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アプリケーションノート

HPLC Separation of Bile Acids Using a CORTECS™ Premier Phenyl Column

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Published on September 01, 2025

Application Brief

This is an Application Brief and does not contain a detailed Experimental section.

Abstract

The determination of bile acids is important in understanding the metabolism and absorption of fats. Additionally, some bile acids have been shown to be associated with colon cancer risk. Six of these compounds were analyzed by LC-MS using a MaxPeak™ Premier CORTECS Phenyl Column. Good separation was achieved for two sets of isobaric compounds present in the panel.

Benefits

- Baseline resolution of isobaric bile acids: taurodeoxycholic acid/taurochenodeoxycholic acid and glycodeoxycholic acid/glycochenodeoxycholic acid

Introduction

Bile acids are endogenous compounds which aid in the digestion, transport, and absorption of fats in the body.

The concentrations of these compounds in blood plasma have been shown to be associated with an increased risk of developing colon cancer.[1] Consequently, the determination of these compounds is important for health monitoring. As such, achieving good chromatographic separation so that they can be accurately measured and monitored is a necessity.

A 2.1 x 50 mm, 2.7 µm CORTECS Premier Phenyl Column was used to separate six bile acids using high-performance liquid chromatography - mass spectrometry (HPLC-MS). MS detection was employed because these analytes do not contain a chromophore. Since some of these compounds are isobaric (have the same molecular weight), the isobars must be resolved chromatographically so that they may be accurately identified and quantified. The CORTECS Premier Phenyl Column employs solid-core silica particles shown to improve column efficiency compared to fully-porous particles of the same size.[2] Additionally, by using MaxPeak Premier Technology, interactions between the analytes and the column hardware are greatly reduced. These interactions can lead to peak tailing, reduced peak area, iron adduct formation, and in rare occasions, metal-catalyzed reactions of the analyte.[3-7]

Results and Discussion

Six bile acids were combined to create a mixture with each compound at a concentration of 50 µg/mL in 90:10 water:acetonitrile, Figure 1. A standard screening gradient was used with formic acid modified mobile phases. A constant concentration (0.1%) of formic acid was maintained throughout the gradient. The starting condition of 5% acetonitrile was maintained for 1.00 minute, followed by a linear gradient to 95% acetonitrile in 6.86 minutes. The 95% acetonitrile concentration was maintained for 1.14 minutes before returning to the starting condition and re-equilibrating for 2.28 minutes. A flow rate of 0.5 mL/min was used, and the column was maintained at 40 °C. Negative ion electrospray ionization MS detection was carried out using a Waters™ ACQUITY™ QDa™ Detector. Shown in Figure 2 is a stacked plot of single ion recordings (SIR) of the four mass-to-charge ratios for the six bile acids. Peaks were identified based on retention times in comparison to individual standards.

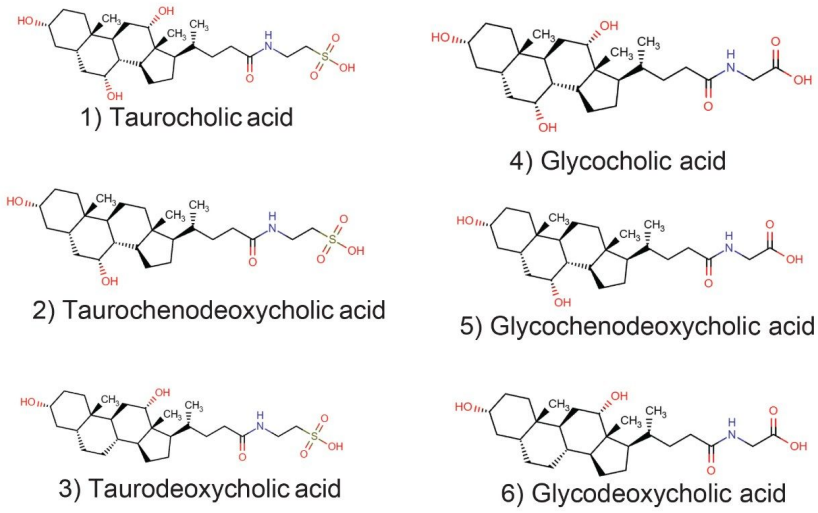

Figure 1. Chemical structures of the bile acids analyzed.

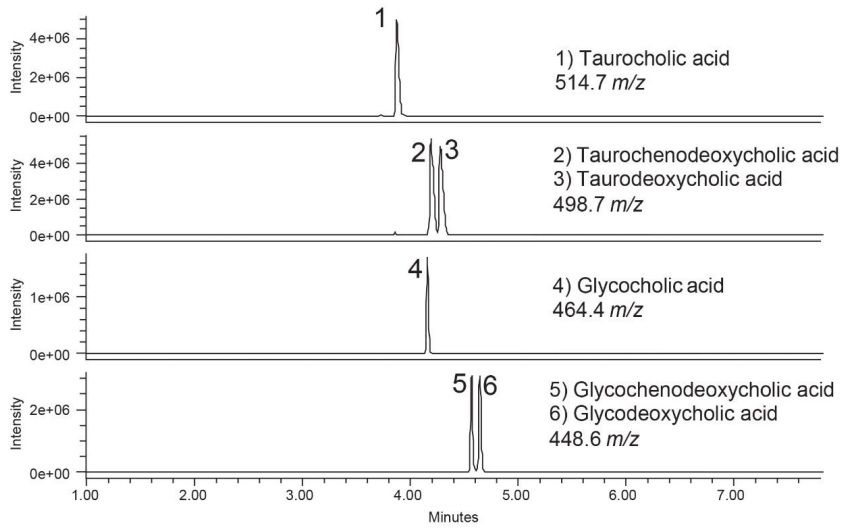

Figure 2. Chromatographic separation of six bile acids using a CORTECS Premier Phenyl Column.

The isobaric pairs taurodeoxycholic acid/taurochenodeoxycholic acid and glycodeoxycholic acid/glycochenodeoxycholic acid are both baseline resolved with good peak shapes. Achieving good results for

taurocholic acid and its deoxy forms is made easier by using a MaxPeak Premier Column. When ionized, the sulfonate group of these compounds is known to interact with metal surfaces in the column hardware causing peak tailing and decreased peak area.[8] However, these results are quite good with no discernible peak area loss or peak tailing.

Conclusion

Bile acids are critically important in the human body as they regulate how fats are metabolized and adsorbed. They may also be associated with the risk of colon cancer. Six bile acids were analyzed using a CORTECS Premier Phenyl Column. Baseline separation of two pairs of isobaric compounds was successfully achieved, allowing their accurate identification based on retention times. MaxPeak Premier Column hardware resulted in minimized interactions between the analytes and the column hardware, while the CORTECS solid-core particles produced high column efficiency. These two features are a match made in analytical heaven for critical separations of challenging analytes.

References

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720008967, September 2025