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Simultaneous Quantification of Lathosterol, Lanosterol and Desmosterol in Human Plasma Using UPLC-Tandem Mass Spectrometry

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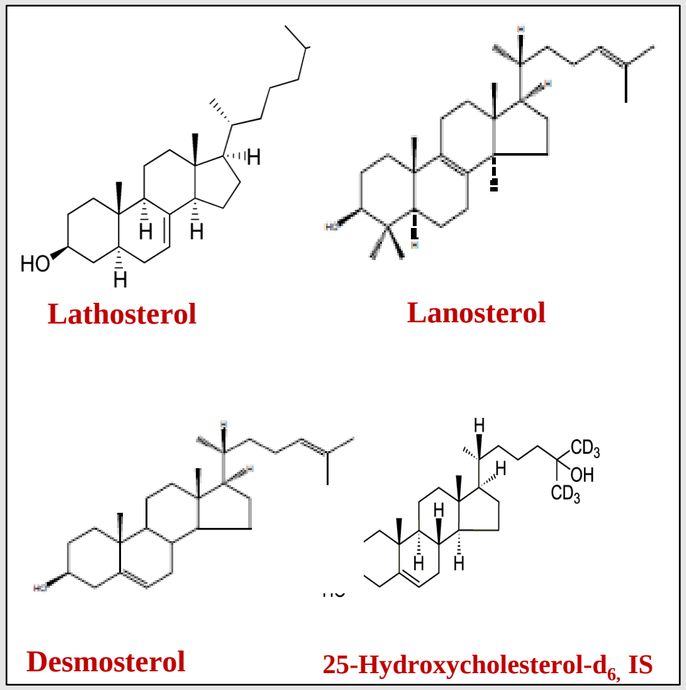
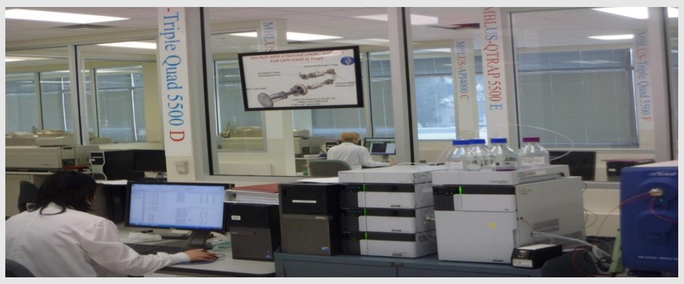
PURPOSE

Lathosterol, lanosterol and desmosterol are precursors in cholesterol biosynthesis pathway. They play significant biological roles in human body. Lathosterol is often used as an indicator of whole-body cholesterol synthesis. Lanosterol could reverse protein aggregation in cataracts, therefore, it can be used for the treatment of cataract patients. While desmosterol is one of several biomarkers for Alzheimer disease. Due to their structural similarities, a tremendous challenge has been encountered for the development of a chromatographic method for quantitation of those sterols. In this study, we used a UPLC-MS/MS system to achieve a good separation of lathosterol, lanosterol, desmosterol, cholesterol, and other analogues. The method has been used for analysis of those sterols to support clinical studies.





METHOD

In this method, lathosterol, lanosterol, desmosterol and the added internal standards were extracted from human plasma using liquid-liquid extraction. The LC-MS/MS analysis was carried out on a Sciex QTAP 5500 mass spectrometer coupled with a Waters UPLC system. The chromatographic separation was achieved on a reversed phase column (Thermo Hypersil GOLD column, 100 × 2.1 mm, 1.9 µm). The mass spectrometer was operated in positive APCI mode. The MRM transitions were monitored at m/z 369.3 → 161.1 for lathosterol, 409.43→ 109.1 for lanosterol, and 367.4 → 147.1 for desmosterol, 375.6 → 95.1 for 25-hydroxycholesterol-d6 (IS). A linear regression (1/concentration^2) calibration curve was established from 0.1 µg/mL to 10 µg/mL for each analyte in human plasma.

Sample Preparation:

Due to endogenous presence of lathosterol, lanosterol, desmosterol (1.37-5.79 µg/mL for lathosterol, 0.303-1.02 µg/mL for lanosterol, 0.461-1.53 µg/mL), a surrogate matrix was used for the preparation of calibration standards and LLOQ and LQC samples, however, MQC and HQC samples were prepared in authentic human plasma. Lathosterol and its internal standard were extracted from an aliquot of 50-µL plasma using liquid-liquid extraction method. The supernatant was dried, reconstituted and transferred to a 96-Well plate for LC-MS/MS analysis.

Liquid Chromatography:

Pump: Waters Acuity BSM Pump. Autosampler: Waters Acuity FTN Autosampler
Analytical Column: Thermo Hypersil GOLD column, 2.1 x 100 mm, 1.9 µm
Isocratic Flow: The analytes were eluted using a Isocratic flow of mobile phase A (0.1% formic acid)/mobile phase B (0.1% formic acid in methanol) (17:83, v:v)
Injection Volume: 12 µL

Mass Spectrometry:

MS System: AB/Sciex QTRAP 5500
Condition: APCI (+) MS/MS,
MRM transition:
Lathosterol: 369.3→1611
Lanosterol: 409.3 → 109.1
Desmosterol: 367.4 → 147.1
25-Hydroxycholesterol-d$_6$: 375.6→95.1

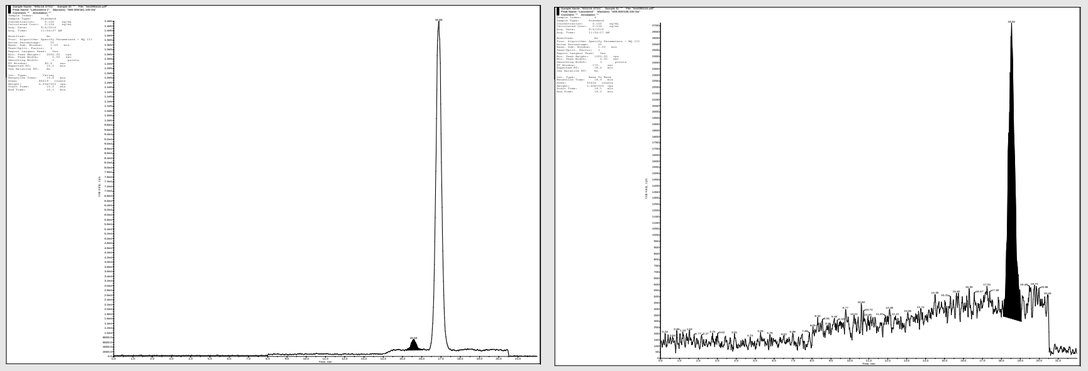


Figure 1. Ion chromatogram of LLOQ sample, the chromatogram is for Lathosterol (MRM transition: 369.4 → 95.1 (left); the chromatogram is for Lanosterol (MRM transition: 409.3 → 109.1(right) .

RESULTS

Excellent linearity was obtained with a correlation coefficient ≥ 0.9900 for all analytes. The high dynamic calibration range was reached due to eliminated background noise and higher specificity. For lathosterol, including LLOQ, the inter-day CV ranged from 2.6% to 7.4% and the biases of the means ranged from -12.7% to 2.1%. For lanosterol, including LLOQ, the inter-day CV ranged from 2.7% to 5.6% and the biases of the means ranged from -9.6% to 4.0%. For desmosterol, including LLOQ, the inter-day CV ranged from 2.2% to 2.8% and the biases of the means ranged from -4.1% to -0.8%. An excellent resolution was obtained between cholesterol and lathosterol, which is necessary to effectively quantitate lathosterol since both lathosterol and cholesterol has the same molecular weight and with similar polarity. The method has been successfully applied to the analysis of the clinical samples

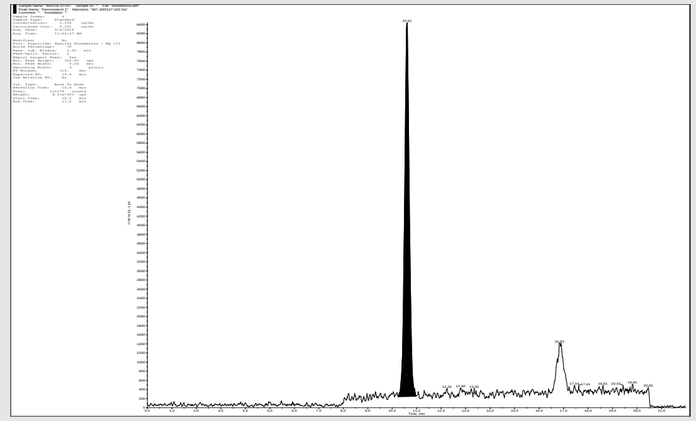


Figure 2. Ion chromatograms of LLOQ sample, the chromatogram is for Desmoosterol (MRM transition: 367.4 → 147.1).

CONCLUSION

An LC-MS/MS bioanalytical method for the quantitation of lathosterol, lanosterol and desmosterol in human plasma has been developed. The results presented in this post demonstrate that HPLC separation of lathosterol, cholesterol, lanosterol and desmosterol, higher specificity and low background noise analysis method for the determination of lathosterol, lanosterol and desmosterol in human plasma using QTRAP 5500 Systems.