0.6	70	30
1.00	0.6	70	30
1.01	0.6	0	100
1.50	0.6	0	100
1.51	0.6	70	30
3.00	0.6	70	30

Max Pressure: 400 bar
Detector MS/MS
Ion Mode: ESI+
Mode: MRM
Instrument UHPLC
Sample Preparation
1. Centrifuge female human urine for 5 min at 4500 rpm, 10 °C.
2. Aliquot 380 µL supernatant. Add 20 µL each of internal standard solution (1 µg/mL in methanol).
3. Load 200 µL of sample on to ISOLUTE SLE+ 200 µL supported liquid extraction plate (part# 820-0200-P01).
4. Apply a pulse of vacuum to initiate flow.
5. Wait 5 min for sample to completely absorb.
6. Extract samples with 1 mL of MTBE. Allow solvent to flow for 5 min under gravity. Apply vacuum for 10-30 sec to complete elution.
7. Evaporate extracts to dryness under a stream of nitrogen.
8. Reconstitute in 200 µL mobile phase A prior to analysis.
9. Vortex to mix.