Development of a New LC-MS/MS Assay to Determine 7α-hydroxy-4-cholesten-3-one (C4), a Bile Acid Synthesis Biomarker in Human Serum

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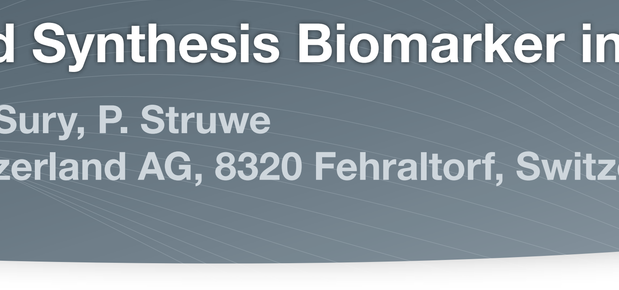
Introduction

Bile acid synthesis is a catabolic pathway of cholesterol taking place in the liver and it affects various physiological processes, such as lipid and glucose metabolism. The imbalance of bile acid synthesis is associated with severe diseases such as non-alcoholic steatohepatitis (NASH), irritable bowel syndrome (IBS) and Crohn's disease. 7α-hydroxy-4-cholesten-3-one (C4) is an intermediate metabolite of the classic bile acid biosynthesis pathway and its level characterizes the activity of the cytochrome P450 7A1 (CYP7A1), the key enzyme in the rate-limiting step of bile acid biosynthesis. Therefore, C4 is an important biomarker of conditions with pathological bile acid synthesis.



LogP: 5.46-6.37

Method

Figure 1. Sample extraction procedure from human serum and surrogate matrix.

- STDs, QC LLOQ and QC S are prepared in surrogate matrix.
- A Hamilton 96-channel liquid handling robot is used for the supernatant transfer and SPE load steps.
- Simplified SPE workflow is applied with the Waters Oasis PRiME HLB µElution plate for desalting and phospholipid removal.

Table 1. Chromatographic conditions and MS/MS parameters.

Chromatographic conditions	
UPLC	Waters ACQUITY UPLC I-Class
Analytical column	Waters ACQUITY UPLC BEH C18 100 × 2.1 mm, 1.7 µm
Mobile phase A **Mobile phase B**	Water/Formic acid 100:0.1 (v/v) Acetonitrile/Formic acid 100:0.1 (v/v)
Flow rate / Column temperature / Injection volume	0.4 mL/min / 40 °C / 10 µL
Total run time	10 min
MS/MS conditions	
Mass spectrometer	SCIEX QTRAP 5500
Source / Polarity	ESI / Positive
Followed MRM transitions	C4: m/z 401.2 → m/z 177.1 C4-d7 (IS): m/z 408.2 → m/z 177.1

Results

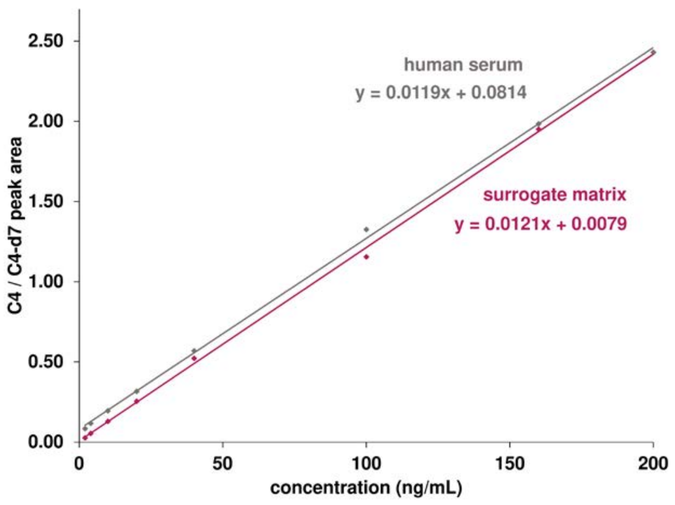


Figure 2. C4 standard curves in human serum and surrogate matrix.

The parallel standard curves demonstrate the suitability of the surrogate matrix.

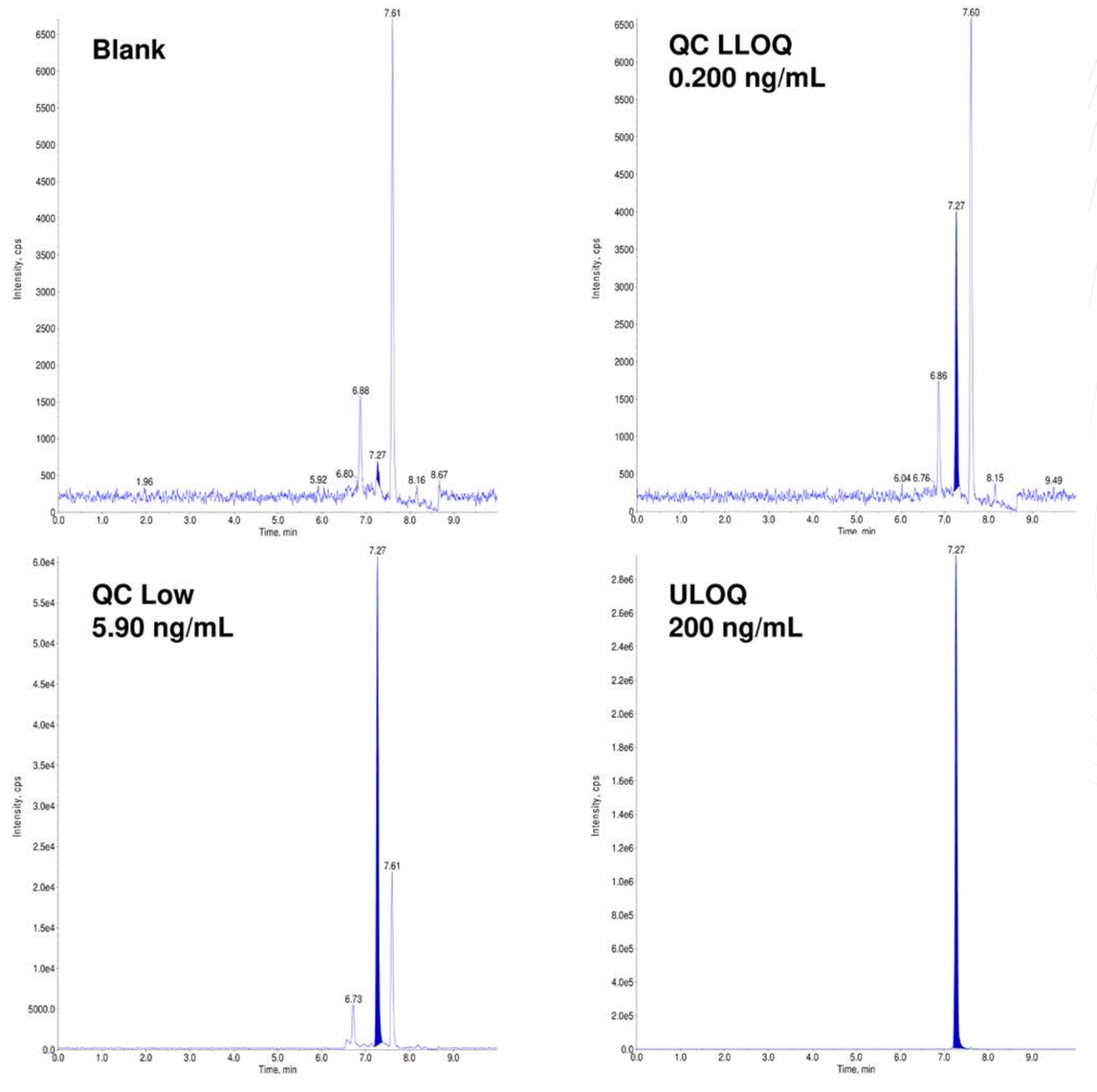


Figure 3. Typical chromatograms of blank, QC LLOQ (in surrogate matrix), QC Low (endogenous concentration of the human serum pool) and ULOQ samples.

Method qualification summary

Table 2. Precision and accuracy results for C4.

Inter-batch precision and accuracy results					
	QC LLOQ 0.200 ng/mL	**QC S 0.600 ng/mL**	**QC Low 5.90 ng/mL**	**QC Mid 75.0 ng/mL**	**QC High 150 ng/mL**
Accuracy (%)	98.0	99.5	104.5	100.1	99.3
CV (%)	10.1	2.6	2.1	3.9	3.3
N	12	12	12	12	12

- Dilution integrity with the surrogate matrix was shown with 5× dilution factor.
- Stress test showed no cross-well contamination.
- Recovery was around 60% for the analyte and IS, consistent between Low, Mid and High QCs.
- Matrix effect was assessed in multiple individual matrices and the overall precisions and accuracies were within acceptance criteria.
- C4 is stable in human serum (cumulative freeze-thaw/benchtop) and in the processed samples.

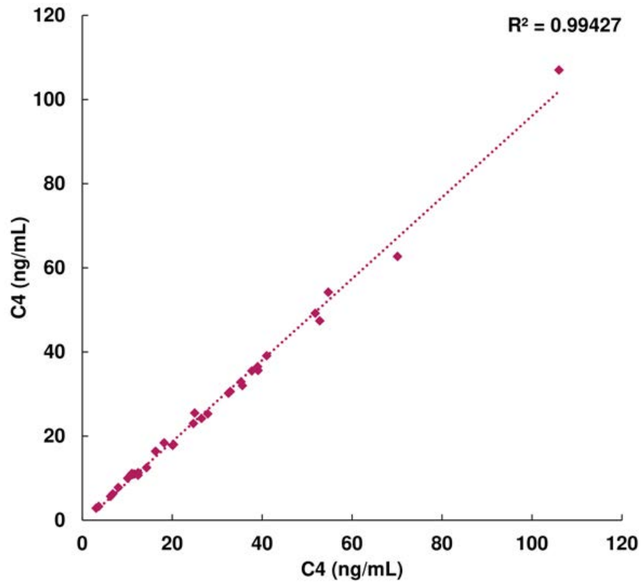


Figure 4. Incurred sample reanalysis (ISR) of 34 individual human serum samples.

C4 was measured in 34 samples in two independent batches. The results show that C4 levels ranged from ~3.00-100 ng/mL. Furthermore, the ISR analysis demonstrated the robustness of the method, as the concentrations in the second batch were <15% different from the first batch.

Conculsions

- The developed method allows the quantitative determination of C4 in human serum in the range of 0.200-200 ng/mL, a suitable range to measure C4 levels in diseases with impaired bile acid synthesis.
- Robustness of the qualified method was demonstrated by the high reproducibility of measured C4 concentrations in human serum samples.

Acknowledgements

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