

응용 자료

MetaboQuan-R for Bile Acids in Human Serum

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Abstract

This application note demonstrates how these compounds can be separated, without compromising throughput, using Waters CORTECS UPLC Technology combined with negative mode electrospray ionization mass spectrometry.

A rapid UPLC-MS/MS methodology has been developed for the analysis of bile acids. This research method has been demonstrated to be suitable for the analysis of physiologically relevant levels of these analytes in human serum. This method utilizes a generic LC-MS platform that can be used for various compound classes (including metabolomics, lipidomics, and proteomics), meaning it can be applied as part of a suite of analyses that are run subsequently as part of a targeted multi-omics workflow.

Benefits

- Simultaneous analysis of 16 bile acids in a single analytical run under three minutes
- Rapid separation of key isomeric compounds, including 11+ unknown isobars
- High throughput analysis means larger sample sets can be analyzed quickly
- A simple analytical workflow that doesn't require derivitization

- Use of a generic LC-MS configuration yields versatility for switching from one compound class to another

Introduction

Bile acids are an important class of biological molecules that are generated in the liver and play a central role in various biological functions, including cholesterol homeostasis. Quantitative analysis for research of bile acids using LC-MS is complicated by the presence of many isomeric compounds. Historically, this has meant that throughput has been compromised in order to separate these isomeric compounds. Here we demonstrate how these compounds can be separated, without compromising throughput, using Waters CORTECS UPLC Technology combined with negative mode electrospray ionization mass spectrometry. This application note is also part of a MetaboQuan-R method package.

Experimental

Human serum sample preparation

100 μ L of human serum was protein precipitated with 400 μ L of methanol and centrifuged for three minutes at 25,000 g. 100 μ L of the resulting supernatant was diluted with 100 μ L of deionized water and mixed. 10 μ L of this was then injected onto the UPLC-MS/MS system.

LC conditions

UPLC separation was performed with an ACQUITY UPLC I-Class System (fixed loop), equipped with a CORTECS T3, 2.7 μ m (2.1 \times 30 mm) analytical column. 10 μ L of sample was injected at a flow rate of 1.3mL/min. Mobile phase A was 0.01% formic acid (_{aq}) containing 0.2 mM Ammonium Formate and mobile phase B was 50% isopropanol in acetonitrile containing 0.01% formic acid and 0.2 mM Ammonium Formate. After an initial 0.1 minute hold at 20% Mobile phase B, the bile acids were eluted from the column and separated with a gradient of 20–55% Mobile phase B over 0.7 minutes, followed by a 0.9 minute column wash at 98% Mobile phase B. The column was then re-equilibrated to initial conditions. The analytical column temperature was maintained at 60 °C.

MS conditions

Multiple Reaction Monitoring (MRM) analyses were performed using a Xevo TQ-S micro MassSpectrometer. All experiments were performed in negative electrospray ionization (ESI-) mode. The ion source temperature and capillary voltage were kept constant and set to 150 °C and 2.0 kV respectively. The cone gas flow rate was 50 L/hr and desolvation temperature was 650 °C.

Informatics

Method information was imported onto the LC-MS system using the Quanpedia functionality within MassLynx. This extendable and searchable database produces LC and MS methods as well as processing methods for use in TargetLynx for compound quantification.

Results and Discussion

The 16 bile acids detailed in Table 1 were separated and detected using the LC-MS platform and extraction protocol described. Some of these compounds give peaks in their MRM trace that are unidentified. These are most probably due to isomeric forms of these analytes. Figure 1 shows an example chromatogram for the separation achieved using the UPLC method detailed above. Quantitative analysis was performed for chenodeoxycholic acid in human serum by standard addition. This data is shown in Figure 2 and demonstrates the separation of chenodeoxycholic acid from deoxycholic acid. This data also shows that the limits of detection for these analytes are in the low ng/mL range.

Bile acid	MRM transition	RT (mins)	Cone voltage (V)	Collision energy (eV)
Lithocholic acid (LCA)	375.25>375.25	0.90	60	32
Deoxycholic acid (DCA)	391.25>391.25	0.83	60	16
Glycolithocholic acid (GLCA)	432.25>74.00	0.82	60	35
Chenodeoxycholic acid (CDCA)	391.25>391.25	0.80	60	16
Taurolithocholic acid (TLCA)	482.25>80.00	0.71	60	60
Glycodeoxycholic acid (GDCA)	448.25>74.00	0.70	60	35
Glycochenodeoxycholic acid (GCDCA)	448.25>74.00	0.67	60	35
Cholic acid (CA)	407.25>343.25	0.66	60	34
Ursodeoxycholic acid (UDCA)	391.25>391.25	0.66	60	16
Taurodeoxycholic acid (TDCA)	498.25>80.00	0.60	60	60
Taurochenodeoxycholic acid (TCDCA)	498.25>80.00	0.57	60	60
Glycocholic acid (GCA)	464.25>74.00	0.55	60	34
Taurocholic acid (TCA)	514.25>80.00	0.46	60	64
Taurohyodeoxycholic acid (THDCA)	498.25>80.00	0.45	60	60
Tauroursodeoxycholic acid (TUDCA)	498.25>80.00	0.44	60	60
Glycoursodeoxycholic acid (GUDCA)	448.25>74.00	0.52	60	35

Table 1. List of MS/MS conditions and retention times for bile acids.

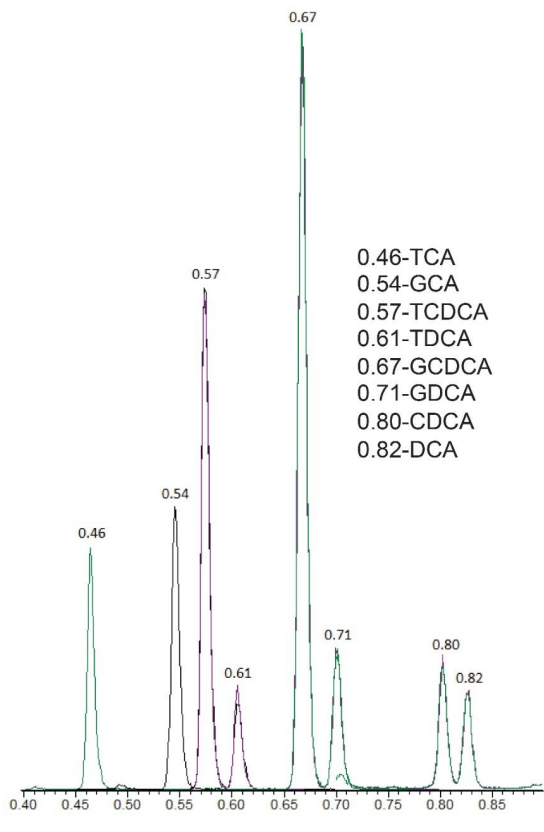


Figure 1. Example chromatogram showing separation achieved for bile acids in human serum.

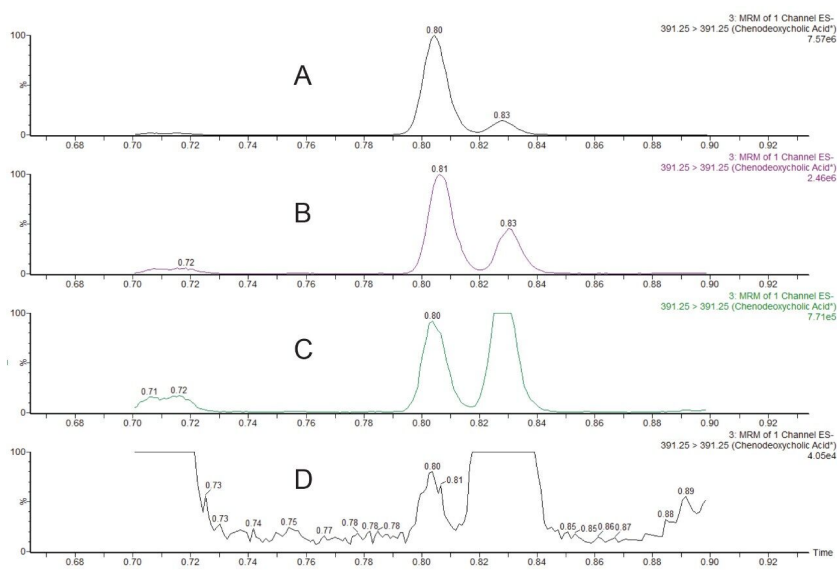


Figure 2. Chromatograms showing separation achieved for a key pair of isobaric bile acids (CDCA and DCA) when performing a standard addition experiment for CDCA in human serum. A. Native CDCA +2500 ng/mL, B. Native CDCA +1000 ng/mL, C. Native CDCA +250 ng/mL, D. Native CDCA (approximately 10 ng/mL).

Conclusion

A rapid UPLC-MS/MS methodology has been developed for the analysis of bile acids. This research method has been demonstrated to be suitable for the analysis of physiologically relevant levels of these analytes in human serum. This method utilizes a generic LC-MS platform that can be used for various compound classes (including metabolomics, lipidomics, and proteomics), meaning it can be applied as part of a suite of analyses that are run subsequently as part of a targeted multi-omics workflow.

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MetaboQuan-R <<https://www.waters.com/waters/Targeted-Omics/nav.htm?cid=134980821>>

Available for purchase online

CORTECS T3 Column, 120Å, 2.7 µm, 2.1 mm X 30 mm <
<https://www.waters.com/waters/partDetail.htm?partNumber=186008481>>

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