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應用手冊

Bile Acid Profiling Using UltraPerformance Convergence Chromatography (UPC2) Coupled to ESI-MS/MS

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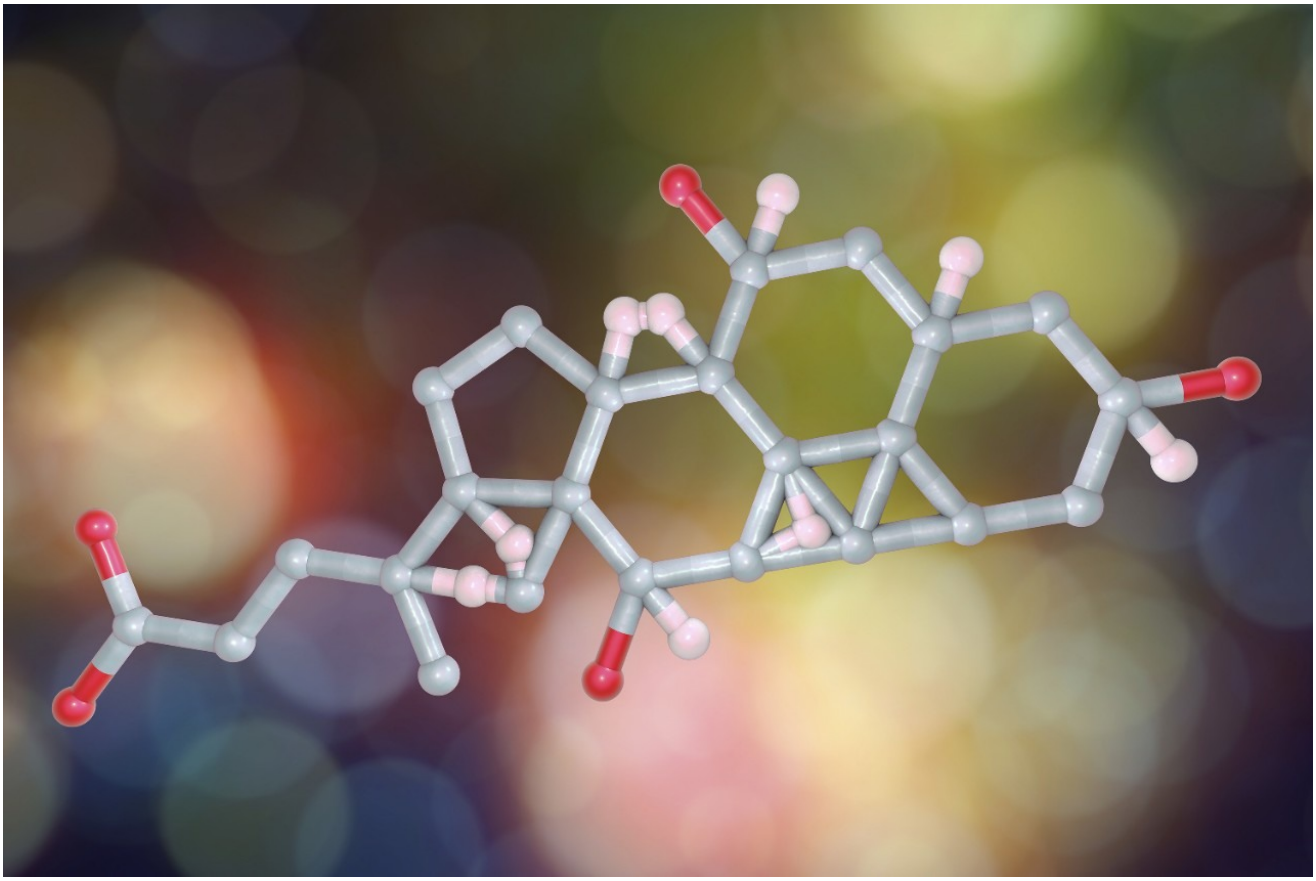

This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application brief describes a novel method for rapid profiling and quantification of bile acids using UltraPerformance Convergence Chromatography (UPC^2).

Benefits

UltraPerformance Convergence Chromatography combined with ESI-MS/MS enables the simultaneous profiling of 25 bile acids within 13 minutes.

Introduction

Bile acids play an important role as the signaling molecules that regulate triglyceride, cholesterol, and glucose metabolism.[1] These signaling pathways have become the source of attractive drug targets for metabolic diseases. Also bile acids are used as biomarkers in serum to interpret liver diseases and the mechanism of bile acid-regulated metabolism.[2–5] Therefore, an analytical method that determines the bile acid profile in the body is beneficial.

The separation of bile acids is complex due to the presence of structural analogues including isomeric forms, and the polarity diversity between unconjugated and conjugated forms. In the past, gas chromatography (GC) and liquid chromatography (LC) have been used to analyze these compounds; however, several limitations have been observed. GC always requires exhaustive derivatization steps which lead to a loss in bile acids at each step. In addition, aliquots of the samples have to be extracted separately to determine the composition or the concentration of conjugated bile acids in GC analysis.

While LC can detect conjugated and unconjugated forms simultaneously, the separation can take up to 30 minutes for a sample. This application note describes a faster UPC^2-MS method for bile acid profiling and quantification.

Results and Discussion

A method using supercritical carbon dioxide ($SCCO_2$)-based mobile phases was developed on the Waters

ACQUITY UPC^2 System to simultaneously separate 25 different bile acids including their conjugates. The use of $SCCO_2$ as the primary mobile phase allows for faster analyte diffusion and lower back pressure, resulting in faster analysis times. The ACQUITY UPC^2 System was connected to a Xevo TQ-S Mass Spectrometer in order to enhance the sensitivity and specificity of the analysis. Separation results for 25 different bile acids (including glycine and taurine conjugates) are shown in Figure 1. All bile acids were separated within 13 minutes, which is approximately two-fold faster than previous methods of analysis in bile acid profiling. This method was optimized through systematic investigation of different stationary phases, column temperature settings, additives, and pH in the modifier. The detection mode using the Xevo TQ-S was ESI negative. No make-up flow resulted in maximum sensitivity.

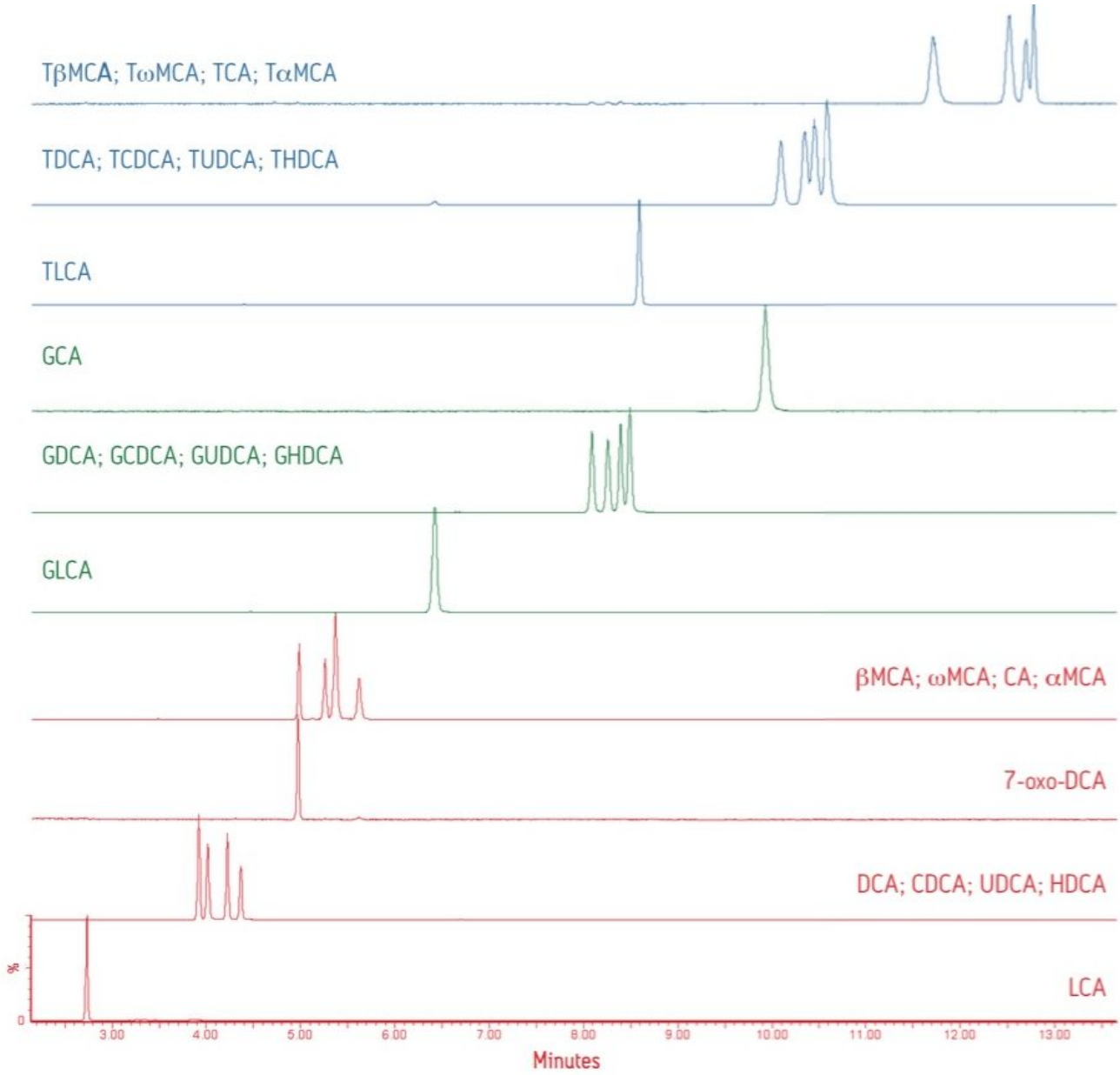

Figure 1. Simultaneous analysis of a 25-bile acids standard mixture including glycine and taurine conjugates was achieved within 13 minutes. The compound name is shown in order of retention time on each MRM chromatogram.

The developed method was applied to the quantitation of bile acids and their conjugates in a real biological sample (rat serum). Commercially purchased rat serum was deproteinized using methanol in a ratio of 3:1 methanol/serum, vortexed, and centrifuged. The supernatant was then injected onto UPC^2-MS/MS in order to determine the amount of each bile acid and their conjugates present in the serum sample. Figure 2 shows the results, indicating that this method can reproducibly determine the levels of individual bile acids and their conjugates in biological fluids.

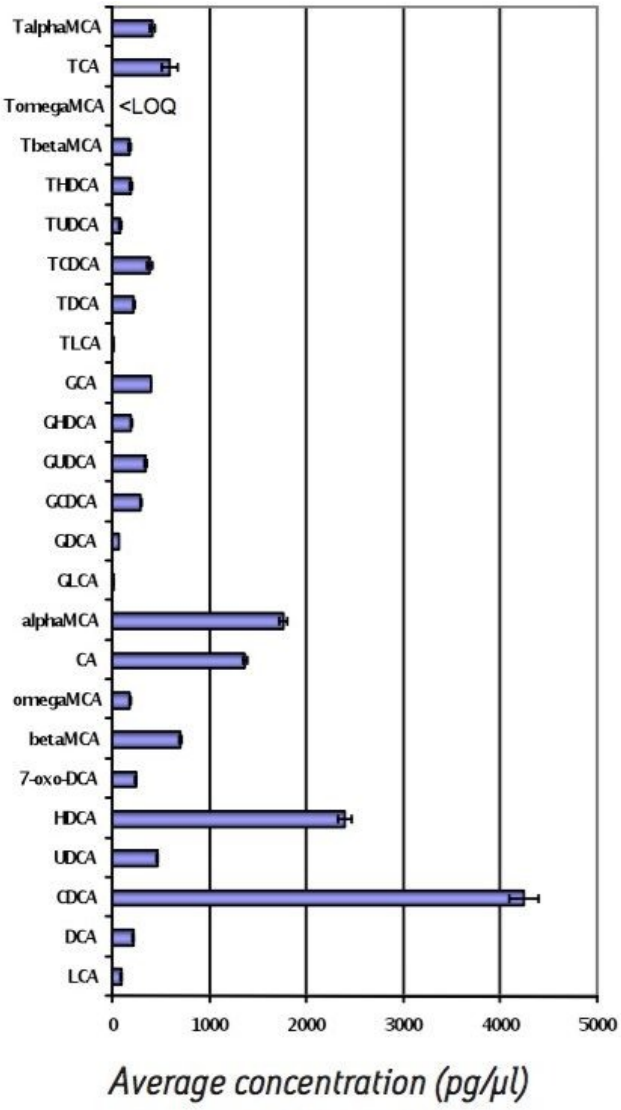

Figure 2. The quantification of bile acids in rat serum by UPC^2-MS (n=6).

Conclusion

The use of UltraPerformance Convergence Chromatography (UPC^2) in conjunction with ESI-MS/MS enables the simultaneous profiling of 25 bile acids including conjugated forms in biological fluids. The profiling is achieved within 13 minutes by a sub-2-µm particle column on the system with acceptable resolution of the analytes. This application demonstrates that this novel separation method is highly suitable for determining the effects of bile acid levels in triglyceride, cholesterol, and glucose metabolism and has a potential to speed up the development of novel drug targets for metabolic diseases.

References

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