Application Note: 505

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 µL) was spiked with 20 ng of Mevalonate-D_7. 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 µL of 0.2% ammonium hydroxide to restore the non-lactonic form. Then 10 µL 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 µL sample was injected directly on a Thermo Scientific BioBasic AX column (150 × 2.1 mm, 5 µm). A gradient LC method used mobile phases A (10 mM ammonium formate, pH 8) and B (acetonitrile) at a flow rate of 200 µL/min.

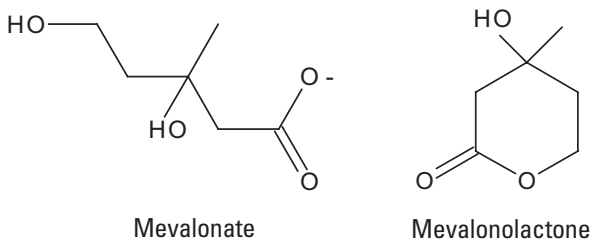

Figure 1. Structure of mevalonate and mevalonolactone

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→ 59 for mevalonate *m/z* 154→ 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.

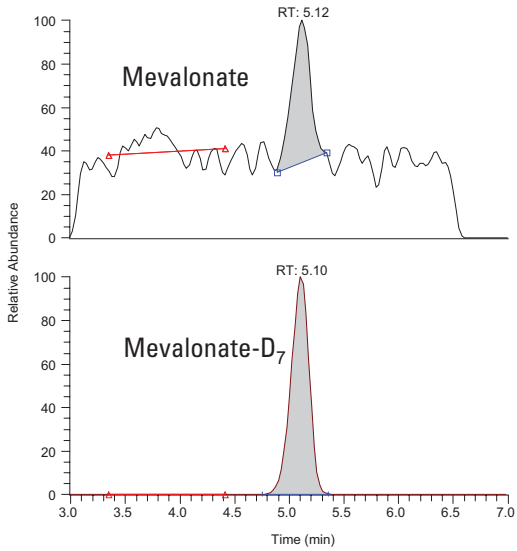

Figure 2. Ion chromatograms of 2.5 ng/mL calibration standard

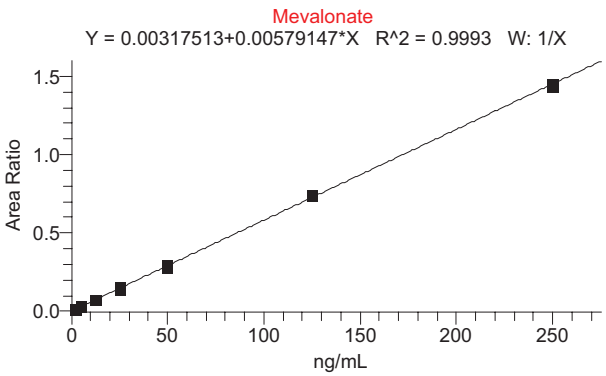

Figure 3. Calibration curve of mevalonate

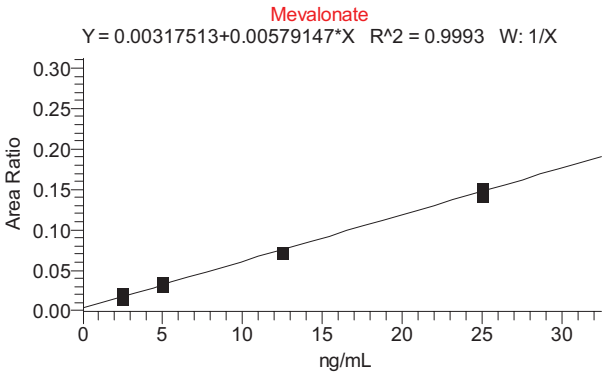

Figure 4. Zoom on low calibration points

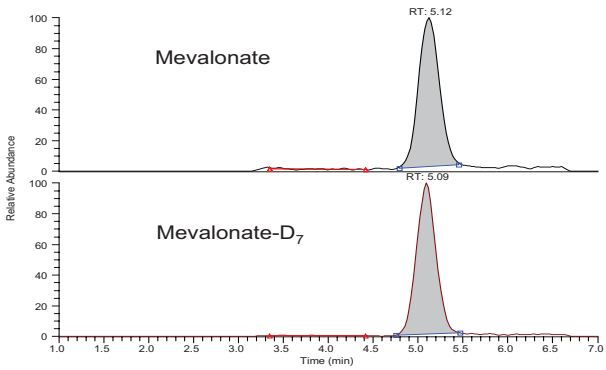

Figure 5. Ion chromatograms of plasma sample containing 24 ng/mL

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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