Rapid Quantitation of 15 Major Bile Acids in Human Serum by UPLC-ESI-MS/MS

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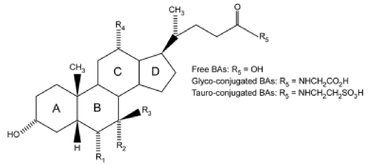
Purpose

Bile acids (BAs) are 24-carbon steroids formed from cholesterol in the liver and they play an important role as the signaling molecules that regulate triglyceride, cholesterol, and glucose metabolism. Bile acids may serve as biomarkers for normal liver function. The purpose of this study is to develop a rapid and reproducible LC-MS/MS method for quantitation of 15 individual bile acids in support of clinical studies.

Introduction

The separation and quantitation of bile acids is very challenging due to the presence of structural analogues including isomeric forms and the polarity diversity between unconjugated and conjugated forms. To support clinical studies, we have developed and validated a robust and specific LC-MS/MS method for the determination of 15 individual bile acids in human serum. The calibration curve ranged from 10.0 to 2500 ng/mL for CA, CDCA, DCA, LCA, UDCA, GCA, GCDCA, GDCA, GLCA, GUDCA, TCA, TCDCA, TDCA, TLCA and TUDCA. Bile acids and the added internal standards, CA-d_4, CDCA-d_4, DCA-d_4, LCA-d_4, UDCA-d_4, GCA-d_4 and GCDCA-d_4 were extracted from human serum and injected to an HPLC system and detected in negative ESI-MRM mode on an API-5500 LC-MS/MS system.

Structure



Hydrophobicity

	$R_1^{(\alpha)}$	$R_2^{(\alpha)}$	$R_3^{(\beta)}$	$R_4^{(\alpha)}$
UDCA	H	OH	H	H
CA	H	OH	OH	OH
CDCA	H	OH	OH	H
DCA	H	H	H	OH
LCA	H	H	H	H

Methods

Sample Preparation:

Due to presence of endogenous bile acids in human serum, double charcoal stripped human serum was used for the preparation of calibration standards and LLOQ samples. LQC, MQC and HQC samples were prepared in authentic human serum. The endogenous concentration of the pooled serum were used as LQC, while MQC and HQC samples were prepared by fortifying 300 ng/mL and 4000 ng/mL of bile acids in the pooled serum, respectively. Bile acids and the added internal standards were extracted from 100 µL of human serum by protein precipitation using acetonitrile. After vortexing and centrifugation, the supernatant was transferred to and diluted in a 96-well plate for LC-MS/MS analysis. LC separation was performed on a reverse phase C18 column (2.6 µm, 100 x 2.1 mm). Analytes were detected by multiple reaction monitoring (MRM) (see table 1) in negative ion electrospray mode on an AB Sciex API-5500 LC-MS/MS analysis.

Liquid Chromatography:

Pump: Shimadzu UFLC LC-30AD
Autosampler: Shimadzu UFLC SIL-30AC
System Controller: Shimadzu CBM-20A
Analytical Column: Accucore C18 , 100x2.0 mm, 2.6µm
Gradient: Analytes were eluted using a gradient with MPA (0.1% formic acid in water) and MPB (0.1% formic acid in ACN/MeOH (40/60 v/v)) in 10 min.
Flow Rate: 0.5 mL/min.
Injection Volume: 10 µL

Mass Spectrometry:

MS System: AB Sciex API-5500
Scan Mode: (-)ESI-MRM

Results and Discussion

Table 1. MRM Transitions of 15 Bile Acids

Chemical Name	MRM transitions	Retention Time (min)	Internal Standard
CA	407.3 → 407.3	5.5	CA-d_4
GCA	464.3 → 464.3	2.7	GCA-d_4
DCA	391.3 → 391.3	7.1	DCA-d_4
CDCA	391.3 → 391.3	6.9	CDCA-d_4
GDCA	448.3 → 74	5.2	GCDCA-d_4
GCDCA	448.3 → 74	4.8	GCDCA-d_4
TCA	514.3 → 514.2	2.1	TCA-d_4
TDCA	498.3 → 498.3	4.6	CA-d_4
UDCA	391.3 → 391.1	4.9	CA-d_4
TCDCA	498.3 → 79.9	4.2	CA-d_4
GUDCA	448.3 → 448.3	1.8	TCA-d_4
LCA	375.3 → 375.1	8.4	LCA-d_4
GLCA	432.3 → 74	6.6	CA-d_4
TLCA	482.3 → 79.9	5.8	CA-d_4
TUDCA	498.3 → 79.9	1.6	TCA-d_4

CA: Cholic acid, CDCA: chenodeoxycholic acid, DCA: deoxycholic acid, LCA: lithocholic acid, UDCA: ursodeoxycholic acid, GCDCA: glycochenodeoxycholic acid, GDCA: glycodeoxycholic acid, TCDCA: taurochenodeoxycholic acid, TDCA: taurodeoxycholic acid

CA-d4: cholic acid-2,2,4,4-d4, CDCA-d4:chenodeoxycholic acid-2,2,4,4-d4, DCA-d4:deoxycholic acid-2,2,4,4-d4, LCA-d4: lithocholic acid-2,2,4,4-d4, UDCA-d4: ursodeoxycholic acid-2,2,4,4-d4, GCA-d4: glycocholic acid-2,2,4,4-d4, and GCDCA-d4: glycochenodeoxycholic acid-2,2,4,4-d4

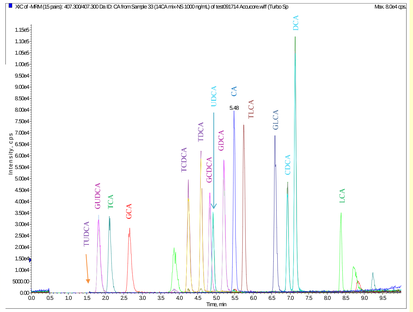


Figure 1. Chromatograms for 15 bile acids

Calibration Range		10 to 5000 ng/mL	
Correlation coefficient(r^2, mean)		>0.9939	
Accuracy & Precision	QC	Conc. (ng/mL)	Accuracy RE% / Precision CV%
Inter-Batch (n=18)	LLOQ	10.0	<7.5 / <8.7
	Low	Endogenous	<-1.9 / <5.1
	Medium	LQC + 300	<-7.0 / 2.1
	High	LQC + 4000	<5.9 / <2.0
Recovery		68.9% to 83.7%	
		Condition	Accuracy RE%
Freeze/Thaw		4 Cycles, <-70 ºC	<3.6
Bench-Top		4 hrs, Room Temperature	<5.2
Autosampler Extract Stability		27 hrs, Room Temperature	<4.5
Long-Term Storage Stability		49 Days, <-70 ºC	<5.1

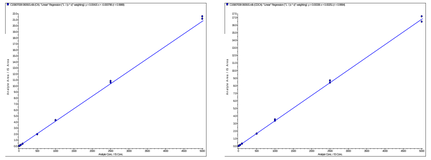


Figure 1. Typical Calibration Curve of CA (left) and CDCA (right) in Charcoal Stripped Human Serum

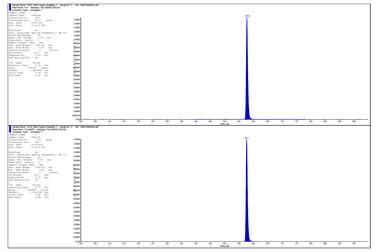


Figure 2. Typical chromatograms for CA in human serum (LQC, 41.7 ng/mL, top) and IS (CA-d4, bottom)

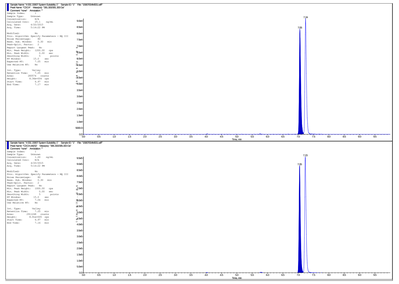


Figure 3. Typical chromatograms for CDCA in human serum (LQC, 67.8 ng/mL, top) and IS (CDCA-d4, bottom)

- This assay was validated within a nominal range of 10.0 to 5000 ng/mL for 15 bile acids in human serum with the correlation coefficients (r^2) ≥0.9939.
- The recovery for bile acids was from 68.9% to 83.7% from human serum.
- Due to the presence of endogenous of bile acids in human serum, calibration curve standards and LLOQ samples were prepared in double charcoal stripped human serum , while the LQC, MQC and HQC samples were prepared in actual human serum. The measured concentrations showed a very good accuracy and precision for those QC samples, indicating the charcoal stripped human serum is a suitable surrogate matrix for preparing calibration standards for the quantitation of bile acids in human serum. No interference was observed from human serum and the charcoal stripped human serum.
- Bile acids in human serum was found stable after storage at approximately -20ºC and -70 °C for 49 days, 4 cycles of freeze and thaw, and 4 hours on the bench-top at room temperature.

Conclusions

A robust, specific and reproducible LC-MS/MS assay for the quantification of 15 bile acids as biomarkers in human serum has been developed and validated. The assay has been used for support of clinical studies.