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Application News

No. C232

LC/MS

Profiling of Bile Acids in Human Plasma Using Triple Quadrupole Mass Spectrometer

Bile acids are produced in the liver by the catabolism of cholesterol, and synthesized to primary bile acids by conjugating with taurine or glycine. Some primary bile acids are modified by intestinal bacteria to produce the secondary bile acids.

These bile acids promote lipid absorption as a surfactant and inhibit metabolic pathways.

For early diagnosis or functional evaluation of hepatic diseases, blood enzyme activity tests (such as those for ALT and AST) and total bile acids test are widely used. Although the total bile acids test allows us to ascertain that a decrease in hepatic functions has occurred, additional tests are required for disease identification. Therefore, to simultaneously identify diseases, such as bile acid metabolic disorder, simultaneous analysis of the primary and other bile acids is required in addition to the total bile acids test.

For many bile acids, it is difficult to generate fragment ions by a triple quadrupole mass spectrometer because of their structures, and these bile acids should be completely separated under optimized LC conditions to facilitate accurate qualitative/quantitative analysis. Therefore, for accurate determination and quantification of these bile acids, good chromatographic separation is required.

This report introduces an example of analysis of 28 bile acids and 10 internal standards in human plasma using "LC/MS/MS Method Package for Bile Acids," a method in which the conditions for liquid chromatography and triple quadrupole mass spectrometry have been optimized.

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Pretreatment

The human plasma sample was collected from an anonymous healthy subject. Fifty µL of plasma was mixed with 10 µL of internal standard solution (10 µmol/L in methanol). This was followed by the addition of 30 µL of aqueous hydrochloric acid (1 mol/L), and then 1000 µL of acetonitrile. After shaking by a vortex mixer for approximately one minute, the sample was centrifuged at 14000 G for 15 minutes. Then, 850 µL of supernatant was transferred to another micro tube, and evaporated to dryness with a vacuum evaporator. Finally, the solution re-dissolved with 100 µL of methanol was sonicated for 10 minutes, centrifuged at 14000 G for 15 minutes, and transferred to a vial with a glass insert for analysis.

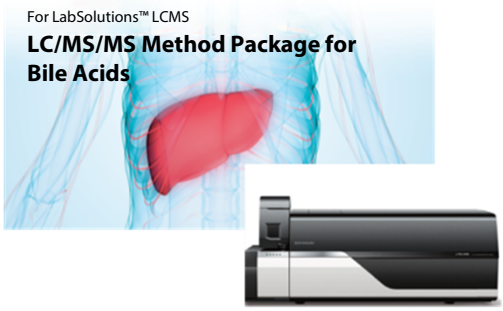

Fig. 1 LC/MS/MS Method Package for Bile Acids and LCMS™-8060 System

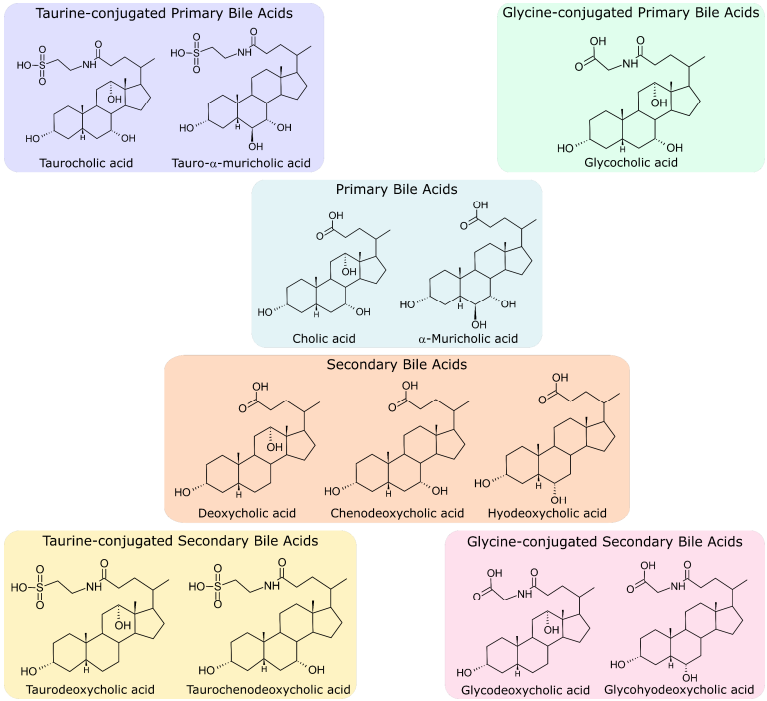

Fig. 2 Structures of Representative Bile Acids

Analytical Conditions

The pretreated plasma was analyzed under the conditions shown in Table 1. In the analysis, "LC/MS/MS Method Package for Bile Acids *1" and LCMS-8060 were used. For quantitative analysis, the stable isotope dilution method was used.

Using this method, 28 compounds consisting of the major primary bile acids, secondary bile acids and their conjugates can be simultaneously analyzed in only 17 minutes. Furthermore, the optimized MS/MS conditions for bile acids and their conjugates are applied, and up to three MRM transitions are set up. Ten stable isotopes for which the MS/MS conditions have been optimized are designated as the internal standards to obtain high quantitative accuracy.

Table 1 Analytical Conditions

UHPLC (Nexera™ X2 system)	
Column	: Shim-pack™ G-BA C18*2
Mobile phase	: A) Acetic acid in water B) Acetonitrile/Methanol
Mode	: Gradient elution
Flow rate	: 0.3 mL/min
Column temp.	: 40 °C

MS (LCMS™-8060)	
Ionization	: ESI (negative mode)
Mode	: MRM
Nebulizing gas flow	: 2.0 L/min
Drying gas flow	: 10.0 L/min
Heating gas flow	: 10.0 L/min
DL temp.	: 250 °C
Block heater temp.	: 400 °C
Interface temp.	: 300 °C

*1: P/N S225-38610-91
*2: P/N 227-30815-01

Results of Analysis

As shown in Table 2, a total of 15 bile acids were detected from human plasma. The detected compounds consist of 1 primary bile acid, 3 secondary bile acids, 6 taurine-conjugated and 5 glycine-conjugated secondary bile acids. Fig. 3 shows the chromatogram of bile acids. Fig. 4 shows the concentrations of bile acids calculated based on the quantitative results of these compounds.

Table 2 Compounds Detected by the Analysis of Bile Acids in Human Plasma

Compound	Abbreviated name	Concentration (pmol/mL)
Tauro-β-murichолic acid	TβMCA	4.28
Tauroursodeoxycholic acid	TUDCA	3.86
Taurocholic acid	TCA	18.8
Taurochenodeoxycholic acid	TCDCA	82.6
Taurodeoxycholic acid	TDCA	41.1
Taurolithocholic acid	TLCA	8.58
Glycocholic acid	GCA	116
Glycoursodeoxycholic acid	GUDCA	241
Glycochenodeoxycholic acid	GCDCA	792
Cholic acid	CA	131
Glycodeoxycholic acid	GDCA	135
Ursodeoxycholic acid	UDCA	58.7
Chenodeoxycholic acid	CDCA	334
Deoxycholic acid	DCA	599
Glycolithocholic acid	GLCA	119

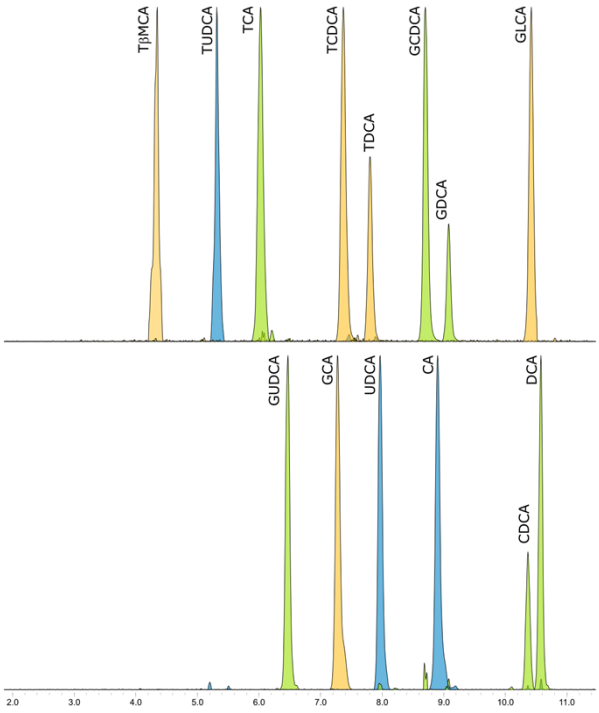

Fig. 3 Mass Chromatogram Obtained by the Analysis of Bile Acids in Human Plasma

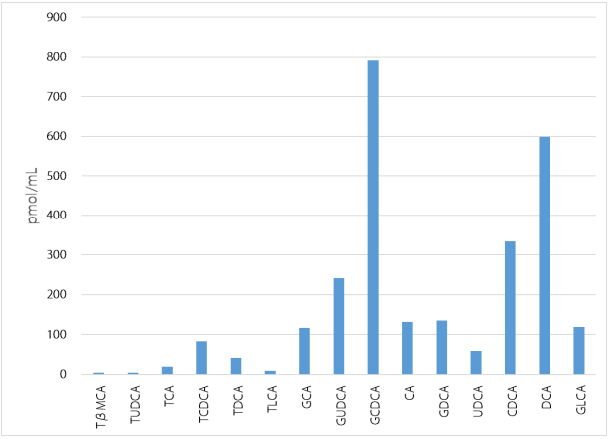

Fig. 4 Concentrations Calculated on the Analysis Results of Bile Acids in Human Plasma (Graph)

Conclusion

As mentioned above, the use of LC-MS/MS and "LC/MS/MS Method Package for Bile Acids," which enables highly sensitive and comprehensive analysis, allows quick detection and quantification of bile acids in human plasma samples. Therefore, this method is expected to be used for intestinal bacterial studies and pathological studies in various phases of clinical research.

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