SHIMADZU

TP097

Liquid-Chromatography coupled to Tandem Mass Spectrometry for 28 Bile Acids Profiling in Serum Samples

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1. Overview

- New LC/MS/MS Method Package for simultaneous analysis of 28 bile acids .
- Method was successfully applied to human plasma and mouse liver samples.
- Useful tool for clinical research or gut microbiome studies.

2. Introduction

Bile acids are primarily produced in mammalians through cholesterol catabolism in the liver. Primary bile acids can be associated to taurine or glycine to form conjugates and transformed by gut bacteria giving secondary forms thereof. They constitute then a large family of molecules with several position isomer groups (see Figure 1).

Their role as digestive surfactants to promote lipids absorption is well-known. But these hormones are also involved in various metabolic pathway regulations.

Blood enzyme activity tests (ALT, AST, etc.) and total bile acid test are widely conducted for the early diagnosis of liver disease and functional evaluation. However, although it can be confirmed that the liver function declines with the evaluation of total bile acid amount, it does not give much information in case of metabolic troubles. Therefore, clinical researchers could get more valuable information with a more precise and larger bile acids profiling.

Due to their molecular structure, these compounds are also difficult to fragment by tandem mass spectrometry. Therefore, a good chromatographic separation is needed for accurate identification and quantification.

A newly developed method package (Shimadzu Corp., Kyoto, Japan) providing liquid-chromatography and tandem-mass spectrometry conditions for the analysis of 28 bile acids and 10 internal standards was applied to human plasma samples.

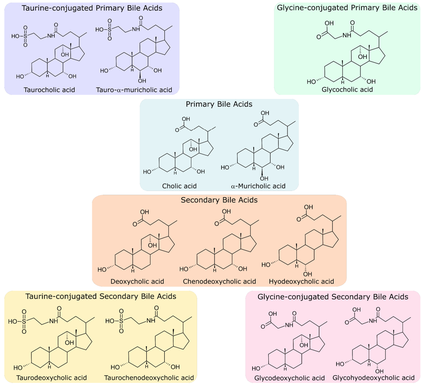

Figure 1 Selected Bile Acid Structures

3. Methods

3-1. Reagents

Analytical standards of targeted bile acids and deuterated internal standards were purchased from Sigma-Aldrich and Steraloids. Individual stock solutions at 100 µM were prepared in methanol and further diluted methanol to make calibration standard. One-point calibration standard at 1µM was prepared and injected 4 times.

All other reagents were of analytical grade and solvents used were of of LC-MS grade (Wako chemicals).

3-2. Sample Preparation

Human plasma sample was a pool of healthy anonymous donor. Fifty microliters of plasma were mixed with ten microliters of internal standard solution (10 µM in methanol). Then 30 µL of aqueous hydrochloric acid solution (1 M) were added and followed by 910 µL of acetonitrile. After 1 minute of vortex, samples were centrifuged at 14 000 g for 15 minutes. 850 µL of supernatant were transferred in a new microtube and evaporated to dryness with a vacuum concentrator. Reconstituted extracts with 100 µL of methanol were sonicated for 10 minutes, centrifuged at 14 000 g for 15 min and transferred in a vial with glass insert for analysis.

3-3. Analytical Conditions

Analysis was performed using a Nexera X2 UHPLC system coupled with LCMS-8060 triple quad mass spectrometer. Parameters are described in Table 1 and 2.

Table 1: LC Conditions

System	: Nexera X2
Column	: GL Sciences Inertsil ODS-4 HP 3µm 150x2mm
Temperature	: 40 ° C
Mobile Phases	: A: Water + 0.05% acetic acid B: Acetonitrile / methanol 40/60
Flow Rate	: 300 µL/min
Injection Volume	: 2 µL
Gradient	: 35 % B (0.5min) to 50%B in 0.1 min. 50%B to 85%B in 8.4 min. 85%B to 100%B in 2 min (2 min).
Total Run Time	: 17 min

Table 2: MS/MS conditions

System	: LCMS-8060
Ionization	: Heated ESI
Probe Voltage	: -3 kV (negative ionization)
Temperature	: Interface: 300 ° C / Desolvation Line: 250 ° / Heater Block: 400 ° C
Gas Flow	: Nebulizing Gas: 2 L/min / Heating Gas: 10 L/Min / Drying Gas: 10 L/min
Dwell Time / Pause time	: 7 ms / 1 ms :
MRM	

Compound	MRM Quant	MRM Qual	
TαMCA / TβMCA / TCA	514.3 > 514.3	514.3 > 124.1	514.3 > 80.1
D5-TCA	519.3 > 519.3	519.3 > 124.1	519.3 > 80.1
TUDCA / THDCA / TCDCA / TDCA	498.3 > 498.3 498.3 > 107.1	498.3 > 124.1	498.3 > 80.1
D4-TCDCA	502.3 > 502.3	502.3 > 80.1	502.3 > 107.1
TLCA	482.3 > 482.3	482.3 > 80.1	482.3 > 107.1
D5-TLCA	487.3 > 487.3	487.3 > 80.1	487.3 > 107.1
GCA	464.3 > 464.3	464.3 > 74.0	464.3 > 402.4
D4-GCA	468.3 > 468.3	468.3 > 74.0	468.3 > 402.4
GUDCA / GHDCA / GCDCA / GDCA	448.2 > 448.2 448.2 > 402.4	448.2 > 74.1	448.2 > 386.2
D4-GDCA	452.2 > 452.2	452.2 > 74.1	452.2 > 404.2
ωMCA / βMCA / αMCA / CA	407.1 > 407.1	407.1 > 343.1	407.1 > 289.3
D4-CA	411.3 > 411.3	411.3 > 343.1	411.3 > 289.3
7-keto-DCA	405.3 > 405.3	405.3 > 289.3	405.3 > 123.2
UDCA / HDCA / CDCA / allo-CDCA / DCA	391.3 > 391.3	391.3 > 345.2	391.3 > 343.3
D4-CDCA	395.3 > 395.3		
D4-DCA	395.3 > 395.3	395.3 > 345.2	395.3 > 343.2
7-keto-LCA / 12-keto-LCA	389.3 > 389.3		
LCA / allo-LCA	375.3 > 375.3		
D4-LCA	379.3 > 379.3		
GLCA	432.3 > 432.3	432.3 > 74.1	432.3 > 388.3
D4-GLCA	436.3 > 436.3	436.3 > 74.1	436.3 > 388.3

4. Results

4-1. Calibration

Response factor of each peak (peak area/ISTD area) was calculated for each compound and averaged.

Peak ratio precision was inferior to 10% for all compounds. Average response factor was used to calculate concentrations in plasma samples.

4-2. Plasma Samples

Human plasma sample from a pool of healthy anonymous donors was processed in triplicate. Each extract was injected twice. As expected, not all the targeted bile acids were detected in human plasma.

Figure 2 shows an example of the mass chromatograms obtained. Calculated concentrations and precision of the assay are shown in Table 3.

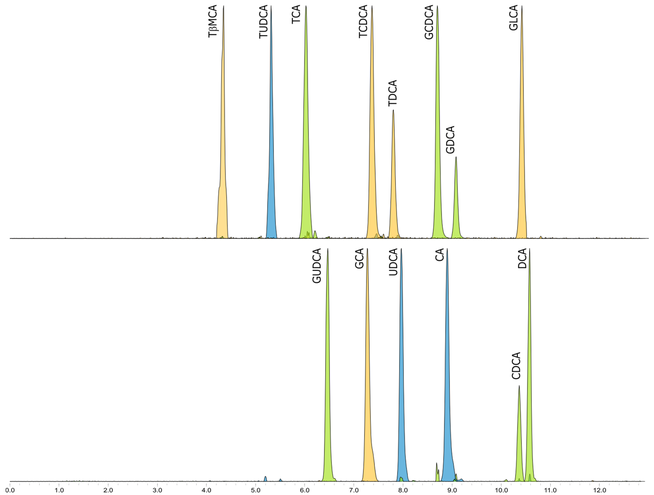

Figure 2 Mass Chromatograms of Detected Bile Acids in Human Plasma Sample (intensities normalized)

Table 3: Calculated Concentrations in Human Plasma

	TβMCA	TUDCA	TCA	TCDCA	TDCA	TLCA	GCA
Mean (pmol/mL)	4.28	3.86	18.8	82.6	41.1	8.58	116
SD (pmol/mL)	1.64	0.582	1.36	5.08	1.90	0.226	0.939
%RSD	38%	15%	7%	6%	5%	3%	0.8%

	GUDCA	GCDCA	CA	GDCA	UDCA	CDCA	DCA	GLCA
Mean (pmol/mL)	241	792	131	135	58.7	334	599	119
SD (pmol/mL)	2.98	25.8	5.17	3.44	2.15	23.2	12.7	15.6
%RSD	1%	3%	4%	3%	4%	7%	2%	13%

5. Conclusion

A newly developed Method Package was applied for the analysis of human plasma samples. The Method Package offers tun-key solution and can be used for clinical research.

As it covers a larger list of targeted bile acids, it can be used with other relevant biological matrices.