

# Analysis of Bile Acids by HPLC with ELSD using the Agilent InfinityLab Poroshell 120 EC-C18 LC Column

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## Abstract

Bile acids, both conjugated and unconjugated, were analyzed by HPLC using Agilent InfinityLab Poroshell 120 EC-C18 columns with 2.7 and 4  $\mu\text{m}$  particle sizes. The InfinityLab Poroshell 120 EC-C18, 4.6  $\times$  250 mm, 4  $\mu\text{m}$  column separated all bile acids in 32 minutes with a gradient method. The method was transferred to an InfinityLab Poroshell 120 EC-C18, 4.6  $\times$  100, 2.7  $\mu\text{m}$  column to achieve a quick separation within 13 minutes.

## Introduction

In this Application Note, 14 bile acids plus cholesterol, listed in Table 1, were separated by HPLC with an InfinityLab Poroshell 120 EC-C18, 4.6 × 250 mm, 4 μm column. To achieve quick separation, the method was transferred to a shorter InfinityLab Poroshell 120 EC-C18, 4.6 × 100 mm, 2.7 μm column. Because unconjugated bile acids lack characteristic chromophores, an ELSD was used for sufficient sensitivity.

## Experimental

### Reagents and chemicals

All reagents were HPLC grade or higher. HPLC grade methanol and acetonitrile were bought from J. T. Baker (Center Valley, PA, USA.). Water was purified using an ELGA PURELAB Chorus system (High Wycombe, UK). Ammonium acetate was from J&K Scientific (Beijing, China). Standards were from Sigma-Aldrich (St. Louis, MO, USA). The standard stock solutions were all produced in methanol to a concentration of 1 mg/mL. The standard mixture solution was made by mixing all individual standard solutions to a concentration of approximately 70 μg/mL.

### Equipment and materials

- **Column inlet:** Agilent InfinityLab Quick Connect LC fitting (p/n 50675965)
- **Column outlet:** Agilent InfinityLab Quick Turn LC fitting (p/n 5067-5966)
- Agilent Captiva Econofilter, PTFE membrane, 13 mm diameter, 0.2 μm pore size (p/n 5190-5265)
- Agilent vial, screw top, amber, write-on spot, certified, 2 mL (p/n 5182-0716)
- Agilent bonded screw cap, bonded blue, PTFE/red silicone septa (p/n 5190-7024)

**Table 1.** Compounds separated in this Application Note.

No.	Compound	Molecular Weight (g/mol)	CAS Number	Structure
1	Glycohyodeoxycholic Acid	449.63	13042-33-6	
2	Taurohyodeoxycholic Acid Sodium	521.69	110026-03-4	
3	Glycocholic Acid	465.62	475-31-0	
4	Sodium Taurocholate	537.68	145-42-6	
5	Hyodeoxycholic Acid	392.57	83-49-8	
6	Cholic Acid	408.57	81-25-4	

- Agilent InfinityLab solvent bottle, amber, 1,000 mL (p/n 9301-6526)
- Agilent InfinityLab Stay Safe cap, GL45, three-port, one-vent valve (p/n 5043-1219)
- Eppendorf pipettes and repeater
- Sonicator (VWR, Radnor, PA, USA)

### Instrumentation

- Agilent 1290 Infinity II high speed pump (G7120A)
- Agilent 1290 Infinity II multisampler (G7167B)
- Agilent 1290 Infinity II multicolumn thermostat (G7116B)
- Agilent 1290 Infinity II ELSD (G7102A)
- Agilent OpenLab CDS, version C.01.07

**Table 1.** Compounds separated in this Application Note (continued).

No.	Compound	Molecular Weight (g/mol)	CAS Number	Structure
7	Glycochenodeoxycholic Acid Sodium	471.61	16564-43-5	
8	Taurochenodeoxycholic Acid Sodium	521.69	6009-98-9	
9	Glycodeoxycholic Acid	449.63	360-65-6	
10	Taurodeoxycholic Acid Sodium	521.69	1180-95-6	
11	Chenodeoxycholic Acid	392.57	474-25-9	

**Table 1.** Compounds separated in this Application Note (continued).

No.	Compound	Molecular Weight (g/mol)	CAS Number	Structure
12	Deoxycholic Acid	392.57	83-44-3	
13	Lithocholic Acid	376.57	434-13-9	
14	Lithocholic Acid Methyl Ester	390.6	1249-75-8	
15	Cholesterol	386.65	57-88-5	

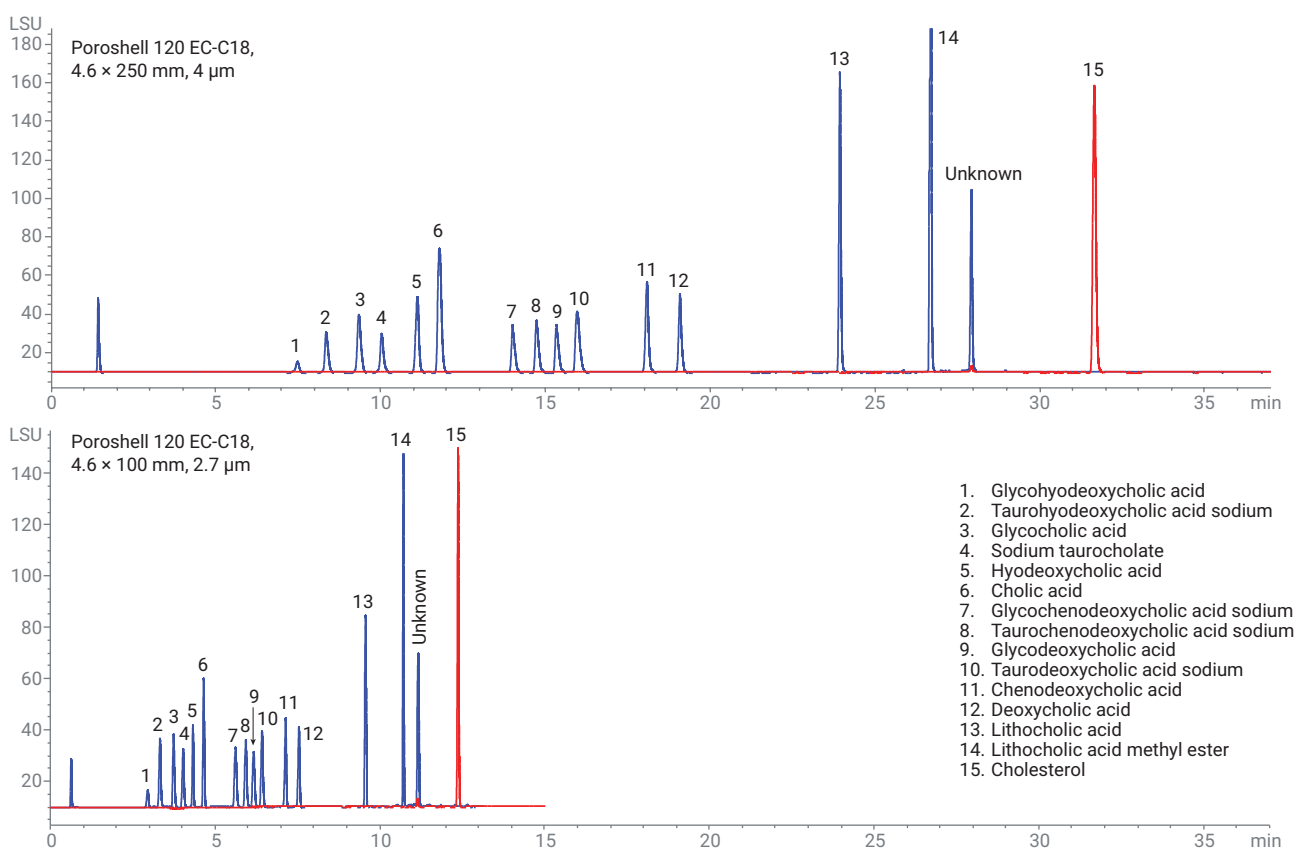
HPLC Conditions		
Column	InfinityLab Poroshell 120 EC-C18, 4.6 x 100 mm, 2.7 µm (p/n 695975-902)	InfinityLab Poroshell 120 EC-C18, 4.6 x 250 mm, 4 µm (p/n 690970-902)
Mobile Phase A	20 mM ammonium acetate in water	
Mobile Phase B	Acetonitrile: methanol (60:40)	
Gradient	0 to 2 minutes, 40 %B; 2 to 8 minutes, 40 to 60 %B; 8 to 10 minutes 100 %B; Stop time: 15 minutes	0 to 5 minutes, 40 %B; 5 to 20 minutes, 40 to 60 %B; 20 to 25 minutes 100 %B; Stop time: 37 minutes
Flow Rate	1.50 mL/min	
Column Temperature	60 °C	
Injection Volume	4 µL	10 µL
ELSD Conditions		
Evaporator Temperature	80 °C	
Nebulizer Temperature	30 °C	
Gas Flow Rate	1.60 SLM	

## Results and discussion

The upper chromatogram in Figure 1 shows the separation of the 15 compounds with the InfinityLab Poroshell 120, 4.6 × 250 mm, 4 μm column. The analysis was performed in 32 minutes with full baseline separation. The method was transferred to a shorter column with a smaller particle size of 2.7 μm. The analysis time was shortened

to only 12.5 minutes, but still with excellent resolution for all compounds. The 4 μm superficially porous particle generated double the efficiency of the traditional 5 μm totally porous particle. The 2.7 μm superficially porous particle delivered UHPLC comparable to sub-2 μm particles, but with relatively low backpressure. The 2 μm frit on the InfinityLab Poroshell 120 EC-C18 column is less likely to plug compared

to the 0.5 μm frit typically used on sub-2 μm UHPLC columns. Therefore, the InfinityLab Poroshell 120 EC-C18 columns are a more rugged column choice for dirty samples. Real samples for bile acids analysis are always biological samples such as bile, serum, and urinary samples. The InfinityLab Poroshell 120 EC-C18 columns are suitable for such samples.

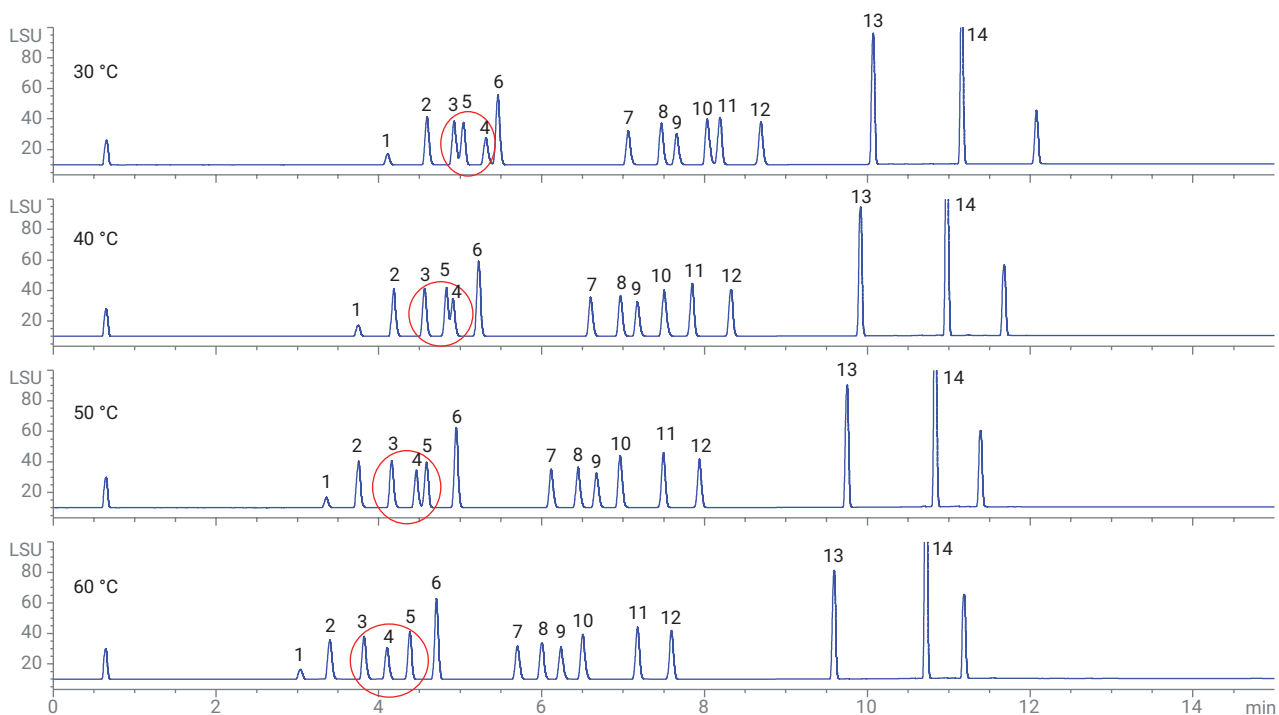


**Figure 1.** Chromatograms of bile acid separation with InfinityLab Poroshell 120 EC-C18, 4.6 × 250 mm, 4 μm and InfinityLab Poroshell 120 EC-C18, 4.6 × 100 mm, 2.7 μm columns.

To achieve better separation, the HPLC parameters including mobile phase composition and column temperature were adapted. In this separation, column temperature was crucial for the resolution. Figure 2 shows separation under different column temperatures. When the temperature was increased, the elution order of peaks 3, 4, and 5 changed, and the resolution was improved.

## Conclusions

Both 4 and 2.7  $\mu\text{m}$  particle size columns provided good resolution for all the bile acids. The shorter column with smaller particle size achieved quick separation with significant time and solvent consumption. The optimized method using HPLC coupled with ELSD was suitable for bile acids analysis.



**Figure 2.** Chromatograms of different column temperature using the InfinityLab Poroshell 120 EC-C18, 4.6 × 100 mm, 2.7  $\mu\text{m}$  column.

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Printed in the USA, March 14, 2019  
5994-0807EN