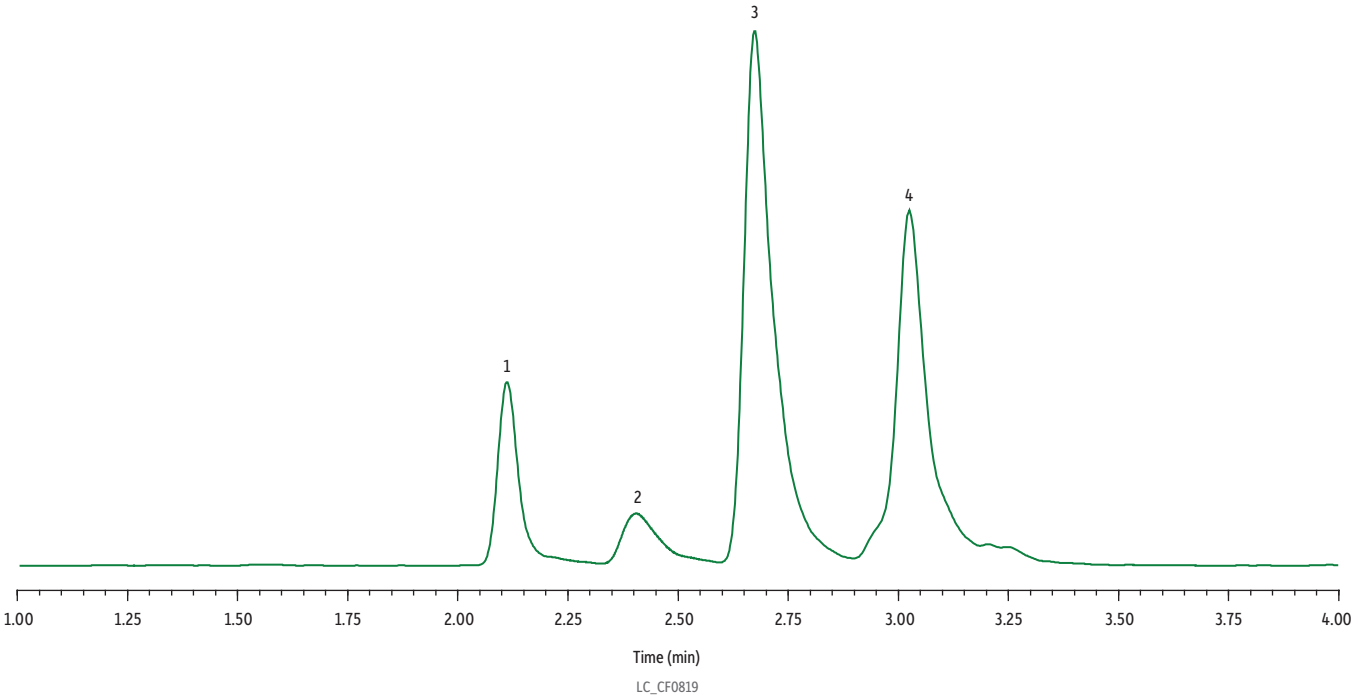
Plasma Matrix Interference Separated from D4-UDCA in Patient Sample on Raptor Inert ARC-18



Peaks	t_R (min)	Precursor Ion	Product Ion
1. Matrix interference	2.11	-	-
2. Matrix interference	2.41	-	-
3. D_4 UDCA	2.67	395.40	395.40
4. D_4 HDCA	3.02	395.40	395.40

Column Raptor Inert ARC-18 (cat.# 9314A12-T)
Dimensions: 100 mm x 2.1 mm ID
Particle Size: 2.7 µm
Pore Size: 90 Å
Guard Column: Raptor Inert ARC-18 EXP guard column cartridge 5 mm, 2.1 mm ID, 2.7 µm (cat.# 9314A0252-T)
Temp.: 50 °C
Standard/Sample Diluent: 60:40 Water:MPB
Inj. Vol.: 5 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	60	40
6.00	0.5	30	70
7.00	0.5	20	80
7.50	0.8*	0	100
8.10	0.8*	0	100
8.20	0.5	60	40
9.50	0.5	60	40

Max Pressure: 365 bar
Detector Shimadzu LCMS-8045 in ESI- mode
Instrument Shimadzu Nexera X2
Sample Preparation For control samples, a 100 µL aliquot of patient sample was added to a microcentrifuge tube. Ten microliters of internal standards was added and vortexed. Samples were protein precipitated using 400 µL of ice-cold acetonitrile. After vortexing and centrifugation at 4200 rpm for 15 minutes, the supernatant was transferred to a glass test tube and dried down under nitrogen. All samples were reconstituted in 200 µL of 60:40 water:MPB. The sample was transferred to a clean 2 mL screw-thread vial (cat.# 21143) with a glass insert (cat.# 21776) and capped with short-cap, screw-vial closures (cat.# 24498).
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 7 minutes to protect the mass spectrometer.