WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.421
Volume 8, Issue 2, 1390-1401 Research Article ISSN 2278 – 4357

METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF SIMVASTATIN IN BULK AND TABLET DOSAGE FORM BY RP-HPLC

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Article Received on
23 December 2018,

Revised on 13 Jan. 2019,
Accepted on 03 Feb. 2019

DOI: 10.20959/wjpps20192-13203

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ABSTRACT

A simple, rapid, and precise reversed-phase high-performance liquid chromatographic method for estimation Simvastatin in bulk and tablet dosage form has been developed and validated as per ICH Guidelines. Chromatographic separation was performed on a Waters ODS (C18) RP Column, 250 mm x 4.6 mm. 5µm column with Acetonitrile: Methanol (70:30) as mobile phase at a flow rate of 1.0 mL min−1. UV detection was performed at 242nm. Total run time was 6.0 minutes is eluted with retention time was found to be 3.816minutes. The method was validated for accuracy, precision, linearity, specificity, and sensitivity in accordance with ICH guidelines. Validation revealed the method is specific, rapid, accurate, precise, reliable, and reproducible. Calibration plots were linear over the concentration ranges 0–50µg mL−1 for Simvastatin. Limit of detection were 0.09µg mL−1 and limit of quantification were 0.26µg mL−1 for Simvastatin. The high recovery and low coefficients of variation confirm the suitability of the method for estimation of Simvastatin in bulk and tablet dosage form. The developed method offers several advantages in terms of simplicity in mobile phase, mode of elution, easy sample preparation steps and comparative short run time which makes the method specific and reliable for its intended use in routine analysis of estimation of Simvastatin in bulk and tablet dosage form.

KEYWORDS: Simvastatin, Method Development, Validation, Accuracy, Precision.

1. INTRODUCTION

Simvastatin is a lipid-lowering agent.[1,2,3] that is derived synthetically from the fermentation of Aspergillus terreus. It is a potent competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase (hydroxyl methyl glutaryl COA reductases), which is the 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. For the treatment of hypercholesterolemia and for the reduction in the risk of cardiac heart disease mortality and cardiovascular events. It can also be used in adolescent patients for the treatment of heterozygous familial hypercholesterolemia. Simvastatin is a prodrug.[4,5] in which the 6-membered lactone ring of simvastatin is hydrolyzed in vivo to generate the beta, delta-dihydroxy acid, an active metabolite structurally similar to HMG-CoA (hydroxymethylglutaryl CoA). Once hydrolyzed, simvastatin competes with HMG-CoA for HMG-CoA reductase, a hepatic microsomal enzyme.[6] Interference with the activity of this enzyme reduces the quantity of mevalonic acid, a precursor of cholesterol. Simvastatin, the methylated form of lovastatin, is an oral antilipemic agent which inhibits HMG-CoA reductase. Simvastatin is used in the treatment of primary hypercholesterolemia,[7,8] and is effective in reducing total and LDL-cholesterol as well as plasma triglycerides and apolipoprotein B. The IUPAC,[9] Name for Simvastatin is (1S,3R,7S,8S,8aR)-8-{2-[(2R,4R)-4-hydroxy-6-oxooxan-2-yl]ethyl}-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2-dimethylbutanoate. The molecular formula,[10] of Simvastatin is $C_{25}H_{38}O_5$. The molecular weight is 418.5662g/mol. The chemical structure of Simvastatin shown in following Fig-1.

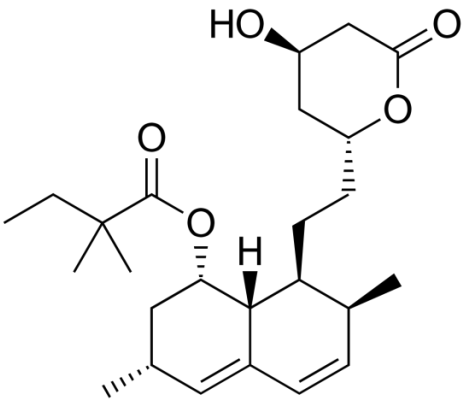

Fig. 1: Structure of Simvastatin.

2. MATERIALS AND METHODS

2.1. Instruments

Table 1: List of Instruments.

S. No.	Instruments/Equipments/Apparatus
1.	**HPLC** with Empower2 Software with Isocratic with UV-Visible Detector.
2.	**ELICO SL-159** UV – Vis spectrophotometer
3.	Electronic Balance **(SHIMADZU ATY224)**
4.	Ultra Sonicator **(Wensar wuc-2L)**
5.	**Waters ODS (C_{18}) RP Column, 250 mm x 4.6 mm. 5µm**
6.	P^H Analyzer **(ELICO)**
7.	Vacuum filtration kit **(BOROSIL)**

2.2. Chemicals / Reagents

Table 2: List of Chemicals.

S. No.	Name	Specifications		Manufacturer/Supplier
		Purity	Grade	
1.	Doubled distilled water	99.9%	HPLC	Sd fine-Chem ltd; Mumbai
2.	Methanol	99.9%	HPLC	Loba Chem; Mumbai.
3.	Acetonitrile	99.9%	HPLC	Loba Chem; Mumbai.
4.	Potassium dihydrogen orthophosphate	99.9%	L.R.	Sd fine-Chem ltd; Mumbai
5.	Triethyl amine	99.9	L.R.	Sd fine-Chem ltd; Mumbai
6.	Glacial acetic acid	99.99	L.R	Sd fine-Chem ltd; Mumbai

3. Method Development

3.1 HPLC Instrumentation & Conditions

The HPLC system employed was **WATERS** with Empower2 Software with Isocratic[11] with UV-Visible Detector.

3.2 Initialization of the instrument

The HPLC instrument was switched on. The column was washed with HPLC water for 45 minutes. The column,[12] was then saturated with mobile phase for 45 minutes. The mobile phase was run to find the peaks. After 20 minutes the standard drug solution was injected in HPLC.[13]

3.3. Mobile Phase Preparation

The mobile phase used in this analysis consists of a mixture of Acetonitrile and Methanol in a ratio of 70:30.

700 ml of Acetonitrile solution was added and properly mixed with 300 ml of Methanol and a homogenous solution is achieved. This mobile phase was filled and sonicated[14] for 15 minutes before using in the experiment.

3.4. Sample & Standard Preparation for the Analysis

25 mg of Simvastatin standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase.

Further dilution was done by transferring 0.3 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase.[15]

3.5 Diluent

Mobile phase can be used as diluent.[16]

3.6. Optimization of Chromatographic Conditions

The chromatographic conditions were optimized by different means. (Using different column, different mobile phase, different flow rate[17], different detection wavelength[18] & different diluents for sample preparation[19] etc.

Table 3: Summary of Optimization of Process.

Column Used	Mobile Phase	Flow Rate	Wave length	Observation	Result
Waters ODS (C18) RP Column, 250 mm x 4.6 mm. 5µm	Methanol : Water = 50 : 50	0.8 ml/min	242 nm	Broad Peak	Method rejected
Waters ODS (C18) RP Column, 250 mm x 4.6 mm. 5µm	Acetonitrile : Water = 60 :40	1.0 ml/min	242 nm	Peak broken at the end	Method rejected
Waters ODS (C18) RP Column, 250 mm x 4.6 mm. 5µm	ACN: Acetate buffer = 60 : 40	1.0 ml/min	242 nm	Splitting of peak	Method rejected
Waters ODS (C18) RP Column, 250 mm x 4.6 mm. 5µm	Acetonitrile: Methanol (50:50)	1.0 ml/ min	242 nm	Splitting of peak	Method rejected
Waters ODS (C18) RP Column, 250 mm x 4.6 mm. 5µm	Acetonitrile: Methanol (60:40)	1.0 ml/min	242 nm	Broad Peak	Method rejected
Waters ODS (C18) RP Column, 250 mm x 4.6 mm. 5µm	Acetonitrile: Methanol (70:30)	1.0 ml/min	242 nm	Good sharp peak	Method accepted

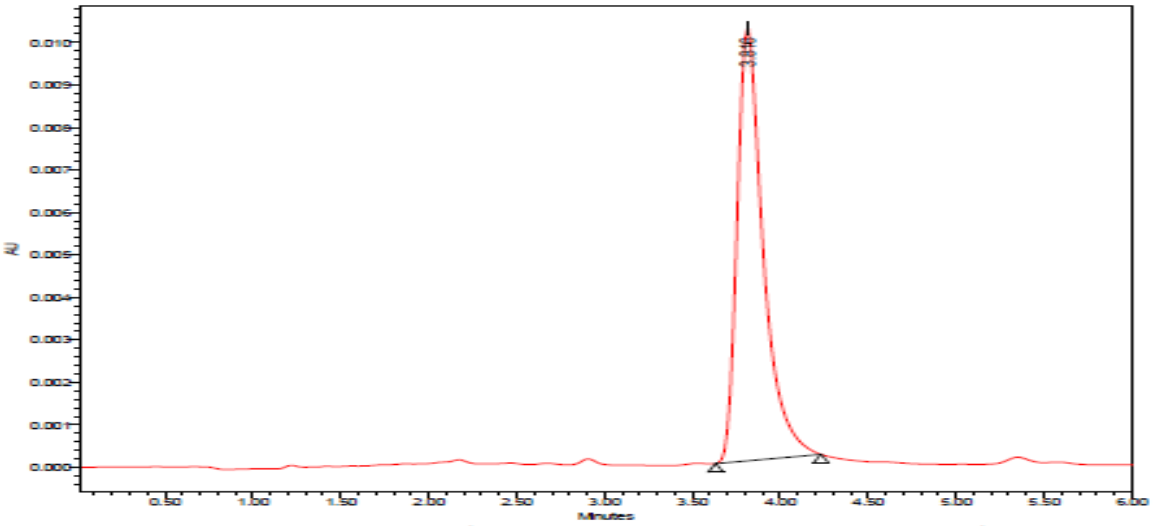

Fig. 2: Optimised Chromatographic Condition.

4. Method Validation

1. Accuracy

Recovery study

To determine the accuracy[20] of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of pure drug of Simvastatin were taken and added to the pre-analyzed formulation of concentration 30µg/ml. From that percentage recovery values were calculated. The results were shown in Table-4.

Table 4: Accuracy Readings.

Sample ID	Concentration (µg/ml)			% Recovery of Pure drug	Statistical Analysis
	Conc. Injected	Conc. Recovered	Peak Area		
S_1 : 80 %	24	24.022	472546	101.916	Mean= 101.3317
S_2 : 80 %	24	23.937	471121	101.736	S.D. = 0.860928
S_3 : 80 %	24	24.206	475612	100.343	% R.S.D.= 0.849614
S_4 : 100 %	30	30.103	574216	100.113	Mean= 101.018
S_5 : 100 %	30	30.521	581211	101.394	S.D. = 0.787478
S_6 : 100 %	30	30.575	582121	101.547	% R.S.D.= 0.779542
S_7 : 120 %	36	36.041	673514	100.858	Mean= 100.2287
S_8 : 120 %	36	36.502	681214	99.737	S.D. = 0.57304
S_9 : 120 %	36	36.557	682132	100.091	% R.S.D.= 0.571732

2. Precision

2.1 Repeatability

The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of six replicates of a fixed amount of drug.

Simvastatin (API) the percent relative standard deviations[21] were calculated for Simvastatin is presented in the Table-5.

Table 5: Repeatability Results of Precision.

HPLC Injection Replicates of Simvastatin	Retention Time (Minutes)	Peak Area
Replicate – 1	3.816	598647
Replicate – 2	3.815	586484
Replicate – 3	3.799	584624
Replicate – 4	3.797	598642
Replicate – 5	3.815	584213
Replicate -6	3.816	579874
Average	**3.809667**	**588747.3**
Standard Deviation	**0.00907**	**7966.538**
% RSD	**0.238081**	**1.353134**

2.2 Intermediate Precision

2.2.1 Intra-assay & inter-assay

The intra & inter day variation of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Simvastatin revealed that the proposed method is precise.[22]

Table 6: Results of intra-assay & inter-assay.

Conc. Of Simvastatin (API) (µg/ml)	Observed Conc. Of Simvastatin (µg/ml) by the proposed method			
	Intra day		Inter day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
24	23.98	1.03	24.02	1.02
30	30.06	0.97	30.09	0.97
36	36.03	0.95	35.93	0.65

3. Linearity and Range

The calibration curve showed good linearity in the range of 10-50µg/ml, for Simvastatin (API) with correlation,[23] coefficient (r^2) of 0.999 (Fig-3). A typical calibration curve has the regression equation of y = 16721x + 70860 for Simvastatin.

To evaluate the linearity, serial dilution of analyte were prepared from the stock solution was diluted with mobile phase to get a series of concentration ranging from 10, 20, 30, 40 and 50µg/ml. The prepared solutions were filtered through whatmann filter paper (No.41). From these solutions, 20µl injections of each concentration were injected into the HPLC system and chromatographed,[28] under the optimized conditions. Calibration curve was constructed

by plotting the mean peak area (Y-axis) against the concentration (X-axis). The results which are given in Table below were within acceptable limits.

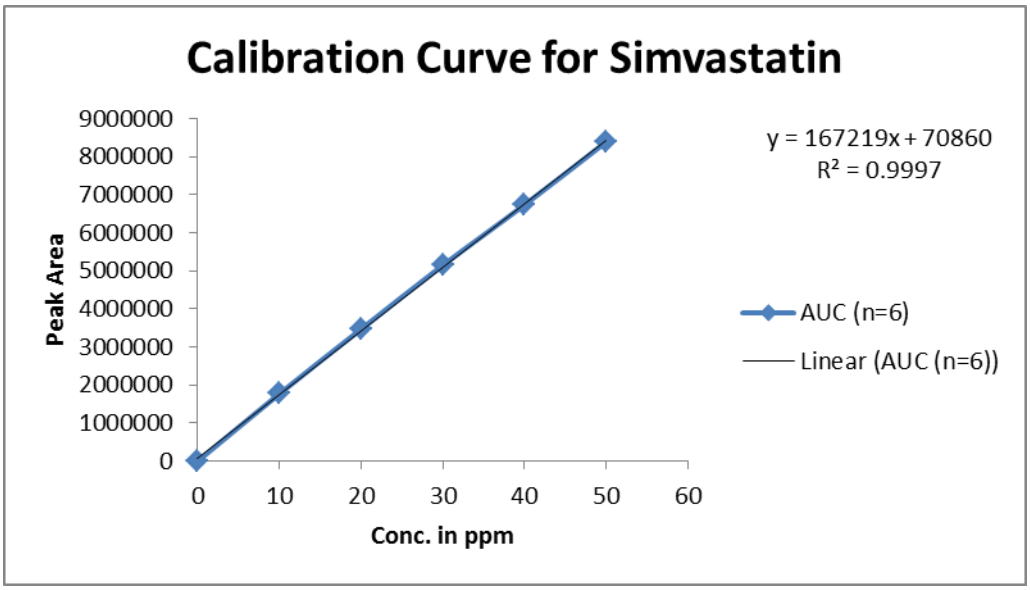

Fig. 3: Calibration curve of Simvastatin (API).

Table 7: Linearity results of Simvastatin.

CONC.	AUC (n=6)
0	0
10	1768452
20	3468421
30	5146243
40	6735124
50	8389756

4. Method Robustness

Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), Temperature (±2^{0}C), Wavelength of detection (±2nm) & Acetonitrile content in mobile phase (±2%) studied to determine the robustness[24] of the method are also in favour of (Table-8, % RSD < 2%) the developed RP-HPLC method for the analysis of Simvastatin (API).

Table 8: Results of Method Robustness Test.

Change in parameter	% RSD
Flow (1.1 ml/min)	0.26
Flow (0.9 ml/min)	0.09
Wavelength of Detection (244 nm)	0.13
Wavelength of detection (240 nm)	0.18

5. LOD & LOQ

Limit of detection is the lowest concentration of analyte in a sample which can be detected, but not necessarily quantitated, as an exact value under the stated experimental conditions.

Limit of quantification is the lowest concentration of analyte in a sample which can be quantitatively determined with acceptable precision and accuracy under the stated experimental conditions.

The LOD and LOQ,[25] were calculated by the use of the equations LOD = 3.3 × σ / S and LOQ = 10 × σ / S where σ is the standard deviation of intercept of Calibration plot and S is the average of the slope of the corresponding Calibration plot.

The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.09 & 0.26µg/ml respectively.

6. Assay of Simvastatin In Dosage Form

Simvastatin 20 mg

Twenty tablets were taken and the I.P. method was followed to determine the average weight. Above weighed tablets were finally powdered and triturated well. A quantity of powder equivalent to 100 mg of drugs were transferred to 100 ml volumetric flask, and 70 ml of HPLC grade methanol was added and solution was sonicated for 15 minutes, there after volume was made up to 100 ml with same solvent. Then 10 ml of the above solution was diluted to 100 ml with HPLC grade methanol. The solution was filtered through a membrane filter (0.45 µm) and sonicated to degas. From this stock solution (3.5 ml) was transferred to five different 10 ml volumetric flasks and volume was made up to 10 ml with same solvent system.[26]

The solution prepared was injected in five replicates into the HPLC system and the observations were recorded.

A duplicate injection of the standard solution was also injected into the HPLC system and the peak areas were recorded. The data are shown in Table-9.

ASSAY

Assay % =

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \text{Avg. Wt} = \text{mg/tab}$$

Where:

AT = Peak Area of drug obtained with test preparation

AS = Peak Area of drug obtained with standard preparation

WS = Weight of working standard taken in mg

WT = Weight of sample taken in mg

DS = Dilution of Standard solution

DT = Dilution of sample solution

P = Percentage purity of working standard

Assay,[27] was performed as described in previous chapter. Results obtained are tabulated below

Table 9: Assay of Simvastatin Tablets.

Brand name of Tablets	Labelled amount of Drug (mg)	Mean (±SD) amount (mg) found by the proposed method (n=6)	Mean (± SD) Assay (n = 6)
Simcard (Cipla Pharmaceuticals Limited)	20	19.86 (±0.06)	99.35 (±0.48)

Result & Discussion: The assay of SIMCARD Tablets containing Simvastatin was found to be 99.35 (±48) %.

5. RESULTS AND DISCUSSION

To develop a precise, linear, specific RP-HPLC method for analysis of Simvastatin different chromatographic conditions were applied & the results observed are presented in the thesis.

Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution.

In case of RP-HPLC various columns are available, but here Waters ODS (C18) RP Column, 250 mm x 4.6 mm. 5µm column was preferred because using this column peak shape, resolution and absorbance were good.

Mobile phase & diluent for preparation of various samples were finalized after studying the solubility of API in different solvents of our disposal (methanol, Acetonitrile, dichloromethane, water, 0.1N NaOH, 0.1NHCl). Simvastatin was found to be soluble in ethanol, DMSO, and dimethyl formamide (DMF).

Detection wavelength was selected after scanning the standard solution of drug over 200 to 800nm. From the U.V spectrum of Simvastatin it is evident that most of the HPLC work can be accomplished in the wavelength range of 215-290 nm conveniently. Further, a flow rate of 0.8 ml/min & an injection volume of 20 µl were found to be the best analysis.

The result shows the developed method is yet another suitable method for assay which can help in the analysis of Simvastatin in different formulations.

6. CONCLUSION

In the present work RP-HPLC method for estimation Simvastatin has been developed. The proposed methods are precise, accurate and do not suffer from any interference due to common excipients.

The validation parameters according to I.C.H Q2B guidelines were studied. The accuracy of the methods was proved by performing recovery studies in newly developed formulations. Values greater than 98% indicate that the proposed method is accurate for the analysis of drug.

A sensitive & selective stability indicting RP-HPLC method has been developed & validated for the analysis of Simvastatin API.

The result shows the developed method is yet another suitable method for assay and stability studies which can help in the analysis of Simvastatin different formulations.

Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility.

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