Application Note: 402

Quantitative Measurement of Simvastatin Using High Speed LC/MS

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Key Words

- Accela
- MSQ Plus
- Simvastatin Quantitation
- UHPLC/MS

Introduction

Simvastatin (together with mevastatin and lovastatin in Figure 1), a hypolipidemic drug belonging to the "statin" family, is commonly used to control elevated cholesterol levels and to prevent cardiovascular diseases. It greatly inhibits the production of mevalonate, which is responsible for the endogenous synthesis of cholesterol, through competitive binding of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. The drug is often administrated orally in tablet form as a lactone containing 5-80 mg simvastatin as the active pharmaceutical ingredient, along with the inactive ingredient including cellulose, lactose, starch, *etc*. The quantitative analyses of statins have been accomplished using gas chromatography/mass spectrometry (GC/MS) and high performance liquid chromatography (HPLC) with UV or fluorescence detection. However, conventional HPLC on 3-5 µm diameter particles is slow, while GC/MS and HPLC/fluorescence require imprecise and time-consuming analyte derivatization.

Ultra high performance liquid chromatography (UHPLC), a new separation technique utilizing sub-2 µm diameter HPLC stationary phases, has brought great attention in the field of separation science. High speed separations with increased sensitivity and resolution allow the analyzing time to be dramatically reduced while excellent separation efficiency and retention factor are maintained. The statin separation by USP 30-NF25 monograph with conventional LC systems requires approximately 10 minutes, while a UHPLC system can accomplish the same separation in less than 2 minutes. MS detection is sensitive without analyte derivatization and provides analyte confirmation. However, the 1-2 s peak widths and relatively high mobile phase flow rates typical of UHPLC methods demand a robust, fast scanning MS detector.

In this application note, the recent approach of using a UHPLC/MS method for high throughput separation, quantitation and confirmation of simvastatin in pharmaceutical dosage form will be described.

Experimental Conditions

Sample Preparation

Simvastatin, mevastatin and lovastatin standards were purchased from EMD Chemicals (San Diego, CA, USA). The stock solutions of the three statins were prepared by dissolving the solid standards in HPLC grade acetonitrile (ACN). The calibration standards were prepared by mixing the three statins with 1:1:1 molecular ratio and diluting to a series of concentrations with ACN:water (50:50) right before LC/MS analysis.

Simvastatin assay solutions were prepared according to USP method. A simvastatin tablet (40 mg) was first dissolved in 2 mL distilled water and then diluted with 10 mM sodium acetate in H_2O:ACN (20:80) buffer (pH 4.0) to 25 mL. This solution was sonicated for 15 min and a portion of the clear supernatant was filtered through a 0.2 µm Milli-Q® (Millipore Co. Ltd.) filter. The filtrate was diluted to about 40 ng/mL with ACN: water (50:50), using 50 ng/mL lovastatin as the internal standard.

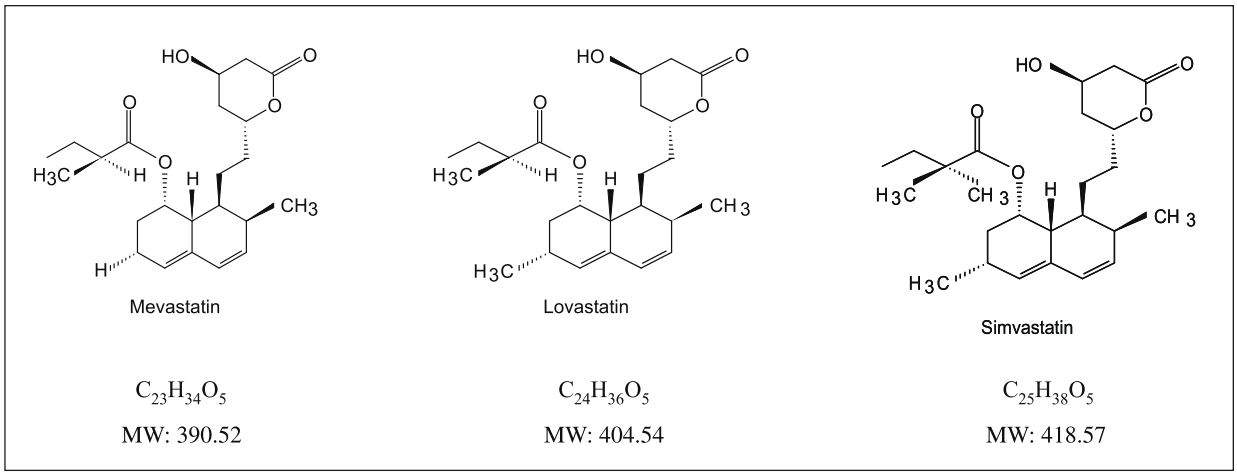

Figure 1: The chemical structures of three statins

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Chromatographic Conditions

Instrument: Thermo Scientific Accela™ UHPLC System (Figure 2)
LC Column: 100 x 2.1 mm Hypersil GOLD™ AQ
Packing: 1.9 µm
Flow Rate: 0.75 mL/min
Mobile Phase: A: Water with 0.1 % Formic acid
B: ACN with 0.1% Formic acid
Gradient: Isocratic, 35% A, 65% B
Injection Volume: 5 µL partial loop injection with a 25 µL loop
Column Temperature: 45 °C

Mass Spectrometer Conditions

Instrument: Thermo Scientific MSQ™ Plus (Figure 2)
Ionization: Electrospray (ESI)
Polarity: Positive
Probe Temperature: 550 °C
Cone Voltage: 80.0 eV
Scan Mode: SIM 453.7, 467.7, 481.8
ESI Voltage: 3.0 kV
Scan Time: 0.2 s

Figure 2: Thermo Scientific Accela-MSQ Plus high speed LC/MS system

Results and Discussion

Separation and MS Detection

Adequate separations were achieved within 2 minutes using the method described in the above section (Figure 3). Mevastatin eluted first at 0.97 min, followed by lovastatin at 1.15 min, and simvastatin at 1.43 min. Lovastatin was chosen as the internal standard for the quantitation of simvastatin in its tablet form. The MS spectra of the statin standards showed $[M+Na]^+$, $[M+ACN+H]^+$ and $[M+ACN+Na]^+$ ion signals. The most abundant ions observed for all the three statins were their adducts with both sodium and ACN $[M+ACN+Na]^+$ at *m/z* 453.7 for mevastatin, *m/z* 467.7 for lovastatin and *m/z* 481.8 for simvastatin. Thus, the ions at *m/z* 453.7, 467.7, 481.8 with a dwell time of 0.2 s were acquired in selective ion monitoring mode (SIM).

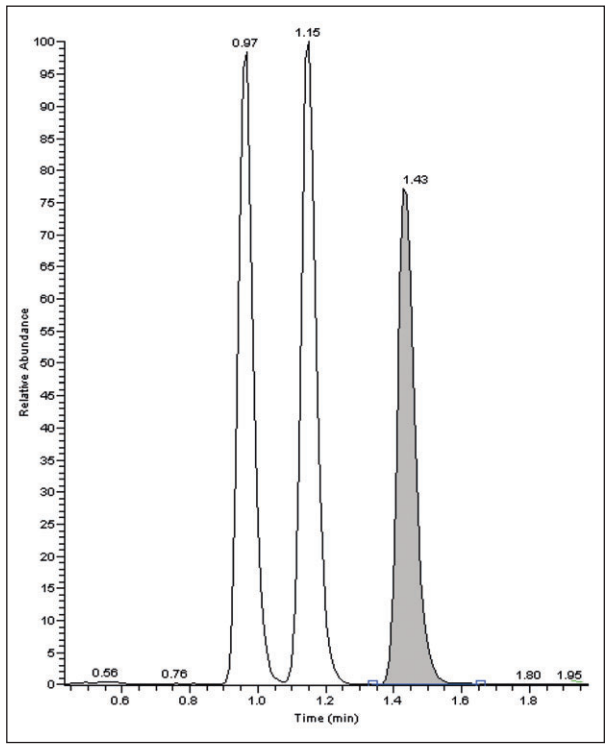

Figure 3: Separation of the statin standards at 82 ng/mL concentration, with elution order of mevastatin, lovastatin and simvastatin

Calibration Curve and Sensitivity

The calibration curve of simvastatin standards was constructed over a concentration range of 0.5-82 ng/mL (equivalent to 2.5-410 pg onto column) (Figure 4). Each calibration level was injected six times and the mean area responses were plotted against the concentrations. A correlation coefficient with $r^2 = 0.9998$ was achieved. The separation of the statin standards was repeated six times at 82 ng/mL. Good reproducibility on both retention time (0.5% RSD) and signal intensity (1.2% RSD) was observed.

The limit of quantitation (LOQ) and the limit of detection (LOD) for simvastatin, based on the calibration curve of signal-to-noise ratio versus concentration, were 0.859 ng/mL and 0.258 ng/mL, respectively.

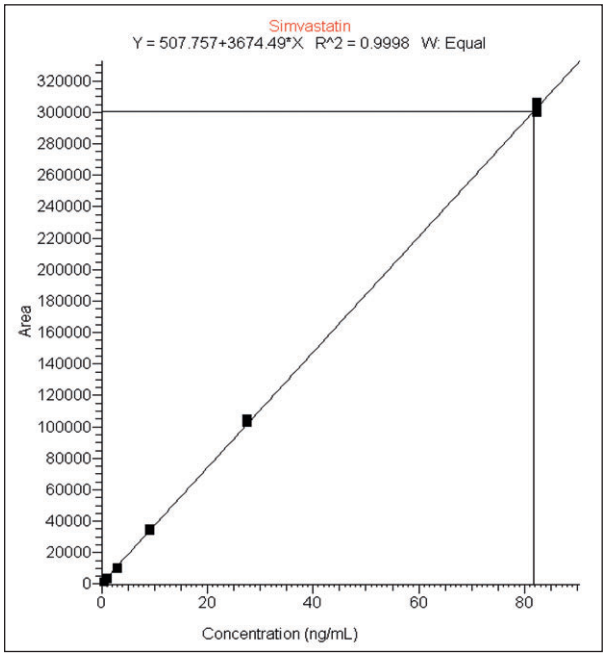

Figure 4: Calibration curve for simvastatin standards over a concentration range of 0.5-82 ng/mL.

Quantitation Using Internal Standard

Matrix effects are the major drawback in quantitative analyses of real samples by LC/MS systems, since the reproducibility and accuracy can be affected by signal suppression or, less frequently, by signal enhancement of co-eluting compounds. These effects can be minimized by using an internal standard. Therefore, lovastatin (50 ng/mL) was added as an internal standard to the freshly prepared simvastatin assay solution (about 40 ng/mL) and four simvastatin standard bracket solutions at 10 ng/mL, 25 ng/mL, 50 ng/mL, and 75 ng/mL. The sequence runs of four calibration levels and one assay sample, with six injections of each level/sample, were repeated in three days. The peak responses for the lovastatin internal standard had very high reproducibility, with an RSD of 2.8% over 24 injections. The concentration of the assay sample was determined to be 40.66 ng/mL based on the calibration curve of the peak area ratio against concentration (Figure 5). The calibration data and assay solution quantitation obtained from three different days were highly reproducible. The percent recoveries from the simvastatin tablet ranged from 101.6% to 101.8% in three days by this method (Table 1).

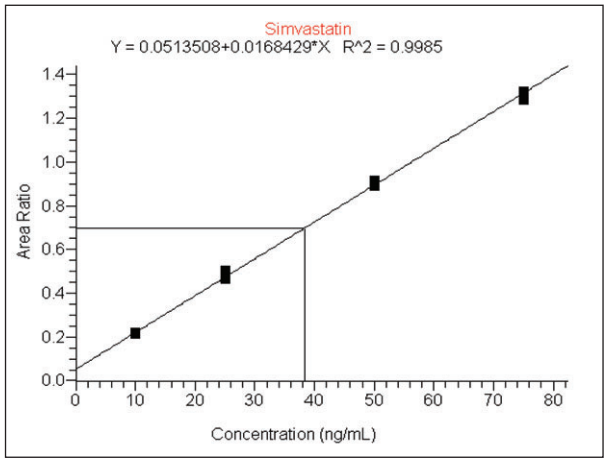

Figure 5: Calibration curve of simvastatin using lovastatin as the internal standard at 50 ng/mL

	Calibration Equation	Calculated Concentration	Reported Concentration	% Recovery	%RSD (n=6)
Day 1	Y = 0.0514 + 0.0168X, r^2 = 0.9985	40.66 ng/mL	40.00 ng/mL	101.66	3.3
Day 2	Y = 0.0475 + 0.0169X, r^2 = 0.9982	40.65 ng/mL	40.00 ng/mL	101.63	2.3
Day 3	Y = 0.0364 + 0.0164X, r^2 = 0.9913	40.73 ng/mL	40.00 ng/mL	101.83	4.2

Table 1: Calibration and simvastatin quantitation in three days using lovastatin as internal standard

Conclusions

A simple, fast, and reliable separation and quantitation method of simvastatin in pharmaceutical dosage form was achieved by high speed LC/MS, with excellent linear response (r^2 = 0.9998) in the range of 0.5-82 ng/mL (equivalent to 2.5-410 pg onto column). The LOD for simvastatin was 0.258 ng/mL and LOQ was 0.859 ng/mL. The simvastatin recovery ranged from 101.6% to 101.8% in three days. These results indicated that this approach can be applied as a reliable separation and quantitation method in the quality control of bulk and commercial tablet manufacturing process of simvastatin.

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