Exploring Different HPLC Column Chemistries for Optimal Separation of 17 Bile Acids by LC-MS/MS

Haley Berkland, Elena Gairloch
Restek Corporation, Bellefonte, PA

Introduction

The analysis of bile acids in human plasma is an important diagnostic tool for the detection of liver disease and can also be used as indicators of potentially harmful side effects of new drugs. There are two main types of bile acids based upon their functional groups: unconjugated and conjugated, primarily with glycine- or taurine-based residues. Quantitation of bile acids in matrix can be very challenging due to several factors. These include structural similarities, varying polarity and stereochemistry, the presence of isomers, limited fragmentation of unconjugated bile acids in a mass spectrometer, high endogenous levels, and matrix effects caused by phospholipids or triglycerides.

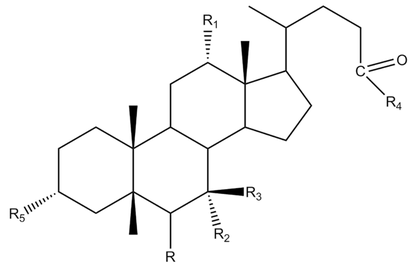
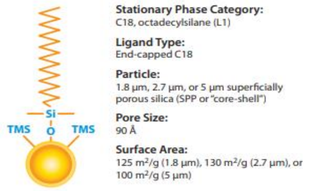
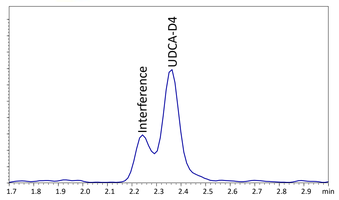


Figure 1. Base chemical structure of bile acids.

In this study, 17 bile acids were analyzed by LC-MS/MS using a Raptor C18 50 x 2.1 mm, 1.8 µm column. Through routine validation, a matrix interference was identified to be co-eluting with one of the bile acids standards and causing issues with quantitation. It was not possible to resolve this interference on the Raptor C18 column, requiring a new method be developed on an alternative column chemistry. The original method conditions as well as the detected matrix interference are shown below.





Column	Raptor C18, 50 x 2.1 mm, 1.8 µm		
MPA	5 mM ammonium acetate, H_2O		
MPB	50:50 MeOH:ACN (v/v)		
Column Temp	60°C		
Inj. Volume	3 µL		
Gradient	Time (min)	Flow Rate (mL/min)	%B
	0.00	0.5	35
	2.00	0.5	40
	2.50	0.5	45
	3.50	0.5	50
	4.60	0.5	55
	5.70	0.5	80
	5.90	0.8	85
	6.50	0.8	85
	6.51	0.5	35
	8.50	0.5	35

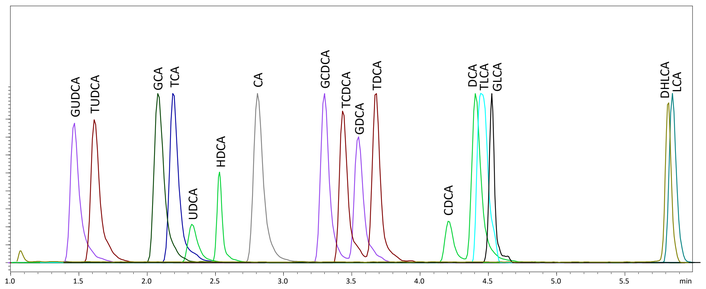
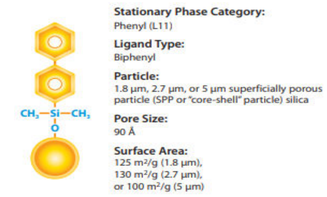


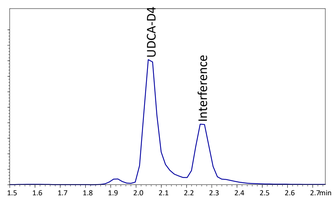
Figure 2. Bile acids analyzed using a C18 column. All analytes, including isomers, are well resolved. A matrix interference with UDCA-D4 is present.

Scouting Methods

Three alternative column chemistries were investigated for the method: **Biphenyl**, **FluoroPhenyl**, and **ARC-18**. A scouting gradient was run to determine compound elution on each stationary phase. Methods were further optimized to determine which was best suited to meet analysis goals. The final method was required to resolve the matrix interference from UDCA-D4 as well as adequately resolve the three pairs of isomers in the analyte list. The scouting gradient started at 10% B, ramped up to 100% B over 8.0 minutes, and then returned to starting conditions and held until 9.5 minutes for ample re-equilibration. Data was collected in ESI- mode.

Results





Column	Raptor Biphenyl, 100 x 2.1 mm, 2.7 µm		
MPA	5 mM ammonium acetate, H_2O		
MPB	50:50 MeOH:ACN (v/v)		
Column Temp	50°C		
Inj. Volume	3 µL		
Gradient	Time (min)	Flow Rate (mL/min)	%B
	0.00	0.5	40
	7.00	0.5	70
	8.00	0.5	100
	8.50	0.5	100
	8.51	0.5	40
	9.50	0.5	40

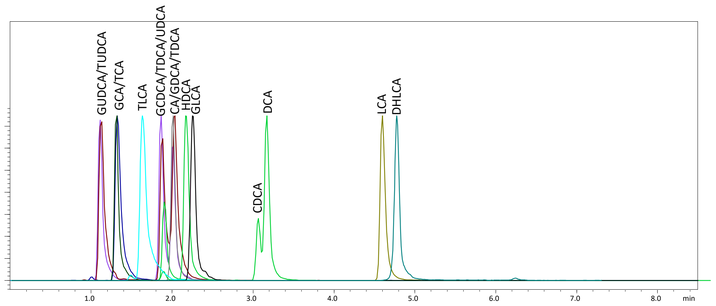
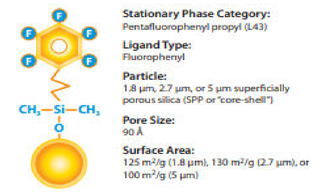
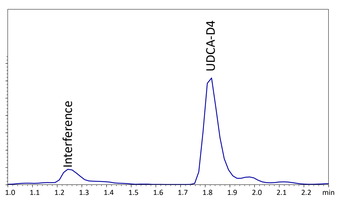


Figure 3. Bile acids analyzed using a Biphenyl column. Some selectivity is observed for glycine/taurine isomers, but limited selectivity is shown for unconjugated isomers with CDCA/DCA unable to be fully resolved. Interference is mostly resolved from UDCA-D4. Due to lack of selectivity for the isomers, this column was not chosen for the final method.





Column	Raptor FluoroPhenyl, 100 x 2.1 mm, 2.7 µm		
MPA	5 mM ammonium acetate, H_2O		
MPB	50:50 MeOH:ACN (v/v)		
Column Temp	50°C		
Inj. Volume	3 µL		
Gradient	Time (min)	Flow Rate (mL/min)	%B
	0.00	0.5	35
	7.00	0.5	60
	8.00	0.5	100
	8.50	0.5	100
	8.51	0.5	35
	9.50	0.5	35

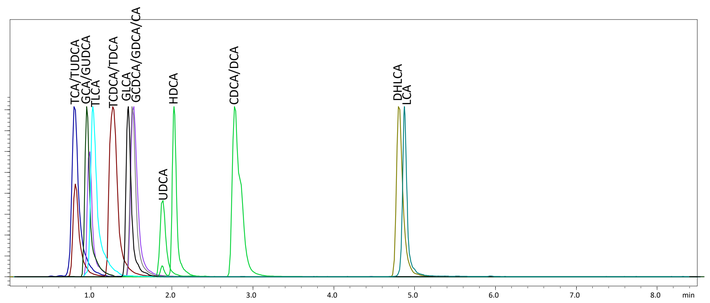
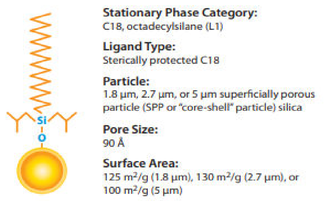
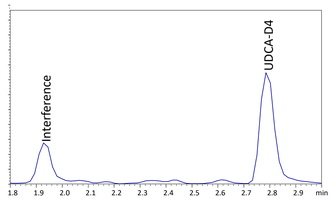


Figure 4. Bile acids analyzed on FluoroPhenyl column. Limited selectivity is observed for the three isomer sets, with GCDCA/GDCA, TCDCA/TDCA, and CDCA/DCA co-eluting. Interference is resolved from UDCA-D4. Due to lack of selectivity for the isomers, this column was not chosen for the final method.

Results





Column	Raptor ARC-18, 100 x 2.1 mm, 2.7 µm		
MPA	5 mM ammonium acetate, H_2O		
MPB	50:50 MeOH:ACN (v/v)		
Column Temp	50°C		
Inj. Volume	3 µL		
Gradient	Time (min)	Flow Rate (mL/min)	%B
	0.00	0.5	40
	7.00	0.5	80
	8.00	0.5	100
	8.50	0.5	100
	8.51	0.5	40
	9.50	0.5	40

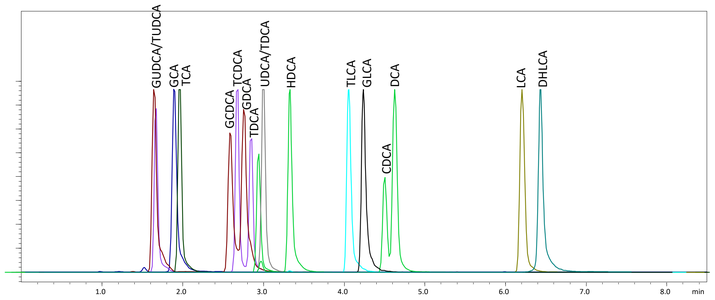
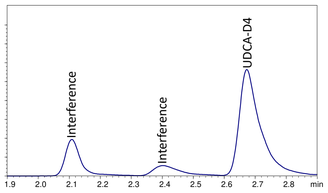


Figure 5. Bile acids analyzed on ARC-18 column. Selectivity shown for all isomer sets but further optimization is needed to fully resolve. Interference is resolved from UDCA-D4.



Column	Raptor Inert ARC-18, 100 x 2.1 mm, 2.7 µm		
MPA	5 mM ammonium acetate, H_2O		
MPB	50:50 MeOH:ACN (v/v)		
Column Temp	50°C		
Inj. Volume	5 µL		
Gradient	Time (min)	Flow Rate (mL/min)	%B
	0.00	0.5	40
	6.00	0.5	70
	7.00	0.5	80
	7.50	0.8	100
	8.10	0.8	100
	8.20	0.5	40
	9.50	0.5	40

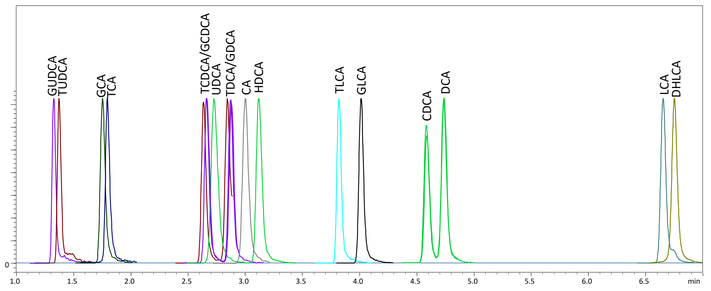


Figure 6. Optimized method on ARC-18 column. All isomers are resolved, as well at the interference with UDCA-D4. An inert column was used to mitigate non-specific binding of the analytes. The flow rate was increased from 0.5 mL/min to 0.8 mL/min from 7.50-8.10 min to flush phospholipids from the column and reduce matrix effects.

Conclusion

While most method developments focus strongly on the analytes of interest, it is also necessary to give attention to potential matrix interferences. Matrix interferences can result in invalid quantitation if not properly resolved. Additionally, this study demonstrates the use of alternative column chemistries to resolve matrix interferences. While the Raptor C18 and Raptor ARC-18 are both C18 ligands, the difference in end-capping allowed increased selectivity to resolve the interference with the ARC-18 phase.