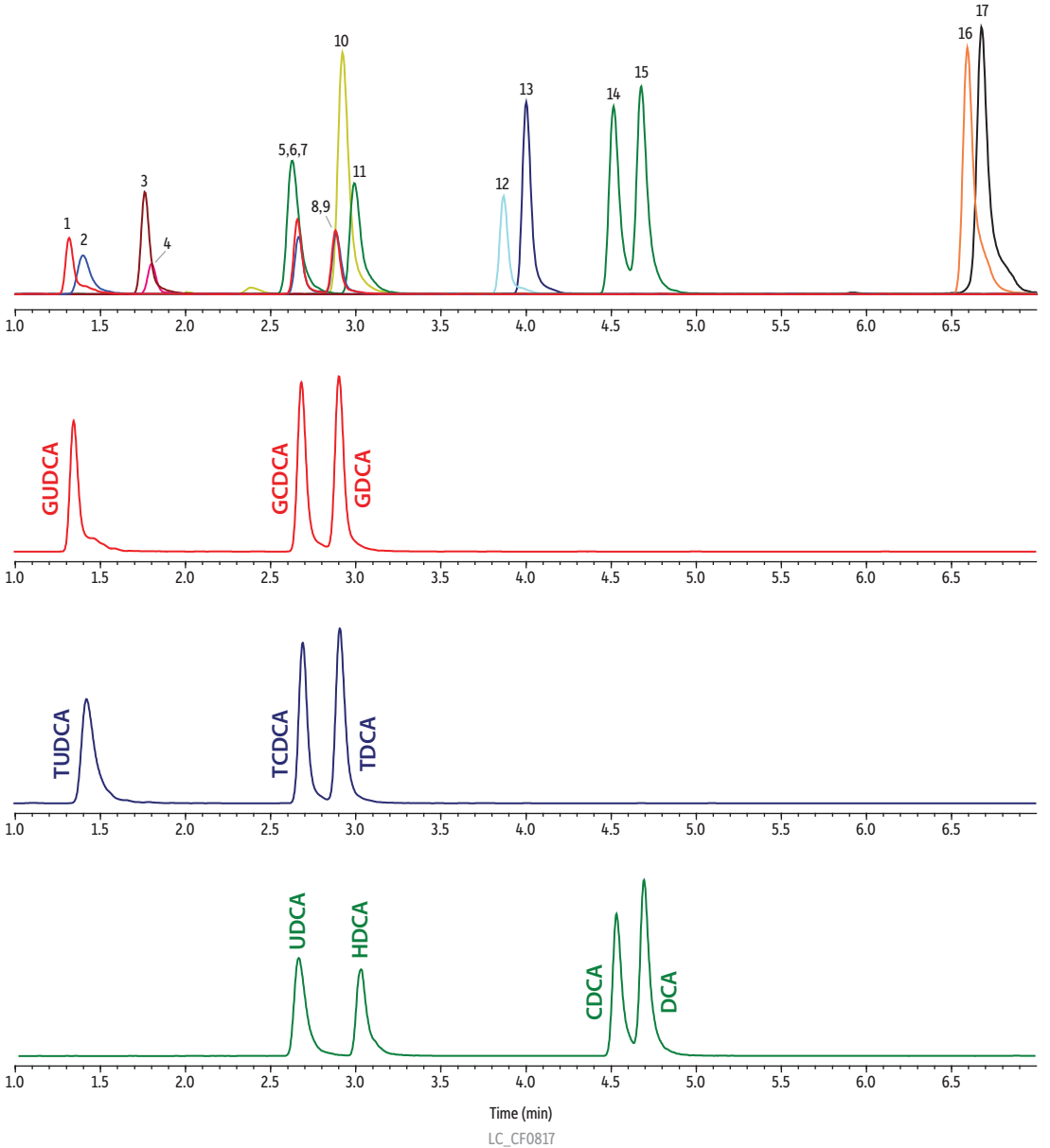
Bile Acids with Isomer Separation in Human Plasma on Raptor Inert ARC-18 by LC-MS/MS



LC_CF0817

Peaks	t_R (min)	Precursor Ion	Product Ion
1. GUDCA	1.32	448.40	74.15
2. TUDCA	1.40	498.10	80.05
3. GCA	1.78	464.10	74.15
4. TCA	1.80	514.20	80.05
5. UDCA	2.63	391.50	391.50
6. GCDCA	2.66	448.40	74.15
7. TCDCA	2.66	498.10	80.05
8. GDCA	2.88	448.40	74.15
9. TDCA	2.88	498.10	80.05
10. CA	2.92	407.20	407.20
11. HDCA	2.99	391.50	391.50
12. TLCA	3.87	482.10	80.05
13. GLCA	4.00	432.20	74.15
14. CDCA	4.51	391.50	391.50
15. DCA	4.68	391.50	391.50
16. LCA	6.59	375.40	375.40
17. DHLCA	6.68	373.20	373.20

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:mobile phase B
Conc.: 5 µM
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 90 µL aliquot of 2x charcoal-stripped plasma (K_2EDTA) was added to a microcentrifuge tube and spiked with 10 µL of calibrator/QC material and vortexed. 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:mobile phase B. 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.