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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR QUANTITATIVE ANALYSIS OF SIMVASTATIN IN PURE AND PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A simple, specific, accurate and precise reverse phase liquid chromatographic method was developed and validated for the determination of Simvastatin in pure and pharmaceutical formulations. A phenomenex C-18, 5 µm column having 250 mm x 4.6 mm internal diameter, isocratic mode with mobile phase containing ethanol: Acetonitrile: Water (30 : 30 : 40 v/v) was used. The flow rate was 1.0 mL/min. and effluents were monitored at 271 nm. The retention time of simvastatin was 2.05 min. The method was validated for parameters as per ICH guidelines. Due to its simplicity and accuracy, the method can be used for routine quality control of Simvastatin in bulk and pharmaceutical formulations.

Key words: Simvastatin, C_{18} column, Reverse Phase, Liquid chromatography, Validation, Specificity.

INTRODUCTION

Simvastatin[1-3] is a typical antihyperlipedimic drug. It is a white to off-white, non-hygroscopic, crystalline powder that is practically insoluble in water, and freely soluble in chloroform, methanol and ethanol[4]. An hypolipidemic drug used to control elevated cholesterol, or hypercholesterolemia, a member of statin class. Primary use of simvastatin is for the treatment of dyslipidemia and the prevention of cardiovascular disease.

Literature survey revealed that various methods have been reported for estimation of Simvastatin in biological matrices such as plasma with help of liquid chromatography[5-9].

Present study involves development of liquid chromatography method using simple mobile phase, which is and rapid for quantitation of simavastatin in tablet dosage forms as well as subsequent validation of developed method according to ICH guidelines.

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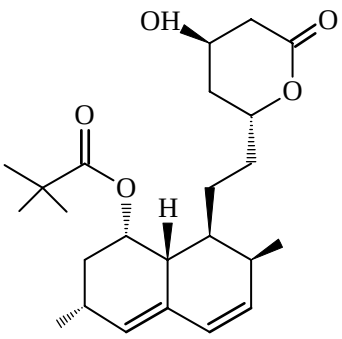


Simvastatin

[(1S,3R,7R,8S,8aR)-8-[2-[(2R,4R)-4-hydroxy-6-oxo-oxan-2-yl]ethyl]-3,7-dimethyl-1,2,3,.7,8,8a-hexahydronaphthalen-1-yl] 2,2-dimethylbutanoate

EXPERIMENTAL

Instrumentation

Quantitative HPLC was performed on a high pressure gradient High Performance Liquid Chromatograph) with two LC-10AT VP pumps, manual injector with loop volume of 20 µL (Rheodyne), programmable variable wavelength UV detector SPD-10A VP, CTO-10AS VP column oven (waters) and Phenomenex C_{18} column (250 mm length, 4.6 mm internal diameter and particle size 5 µm).

Standards and chemicals used

Simvastatin was provided Ardemin Biotech, Inematala Hyderabad. All the chemicals were HPLC grade: ethanol, acetonitrile, orthophosphoric acid and water.

Commercial tablets of Simvastatin were purchased from local market. Zocor product by Merck sharp and Dohme, limited 80 mg tablets.

Preparation of mobile phase

Into a 1000 mL cleaned volumetric flask, ethyl alcohol 300 mL, acetonitrile 300 mL and water 400 mL (which is filtered through 0.25 mm membrane filters by vacuum filtration) was slowly added, thoroughly mixed and then sonicated upto 20 min. Cool the above solution and pH was adjusted to 5.9 with orthophosphoric acid. This solution was again sonicated for about 10 min. Cool the solution to room temperature and used for chromatography method.

Preparation of standard drug solutions

100 mg of Simvastatin was accurately weighed and dissolved in few mL of mobile phase. After dissolving, volume was made upto 100 mL with mobile phase to obtain 1 mg/mL (free base) stock solution. This solution is further diluted with the same mobile phase to obtain required working standard concentrations.

Sample Preparations

20 commercial tablets (Zocor 80 mg tablets) of Simvastatin were finely powdered and the powder equivalent to 100 mg of simvastatin accurately weighed to a 100 mL volumetric flask and dissolved in few mL of mobile phase. The above solution was subjected to sonication for 15 min. After getting clear solution, the solution was filtered through 0.25 µm membrane filters and the solution was made upto 100 mL with mobile phase. The solution of concentration 1mg/1mL was obtained. This solution was further suitably diluted with the same solvent to obtain required concentrated solution of simvastatin pharmaceutical dosage form.

Recommended procedures

For bulk samples

The HPLC system was stabilized for thirty min. by passing mobile phase detector was set at 271 nm, flow rate of 1.0 mL/min to get a stable base line. One blank followed by six replicates of a single standard solution was injected to check the system suitability. Six replicates of each standard solutions 50, 60, 70, 80, 90 and 100 µg/mL were injected. The retention time and average peak areas were recorded. Calibration graph was plotted by taking concentration of Simvastatin on X-axis and peak areas on Y-axis. The amount of drug present in sample was computed from the calibration graph.

For pharmaceutical formulations

The content of twenty tablets was transferred into a mortar and ground to a fine powder. From this tablet powder, equivalent to 100 mg was taken and the drug was extracted with 100 mL of mobile phase. The resulting solution was filtered through whatmann filter paper and degassed by sonication. This solution was further suitably diluted for chromatography. Working sample solutions were prepared and the procedure described under bulk samples was followed.

Optimized chromatographic conditions

Table 1: Chromatographic conditions for Simvastatin

S. No.	Test	Result
1	Type of Elution	Isocratic
2	Stationary Phase	Phenomenex C 18 column (length: 250 mm, Internal diameter 4.6 mm, Particle size: 5 µm)
3	Mobile Phase	EtOH : CAN : Water (30 : 30 : 40)
4	Flow Rate	1 mL/min
5	Runtime	7 min.
6	Column Temperature (^oC)	Ambient
7	Volume of injection	20 µL
8	Detection Wave Length	By UV at 271 nm
9	Retention Time	2.05

RESULTS AND DISCUSSION

The goal of this study is to develop rapid HPLC methods for the analysis of Simvastain in bulk drug samples and tablet formulations using the most commonly employed column (C_{18}) with UV detection at appropriate wavelength. The representative chromatogram indicating the Simvastatin is shown in Fig. 1.

Parameter fixation

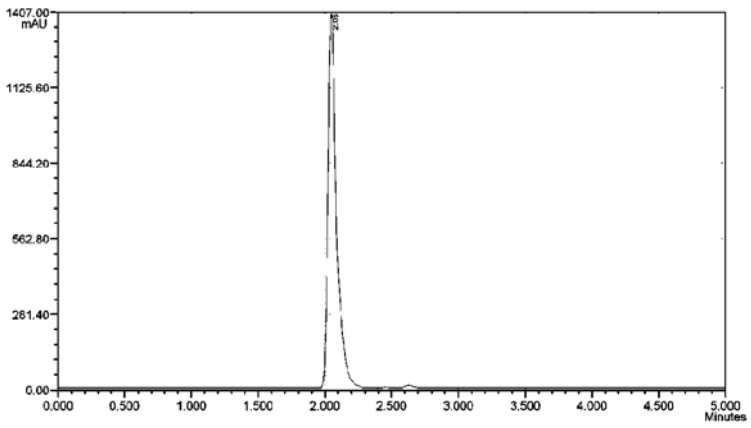
In developing these methods, a systemic study of effects of various parameters was undertaken by varying one parameter at a time and controlling all other parameters. The following studies were conducted for this purpose.

Stationary phase characteristics

Simvastatin is having solubility in aqueous solvents buffered to pH 5.9. Based on the solubility characteristics, the reverse Phase mode of HPLC was selected for chromatography. Among the different RP-HPLC stationary phases tried C_{18} was found to be optimum.

Mobile phase characteristics

In order to get narrow peaks and base line, the authors have carried out number of experiments by varying different components like percentage of components in mobile phase and flow rate by changing one at a time and keeping all other parameters constant.



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1		2.050	141132	648170.9	100.000	1.47	3971
	Sum:		141132	648170.9	100.0000		

Fig. 1: Chromatogram of Simavastatin 80 µg/mL

Method validation

An Integral part of analytical method development is validation. Once the method has been devised, it is necessary to evaluate under the conditions expected for real samples before being used for a specific purpose. The following parameters should be evaluated.

Specificity

The effect of wide range of excipients and other additives usually present in the formulation of simvastatin in the determinations under optimum conditions was investigated. In fact, many have no absorption at this UV maximum. Chromatographic parameters maintained are specific for simvastatin.

Precision

The precision of the method was ascertained from the peak area of simvastatin obtained by determination of six replicates of fixed amount of Simvastatin. The percent relative standard deviation were calculated and were presented in Table 2.

Table 2: Simvastatin precision values

Test	Precision (Day 1)	Precision (Day 2)
Injection	**Area**	**Area**
1	648170	646008
2	648190	646159
3	648693	646329
4	648761	646812
5	647864	639518
6	647947	639620
	$R.S.D_1 = 0.058$	$R.S.D_2 = 0.534$

Linearity

The linearity graphs for the proposed assay methods were obtained over the concentration range of 50-100 µg/mL Simvastatin method of least square analysis was carried out for getting the slope, intercept and correlation coefficient values and the results were presented in Table 3.

Table 3: Linearity range for simvastatin

S. No.	Conc. (µg/mL)	Area
1	50	393713
2	60	471344
3	70	554767
4	80	648170
5	90	714206
6	100	780594

Recovery

Percentage recoveries of drug from spiking known amounts of standard in to the solution. The recoveries of standard drug was shown in Table 4.

Table 4: Simvastatin recovery values

Recovery	Conc. of sample	Recovery	% of recovery
50 %	50 µg/mL	50.0013	100.005
75 %	75 pm	75.09	100.12
100%	100 µg/mL	99.834	99.834

Robustness

Percent recoveries of Simvastatin was good under most conditions and didn't show any significant change, when the critical parameters were modified. The tailing factor for Simvastatin was always less than 2.0 and the component is well eluted under all the changes carried out. Considering the modifications in the system suitability parameters and the specificity of the method, as well as carrying the experiment at room temperature may conclude that the method conditions were robust.

Detection limit and quantitation limit

A Calibration curve was prepared using concentrations in the range of 50-100 µg/mL (expected detection limit range). The standard deviation of Y-intercepts of regression line was determined and kept in following equation for the determination of Detection limit and Quantitation limit.

Table 5: Simvastatin LOD and LOQ Values

Test	Values
L.O.Q	2.5 µg/mL
L.O.D	0.75 µg/mL

CONCLUSION

Statistical analysis of the results shows that the proposed RP- HPLC procedure has good precision and accuracy. Results of analysis of pharmaceutical formulations reveal that the proposed method is suitable for their analysis with virtually no interference of the usual additives presented in pharmaceutical formulations. This method can be adopted for routine quality control analysis of Simvastatin in pure and pharmaceutical formulations.

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