MEDPACE
Bioanalytical Laboratories

Quantification of Lathosterol as a Biomarker in Human Plasma Using UPLC-MS/MS System

Guangchun Zhou, Tian-Sheng Lu, Nicole Greer, and Yong-Xi Li

Medpace Bioanalytical Laboratories, 5365 Medpace Way, Cincinnati, OH 45227

Overview

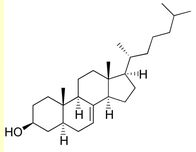
A rapid, sensitive and specific analytical method has been developed and validated to quantify lathosterol as a biomarker in human K_2EDTA plasma for clinical studies.

In this method, lathosterol was extracted from 0.05 mL of human K_2EDTA plasma using simple extraction procedures. Separation was performed on a reverse phase column (Thermo Hypersil C18 Gold). Detection was achieved using a AB/SCIEX QTRAP 5500 system in the positive ion mode with multiple reaction monitoring (MRM). The lower limit of quantitation was 0.1 µg/mL .

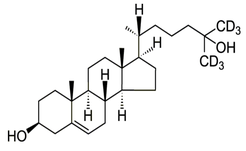
Introduction

Lathosterol, the direct precursor of chlosterol, is an indicator of whole-body cholesterol synthesis in humans. The ratio of lathosterol to cholesterol in serum or plasma is often used for predicting the effect of cholesterol-lowering drugs in clinical studies. GC or GC-MS methods were commonly used for lathosterol and other sterols analysis, however, the extraction procedure was very tedious and GC separation was very time-consuming. Some LC-MS/MS methods were reported but separation of lathosterol from cholesterol was an issue since both lathosterol and cholesterol have the same molecular weight and very similar structures. Cholesterol could camouflage lathosterol peak since cholesterol is in a much higher level in plasma (cholesterol:lathosterol>1000) . In this study, we used a UPLC system to achieve a good separation of lathosterol, cholesterol and other analogues, coupled with a tandem mass spectrometry in positive APCI mode for the quantitation of lathosterol in human plasma.

Structure



Lathosterol



25-Hydroxycholesterol-d_6

Methods

Sample Preparation:

Due to endogenous presence of lathostrerol, 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: Shimadzu UFLC LC-30AD
Autosampler: Shimadzu UFLC SIL-30AC
System Controller: Shimadzu CBM-20A
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: 5 µL

Mass Spectrometry:

MS System: AB/Sciex QTRAP 5500
Condition: APCI (+) MS/MS,
MRM transition:
Lathosterol: 369.4→95.1
25-Hydroxycholesterol-d_6 375.6→95.1

Results and Discussion

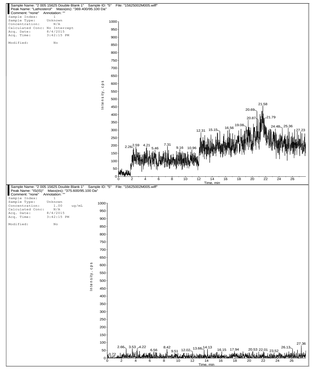


Figure 1. Ion chromatograms of blank plasma, upper chromatogram is for Lathosterol (MRM transition: 369.4 → 95.1), and the lower chromatogram is for 25-hydroxycholesterol-d_6 (IS) (MRM transition: 375.6 → 95.1)

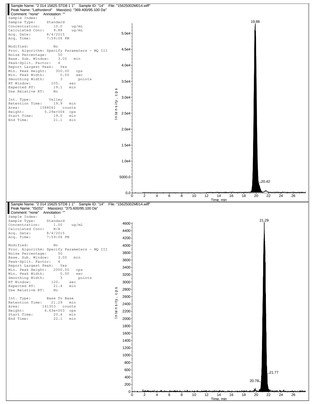


Figure 2. Ion chromatograms of ULOQ plasma sample, upper chromatogram is for Lathosterol (MRM transition: 369.4 → 95.1), and the lower chromatogram is for 25-hydroxycholesterol-d_6 (IS) (MRM transition: 375.6 → 95.1)

Table I. Validation Data Summary for Lathosterol in Human Plasma

Human Plasma				
Calibration Range			0.1 to 10 µg/mL	
Correlation Coefficient (r^2, mean)			0.9922	
Accuracy and Precision			Precision	Accuracy
	QC	Conc. (µg/mL)	CV%	Bias%
Inter-Batch (n=18)	LLOQ	0.1	5.1	-8.0
	Low	0.3	9.6	-10.6
	Medium	0.7	6.9	-3.0
	High	400	13.4	-0.8
		Condition		Accuracy Bias%
Freeze/Thaw		3 Cycles, -70° C		< 2.7
Bench-Top		24 hrs, Room Temperature		< -1.4
Long-Term Storage Stability		32 Days, -70° C		< -5.7

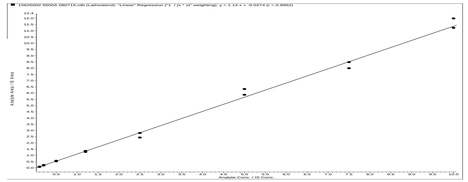


Figure 3. Typical Calibration Curve of Lathosterol in Human Plasma

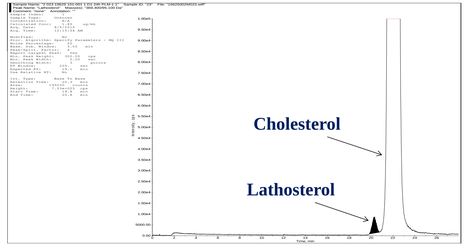


Figure 4. Ion chromatograms of clinical plasma sample, the chromatogram is for Lathosterol (MRM transition: 369.4 → 95.1, cholesterol is in a much higher level in plasma, cholesterol:lathosterol >1000:1).

- Excellent linearity was obtained with a correlation coefficient ≥ 0.9913 for Lathosterol. The high dynamic calibration range was reached due to eliminated background noise. (Figures 1 to 4).
- For Lathosterol, including LLOQ, the inter-day CV ranged from 5.1% to 13.4% and the biases of the means ranged from -10.6% to -0.8%. In addition, Lathosterol was found to be stable in human plasma after 24 hours at ambient temperature, through 3 freeze/thaw cycles from -70 ° C, and after 32 days long-term stored in freezer at -70° C (Table I). These results also indicate that the liquid-liquid extraction method is suitable for lathosterol, as well as its related sterols analysis.
- Lathosterol has the same molecular weight as that of cholesterol and the high ratio of cholesterol:lathosterol (>1000:1) is in blood stream, our LC-MS/MS condition was used to achieve a good separation of lathosterol, cholesterol and other analogues.

Conclusions

A rapid, simple and specific LC-MS/MS method has been developed and validated for quantifying Lathosterol with a lower limit of quantitation of 0.1 µg/mL using 0.05 mL plasma sample.