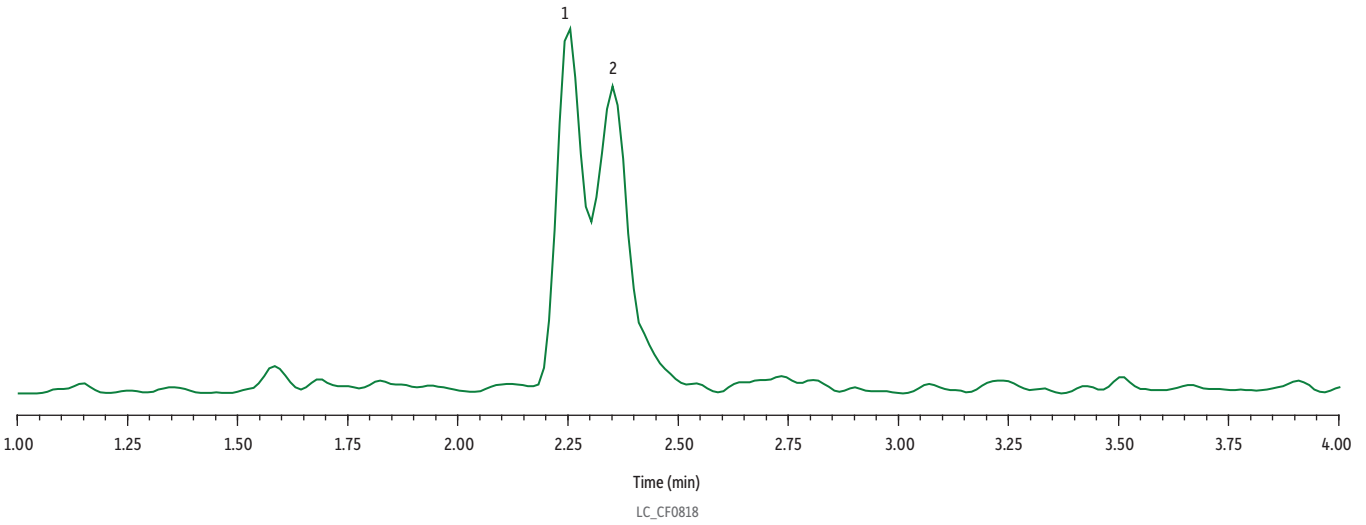
Plasma Matrix Interference Co-Eluting with D4-UDCA in Patient Sample on Raptor C18



Peaks	t_R (min)	Precursor Ion	Product Ion
1. Matrix interference	2.25	-	-
2. D_4 UDCA	2.35	395.40	395.40

Column	Raptor C18 (cat.# 9304252)
Dimensions:	50 mm x 2.1 mm ID
Particle Size:	1.8 µm
Pore Size:	90 Å
Guard Column:	UltraShield UHPLC precolumn filter 0.2 µm (cat.# 25810)
Temp.:	60 °C
Standard/Sample	
Diluent:	65:35 Water:methanol
Inj. Vol.:	3 µL
Mobile Phase	
A:	5 mM ammonium acetate in water, pH unadjusted
B:	Methanol:acetonitrile (v/v, 50:50)

Time (min)	Flow (mL/min)	%A	%B
0.00	0.5	65	35
2.00	0.5	60	40
2.50	0.5	55	45
3.50	0.5	50	50
4.60	0.5	45	55
5.70	0.5	20	80
5.90	0.8*	5	95
6.50	0.8*	5	95
6.51	0.5	65	35
8.50	0.5	65	35

Max Pressure:	275 bar
Detector	Shimadzu LCMS-8045 in ESI- mode
Instrument	Shimadzu Nexera X2
Sample Preparation	A 50 µL aliquot of patient sample was added to a microcentrifuge tube. Fifty microliters of internal standards was added to the tube and vortexed. The samples were protein precipitated using 800 µL of ice-cold acetonitrile. After vortexing and centrifugation at 4200 rpm for 10 min, the supernatant was transferred to a new vial and dried down at 60 °C under nitrogen. All samples were reconstituted in 200 µL of 35% methanol in water.
Notes	*The flow rate was increased to 0.8 mL/min to more thoroughly flush phospholipids from the analytical column, thereby reducing matrix effects. The flow was diverted to waste before 1 minute and after 6 minutes to protect the mass spectrometer.