

Quantitative Analysis of Mevalonate in Plasma Using LC-MS/MS

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Key Words

- LTQ Ion Trap
- Clinical Research
- Cholesterol Synthesis

Introduction

Cholesterol is synthesized *in vivo* through a multiple step pathway. Because mevalonate is the key intermediate of this process, its plasmatic levels are an indirect measure of *in vivo* cholesterol synthesis and, therefore, facilitate clinical research into pharmacological activity of anti-hypercholesterolemic drugs such as statins.

Goal

To develop a reliable and fast analytical method for the quantitative determination of mevalonate in plasma using a Thermo Scientific LTQ linear ion trap mass spectrometer.

Experimental

Sample Preparation

The plasma sample (500 μ L) was spiked with 20 ng of Mevalonate-D₇. Samples were acidified with hydrochloric acid allowing the conversion of mevalonate to mevalonolactone (Figure 1). After purification through solid phase extraction (SPE), samples were dried and dissolved in 400 μ L of 0.2% ammonium hydroxide to restore the non-lactonic form. Then 10 μ L were injected.

Quantitative analysis was performed on the basis of calibration curves, ranging from 2.5 to 250 ng/mL.

HPLC Conditions

High performance liquid chromatography (HPLC) analysis was performed using a Thermo Scientific Surveyor autosampler and pump. The 10 μ L sample was injected directly on a Thermo Scientific BioBasic AX column (150 \times 2.1 mm, 5 μ m). A gradient LC method used mobile phases A (10 mM ammonium formate, pH 8) and B (acetonitrile) at a flow rate of 200 μ L/min.

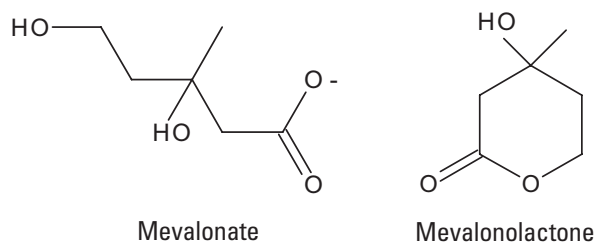


Figure 1. Structure of mevalonate and mevalonolactone

Mass Spectrometry

MS analysis was carried out on a LTQ™ linear ion trap mass spectrometer equipped with a Thermo Scientific Ion Max source with an electrospray ionization (ESI) probe.

Ion polarity:	Negative
Spray voltage:	2 kV
Sheath/Auxiliary gas:	Nitrogen
Sheath gas pressure:	40 (arbitrary units)
Auxiliary gas pressure:	10 (arbitrary units)
Sweep gas pressure:	5 (arbitrary units)
Ion transfer tube temperature:	300 °C
Scan type:	Full Scan MS/MS
Collision gas:	Helium
Collision energy:	30%
Divert valve:	3.0-6.5 min to source
Selected ions for quantification:	m/z 147 \rightarrow 59 for mevalonate m/z 154 \rightarrow 59 for mevalonate-D ₇

Results and Discussion

Figure 2 shows the ion chromatograms of a lower sample of the calibration curve. Excellent linearity ($r^2 = 0.999$) fits for the calibration curve were observed over the range of 2.5 - 250 ng/mL plasma (Figures 3 and 4). The intraday CV% ($n=3$) was in the range 0.5% - 4%. The limit of detection (LOD) was 2 pg, and the limit of quantification (LOQ) was 2.5 ng/mL.

Figure 5 reports an ion chromatogram of a plasma sample of a healthy volunteer (24 ng/mL plasma), extracted and analyzed as described.

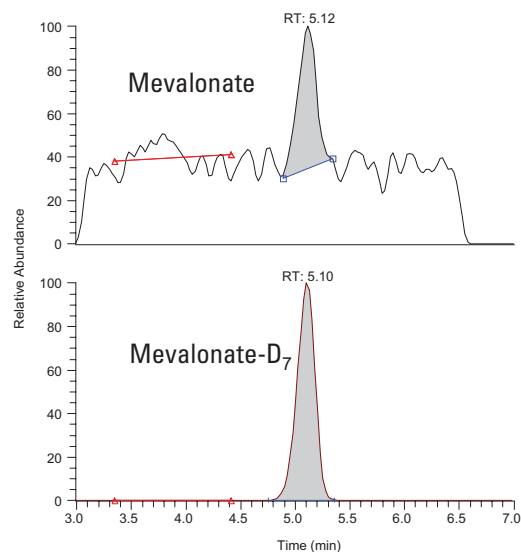


Figure 2. Ion chromatograms of 2.5 ng/mL calibration standard

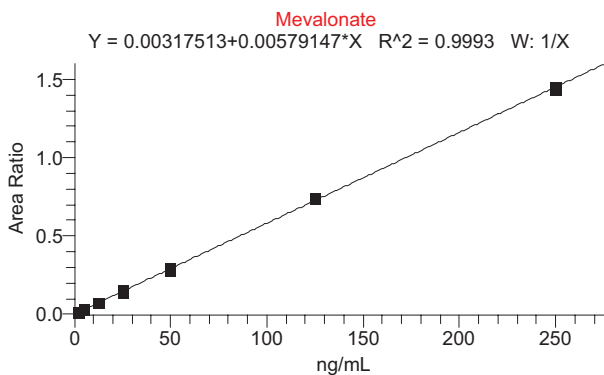


Figure 3. Calibration curve of mevalonate

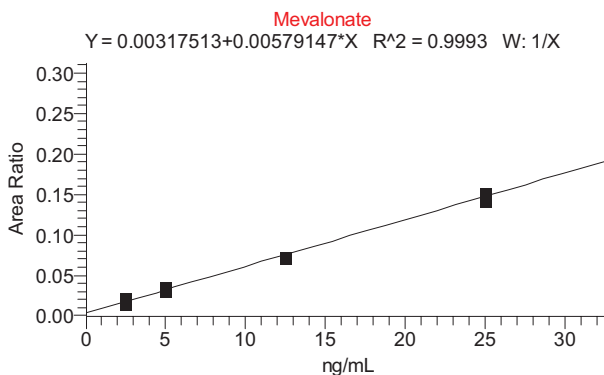


Figure 4. Zoom on low calibration points

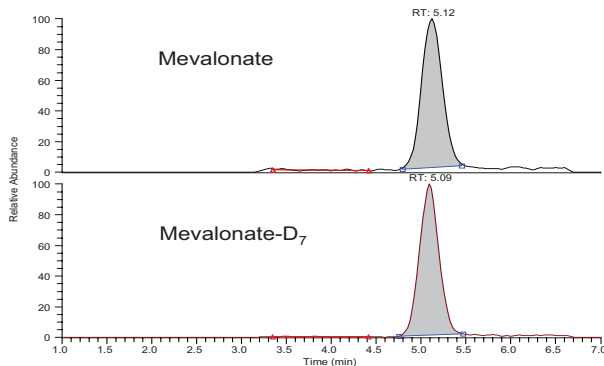


Figure 5. Ion chromatograms of plasma sample containing 24 ng/mL

Conclusion

A robust 10-minute method for the quantification of mevalonate with a dynamic range of 2.5 - 250 ng/mL plasma has been developed for clinical research using fast SPE purification and the LTQ linear ion trap mass spectrometer.

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