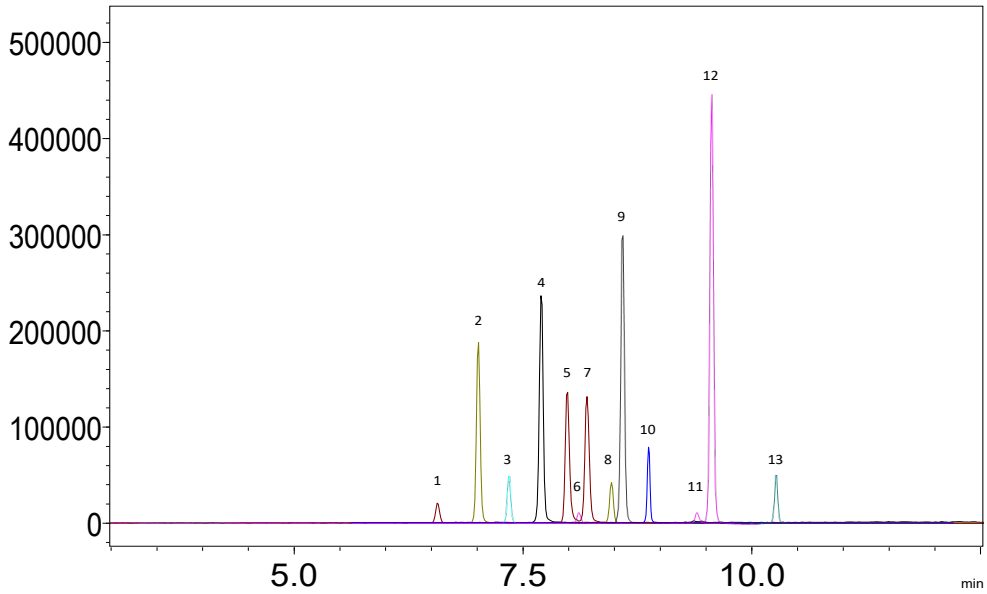
HALO®

CLINICAL / TOXICOLOGY

LCMS Separation of Bile Acids Using HALO® C18

232-A



PEAK IDENTITIES, MRM TRANSITIONS, COLLISION ENERGIES, AND LINEARITIES

Peak Number	Analyte	MH^-	Transition	CE	R^2
1	Sodium-tauroursodeoxycholate (TUDC)	498.7	498.7>124.1	51	0.9998
2	Glycoursodeoxycholic acid (GDC)	448.2	448.2>74.1	34	0.9988
3	Taurocholic acid sodium salt hydrate (TCA)	514.3	514.3>80.0	35	0.9986
4	Glycocholic acid hydrate (GCA)	464.2	464.3>402.3	34	0.9978
5	Sodium taurochenodeoxycholate (TCDC)	498.7	498.7>124.1	52	0.9982
6	Ursodeoxycholic acid (UDC)	391.5	391.5>391.5	8	0.9993
7	Sodium-taurodeoxycholate hydrate (TDC)	498.2	498.7>124.1	52	0.9971
8	Sodium glycochenodeoxycholate (GCDC)	448.2	448.2>74.1	30	0.9981
9	Cholic acid (CA)	407.5	407.5>407.5	8	0.9957
10	Sodium-taurolithocholate (TLC)	482.2	482.2>124.1	50	0.9973
11	Chenodeoxycholic acid (CDC)	391.5	391.5>391.5	8	0.9955
12	Deoxycholic acid (DC)	391.5	391.5>391.5	8	0.9986
13	Lithocholic acid (LC)	375.5	375.5>375.5	8	0.9987

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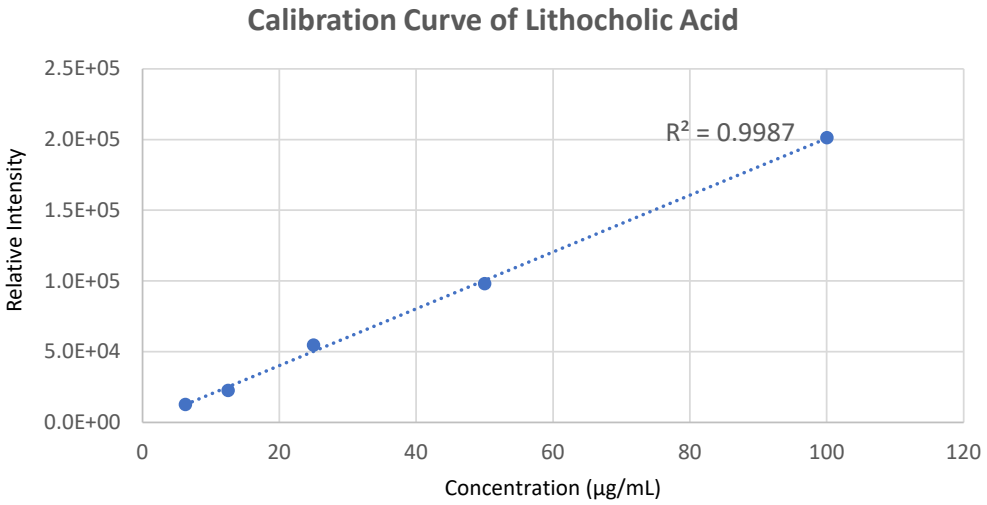

TEST CONDITIONS:

Column: HALO 90 Å C18, 2.7 µm, 2.1 × 150 mm 92812-702
Mobile phase A: 5 mM ammonium formate and 0.012% formic acid in water
Mobile Phase B: 5 mM ammonium formate and 0.012% formic acid in methanol

Gradient:

Time	%B
0.00	30
10.00	95
15.00	95
15.10	30
18.00	30
18.00	End

Flow Rate: 0.4 mL/min
Pressure: 185 bar
Temperature: 40 °C
Injection: 1.0 µL (12.5 µg/mL, in SigMatrix Serum Diluent)
Instrument: Shimadzu Nexera

MS TEST CONDITIONS:

Mass Spectrometer: Shimadzu 8040
Ion mode: Negative Electrospray
Heat Block Temperature: 400 °C
Drying line: 300 °C
Nebulizing Gas Flow: 3 L/min
Drying Gas Flow: 18 L/min
Spray Voltage: -4000 V
Q1/Q2 Resolution: High

An LC MS/MS method was developed for the analysis of bile acids on a HALO® C18 column. The column demonstrated excellent performance in the separation of multiple isobaric compounds and rugged reliability with excellent linearity, enabling clinically relevant concentrations to be analyzed. The main limitation with identification by MSMS is associated to indistinguishable transitions, so the chromatographic separation is paramount for identification. The resolution, precision and narrow peak widths provided by the HALO® C18 column allows for these acids to be clearly separated and identified, and the linearity shows that these acids can be detected and quantitated at clinically relevant levels.