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LCMS-8060 Liquid Chromatograph Mass Spectrometer

Application News

Measuring Bile Acid Levels in Human Plasma Using a Triple Quadrupole Mass Spectrometer

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User Benefits

- Comprehensive measurement of bile acids in plasma
- Stable analysis in the long term without system tuning

■ Introduction

Bile acids play an important role in fat absorption in the small intestine and are also involved in regulating cholesterol metabolism through the conversion of cholesterol into bile acids.[1)] Primary bile acids are produced by cholesterol catabolism in the liver, most of which conjugate with taurine or glycine to produce conjugated bile acids. Some primary bile acids are also modified later by intestinal bacteria to produce secondary bike acids.

Total bile acid concentration in peripheral blood is a marker for liver dysfunction in humans and blood enzyme activity (ALT, AST, etc.) and total bile acid (TBA) tests are routine. Monitoring levels of multiple bile acids individually may also allow the identification of various liver disorders, and based on this the simultaneous analysis of bile acids has been attracting interest. This article describes a quantitative analysis of 22 bile acids in human plasma performed using 9 internal standards. Analysis was performed using the LC/MS/MS Method Package for Bile Acids that contains various LC and MS setup conditions optimized for LC/MS/MS. The analytical system used was the LCMS-8060 (Fig. 1) high-performance liquid chromatograph-mass spectrometer.

Fig. 1 LCMS-8060 High-Performance Liquid Chromatograph-Mass Spectrometer

■ Sample Preparation and Analytical Conditions for Bile Acids in Human Plasm

Fig. 2 shows the sample pretreatment protocol used for LC/MS/MS analysis. Quantitative analysis was performed using an internal standard method. Table 2 shows the composition of a methanol extract solution containing internal standards that was used in sample preparation. Parameters for LC conditions, MS conditions, and MRM transitions were set according to the LC/MS/MS Method Package for Bile Acids (Table 1). Calibration samples were prepared by diluting bile acid internal standards with methanol to 0.5, 1, 10, 100, 200, and 500 nmol/L and calibration curves were prepared for each bile acid.

(1) Dispense 50 µL of plasma and 50 µL of water in a 1.5 mL tube
↓
(2) Add 350 µL of methanol extract solution containing internal standards
↓
(3) Agitate for 10 seconds
↓
(4) Incubate on ice for 10 minutes
↓
(5) Separate by centrifugation (16000 g, 5 min, 4 °C)
↓
(6) Dispense 320 µL of supernatant into a separate microtube
↓
(7) Concentrate by centrifugation for 1 hour
↓
(8) Lyophilize (overnight)
↓
(9) Add 200 µL of methanol
↓
(10) Agitate for 1 minute
↓
(11) Apply ultrasonic vibration for 5 minutes
↓
(12) Separate by centrifugation (16000 g, 5 min, 4 °C)
↓
(13) Dispense supernatant in a vial

Fig. 2 Preparation Protocol for Human Plasma Samples

Table 1 LC and MS Analytical Conditions

Liquid Chromatograph	
System	: Nexera™ X2
Column	: Reversed-phase column
Mode	: Gradient elution
Injection Volume	: 2 µL
Mobile Phase A	: 0.05 % acetic acid in Water
Mobile Phase B	: Acetonitrile : Methanol = 50:50
Flowrate	: 0.3 mL/min

Mass spectrometer	
System	: LCMS-8060
Ionization	: ESI (negative)
Nebulizing Gas	: 2 L/min
Drying Gas	: 10 L/min
Heating Gas	: 10 L/min
DL Temp.	: 250 °C
Heat Block Temp.	: 400 °C
Interface Temp.	: 300 °C

Table 2 Bile Acids Used as Internal Standards (9 Components)

No.	Compound Name	Abbr.	Concentration in Extract (nmol/L)
1	D5_Taurocholic acid	D5_TCA	10
2	D4_Taurochenodeoxycholic acid	D4_TCDCA	10
3	D5_Taurolithocholic acid	D5_TLCA	1
4	D4_Glycocholic acid	D4_GCA	100
5	D4_Cholic acid	D4_CA	10
6	D4_Glycodeoxycholic acid	D4_GDCA	100
7	D4_Chenodeoxycholic acid	D4_CDCA	100
8	D4_Deoxycholic acid	D4_DCA	50
9	D4_Lithocholic acid	D4_LCA	10

■ Consecutive Analysis of Clinical Samples and QC Sample

This analysis used commercially available human plasma as the QC sample and alternatively analyzed the QC sample and 8 clinical samples repeatedly. The QC sample was also the last sample analyzed at the end of each day. Thirty-four QC samples and 272 clinical samples were analyzed over a period of 19 days. Table 3 lists the bile acids detected in the QC sample. Twenty-two components present in the LC/MS/MS Method Package for Bile Acids were detected.

Table 4 shows the concentration of each bile acid and concentration %RSD upon analyzing 34 QC samples. GHDCA was detected with a small peak area and a concentration %RSD of 49.9 %, but results for the other 21 bile acids were very stable with concentration %RSD ranging from 2.8 to 12.4 %. Fig. 3 also shows variation in the peak area of D4_TCDCA, D4_GCA, D4_CA, and D5_TCA upon repeated analysis of human plasma. No equipment tuning was performed and the analytical column was not changed during the entire 19 days of analysis (272 samples in total). The peak area %RSD of internal standards ranged from 4.9 to 6.0 % for these consecutive analyses. These results show stable consecutive analysis was achieved not just for the QC sample but also the clinical samples.

Table 3 Bile Acids Detected in the QC Sample (22 Components)

No.	Compound Name	Abbr.
1	Tauro-ω-muricholic acid	TaMCA
2	Tauroursodeoxycholic acid	TUDCA
3	Taurocholic acid	TCA
4	Taurochenodeoxycholic acid	TCDCA
5	Taurodeoxycholic acid	TDCA
6	Taurolithocholic acid	TLCA
7	Glycocholic acid	GCA
8	Glycoursodeoxycholic acid	GUDCA
9	Glycohyodeoxycholic acid	GHDCA
10	α-Muricholic acid	aMCA
11	7-Ketodeoxycholic acid	7keto_DCA
12	Glycochenodeoxycholic acid	GCDCA
13	Cholic acid	CA
14	Glycodeoxycholic acid	GDCA
15	Ursodeoxycholic acid	UDCA
16	Hyodeoxycholic acid	HDCA
17	7-Ketolithocholic acid	7keto_LCA
18	12-Ketolithocholic acid	12keto_LCA
19	Chenodeoxycholic acid	CDCA
20	Deoxycholic acid	DCA
21	Glycolithocholic acid	GLCA
22	Lithocholic acid	LCA

Table 4 Bile Acid Concentrations in QC Sample (nmol/L) and Concentration %RSD After Measuring QC Sample 34 Times

Compound Name (Abbr.)	Concentration (nmol/L)	Concentration %RSD (n = 34)
TaMCA	5.0	5.4
TUDCA	7.7	4.0
TCA	69.3	3.4
TCDCA	161.1	2.8
TDCA	51.9	3.2
TLCA	2.3	8.5
GCA	507.7	4.0
GUDCA	203.7	3.7
GHDCA	3.7	49.9
aMCA	31.4	7.9
7keto _DCA	3.4	12.4
GCDCA	1890.2	3.5
CA	67.4	3.9
GDCA	497.1	3.2
UDCA	179.4	4.6
HDCA	53.5	5.0
7keto _LCA	18.7	6.3
12keto _LCA	14.3	8.1
CDCA	571.4	9.5
DCA	362.0	4.3
GLCA	29.5	3.8
LCA	23.0	9.5

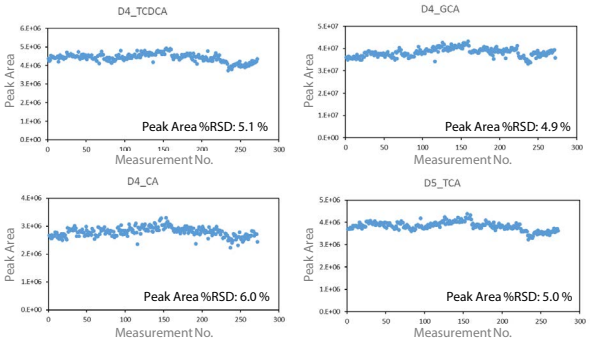

Fig. 3 Variation in Peak Area of 4 Stable Isotope Components during 19 Days of Repeated Analysis of Human Plasma (272 Samples)

■ Conclusion

The LCMS-8060 high-performance liquid chromatograph mass spectrometer was used to perform simultaneous analysis of bile acids in human plasma. Results were very stable with the concentration %RSD for 21 components ranging from 2.8 to 12.4 % in a QC sample after 34 measurements. Analysis of clinical samples over a period of 19 days (272 samples in total) also detected D4_TCDCA, D4_GCA, D4_CA, and D5_TCA with peak area %RSD ranging from 4.9 to 6.0 %.

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■ References

1) Takashi Nakanishi et. al., "Effect of a High-Fat Diet on the Small-Intestinal Environment and Mucosal Integrity in the Gut-Liver Axis", Cells. 2021, 10(11), 3168.

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