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Development and Validation of Simvastatin by RP-HPLC Method In Bulk Drug and Pharmaceutical Dosage Forms

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ABSTRACT

A new, simple, specific, sensitive, rapid, accurate and precise RP-HPLC method was developed for the estimation of Simvastatin in bulk and pharmaceutical formulations. Simvastatin was chromatographed on a hypersil C18 column (250x4.6mm I.D., particle size 5 µm) in a mobile phase consisting of sodium dihydrogen phosphate and Acetonitrile in the ratio 40:60 v/v. The mobile phase was pumped at a flow rate of 1.0 ml/min with detection at 239 nm. The detector response was linear in the concentration of 10-200 µg/ml. The intra and inter day variation was found to be less than 2%. The mean recovery of the drug from the solution was 99.39%. The proposed method is simple, fast, accurate, precise and reproducible hence, it can be applied for routine quality control analysis of Simvastatin in bulk and pharmaceutical formulations.

Keywords: RP-HPLC, Simvastatin, precision, accuracy and formulation.

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INTRODUCTION

Simvastatin is 2, 2 –dimethyl butanoic acid (1S, 3R, 7S, 8S, 8aR) – 1, 2, 3, 7, 8, 8a-hexahydro-3, 7-dimethyl-8-[2- [(2R,4R) tetrahydro -4- hydroxyl- 6 -oxo-2H pyran-2- yl]ethyl-1-naphthalenyl ester1,2 belongs to the group of cholesterol-lowering lactones known as statins which, in 2011-12, were identified as being the most widely prescribed drugs in the world.[1, 2] Statins lower cholesterol by inhibiting the synthesis of mevalonic acid, which is a key precusor in cholesterol synthesis. Simvastatin, a lipid lowering agent that is derived synthetically from a fermentation product of Aspergillus terreus has been found to lessen both normal and elevated LDL-C concentrations. Literature survey reveals that, only few spectrophotometric methods and few analytical methods have been reported for the quantitative estimation of Simvastatin in bulk drug and pharmaceutical formulation.[3-6] Hence an attempt has been made to develop new HPLC methods for its estimation in bulk and pharmaceutical formulation with good precision, accuracy, linearity and reproducibility.[7-10]

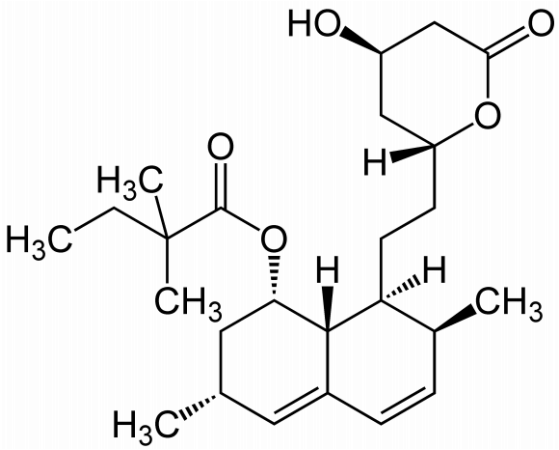


Figure 1: Structure of Simvastatin

MATERIALS AND METHODS

Instrumentation

An isocratic high pressure liquid chromatography (shimadzu HPLC winchrome - ES Series) with one LC-10 AT VP pumps, with UV/VIS detector SPD-10A VP, CTS-10 AS VP column oven (shimadzu), and a hypersil C-18 Column 250 mm x 4.6 mm i.d. particle size 5 µm) was used. The HPLC system was equipped with the software class class-vp (Shimadzu).

Chemicals and reagents

Simvastatin was obtained as a gift sample from Pharmatrain, an analytical testing centre, Hyderabad. Acetonitrile and water used were of HPLC grade (Qualigens), sodium dihydrogen phosphate, and sodium hydroxide were of analytical grade and supplied by SD Fine chem

limited, Mumbai. Commercially available Simvastatin tablets were procured from local market.

Chromatographic conditions

Mobile phase consists of buffer and acetonitrile in the ratio of 40:60. Buffer was prepared by dissolving 2.5 mg of sodium dihydrogen phosphate in 1000 ml HPLC water and the pH is adjusted to 6.8 with sodium hydroxide and filtered through 0.45µ membrane filter. The mobile phase was pumped from the solvent reservoir in the ratio of 40:60 to the column at a flow rate 1ml/min, whereas runtime was set to 14 min. The column was maintained at ambient and the volume of each injection was 20µl. prior to injection of the solutions, column was equilibrated for at least 30 min with mobile phase flowing through the system. The eluent were monitored at 239 nm.

Standard preparation

10 mg of Simvastatin was weighed and transferred into 10 ml volumetric flask containing 7 ml of mobile phase. The solution was sonicated for 15 min to dissolve the drug completely and the volume made up with mobile phase to get the concentration of 1 mg/ml solution. Further pipette out 1 ml of the above stock solution into 10 ml volumetric flask and dilute up to the mark with diluents.

Sample preparation

Weigh about five Simvastatin tablets and calculate the average weight. Accurately weigh and transfer the sample, equivalent to 10 mg of Simvastatin into 10 ml volumetric flask. Add about 7 ml of diluents and sonicate to dissolve it completely and make volume up to the mark with mobile phase. Mix well and filter through 0.45µm filter.

Linearity and calibration curve

To establish the linearity of analytical method, a series of dilutions ranging from 10-200 µg/ml were prepared in the same manner as described in earlier section. All the solutions were filtered through 0.22 µ membrane filter. The solutions were injected in triplicate into the HPLC column, keeping the injection volume constant (20 µl). Chromatograms (Figure 3) were recorded at 239 nm and calibration curve was plotted between the mean peak area vs. respective concentration (Figure 2)

Assay

25 tablets each containing 5mg, 10mg, 15mg of Simvastatin weighed accurately and powdered. A quantity equivalent to 1 mg of Simvastatin was weighed accurately and transferred to 100 ml volumetric flask containing approximately 50 ml of mobile phase. The contents were sonicated for 20 min and volume was made upto the mark with the mobile phase. The resulting solution

was filtered through a membrane filter. The solution obtained was then diluted with the mobile phase so as to obtain a concentration of 100 µg/ml. Sample solution was injected under the same chromatographic conditions and the chromatogram was recorded in triplicate. The amount of Simvastatin present in tablet formulation was determined by comparing the peak area from the standard. The results are furnished in Table 4.

METHOD VALIDATION

Validation of optimized HPLC method was done with respect to following parameters.

Linearity

The linearity of the developed method was performed with a concentration range of 10 to 200 µg/ml at RT 9.5.

Accuracy

Accuracy was evaluated by fortifying a mixture of degraded solution with three known concentrations of the drug. The recovery of added drug was determined by calculating the pre-analyzed drug concentration and correlating with the concentration of spike drug. In Table 1, shows that excellent recoveries were made at each added concentration, despite the fact that the drug was fortified to a mixture that contained drug as well as the degradation products, formed under various stress conditions.

Precision:

Intra-day precision: The procedure precision (intra-day repeatability) was established by analyzing three replicates over three concentrations of Simvastatin shown in Table 2.

Inter-day precision: As shown in Table 2 day to day precision (inter-day) was carried out by three concentrations with three replicates.

Robustness

The robustness study was done by making small changes in composition of mobile phase in the optimized method. There was no significant impact on the retention time and tailing factor. The data of robustness is given in Table 3.

Specificity and selectivity

Specificity is the ability of a method to discriminate between the intended analyte and other components in the sample. Specificity of from other potential components such as impurities, degradants, or excipients. The purity data of developed method is given in Table 3.

LOD and LOQ

The limit of detection and limit of quantification of the developed method were determined by injecting progressively low concentrations of the standard solutions found to be 0.018µg/ml and

0.06µg/ml respectively. The LOD and LOQ values reveal that the developed method shows very good sensitivity.

RESULTS AND DISCUSSION

Optimization of the chromatographic conditions were carried out with various combinations of buffer and Acetonitrile and by observing the peak parameters, the run time of the method was set at 14 min, Simvastatin appeared on the typical chromatogram at 9.5 min, which indicates a good base line. When the same drug solution was injected 3 times, the retention time of the drug was same. Linearity range was observed in the concentration range of 10-200 µg/ml. System suitability was determined by calculating the percent relative standard deviation (RSD) for area and retention time for six replicates injections of 100 µg/ml Simvastatin standard.

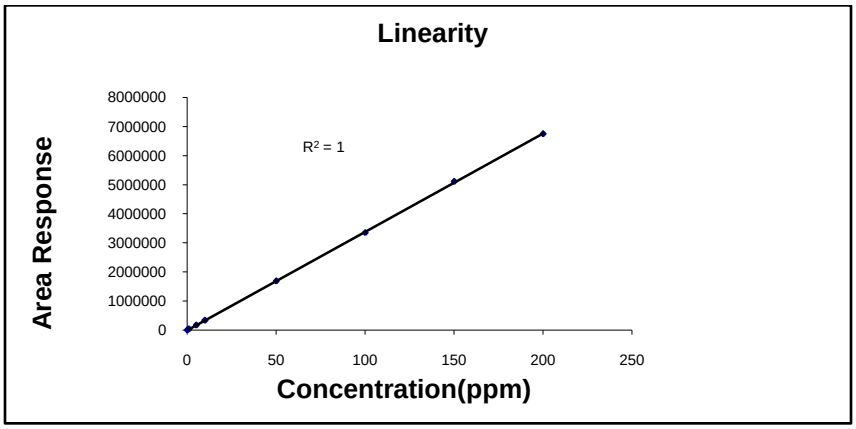
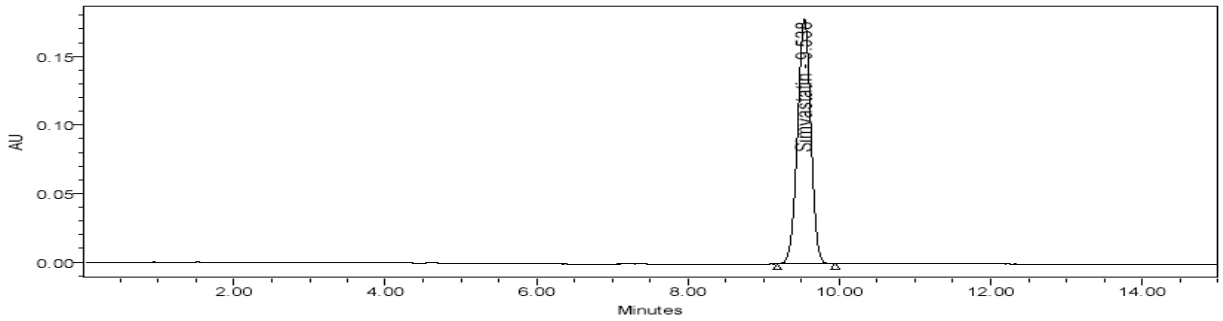


Figure 2: linearity curve

Table-1: Accuracy data for the HPLC assay of Simvastatin

Concentration/ Sample ID	**Amount Added(mg)**	**Amount Found(mg)**	**% Recovery**	**Statistical Analysis**	
80%-Sample 1	81.2	81.09	99.86	Mean	99.72
80%-Sample 2	81.6	81.13	99.43	SD	0.25
80%-Sample 3	82	81.9	99.87	% RSD	0.25
100%-Sample 1	101.6	101.34	99.74	Mean	99.32
100%-Sample 2	101	100.86	99.86	SD	0.82
100%-Sample 3	100.9	99.3	98.38	% RSD	0.82
120%-Sample 1	121.6	120.5	99.11	Mean	99.64
120%-Sample 2	121.3	121.2	99.91	SD	0.46
120%-Sample 3	120.9	120.8	99.91	% RSD	0.46
Overall Statistical Analysis					
Mean	SD		% RSD	Confidence Interval	
99.56	0.21		0.21	0.48	



	Peak Name	RT	Area	% Area	Height
1	Simvastatin	9.538	2161220	100.00	178526

Figure 3: Chromatogram for the estimation of Simvastatin

Table-2: Precision data for the HPLC assay of Simvastatin

Concentration	Sample	Area	Statistical Analysis	
100ppm	sample – 1	2199078	Mean	2212089
	sample – 2	2216055	SD	13997.1
	sample – 3	2233504	%RSD	0.6
	sample – 4	2200223	95% Confidence Interval	2197364
	sample – 5	2221713		
	sample – 6	2201963		

Table-3: Summary of validation parameters for the proposed method

SPECIFICITY	No Interference was found W.r.t. excipients
LINEARITY (R)	1
RANGE	80 – 120 % of test concentration
PRECISION (RSD)	
a) Repeatability (n=6) (system precision)	0.14
b) Intermediate precision (inter-analyst) (n=6)	0.32
c) Method precision (n=6)	0.6
ACCURACY (% Recovery)	99.56 ± 1.052
ROBUSTNESS(overall RSD)	
a) Change in wavelength	
• - 2nm	0.36
• + 2nm	0.45
a) Change in flow rate	
• - 0.02 units	0.35
• + 0.02 units	0.02
a) Change in column temp	
• - 2˚ C	0.59
• + 2˚ C	0.2
a) Change in organic conc	
• - 2 %	0.35
• + 2 %	0.51
a) Change in pH of buffer	
• - 0.1 units	64
• + 0.1 units	0.44
RUGGEDNESS (overall RSD)	0.44

Table 4: Results of Analysis of Commercial Tablets of Simvastatin

Tablet Formulation	label claim (mg)	% Label claim estimated* (Mean ± S.D.)	% Coeff. of Variation	Standard error
SIM I	5	99.435 ± 1.243	1.365	0.514
SIM II	10	99.754 ± 1.509	1.523	0.625
SIM III	15	99.246 ±1.427	1.305	0.613

*Average of six determinations

The area % RSD was calculated to be 0.6 and the retention time % RSD was calculated to be 0.209.). The proposed HPLC method was also validated for intra-day and inter-day variation. The accuracy result shows that the method gives % RSD of < 1. Robustness is the measure of the performance of a method when small, deliberate changes are made to the specified method parameters. The intention of robustness is to identify critical parameters for the successful implementation of the method. Robustness is partially evaluated during method development when conditions are optimized to improve resolution and other method performance criteria (e.g. peak shape, sensitivity). The % RSD values for precision (intra-day and inter-day) is less than 1%, indicating that the method is sufficiently precise. The limit of detection (LOD) and limit of quantitation (LOQ) of developed method were found to be 2 µg/ml and 10 µg/ml respectively. The method demonstrated linearity over a large range of concentration of 10-200 µg /ml. The use of buffer and Acetonitrile in the ratio of 40:60 v/v resulted in peak with good shape and resolution. The high percentage of recovery of Simvastatin ranging from 99.24-99.75 indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram indicating that excipients used in tablet formulation did not interfere with the estimation of the drug by proposed HPLC method.

CONCLUSION

The proposed HPLC method was found to be simple, rapid, sensitive, precise and accurate for the estimation of Simvastatin in pharmaceutical formulations. Hence, this method can easily and conveniently be adopted for routine quality control analysis of Simvastatin in bulk and pharmaceutical formulations.

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