A Simple and Sensitive LC-MS/MS Method for Quantification of 7α-hydroxy-4-cholesten-3-one (C4) in Human Serum

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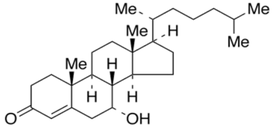
Overview

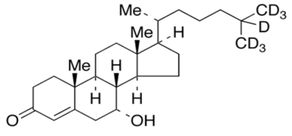
7α-Hydroxy-4-cholesten-3-one (C4) is an intermediate in the biochemical synthesis of bile acids from cholesterol. The serum concentration of C4 is a useful tool for diagnosis of bile acid malabsorption (BAM), especially for patients with chronic diarrhea and irritable bowel syndrome (Brydon et al, 1996; Hofmann et al, 2009). In this study, we developed and validated a sensitive, high-throughput LC-MS/MS method to quantify C4 in human serum to support clinical study.

Materials

7α-Hydroxy-4-cholesten-3-one (C4) and Internal Standard, 7α-hydroxy-4-cholesten-3-one-d_7 used in this study were purchased from Toronto Research Chemicals Inc.



7α-hydroxy-4-cholesten-3-one



7α-hydroxy-4-cholesten-3-one-d7 (IS)

Figure 1. Chemical Structures

Methods

Sample Preparation:

C4 and its internal standard (7α-hydroxy-4-cholesten-3-one-d_7) were fortified into surrogate matrix (Mass Spect Gold Human Serum) to prepare calibration curve standards and LLOQ QC samples. Multiple serum lots were pre-screened. The lot(s) with lowest endogenous C4 level was selected to prepare QC samples.

An aliquot of 100 µL of human serum or surrogate matrix was fortified with 10.0 µL of internal standard working solution, and 300 µL of acetonitrile was added, vortex for approximately 5 minutes and then centrifuge for 10 minutes at 4°C. Transferred 200 µL of supernatant to a new 96-well plate for LC-MS/MS analysis.

Liquid Chromatography:

Pump: Shimadzu LC-30AD
Autosampler: Shimadzu SIL-30AC
Column Heater: Shimadzu CTO-30A

Analytical Column:
Kinetex F5 column, 50 x 2.1 mm, 2.6 µm
Column temperature: 40ºC

Mobile phase:
A: 0.1% formic acid in water
B: 0.1 % formic acid in methanol:acetonitrile 60:40 (v:v)

Mass Spectrometry:

MS System: Sciex 5500 Triple-Quad
Condition: LC/(+)ESI-MS/MS
MRM transitions:
C4: m/z 401.2 → m/z 177.2
C4-d_7: m/z 408.3 → m/z 177.2

Results and Discussion

- Due to presence of endogenous C4 in human serum, surrogate matrix (Mass Spect Gold Human Serum) was used for the preparation of calibration standards and LLOQ. Other QC samples were prepared in authentic human serum.
- This assay was validated in a nominal range from 0.250 - 250 ng/mL with correlation of coefficients > 0.9947.
- Mass Spect Gold Human Serum (Golden West Diagnostics) used as surrogate matrix did not show significant interference for C4, therefore it was suitable for the preparation of calibration standards. Typical calibration curves are depicted in Fig. 2. Typical Chromatogram of an Extracted Blank Sample and an Extracted LLOQ Sample are shown in Fig. 3 and Fig. 4, respectively.

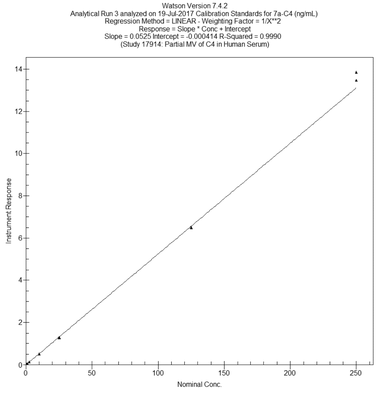


Figure 2. Representative Calibration Curves

Table 1. Assay Performance Summary

Analyte	C4
LLOQ Intra %CV	5.1% to 8.6%
LLOQ Intra %RE	-3.2% to 1.2%
QC Intra %CV	1.2% to 8.7%
QC Intra %RE	0.9% to 10.1%
LLOQ Inter %CV	7.1
LLOQ Inter %RE	-0.8
QC Inter %CV	1.6% to 6.4%
QC Inter %RE	2.3% to 6.8%
%Recovery	83.3% – 100.9% for C4 71.4% for C4-d_7
Carryover	No significant carryover observed
Hemolysis Effect	No significant impact
F/T Stability	3 cycles at -20/-70°C
Bench-Top Stability	Up to 16 hours at ambient
Extract Stability	Up to 72 hours at 4°C
Stock Solution Stability	Up to 6 hours at ambient up to 605 days at -20°C
Working Solution Stability	Up to 186 days at -20°C
Long-Term Storage Stability	Up to 434 days at -20/-70°C
Batch Size	144 injections

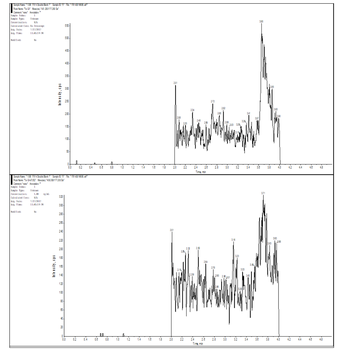


Fig. 3. Representative Chromatogram of an Extracted Blank Sample.

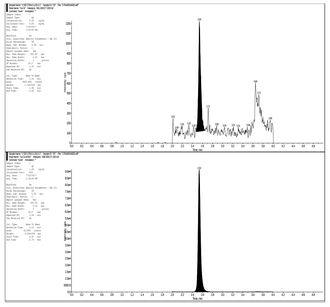


Fig. 4. Representative Chromatogram an Extracted LLOQ Sample.

Conclusions

- A rapid, simple and specific LC-MS/MS method has been developed for for the measurement of C4 in human serum.
- This assay was validated for GCP clinical studies.
- This assay has been used to analyze thousands of clinical samples.