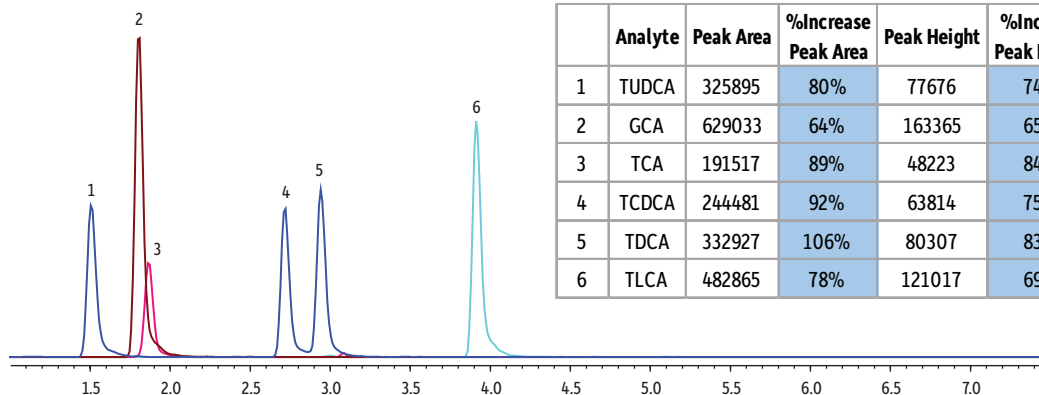


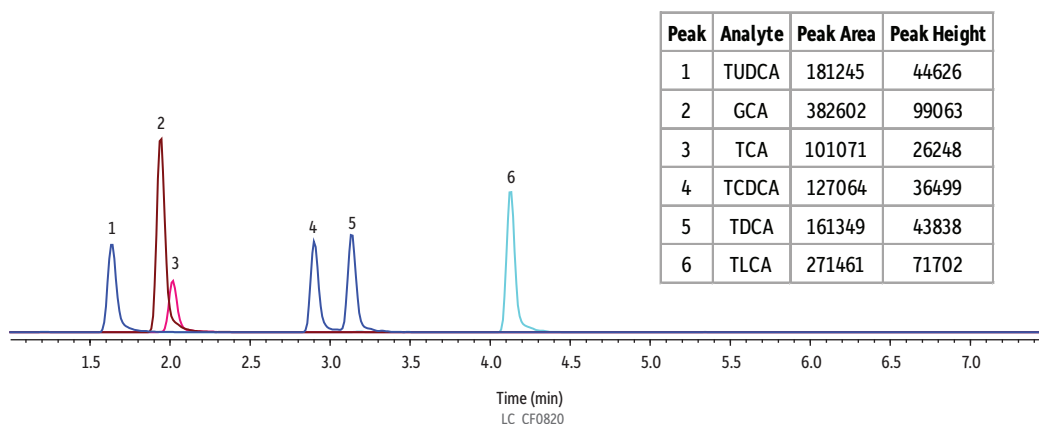
# Comparison of Bile Acids on Raptor Inert ARC-18 and Raptor ARC-18

## Raptor Inert ARC-18



	Analyte	Peak Area	%Increase Peak Area	Peak Height	%Increase Peak Height
1	TUDCA	325895	80%	77676	74%
2	GCA	629033	64%	163365	65%
3	TCA	191517	89%	48223	84%
4	TCDCa	244481	92%	63814	75%
5	TDCA	332927	106%	80307	83%
6	TLCA	482865	78%	121017	69%

## Raptor ARC-18



Peak	Analyte	Peak Area	Peak Height
1	TUDCA	181245	44626
2	GCA	382602	99063
3	TCA	101071	26248
4	TCDCa	127064	36499
5	TDCA	161349	43838
6	TLCA	271461	71702

Peaks	Precursor Ion	Product Ion
1. TUDCA	498.10	80.05
2. GCA	464.10	74.15
3. TCA	514.20	80.05
4. TCDCa	498.10	80.05
5. TDCA	498.10	80.05
6. TLCA	482.10	80.05

**Column** See notes.  
 Dimensions: 100 mm x 2.1 mm ID  
 Particle Size: 2.7 µm  
 Pore Size: 90 Å  
 Temp.: 50 °C  
**Standard/Sample**  
 Diluent: 60:40 Water:mobile phase B  
 Conc.: 0.25 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:** 300 bar  
**Detector** Shimadzu 8045-LCMS 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 were 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.

Columns are:  
 • Raptor Inert ARC-18 (cat.# 9314A12-T)  
 • Raptor ARC-18 (cat.# 9314A12)

