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Simultaneous Measurement of Four Oxysterols in Human Serum by UPLC-APCI-MS/MS

Aiping Zhu, Wanqing Lu, Nelson Santiago, Tian-Sheng Lu and Yong-Xi Li
Medpace Bioanalytical Laboratories, 5365 Medpace Way, Cincinnati, OH 45227

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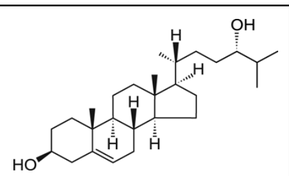
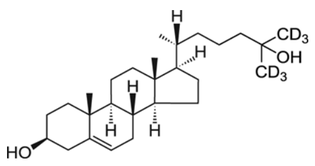
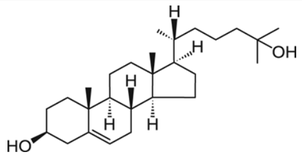
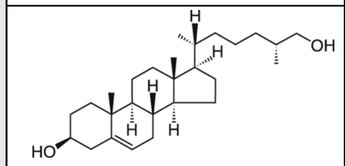
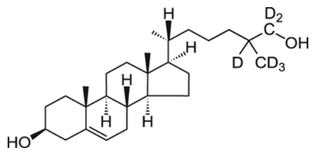
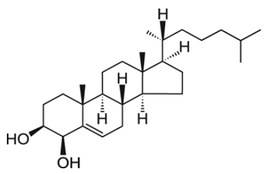
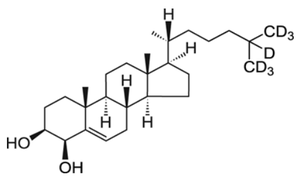
PURPOSE

Oxysterols are oxidized derivatives of cholesterol, which may be important in many biological processes, including cholesterol homeostasis, atherosclerosis, and sphingolipid metabolism, etc. 4β-Hydroxycholesterol is an endogenous biomarker for cytochrome P450 3A4/5 activity. 24S-Hydroxycholesterol and 27-hydroxycholesterol could serve as markers for Alzheimer disease. In addition, 27-hydroxycholesterol and 25-hydroxycholesterol are known to downregulate the cholesterol biosynthetic pathway. The separation and quantitation of oxysterols is very challenging due to their structural similarities. Most reported LC-MS methods required either tedious derivatization procedures or long analysis times. The purpose of this study is to develop a rapid and reproducible LC-MS/MS method for simultaneous quantitation of 4β-, 24S-, 25-, and 27-hydroxycholesterols in human serum without derivatization to support clinical studies.

Analytes	Internal Standards
 24S-hydroxycholesterol	 25-hydroxycholesterol-d6
 25-hydroxycholesterol	
 27-hydroxycholesterol	 27-hydroxycholesterol-d6
 4β-hydroxycholesterol	 4β-hydroxycholesterol-d7

METHOD

Sample Preparation:

Due to endogenous presence of oxysterols in human serum, a surrogate matrix (0.1% Tween 20 in water) was used for the preparation of calibration standards and LLOQ samples, however, LQC, MQC and HQC samples were prepared in authentic human serum. 4β-, 24S-, 25-, and 27-hydroxycholesterols and their internal standards were extracted from an aliquot of 50 µL serum by liquid-liquid extraction with hexane. The organic layer was evaporated and reconstituted in acetonitrile for LC-MS/MS analysis.

Liquid Chromatography:

UPLC: Waters Acquity I
Analytical Column: C18 150 x 2.1 mm, 2µm column
Column Temperature: 45°C
Mobile phase A: 0.1% formic acid in water
Mobile phase B: Methanol: Acetonitrile 50:50 v:v
Flow Rate: 0.3 mL/min
Injection Volume: 10 µL

Mass Spectrometry:

MS System: AB/Sciex Triple Quad 5500
Condition: APCI (+) MS/MS,

Analytes	MRM Transitions	Internal Standards	MRM Transitions
25-hydroxycholesterol	367.3 → 95.1	25-hydroxycholesterol-d6	391.4 → 373.3
24S-hydroxycholesterol	385.4 → 367.3		
27-hydroxycholesterol	385.3 → 135.2	27-hydroxycholesterol-d6	391.3 → 141.2
4β- hydroxycholesterol	385.4 → 109.1	4β- hydroxycholesterol-d7	391.4 → 115.1

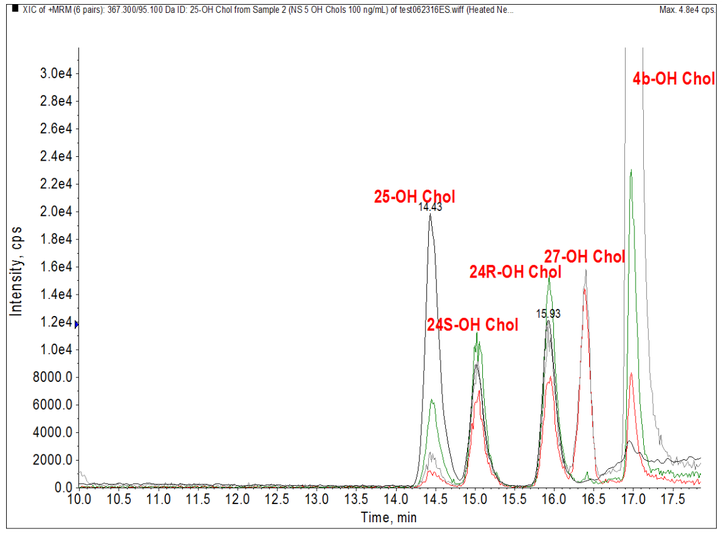


Figure 1. Typical chromatograms of 4β-, 24S-, 24R-, 25-, and 27-hydroxycholesterols

RESULTS

The four structurally similar oxysterols were well resolved within 20 minutes, which provided a reliable way for the quantification of 4β-, 24S-, 25-, and 27-hydroxycholesterols in human serum by LC-MS/MS. This assay was validated in a nominal range of 10.0 to 1000 ng/mL for 4β-, 24S-, and 27-hydroxycholesterols, 5.00 to 500 ng/mL for hydroxycholesterol for 25-hydroxycholesterol with correlation coefficients (r2) ≥0.9981. The intra-day precision CV% ≤ 7.3% and accuracy (bias %) ranged from -2.5% to 10.0%. Inter-day precision CV% ≤ 9.0% and accuracy (bias %) ranged from -3.0% to 8.4%. The four oxysterols were found to be stable in human plasma at least 6 hours at ambient, 3 freeze/thaw cycles at ~-70^{0}C, and at least 45 days in a ~-70^{0}C freezer. The method has been successfully applied to the analysis of the clinical samples.

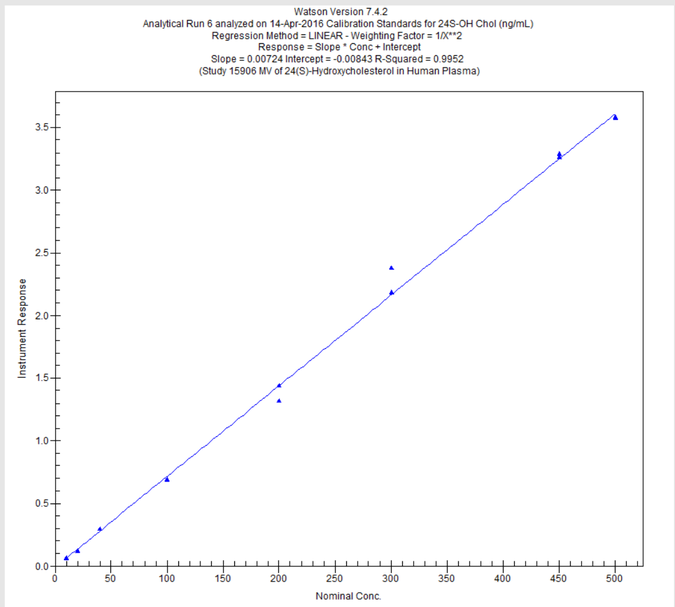


Figure 2. Typical Calibration Curve of 24S-hydroxycholesterol

CONCLUSION

A rapid UPLC-APCI MS/MS method has been developed for simultaneous quantitation of 4β-, 24S-, 25-, and 27-hydroxycholesterol in human serum. The assay provided a sensitive, reproducible and selective for the accurate measurement of 4β-, 24S-, 25-, and 27-hydroxycholesterol in human serum.