Development of an LC-MS/MS method for the quantification of 7-Alpha-hydroxy-4-cholesten-3-one (C4) in human serum

L. Huyghebaert, M. Janssen, E. Cavalier, C. Le Goff
Clinical Chemistry Department, University and CHU of Liège, Belgium

Contact : c.legoff@chuliege.be

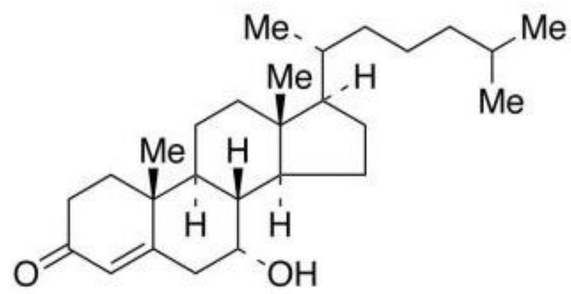

Figure 1 : C4 structure

Background-Aim:

Bile acids malabsorption (BAM) is a bowel disorder marked by chronic diarrhoea, abdominal pain and weight loss. Bile acids are synthesised in the liver, stored in the gallbladder and released into the intestine during meals. They are reabsorbed in the ileum and recycled through an enterohepatic cycle (1). BAM occurs when malabsorption allows bile acids to reach the colon, triggering symptoms. BAM can arise from Crohn's disease or ileal resection (type 1 BAM), idiopathic origins (type 2 BAM), or conditions such as celiac disease, chronic pancreatitis or bacterial overgrowth (type 3 BAM) (2). In our institution, diagnosis currently relies on the SeHCAT retention test, which will soon be phased out. To replace it, we are developing a method to measure a BAM-specific biomarker: 7-alpha-hydroxy-4-cholesten-3-one (C4), reflecting CYP 7A1 activity in bile acids biosynthesis (3). This study aim to develop and validate an LC-MS/MS method for the quantification of C4 (figure 1) in human serum.

Methods:

Quantification was achieved on a Nexera X2 UPLC (Shimadzu® Corporation) coupled to a QT6500 mass spectrometer (Sciex®). Chromatographic separation was achieved using an Xbridge Premier BEH C18 column (2.1x50 mm, 2.5 µm, Waters®) with water and acetonitrile both containing 0,1% formic acid (FA) as mobile phases. Samples were extracted using a double extraction procedure follow by addition of internal standard C4-d7.
The preliminary validation included single-replicate analyses of five quality control (0.5 - 100 ng/mL) over three days. Integrated peak area ratio between C4 to C4-d7 was used for quantification.

Results:

Based on the results obtained with the five levels of quality control: 0.5 – 1 – 3 – 50 – 100 ng/mL, the assay performance characteristics were assessed. The assay demonstrated inter-run precision ≤ 9% and accuracy from 95 % to 108 %. The lower limit of quantification was determined at 0.5 ng/mL. Calibration curves were linear across the tested range (figure 2), with excellent reproducibility. These results confirm the method's robustness for C4 quantification.

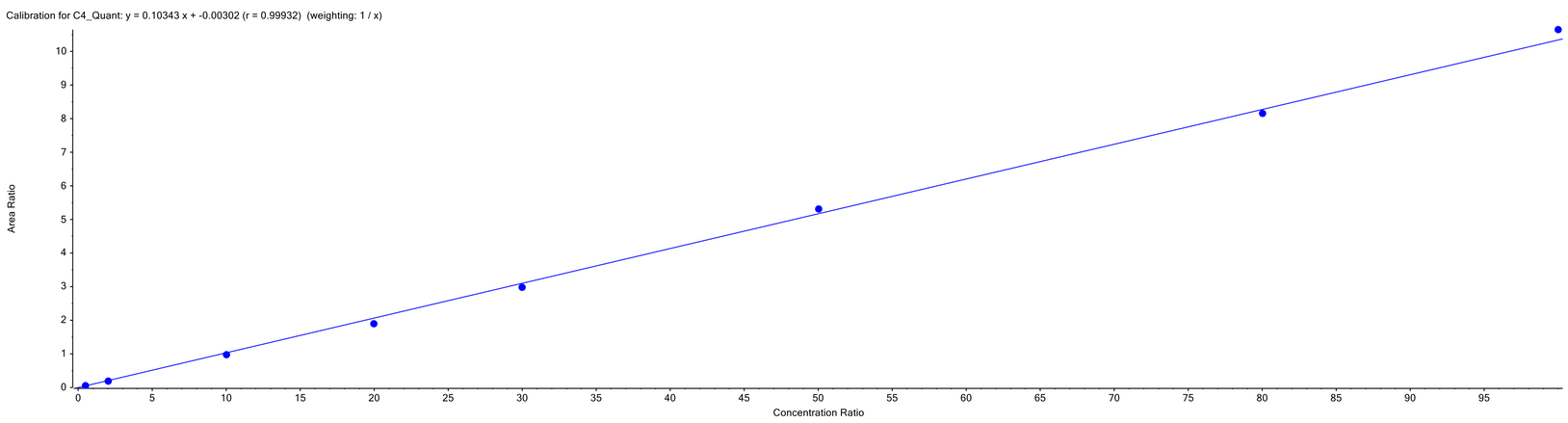

Figure 2 : C4 Calibration curve from 0,5 to 100 ng/mL

Conclusion:

Our results confirm the reliability of the method, supporting its potential as an alternative to the SeHCAT test. Full validation, following CLSI guidelines is underway to enable routine implementation.

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(2) Barkun A, Love J, Gould M, Pluta H, Steinhart AH. Bile acid malabsorption in chronic diarrhea: Pathophysiology and treatment. Can J Gastroenterol. 2013 Nov;27(11):653-659.
(3) Steiner C, von Eckardstein A, Rentsch KM. Quantification of the 15 major human bile acids and their precursor 7α-hydroxy-4-cholesten-3-one in serum by liquid chromatography–tandem mass spectrometry. J Chromatogr B. 2010;878(28):2870-2880. doi:10.1016/j.jchromb.2010.08.045.