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Validation of Assay Indicating Method Development of Simvastatin in Bulk and its Tablet Dosage form by RP-HPLC

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ABSTRACT

A novel, simple and economic reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for the estimation of Simvastatin in bulk and tablet dosage form with greater precision and accuracy. Separation was achieved on Develosil ODS HG-5 RP C_{18}, (150cmx4.6mm i.d. 5µm) column in isocratic mode with mobile phase consisting of acetonitrile :phosphate buffer(pH 3.0) (85:15) with a flow rate of 1 mL/min. The detection was carried out at 236 nm. The retention time of Simvastatin was found to be 5.84 min. The method was validated as per ICH guidelines.Linearity was established for Simvastatin in the range 10 – 100 µg / ml with R^2 value 0.99. The percentage recovery of Simvastatin was found to be in the range 99.19-99.67 %. The high recovery and low relative standard deviation confirm the suitability of the proposed method for the estimation of the drug in bulk and tablet dosage forms. The LOD and LOQ were found to be 0.341 and 1.023 µg/ml respectively.Validation studies demonstrated that the proposed RP-HPLC method is simple, specific, rapid, reliable and reproducible for the determination of Simvastatin for Quality Control level.

INTRODUCTION

Simvastatin is chemically (1S,3R,7S,8S,8aR)-1,2,3,7,8, 8a-Hexahydro-3, 7-dimethyl-8-{2-[(2R,4R)-tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl] ethyl}-1-naphthyl-2,2-dimethyl butyrate (Fig.1) is obtained from the fermentation of Aspergillus terreus. This compound, acts as a highly potent and effective cholesterol-lowering agent, is being used in the control of hypercholesterolemia.

It exhibits a very important hepatic first-pass metabolism, acting by blocking the 3-hydroxy-3- methylglutaryl coenzyme A reductase (HMG-CoA), and thereby reducing the low-density lipoproteins. Simvastatin is a potent inhibitor of HMG-CoA reductase, which is a rate limiting enzyme in cholesterol bio-synthesis (Srinivas *et al.*, 2012). Several methods based on different techniques have been reported for the determination of Simvastatin in biological fluids. These methods include high-performance liquid chromatography/mass spectrometry (LC/MS) (Barrett *et al.*, 2006; Ramakrishna et al., 2007; Basavaiah and Devi, 2008; Yang *et al.*, 2005; Bhavin *et al.*, 2008) and gas chromatography/mass spectrometry (GC/MS) (Takano *et al.*,1990) and high performance liquid chromatography (HPLC) (Fabio *et al.*,2009; Lucie *et al.*, 2008; Carlucci *et al.*,1992; Nagaraju and Vishnuvardhan,2010; Ochiai *et al.*, 1997; Muhammad *et al.*,2008; Muhammad *et al.*,2007). Although these methods are sensitive to permit their use in determination of Simvastatin in urine, plasma or serum, but only few methods are reported for assay of Simvastatin in pharmaceutical formulations. Among them, HPLC methods have been described using expensive reagents or buffers in the mobile phase (Sushil *et al.*, 2011; Kavitha *et al.*, 2011; Abu *et al.*, 2006; Pranav *et al.*, 2011; Kanakapura and Kalsang, 2008) Based on the facts the study was aimed to develop, validate and compare simple, economic and fast analytical methods which can be easily applied in routine analysis for the determination of Simvastatin in lipid based systems.

EXPERIMENTAL

Materials

Pure Simvastatin used as working standards, was obtained from Aurobindo pharma, Hyderabad, India. Tablet containing

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Simvastatin (Biosim 20) was obtained from Apollo Pharmaceuticals Pvt. Ltd, Visakhapatnam, India and used within its shelf life period. Acetonitrile and water (HPLC-grade) were purchased from Loba chem.., Mumbai, India. All other chemicals and reagents employed were of analytical grade, and purchased from Desai chemicals, Visakhapatnam India.

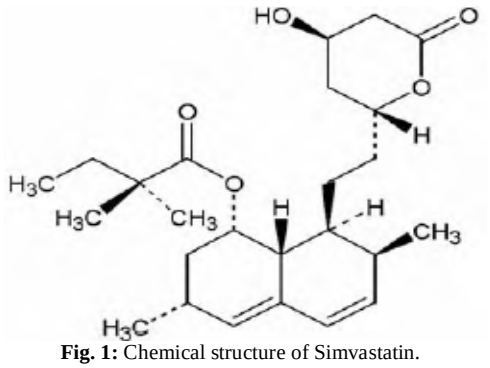

Fig. 1: Chemical structure of Simvastatin.

INSTRUMENTATION

The chromatographic system (Model No.1575) used comprised of Hitachi L2130 with UV-Vis detector. Data integration was carried out using A-4000 version software. Samples were injected into Develosil ODS HG-5 RP C_{18} (5µm, 15cmx4.6mm i.d) column. An Analytical technologies ltd.sonicator was used for enhancing the dissolution of the compounds. A Wenster digital pH meter was used for pH adjustment.

CHROMATOGRAPHIC CONDITIONS

The high performance liquid chromatographic (HPLC) system used was operated isocratically with the column temperature maintained at 30̊ C, using a mobile phase composition of acetonitrile and phosphate buffer (pH adjusted to 3.0 with O-Phosphoric acid) in the ratio of 85:15 v/v at a flow rate of 1.0 mL/min within a run time of 10 min. Prior to use, the mobile phase was degassed by an ultrasonic bath and filtered by a Millipore vacuum filter system equipped with a 0.45 µm high vacuum filter. The drug was detected and quantified at 236 nm.

PREPARATION OF STANDARD SOLUTIONS

The stock solution was prepared by transferring 100 mg of Simvastatin into 100 mL volumetric flask. Then it was added with small amount of diluent, and the mixture was sonicated to dissolve and made up to volume with mobile phase. From this stock solution different concentrations were prepared to give final concentrations of 10 – 100 µg/mL for calibration of standard curve.

ASSAY OF SIMVASTATIN FROM MARKETED TABLETS

Twenty tablets were accurately weighed and crushed to a fine powder in a mortar for the marketed formulation. An amount of the powder equivalent to 100 mg was transferred into a 100 mL volumetric flask and 15 mL of diluent was added to it followed by 10 ml of methanol. The mixture was sonicated to dissolve the exipients and then made up to volume with mobile phase. Following 15 min of mechanical shaking, it was kept in an ultrasonic bath for 15 mins, and the solution was filtered through a 0.45 µm filter paper. Suitable aliquots of the filtered solution were transferred to a volumetric flask and made up to volume with mobile phase to yield six concentrations of Simvastatin (30 µg/mL). A 20 µL volume of the sample solution was injected into the chromatographic system, six times, under optimized chromatographic conditions. The peak areas were measured at 236 nm and concentrations in the samples were determined by interpolation from calibration plots of each drug previously obtained.

METHOD VALIDATION

The method was validated in accordance with ICH guidelines (ICH, 1996). The parameters assessed were linearity, accuracy, and limit of detection (LOD), limit of quantification (LOQ), precision, reproducibility, robustness and system suitability.

Accuracy

Accuracy was best determined by the standard addition method. Previously analyzed samples of Simvastatin API were added with standard drug solutions and are analyzed by the proposed method. Recovery (%), RSD (%) was calculated for each concentration.

Precision

Precision was determined as both repeatability and intermediate precision, in accordance with ICH guidelines. Repeatability of sample injection was determined as intraday variation and interday variation. For these determinations, single concentration (30 µg/ml) at different time intervals and different days, of the solution of Simvastatin API was used.

Robustness

The concept of robustness of an analytical procedure has been defined by the ICH as “a measure of its capacity to remain unaffected by small but deliberate variations in method parameters”. To determine the robustness of the method experimental conditions are purposely altered and chromatographic characters are evaluated. Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), wavelength of detection (±2nm) and acetonitrile content in mobile phase (±2%) were studied to determine the robustness of the method.

Limit of detection (LOD)

The limit of detection (LOD) of an analytical method may be defined as the concentration, which gives rise to an

instrument signal that is significantly different from the blank. For spectroscopic techniques or other methods that rely upon a calibration curve for quantitative measurements, the IUPAC approach employs the standard deviation of the intercept (Sa), which may be related to LOD and the slope of the calibration curve, b, by

$$LOD = 3\ Sa / b$$

Limit of quantitation (LOQ)

The LOQ is the concentration that can be quantitated reliably with a specified level of accuracy and precision. The LOQ represent the concentration of analyte that would yield a signal-to-noise ratio of 10.

$$LOQ = 10\ Sa / b$$

Where, Sa is the standard deviation of the peak area ratio of analyte to IS (6 injections) of the drugs and b is slope of the corresponding calibration curve.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

The chromatographic conditions were optimized by different means i.e. using different column, different mobile phase, different flow rate, different detection wavelength and different diluents for standard drug and marketed tablets are summarized in Table 1 and Fig.3.

Linearity & Range

The calibration curve (Fig 2) showed good linearity in the range of 10-100 µg/ml for Simvastatin API with correlation coefficient (r^2) of 0.99. A typical calibration curve has the regression equation of y = 38340x - 10101 for Simvastatin.

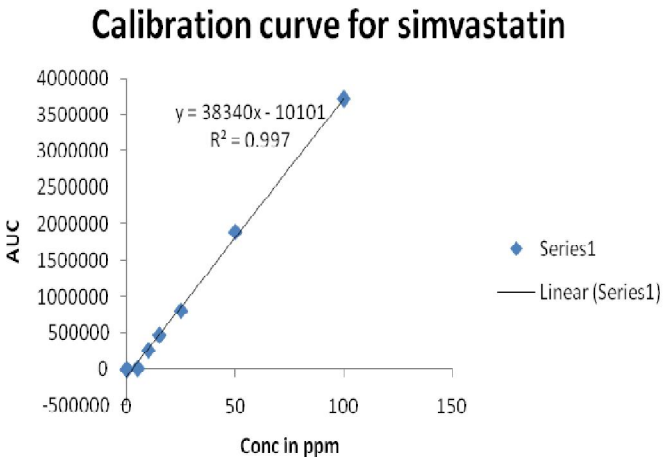

Fig. 2: Calibration curve for Simvastatin

Accuracy

Recovery study

The recovery of the method, determined by adding a previously analyzed test solution with additional drug standard solution at three levels of concentration, was 99.19- 99.67%. The values of recovery (%) and RSD listed in Table 2 indicate the method is accurate.

Precision

Intra-assay & inter-assay

The Repeatability and intra & inter day variation of the method were carried out and the high values of mean assay and low values of standard deviation and % RSD (% RSD < 2%) within a day and day to day variations for Simvastatin revealed that the proposed method is precise (Table 3 & 4).

Robustness

Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), Wavelength of detection (±2nm) & acetonitrile content in mobile phase (±2%) studied to determine the robustness of the method are also in favor of (Table 5, % RSD < 2%) the developed RP-HPLC method for the analysis of Simvastatin API.

LOD & LOQ

The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.341 & 1.023µg/ml respectively.

ASSAY OF SIMVASTATIN IN TABLET DOSAGE FORMS

Assay was performed by using the regression equation (y = 38340x - 10101, R^2=0.997) obtained from the standard curve of Simvastatin API. Results obtained are given in table 6. The assay of Biosim 20 tablet containing Simvastatin was found to be 100.5±0.17 as per the method. The chromatogram is represented in Fig.4.

CONCLUSION

A New RP-HPLC method indicating assay of Simvastatin in bulk and in pharmaceutical dosage forms is established. This method is simple, reliable, linear, accurate, sensitive and reproducible as well as cost effective for the effective quantitative analysis of Simvastatin in bulk and tablet formulations.

The method was completely validated showing satisfactory data for all the method validation parameters tested and method is free from interference of the other active ingredients and additives used in the formulations. Therefore the method is suitable for use of the routine quality control analysis of Simvastatin in API or in pharmaceutical dosage forms.

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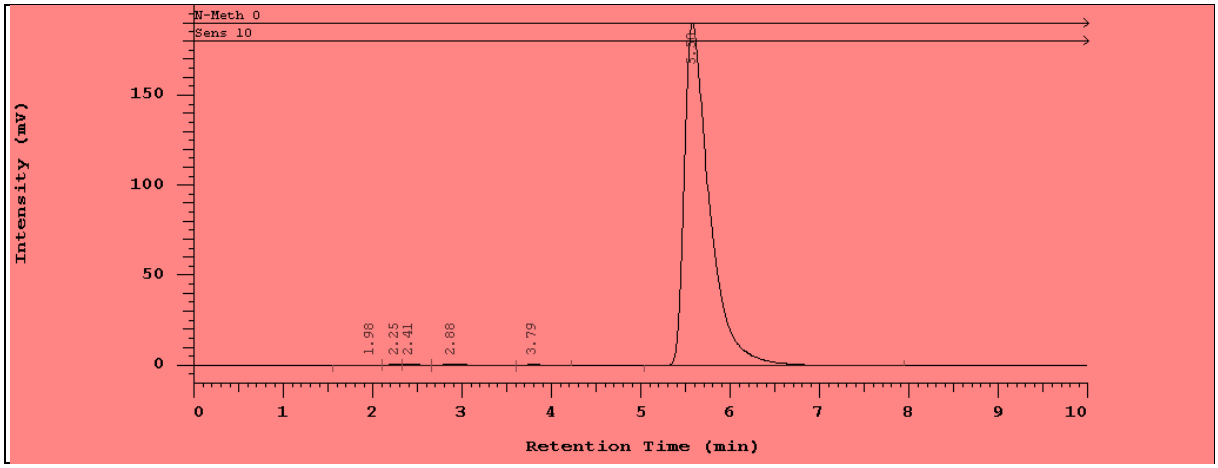

Fig. 3: RP-HPLC Chromatogram of Simvastatin standard drug.

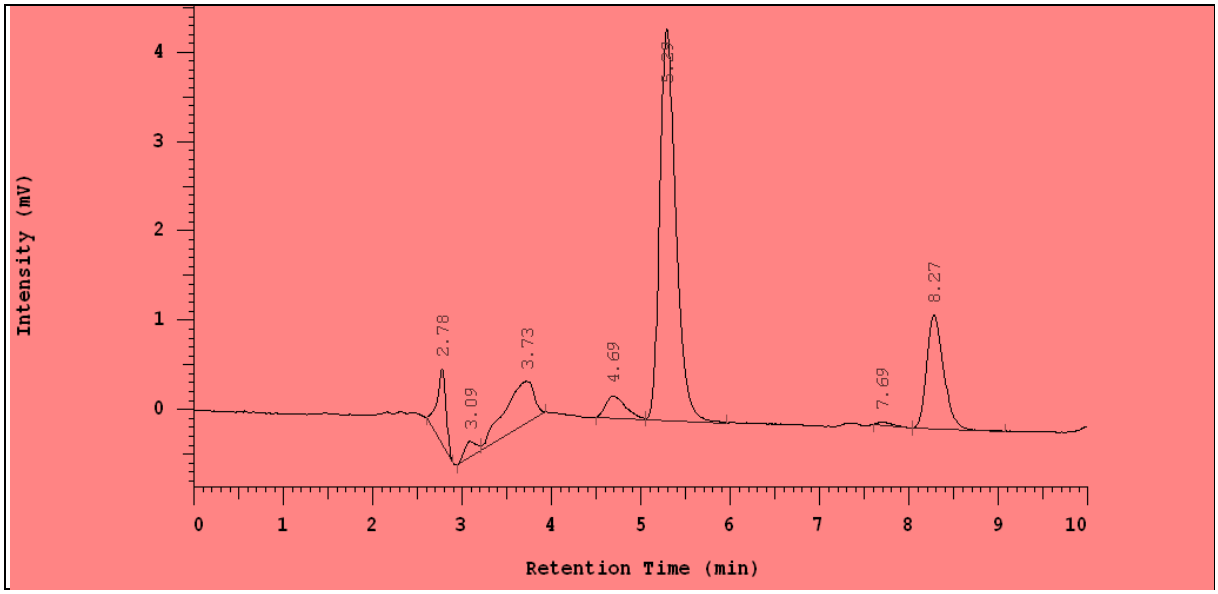

Fig. 4: RP-HPLC Chromatogram of Biosim 20 Tablet.

Table 1: Results of Optimization

Column Used	Mobile Phase	Flow Rate	Wave length	Observation	Result
Microbondapak C_{18}, 5□m, 50x4.6mm i.d.	Acetonitrile only	0.5 ml/min	254 nm	No peak	Method rejected
Phenomenex RP- C_{18}, Luna5□m, 250 x 4.6mm i.d.	Acetonitrile:Water(80:20) only	1ml/min	254 nm	Resolution but not satisfied	Method rejected
Microbondapak C_{18}, 5µm, 50x4.6mm i.d.	Acetonitrile : phosphate buffer(pH7.8)= 70:30	1ml/min	260 nm	Poor resolution	Method rejected
Phenomenex RP- C_{18}, Luna5□m, 250 x 4.6mm i.d.	Acetronitrile : phosphate buffer(pH 4.0 = 80:20	1ml/min	254 nm	Poor resolution	Method rejected
Develosil ODS HG-5 RP C_{18}, 5µm, 150cmx4.6mm i.d.	Acetonitrile : phosphate buffer(pH 3.0) (85:15)	1ml/min	236 nm	Good resolution and sharp peak	Method accepted

Table. 2: Results of recovery study.

Sample ID	Concentration (µg/ml)		%Recovery of	Statistical Analysis
	Pure drug	Formulation	Pure drug	
S_1 : 80 %	8	10	99.63	Mean= 99.67667%
S_2 : 80 %	8	10	99.92	S.D. = 0.223681
S_3 : 80 %	8	10	99.48	% R.S.D.= 0.224407
S_4 : 100 %	10	10	99.19	Mean= 99.19%
S_5 : 100 %	10	10	99.25	S.D. = 0.06
S_6 : 100 %	10	10	99.13	% R.S.D.= 0.06049
S_7 : 120 %	12	10	99.25	Mean= 99.49%
S_8 : 120 %	12	10	99.54	S.D. = 0.219317
S_9 : 120 %	12	10	99.68	% R.S.D.= 0.220441

Table . 3: Repeatability.

HPLC Injection Replicates of SIMVASTATIN	Area	Retention Time
Replicate – 1	482414	5.77
Replicate – 2	483451	5.84
Replicate – 3	472415	5.85
Replicate – 4	487569	5.87
Replicate – 5	485120	5.85
Average	482193.8	5.836
Standard Deviation	5803.219	0.038471
% RSD	1.203503	0.795508

Table. 4: 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
10	10.005	1.05	10.006	0.24
30	30.003	0.55	30.084	0.41
100	99.84	0.18	99.95	0.18

Table. 5: Results of robustness test.

Change in parameter	% RSD(n=6)
Flow (0.9 ml/min)	0.17
Flow (1.1 ml/min)	0.14
Wavelength of Detection (234 nm)	0.11
Wavelength of detection (238 nm)	0.21
Acetonitrile :Phosphate buffer (87:13)	0.08
Acetonitrile :Phosphate buffer (83:17)	0.16

Table. 6: Assay of Simvastatin Tablet.

S. No	Formulations (Biosim 20)	Standard Peak area	Sample Peak area	Labelled Amount(mg)	Amount Found(mg)	%Assay ±RSD
1	30µg/ml	482193	481998	20	19.99	99.95±0.17
2	30µg/ml	482193	490023	20	20.32	101.6±0.17
3	30µg/ml	482193	482114	20	19.99	99.95±0.17
4	30µg/ml	482193	482099	20	19.99	99.95±0.17
5	30µg/ml	482193	490039	20	20.32	101.6±0.17
6	30µg/ml	482193	482106	20	19.99	99.95±0.17
Avg.	**30 µg/ml**	**482193**	**484729.83**	**20**	**20.1**	**100.5±0.17**

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