Quantification of Bile Acid by Reversed Phase HPLC with Evaporative Light Scattering Detection

Application Note

Author

Stephen Ball
Agilent Technologies, Inc.

Introduction

Cholic acid, deoxycholic acid, and chenocholic acid (in the bile) are esterified at the terminal carboxyl group with either glycine to give glycocholic acid or taurine to yield taurocholic acid. After synthesis in the liver, they are concentrated and stored in the gall bladder before being secreted into the small intestine where the salts aid the hydrolysis of neutral fats by acting as emulsifying and stabilizing agents. The bile acid derivatives that are of physiological importance do not have specific UV chromophores and hence RI detection is commonly used so limiting the analysis to isocratic HPLC methods.

However, these acid salts are not volatile and so could be detected using the 380-LC evaporative light scattering detector which can be used with both isocratic and gradient HPLC. The 380-LC offers greater sensitivity than UV detection, particularly for bile acids that have no UV chromophore. Solvent peaks are absent and excellent baseline stability is present. The 380-LC is renowned for its rugged design and ability to deliver high performance for demanding HPLC or GPC applications. Reversed phase PLRP-S columns are ideally suited to the analysis of low molecular weight compounds because the small pore sizes have extremely high surface areas available to the solutes. Coupling PLRP-S columns with the 380-LC evaporative light scattering detector is an excellent system for separating bile acids.

Agilent Technologies

Instrumentation

Column: PLRP-S 100Å 5 µm, 150 x 4.6 mm (p/n PL1111-3500)
Detector:380-LC (neb=85 °C, evap=60 °C, gas=1.0 SLM)

Materials and Reagents

Eluent A:0.058 M Ammonium acetate, 58% methanol, 3% isopropanol, pH 5.3
Eluent B:THF

Conditions

Flow Rate:	0.5 mL/min
Gradient:	0-15% B in 20 min

Results and Discussion

The gradient HPLC method, using a polymeric reversed phase column separated five conjugated bile acid salts, as shown in Figure 1. The bile acid calibration curves generated by the detector are shown in Figure 2.

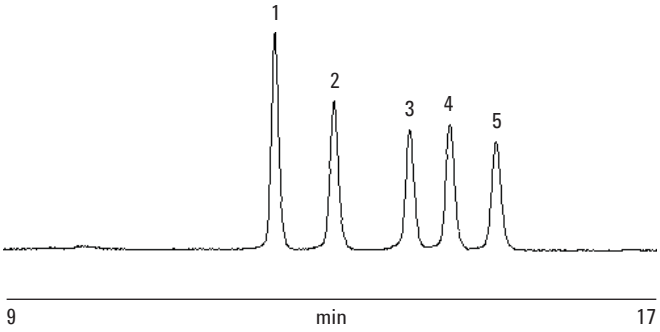

Figure 1. Resolution of conjugated bile acids by reversed phase HPLC using a PLRP-S 100Å column.

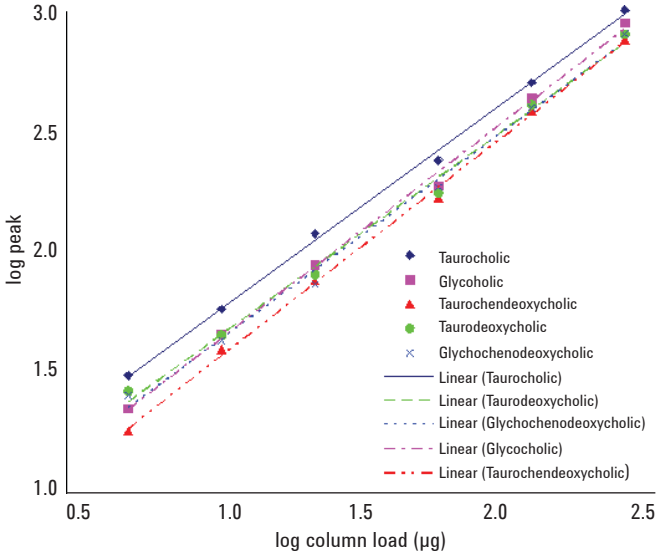

Figure 2. Calibration plots of five bile acids from the 380-LC evaporative light scattering detector.

Conclusion

Reversed phase HPLC with the 380-LC evaporative light scattering detector and PLRP-S columns successfully quantified bile acids.

PLRP-S columns are the preferred choice for the analysis of many small molecules. These columns are more retentive for small molecules than the majority of alkyl bonded silicas. PLRP-S media possess a much greater surface area than alkyl bonded silicas and therefore even polar molecules may be retained for much longer, resulting in greater resolution.

PLRP-S columns and the 380-LC make an ideal combination for the separation of low molecular weight compounds that have no chromophores, under isocratic or gradient conditions.

www.agilent.com/chem

Published in UK, August 25, 2015
5990-8195EN

Agilent Technologies