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A novel fast and simple quantification method for bile acids in human serum by LC-MS/MS

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Introduction

Bile Acids (BAs) are steroid acids of the bile. They are fat substances solubilizers of the enterohepatic cycle and are key players of lipid and energy metabolism. Abnormal BAs profiles may be used as biomarkers of several liver diseases. Despite the clinical need of measuring BAs concentration in plasma/serum, reference separations were proved to be unsuitable for simple and rapid analysis. Here is proposed a new LCMS method for the simultaneous high sensitive quantification of 27 BAs. A quick sample preparation technique based on proteins precipitation was applied. Good repeatability was obtained (RSD<5%). Linearity was tested for 16 BAs and confirmed for all in the range 1 to 100 ng/ml. The r^2 coefficients were above 0.99, with S/N>10 for LLOQ levels.

Materials and Methods

The quantitative analysis of BAs was performed using commercial serum (Seralab, double charcoal stripped) implemented with bile acids standards. Sample preparation procedure (Figure 1.) consists of protein precipitation of the serum sample (100 µL) with 5% ammonium hydroxide in acetonitrile, followed by centrifugation at 10000 g for 10 min, nitrogen flow evaporation to dryness and take-up (100 µL). The analytes were monitored using UHPLC-MS/MS system (Nexera X2 and LCMS-8060, Shimadzu, Kyoto). The HPLC separation uses a typical C8 reverse phase column (2.7um 2.1x100mm), with 0.2% formic acid in water as mobile phase A, and methanol : isopropanol 1/1 (v/v) as mobile phase B. Specific external and internal needle rinsing, performed in parallel of the analytical run, ensure the total absence of carryover. MS detection is carried out in negative electrospray ionization, using MRM mode. One to four MRM transitions are monitored per compound, with dwell times between 5 and 32 msec. This provides a minimum of 30 points per chromatographic peak for all BAs. The total analysis run time is 15 min.

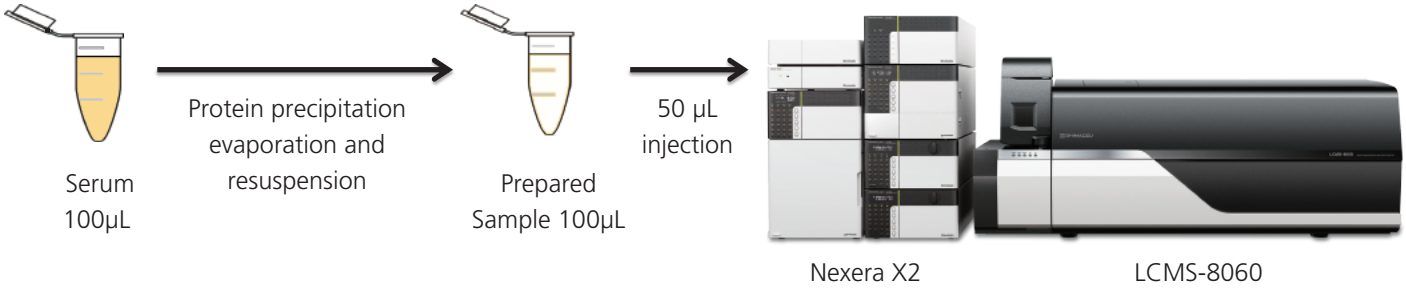

Figure 1. Sample workflow overview.

Typical Chromatograms

A total of 27 bile acids were simultaneously quantified in a total analysis time of 15 min (Figure 2.). The absence of carryover was also confirmed (residual signal < 0.1% of LLOQ signal in blank sample).

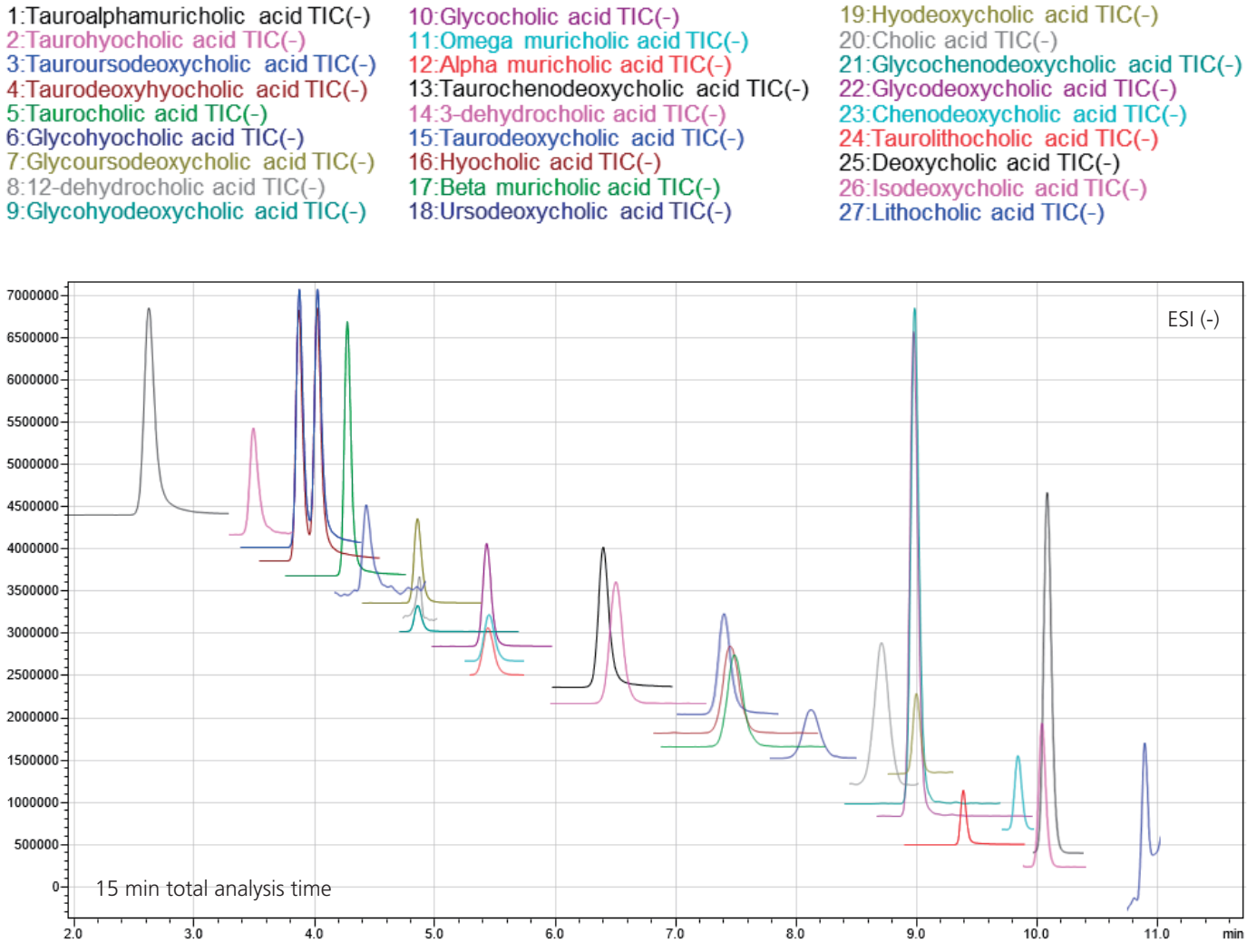

Figure 2. Typical chromatograms (TIC) for the analysis of 27 bile acids at 1ng/mL in serum

Limits of quantification in serum

Lower limit of quantification (LLOQ) in standard sample was confirmed to be equal or below 1 ng/mL for all 27 BAs (S/N > 10). In serum (Table 1.), LLOQ for 12-dehydrocholic acid is 2.5 ng/mL and is below 1 ng/mL for all other BAs. Good repeatability was obtained at LLOQ level (RSD < 10%).

Table 1. LLOQ of the 27 BAs in serum, and associated signal to noise ratios (S/N) and %RSD values (n=3).

Compound	LLOQ	S/N	% RSD (n=3)
Tauroalphamuricholic acid	0,01	15	4,3
Taurocholic acid	0,01	18	4,4
Taurohyocholic acid	0,02	17	3,6
Taurochenodeoxycholic acid	0,01	13	7,5
Taurodeoxycholic acid	0,02	11	7,1
Taurodeoxyhyocholic acid	1,0	11	5,2
Tauroursodeoxycholic acid	1,0	12	4,0
Taurolithocholic acid	0,01	17	9,8
Glycocholic acid	0,01	15	3,7
Glycohyocholic acid	0,01	15	2,8
Glycochenodeoxycholic acid	0,02	17	6,1
Glycodeoxycholic acid	0,02	17	4,6
Glycohyodeoxycholic acid	0,02	15	3,7
Glycoursodeoxycholic acid	0,01	13	2,5
Alpha muricholic acid	0,02	15	2,9
Beta muricholic acid	0,03	11	3,1
Cholic acid	0,2	19	2,9
Hyocholic acid	0,05	12	3,0
Omega muricholic acid	0,03	11	2,9
12-dehydrocholic acid	2,5	10	10
3-dehydrocholic acid	0,02	10	2,6
Chenodeoxycholic acid	0,4	10	2,9
Deoxycholic acid	0,3	10	3,8
Hyodeoxycholic acid	0,05	11	2,7
Isodeoxycholic acid	0,02	13	3,2
Ursodeoxycholic acid	0,04	11	2,1
Lithocholic acid	1,0	12	8,9

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Linearity

Linearity in serum was tested for 16 BAs and confirmed for all in the range: 1 to 100 ng/mL. Excellent repeatability was obtained (RSD < 5%), as well as good accuracies (85-115%). The r^2 of linearity models were above 0.99 (Figure 3.).

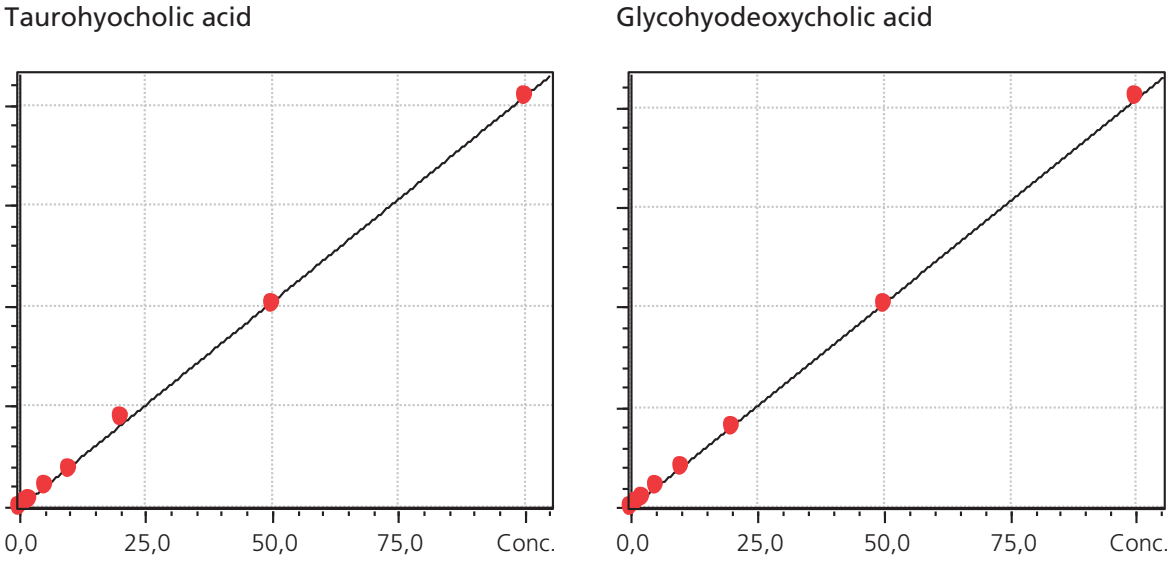

Figure 3. Examples of standard addition calibration curve obtained for taurohyocholic acid and glycohyodeoxycholic acid.

Conclusion

A novel LC-MS/MS method was developed for the simultaneous and high sensitive quantification of 27 BAs in human serum, with simple a sample preparation. BAs were quantified in a total analysis time of 15 min with a good distinction between isomers. The method showed good repeatability, linearity and accuracy. The method proved its fits for purpose to monitor changes of BAs profile in metabolism.

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