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RESEARCH ARTICLE

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMVASTATIN USING LOVASTATIN AS INTERNAL STANDARD

Raghuram Reddy Adidala* Mounika Arrabelli, Jayanth Regula, Srinivas Nakka,

Synapse Life Sciences, Hanamkonda, Warangal, A.P., India.

ABSTRACT:

Simvastatin is a potent competitive inhibitor of 3hydroxy-3-methylglutaryl coenzyme A reductase, which is a rate-limiting enzyme in cholesterol biosynthesis. It may also interfere with steroid hormone production due to the induction of hepatic LDL receptors, it increases breakdown of LDL cholesterol. We have established a sensitive and accurate High performance liquid chromatographic method for determination of Simvastatin as per the ICH guidelines using specificity, linearity, limit of detection, limit of quantification, Precision and accuracy. Chromatography was performed with an analytical Inspire C_{18} column (250 mm x 2.0 mm, 5 µm), Shimadzu HPLC model with LC 10AD HPLC Pump and SPD 10A HPLC UV-Detector, and using acetonitrile: 0.1% glacial acetic acid (80: 20 % v/v) as the mobile phase. The linearity of Simvastatin is 0.999 over a concentration range of 0.1 to 10 µg/ml. Interday and intraday variability was < 10%.
Keywords: Simvastatin, Lovastatin, HPLC.

1. INTRODUCTION:

Simvastatin belongs to the statin drug family, the members of which are used as cholesterol-lowering agents for patients with hypercholesterolemia. This semisynthetic drug (Fig. 1) exhibits a very important hepatic first-pass metabolism by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA) and reduces low-density lipoproteins. Several methods can be employed in separation, purification and determination of these compounds[1]. The aim of our work is to develop simple, easy, cost effective and isocratic method for quantifying the Simvastatin after extraction from biological samples.

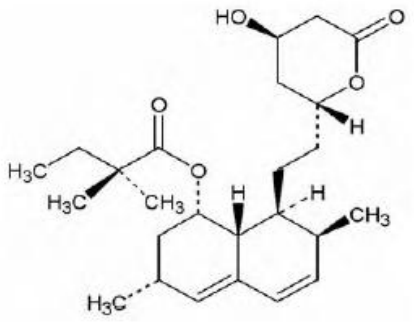

Figure 1: Structure of Simvastatin.

2. AIMS AND OBJECTIVES:

1. Preparation of standard solutions of drug and internal standard.

2. Recording chromatograms of the prepared standard solutions.

3. Method development and validation of RP-HPLC method for quantification of Simvastatin.

3. MATERIALS AND METHODS[2-7]:

3.1. Instrumentation:

HPLC method was performed using isocratic Shimadzu LC 10AD Pump with Shimadzu SPD 10 UV Detector operated by using Inspire C_{18} (250 x 4.5 mm, 5 µm) column with 20µL rheodyne injection volume.

3.2. Reagents and Chemicals:

Acetonitrile, Water and Glacial acetic acid were of HPLC grade obtained from Finar Chemicals (Ahmedabad, India). Simvastatin and Lovastatin were procured from Aurobindo Pharma ltd., and Lupin ltd., respectively as gift samples.

3.3. Mobile phase:

The mobile phase consists of acetonitrile and 0.1% glacial acetic acid in the ratio of 80:20 % v/v which was filtered through 0.45µ membrane filter and degassed before use and pumped into column at a flow rate of 1 ml/min. The detection wavelength was set to 240 nm and the total run time was 15 min.

* **Corresponding author:**

Raghuram Reddy Adidala
Head, Analysis Division, Synapse Life Sciences, Warangal, A.P., India. 506001.
Contact number- +91-9949783347
Email ID: - raghukpl@yahoo.com

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3.4. Standard Solutions:

Primary stock solutions of Simvastatin and Lovastatin (Internal standard) were prepared in methanol at a concentration of 1 mg/ml and stored at -20^0 C.

3.4.1. Standard Solutions:

From 1mg/ml solution of Simvastatin is pipetted out and diluted to different concentrations to obtain final concentrations of 0.1, 0.25, 0.5, 1, 2.5, 5 and 10 µg/ml and add 0.1 µg/ml of internal standard in common to all final concentrations.

3.5. Method Validation[8-11]:

Specificity, Linearity, Limit of Detection, Limit of Quantification, Precision and Accuracy was determined for the developed method.

a. Specificity:

The specificity method was used to determine any interference in the chromatogram during the retention time of both Simvastatin and Lovastatin (Internal standard).

b. Linearity:

The developed method was used for determining the linearity of Simvastatin ranging from the concentrations of 0.1 µg/ml to 10 µg/ml. Calibration curves were plotted by taking Peak area of Simvastatin on Y-axis and Concentration of corresponding Simvastatin on X-axis. The linearity was evaluated by linear regression analysis using least square method. The linearity was accepted if the r^2 was greater than 0.99.

c. LOD & LOQ Determination:

Lower limit of detection and lower limit of quantification were determined by using the parameters of standard error of estimate and slope calculated from linearity data of Simvastatin.

d. Precision and Accuracy:

In order to assess the intra- and inter-day precision and accuracy, Simvastatin at low (40ng/ml), medium (400 ng/ml) and high (4000 ng/ml) concentrations were prepared as described above. The intra-day precision of the assay was assessed by calculating the coefficient of variation (CV) for the analysis of samples in three replicates. And inter-day precision was determined by the analysis of samples on three consecutive days. Accuracy was calculated by comparing the measured values and the true values and expressed in percent.

4. RESULTS AND DISCUSSION:

4.1 Specificity:

Chromatograms of Simvastatin and I.S. (Lovastatin) were represented in the figure 2. No interference of endogenous peaks with the Simvastatin or Lovastatin at their respective retention time of Simvastatin was 6.5 min, Lovastatin was 5.4 min respectively.

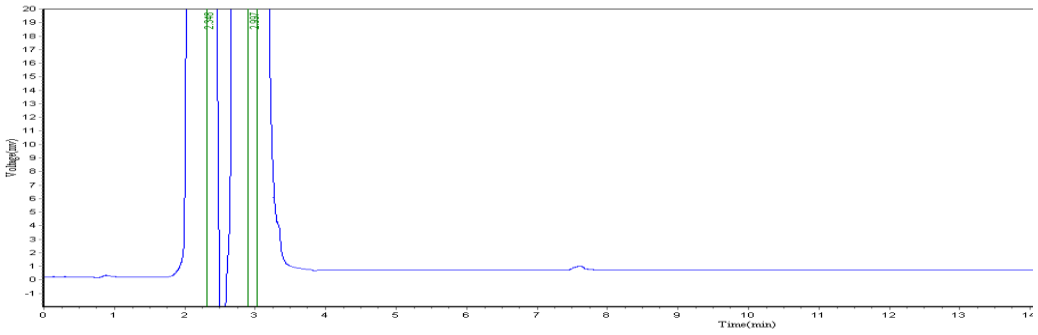

Figure 2: Blank sample

4.2 Linearity of calibration curve:

Linearity of Simvastatin ranging from the concentrations of 0.1 µg/ml to 10 µg/ml was determined. The regression equation was y= 0.214x + 0.059 with correlation coefficient r^2= 0.992, which shows that the method was linear represented in table-1 and figure-3.

Table 1: Linearity data of Simvastatin

Conc. (µg/ml)	Peak area
0.1	0.07635
0.25	0.09882
0.5	0.1659
1	0.2949
2.5	0.592
5	1.129
10	2.1977

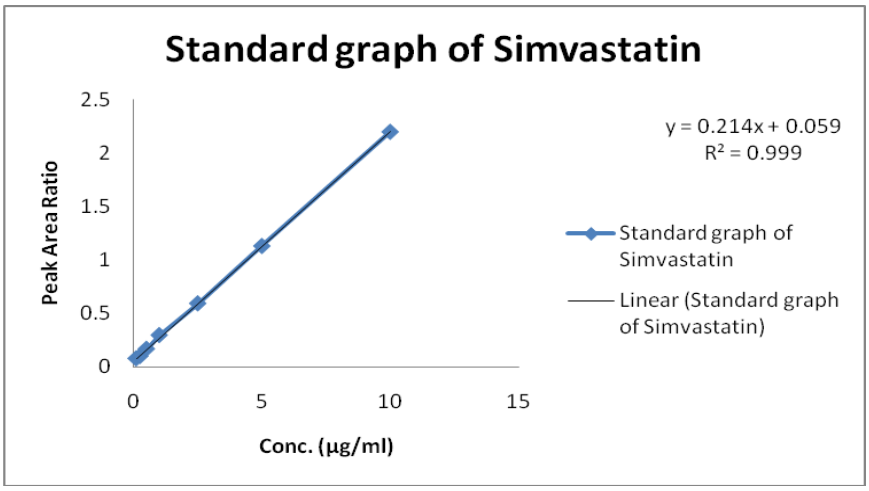

Figure 3: Linearity graph of Simvastatin.

4.3. LOD and LOQ:

The LOD and LOQ were calculated using the formula and the LOD and LOQ of Simvastatin was found to be 0.18 µg/ml and 0.54 µg/ml respectively.

4.4. Precision and Accuracy:

Intra- day accuracy of 40, 400 and 4000 ng/ml was found to be 92.95, 93.75 and 97.77 respectively and inter- day accuracy was found to be 89.17, 91.47 and 97.04 respectively. Therefore, the intra- and inter- day precision (% deviation) were within < ±6% for the LOQ. The intra- and inter-day assay precision (CV) ranged from 4.36 to 1.58 and 5.55 to 2.67 % respectively. These results showed in table-2 indicated that the present assay has very good accuracy and precision.

Table 2: Inter- and Intra-day variation of selected samples

INTRA DAY	*Trial 1*	*Trial 2*	*Trial 3*	*MEAN**	*SD**	*Accuracy %*	*CV%*
40	37.99	35.32	38.25	37.18	1.62	92.95	4.36
400	383	377	365	375	9.16	93.75	2.44
4000	3975.23	3899.19	3852.34	3908.92	62.02	97.77	1.58
INTER DAY							
40	36.37	33.44	37.22	35.67	1.98	89.17	5.55
400	377.33	368.11	352.23	365.89	12.69	91.47	3.47
4000	3985.22	3882.26	3777.2	3881.56	104.01	97.04	2.67

**Mean, SD (n = 3)*

5. CONCLUSION:

The HPLC method was developed using Lovastatin as an internal standard. The method was cost effective and easy method with high resolution and sharp peaks. Developed method was validated as per the ICH guidelines using specificity, linearity, limit of detection, limit of quantification, Precision and accuracy. As the developed method was found to be satisfactory and complies with all validated parameters, this method can be used for evaluation of Simvastatin.

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