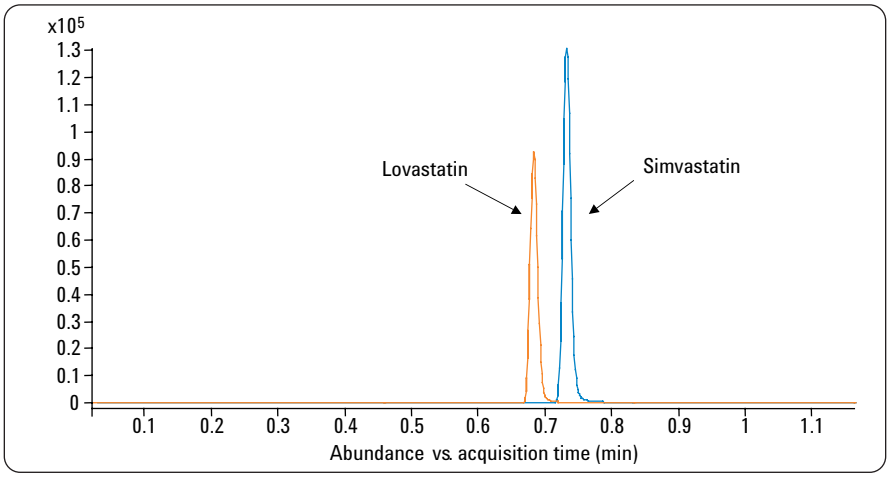
Quantification of lovastatin in human plasma by LC/ESI/MS/MS using the Agilent 6410 Triple Quadrupole LC/MS system

Application Note

Manufacturing QA/QC



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Abstract

Lovastatin was the first specific inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG CoA reductase) approved for the treatment of hypercholesterolemia. This Application Note describes an easy, sensitive quantification method for lovastatin in human plasma, based on a liquid chromatography-electrospray ionization-tandem mass spectrometry (LC/ESI/MS/MS) technique. Solid phase extraction was used for the extraction of lovastatin from plasma, and simvastatin was used as the internal standard. For quantification, the triple-quadrupole MS was used in multiple reaction monitoring (MRM) mode. The monitored transitions were *m/z* 405.4 to 199.2 for lovastatin and *m/z* 419.1 to 199.2 for simvastatin. A linearity coefficient of $R^2 > 0.99$ was observed over a wide range of 0.1 ppb to 50 ppb of lovastatin in human plasma.

Several methods are currently available for the quantification of this statin, but sensitivity and shorter runtime were the major challenges in those methods. The advantages of the method described here are simplicity, short runtime (1.2 minutes) and better sensitivity through MRM.

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Introduction

Lovastatin (lactone form of 1′,2′,6′,7′,8′,8a′-hexahydro-3,5-dihydroxy-2′,6′-dimethyl-8′(2″,2″-dimethyl-1″-oxobutoxy)-1′-naphthaleneheptanoic acid) lowers plasma cholesterol by inhibiting 3-hydroxy-3-methylglutaryl-CoA reductase, and was the first of this class of agents approved for clinical use. Simvastatin, a chemically modified form of lovastatin, was used as the internal standard for this study. Both of these statins are inactive lactone prodrugs which, when converted to their respective dihydroxy acid forms, show inhibitory activity.[1,2] Lovastatin levels in plasma after therapeutic oral doses are reported to be very low compared with levels observed after intravenous dosing. The results in the existing literature show that only 5 % of the drug is bioavailable when given orally.[3] This explains the need for sensitive and selective methods for the determination of lovastatin in plasma for therapeutic monitoring of drug levels. This Application Note describes a simple, rapid, and sensitive LC/ESI/MS/MS method for direct quantification of lovastatin from a concentration range of 0.1 ng/mL to 50 ng/mL in human plasma.

Experimental

Materials

Lovastatin (LVF-05311, molecular mass = 404.5) and simvastatin (PS-07306, molecular mass = 418.5) were purchased from VARDA Biotech. All solvents used in this study were of HPLC grade; acetonitrile was purchased from Merck and formic acid from Fluka. Millipore deionized water was used.

The chemical structures of simvastatin and lovasatin are shown in figure 1.

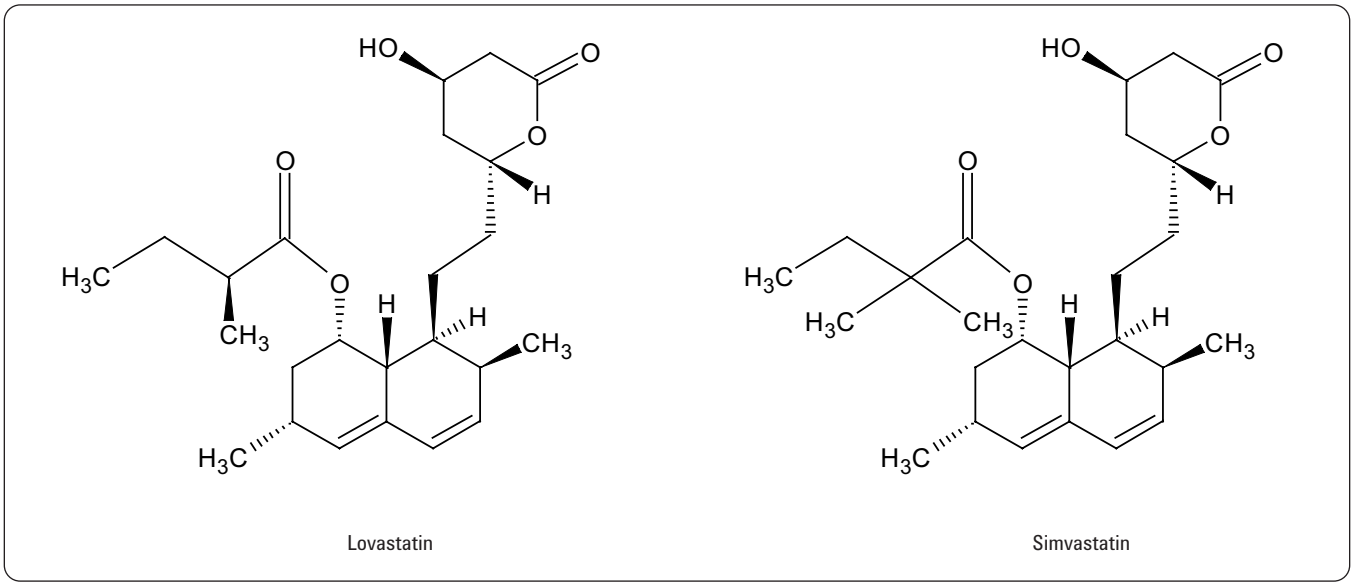
Instrumentation and conditions

All analyses were performed using the Agilent 6410A Triple Quadrupole LC/MS system coupled with an Agilent 1200 Series Rapid Resolution (RRLC) system. The system components included an Agilent 1200 Series binary pump SL with degasser, an Agilent 1200 Series autosampler SL, and an Agilent 1200 Series thermostatted column compartment SL. The Agilent MassHunter Workstation software was used for system control and data acquisition.

An Agilent ZORBAX Eclipse Plus C18 column (1.8 µm particles, 2.1 mm i.d. x 30 mm length) was used for all chromatographic separations. Mobile phase A was water containing 0.1 % formic acid and mobile phase B was acetonitrile with 0.1 % formic acid. The linear gradient conditions are shown in table 1.

Time (min)	% Mobile phase A	% Mobile phase B
0	60	40
0.5	5	95
1.0	5	95
1.2	60	40

Table 1
Gradient used for experiments.



Lovastatin

Simvastatin

Figure 1
Structures of lovastatin and simvastatin.

The LC system was operated at 700 µL/min, and the column temperature was 45 °C. The column eluent was introduced into the electrospray ionization source. The nebulizing gas flow-rate was set at 11 mL/min, drying gas temperature at 325 °C, and the capillary voltage at 4000 V. The injection volume was 4 µL and represented no more than 10 % of the total sample available for injection.

The responses of lovastatin and simvastatin were measured by MRM in the positive ionization mode with a fragmentor voltage of 100 V and a collision energy of 7 V. The ion monitored for lovastatin was the transition from *m/z* 405.4 to 199.2 and that for simvastatin was the transition from *m/z* 419.1 to 199.2; both ions had a dwell time of 20 msec/ion. The fragmentation patterns for lovastatin and simvastatin are shown in figures 2 and 3.

Preparation of stock solution and calibration standard

Stock solutions of lovastatin (I) and simvastatin (II) of concentration 100 µg/mL were prepared in 60/40 % acetonitrile/water (diluent) and were diluted with diluent to obtain the desired concentrations.

Various concentrations of lovastatin were spiked into 250 µL of plasma as per table 2. After the addition of lovastatin, constant volumes of 50 µL of 5 ppb (5 ng/mL) simvastatin (as internal standard) were added to all the samples, followed by 400 µL of water.

The theoretical concentrations of lovastatin calibration standards were 0.1, 0.2, 0.4, 0.5, 1.0, 2.5, 5.0, 10.0, 25.0, and 50.0 ng/mL, after extraction from plasma and reconstitution in mobile phase.

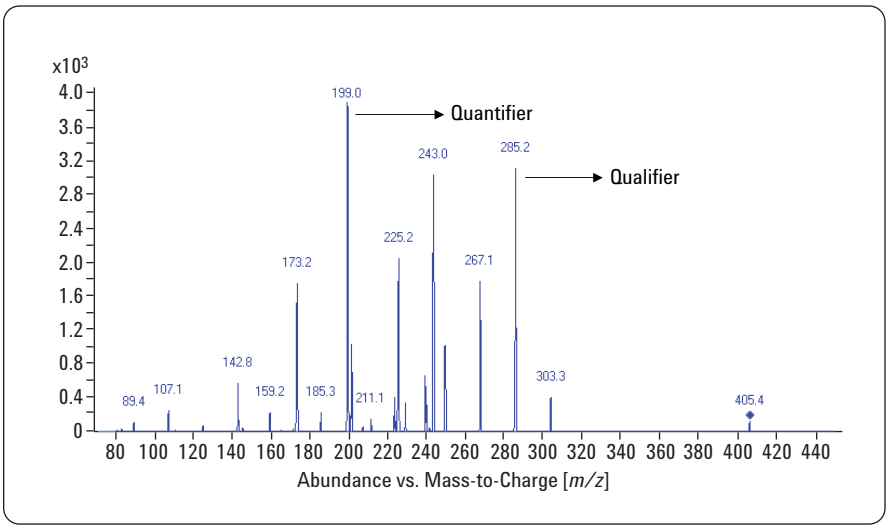


Figure 2
Fragmentation pattern for lovastatin, showing qualifier and quantifier ions that were used for the analyses.

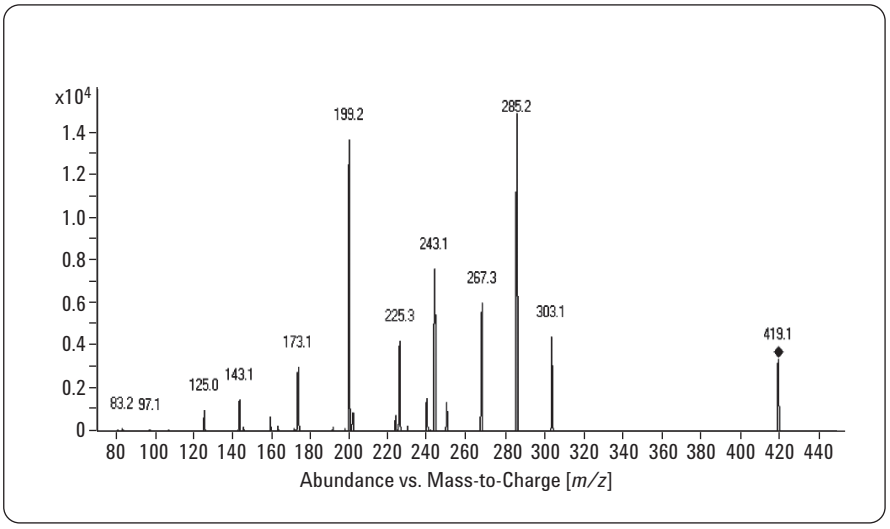


Figure 3
Fragmentation pattern for simvastatin.

Expected lovastatin concentration after extraction and reconstitution	Amount of lovastatin spiked
Blank	50 µL of water
Lovastatin 50 ppb	50 µL of 100 ppb
Lovastatin 25 ppb	50 µL of 50 ppb
Lovastatin 10 ppb	50 µL of 20 ppb
Lovastatin 5 ppb	50 µL of 10 ppb
Lovastatin 2.5 ppb	50 µL of 5 ppb
Lovastatin 1 ppb	50 µL of 2 ppb
Lovastatin 0.5 ppb	50 µL of 1 ppb
Lovastatin 0.4 ppb	50 µL of 0.8 ppb
Lovastatin 0.2 ppb	50 µL of 0.4 ppb
Lovastatin 0.1 ppb	50 µL of 0.2 ppb

Table 2
Preparation of plasma concentrations.

Extraction procedure

AccuBond C18 100 mg/1 mL cartridges were preconditioned with 1 mL of water, followed by 1 mL of methanol, followed by another 1 mL of water. The samples prepared as described earlier were passed through the cartridge immediately after spiking. After loading the sample in plasma, the cartridges were washed with 1 mL water, 1 mL of 0.5 % formic acid in water, and finally again with 1 mL of water. The samples were eluted with 2 mL acetonitrile; the organic fraction was collected separately and evaporated to dryness in a Rapid Vap Vacuum Evaporation System at 45 °C. The reconstitution was done with 100 µL of mobile phase (mobile phase A:mobile phase B; 60:40), and the resulting sample was injected into the LC/ESI/MS/MS system.

Three replicates of each standard in plasma were analyzed and calibration curves were plotted. The ratios of the peak areas of lovastatin to the peak areas of simvastatin were calculated. The calibration curves were constructed by weighted (1/*x*) least-squares linear regression analysis of the peak area ratios of lovastatin to simvastatin versus the concentrations of lovastatin. Calibration curve equations were used to calculate the concentrations of lovastatin in the samples based on their peak area ratios.

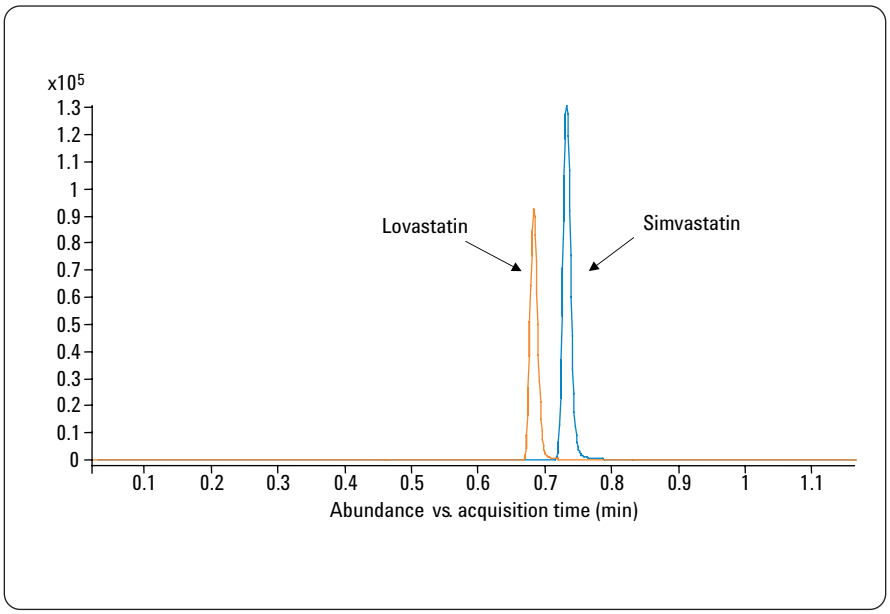


Figure 4
Overlay of lovastatin and simvastatin from MRM analysis.

Parameter	Set value
Ionization mode	Positive
Dry gas temperature	325 °C
Dry gas flow	11 mL/min
Nebulizer	50 psi
Capillary voltage	4000 V
Vacuum	3.0 x 10^{-5} Torr
Fragmentor voltage	100 V
Collision energy	7
Dwell time	20 msec/ion
MS1	Wide
MS2	Unit

Table 3
Final MS/MS parameters used in the experiments.

Results and discussion

Chromatographic and detection parameters

Short run times of about 1.2 minutes were achieved for both lovastatin and simvastatin. Lovastatin was eluted at 0.68 minutes and simvastatin at 0.73 minutes. The peaks of interest were free from interfering peaks at their respective retention times.

To minimize undesirable fragmentation and achieve highest response, various fragmentor voltages were tested from 80 to 220 V. At 100 V, the MS response of lovastatin and simvastatin showed both minimal undesirable fragmentation and highest response. The protonated molecular ion of lovastatin $[M+H]^+$ (*m/z* 405) was tested to give the highest sensitivity. Based on the optimization results, *m/z* 199 was selected as the quantifier ion and *m/z* 285 as qualifier. A representative overlay of lovastatin and simvastatin from the MRM study is shown in figure 4.

Other mass spectrometric parameters (gas temperature, gas pressure, and gas flows) were adjusted to get a maximum signal for lovastatin, with the results shown in table 3.

Linearity
Calibration curves were plotted as the peak area ratio (drug/IS) versus drug concentration. Results for the calibration curve showed good linearity ($R^2 > 0.99$) over the concentration range of 0.1 to 50 ppb lovastatin in the solution that was injected.

The average signal to noise and recovery results are shown in table 4.

The mean recovery of lovastatin from human plasma was 101.74 ±14.4 % (range 87.33 to 116.15 %). These results indicate that the method is reliable over a concentration range of 0.1 ppb to 50 ppb in the final extract. A lovastatin concentration of 0.1 ppb with a signal-to-noise ratio of 12.99 (limit of quantification) can be easily quantified using this instrument configuration.

Conclusion

In conclusion, the use of LC/ESI/MS/MS allows for accurate and reliable measurement of lovastatin concentrations in human plasma. The method has proven to be fast and simple, with each sample requiring 1.2 minutes of analysis time. The major advantages of this method are the simple sample preparation procedure, fast LC separation, and sensitive MRM detection.

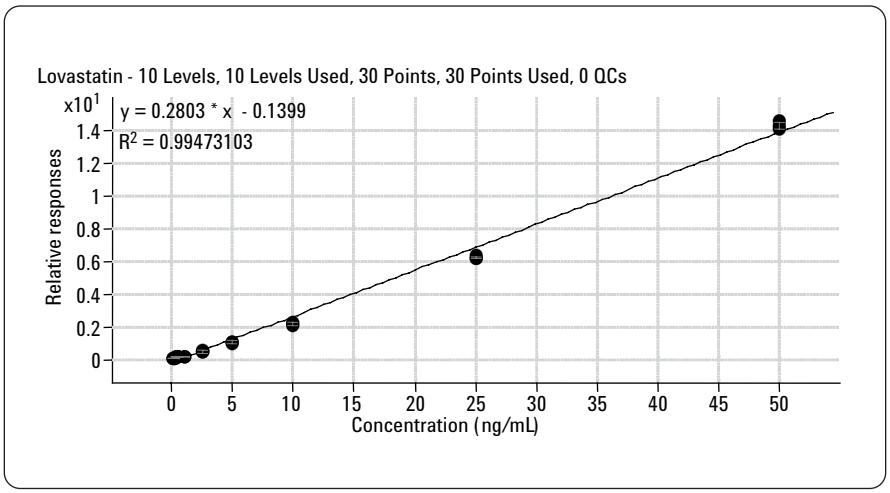


Figure 5
Linearity plot for lovastatin using simvastatin as IS.

Sample name	Signal-to-noise ratio	Recovery (%)
Lovastatin 0.1 ppb	12.99	92.34
Lovastatin 0.2 ppb	16.25	87.33
Lovastatin 0.4 ppb	19.09	116.15
Lovastatin 0.5 ppb	22.86	84.91
Lovastatin 1.0 ppb	38.12	94.91
Lovastatin 2.5 ppb	69.25	99.41
Lovastatin 5.0 ppb	71.99	97.10
Lovastatin 10.0 ppb	72.84	100.77
Lovastatin 25.0 ppb	77.86	99.94
Lovastatin 50.0 ppb	304.99	98.89

Table 4
Results showing the responses and recoveries.

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1.
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