RP-HPLC Determination of Simvastatin and Its Related Substance Lovastatin

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ABSTRACT: OBJECTIVE To establish a RP-HPLC method for determination of simvastatin and its related substance lovastatin. **METHODS** The chromatographic conditions were: a Waters Symmetry C_{18} column (250 mm × 4.6 mm, 5 μm), a mixture of acetonitrile-sodium dihydrogen phosphate (pH 5.4) (65∶35) as mobile phase, flow rate 1.0 mL • min^{-1}, and detected at 238 nm. **RESULTS** The linear ranges of lovastatin and simvastatin were 0.3 ~ 3.0 μg • mL^{-1}, 0.03 ~ 0.30 mg • mL^{-1}, respectively. The average recovery were 100.2% (RSD = 1.5%) and 99.4% (RSD = 1.7%), respectively. **CONCLUSION** The method is simple, quick, sensitive, accurate, and reproducible. It can be used to the quality control of synthetic simvastatin products.

KEY WORDS: HPLC; simvastatin; lovastatin; determination

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反相高效液相色谱法测定辛伐他汀及其有关物质洛伐他汀的含量

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摘要：目的 建立测定辛伐他汀及其有关物质洛伐他汀的含量的反相高效液相色谱方法。**方法** 采用 Symmetry C_{18}（250 mm×4.6 mm，5 μm）色谱柱，流动相为乙腈-磷酸二氢钠缓冲液（pH 5.4）（65∶35），流速 1.0 mL·min^{-1}，检测波长为 238 nm。**结果** 洛伐他汀和辛伐他汀的线性范围分别为：0.3～3.0 μg·mL^{-1}，0.03～0.30 mg·mL^{-1}。平均加样回收率分别为：100.2%（RSD=1.5%）、99.4%（RSD=1.7%）。**结论** 本法简便快速，灵敏准确，可作为辛伐他汀生产过程质量控制方法。

关键词：高效液相色谱；辛伐他汀；洛伐他汀；含量测定

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Cardiovascular disease, in particular coronary heart disease (CHD), is the principal cause of morbidity and mortality. Elevated plasma total cholesterol and low-density lipoprotein cholesterol levels have been shown repeatedly to be predictive of premature CHD. The "statin" class of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors is among the most frequently prescribed agents for reducing morbidity and mortality related to CHD[1]. Lovastatin and simvastatin are two of HMG-CoA reductase inhibitors presently available. Lovastatin is a natural product which is derived from the fungus Aspergillus terreus. Simvastatin is produced by semi-synthetic processes from lovastatin. It is necessary to determine the content of lovastatin in the simvastatin bulk drug for the United States Pharmacopeia (USP29) limites the percentage of lovastatin in the bulk drug of simvastatin to be no more than 1%. A micellar electrokinetic chromatographic (MEKC) method has been developed for the quantification of lovastatin and simvastatin in pharmaceutical dosage forms[2]. Yang *et al.*[3] has reported a high performance liquid chromatographic procedure to determine the lovastatin impurity in the crude simvastatin product with O_{18}-zirconia column, CH_3OH/H_2O (70∶30) as mobile phase. To control the simvastatin quality in the process of synthesis, the RP-HPLC method was developed to simultaneously determine lovastatin and simvastatin in the simvastatin bulk drug on a common C_{18} column.

1 Apparatus and Chemicals

1.1 Instruments

The HPLC system consisted of Water 1525 binary pump and Waters 2487 dual wavelength UV detector, equipped with injection syringe of 20μL loop and a thermostat oven compartment. The data acquisition and peak integration were performed using the Breeze 1525 workstation.

1.2 Chemicals and reagents

Reference standards of lovastatin and simvastatin were obtained from Henan Topfond Pharmaceutical Co., Ltd. Acetonitrile (HPLC grade) was purchased from Tedia (USA). Sodium dihydrogen phosphate and sodium hydroxide were procured from Sinopharm Chemical Reagent Co., Ltd. De-ionized water was prepared through the self-made Ultra-Pure water system.

2 Methods and Results

2.1 Chromatographic conditions

The HPLC was performed on a Symmetry C_{18} column (4.6 mm×150 mm, 5 μm) with a liquid flow rate of 1.0 mL·min^{-1} under ambient condition. The mobile phase was the mixture of acetonitrile and 0.2 mol·L^{-1} sodium dihydrogen phosphate buffer (pH adjusted to 5.4 by NaOH) (65∶35). The detection wavelength was set at 238 nm. The column temperature was maintained at 25℃.

2.2 Solution preparation

Composite stock solution of lovastatin and simvastatin was prepared by dissolving accurately weighed amounts of the compounds in mobile phase to obtain a solution with concentrations of about 0.3 mg·mL^{-1} of simvastatin and 0.003 mg·mL^{-1} of lovastatin. Accurately weighed quantities of simvastatin bulk drug was dissolved in mobile phase and diluted to obtain the sample solution with a concentration of 0.1 mg·mL^{-1}. These solutions were filtrated with 0.45μm microfiltrate membrane before use.

2.3 Specificity and suitability of the method

No interfering peaks were observed in the chromatograms for both individual statins at the retention times of the analytes under destructive tests. The resolution R between simvastatin and lovastatin is about 2.24; the column efficiency is not less than 1000 theoretical plates; the tailing factor T is about 1.03.

2.4 Limit of detection (LOD) and limit of quantitation (LOQ)

The diluted composite stock solutions of lovastatin and simvastatin were injected and the lovastatin concentration of 5 ng·mL^{-1} resulted in the peak height of lovastatin approximately three times higher than baseline noise while 15 ng·mL^{-1} resulted in approximately 10 times higher than baseline noise. So the LOD and LOQ for lovastatin are 5 ng·mL^{-1} and 15 ng·mL^{-1}, respectively.

2.5 Calibration curve and linearity

Transfer 1.0, 3.0, 5.0, 7.0, 9.0, and 10.0 mL of composite stock solution of lovastatin and simvastatin to separate 10-mL volumetric flasks, and dilute with mobile phase to volume to obtain serials of standard solutions, respectively. Peak areas (y) of the lovastatin and simvastatin were measured and plotted against the concentration (x) of each statin. Each was repeated five times to get the average. The regression equations for lovastatin and simvastatin were $y = 8327.7x + 137$ ($r = 0.999\ 4$) and $y = 459153x - 1019$ ($r = 0.999\ 9$). The linearity of the calibration curves was in the range from 0.3 ~ 3.0 μg • mL^{-1} and 0.03 ~ 0.30 mg • mL^{-1} for lovastatin and simvastatin, respectively.

2.6 Precision tests of repeating injections

The sample solution of simvastatin was used for precision studies. Intra-assay variability and inter-assay variability were determined by analyzing samples for five times within the same day and over five consecutive days. The relative standard deviations (RSD%) were calculated for evaluation of the precision. The within-and between-day coefficients of variation were 0.65% and 0.79% ($n = 5$) for lovastatin, respectively, and 1.1% and 1.5% ($n = 5$) for simvastatin. This showed that the precision of this method for analysis of lovastatin and simvastatin in simvastatin bulk drug was satisfied.

2.7 Repeatability analysis

Six aliquots were prepared from the same batch of simvastatin sample according to the procedure described under item 2.2. The concentration of lovastatin and simvastatin were measured by recording and calculating the peak areas of HPLC chromatograms. The RSD of peak areas are 0.7% and 1.3%, for lovastatin and simvastatin, respectively. The results show that the HPLC method has good reproducibility.

2.8 Sample Stability test

The concentrations of lovastatin and simvastatin in the same batch of simvastatin sample were measured at 0, 4, 8, 12, and 16 h. The RSD of peak areas are 0.9% and 1.6%, for lovastatin and simvastatin respectively. The results indicate that the simvastatin samples are stable for at least 16 h.

2.9 Recovery studies

The simvastatin sample with known content of lovastatin and simvastatin was spiked with three different concentrations of lovastatin and simvastatin, respectively. Three aliquots for each concentration were processed as described under item 2.2. The peak areas of lovastatin and simvastatin were calculated with those obtained by calibration curves under item 2.5 and the recovery was obtained by compare the determined and added amount of each statin. The average recovery of this analytical method was 100.2% (RSD = 1.5%, $n = 9$) for lovastatin and 99.4% (RSD = 1.7%, $n = 9$) for simvastatin, respectively.

2.10 Assay of lovastatin and simvastatin in bulk drug

Under the optimum conditions, the developed RP-HPLC method has been applied to the determination of lovastatin and simvastatin in simvastatin bulk drug according to the procedure described above. The results were showed in Tab 1.

Tab 1 Results of sample determination ($n = 5$)

表 1 样品测定结果($n = 5$)

Batch number	Lovastatin/%	RSD/%	Simvastatin/%	RSD/%
20060306	1.15	1.3	97.96	1.2
20060307	0.97	1.9	98.32	1.1
20060308	0.72	2.7	98.49	1.7

3 Discussions

Both lovastatin and simvastatin tend to suffer from pH higher than 7, which can lead them to convert to their β-hydroxy acids easily. So the phosphate buffer with pH 5.4 was chosen to insure the stability and good peak shape in the course of determination. In fact, the assay results of the method are not influenced by variation of chromatographic conditions, including different columns, mobile phases with different pH (3.5 ~ 6.5).

The feasibility of different mixture of acetonitirile and phosphate buffer was tested for complete chromatographic resolution of two statins. Higher proportion of acetonitirile in the mobile phase resulted in a shorter retention time. Baseline separation of lovastatin and simvastatin was achieved within 40min (with retention time of 26.20 and 39.94min respectively), with mobile phase consisting of acetonitrile-buffer (50∶50). While using the mobile phase of acetonitrile-buffer (70∶30), two analytes could be separated in 10 minutes (with retention time of 6.47 and 8.63 min, respectively) with satisfactory resolution. To guarantee the optimum results the mobile phase of acetonitrile-buffer (65∶35) is suitable in this method.

The method is simple, sensitive, accurate, and repeatable with good robustness. It can be used to the quality control of synthetic simvastatin products.

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