Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2016, 8 (7):230-236
(http://scholarsresearchlibrary.com/archive.html)

Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

A novel analytical method development and validation for the estimation of simvastatin in bulk and pharmaceutical dosage forms by RP-HPLC

B. Venkateswara Rao*, S. Vidyadhara, S. Siva Prasad and D. Asheer

Department of Pharmaceutical Analysis, Chebrolu Hanumaiah Institute of Pharmaceutical Sciencies, Chandramoulipuram, Chowdavaram, Guntur-522019

ABSTRACT

A novel, simple, rapid and precise reverse phase isocratic high performance liquid chromatographic (RP-HPLC) method has been developed for the estimation of Simvastatin in marketed formulations. Estimation of drug in the formulation was done with a C18 column[Agilent ODS UG column, 250mm×4.5mm]using mobile phase of composition Acetonitrile:Methanol:Phosphate buffer pH 3.0 in the ratio of (50: 40: 10v / v) and flow rate was 1.0 mL/min and effluent was monitored with UV detection at 238 nm. The retention time of Simvastatin was observed at 4.3min. The developed method was found to be linear over a concentration range of 5-25µg/mL for Simvastatin. The method was validated according to the guidelines of International Conference on Harmonization (ICH) for specificity, linearity, precision, accuracy and robustness. The proposed method was successfully applied in the estimation of commercial formulations.

Keywords: Simvastatin, RP-HPLC, Method validation

INTRODUCTION

Simvastatin belongs to a group of cholesterol lowering lactones known as statins which are among the world's most widely prescribed drugs. Statins lower cholesterol by inhibiting the synthesis of Mavalonic acid, which is a key precursor in cholesterol synthesis [1-3].

Simvastatin is official in Indian Pharmacopoeia. Many analytical methods were traced for the estimation of Simvastatin in combination with other cardiovascular drugs but very few methods were reported on the individual estimation of Simvastatin [4-14]. In present study a simple, sensitive, specific, accurate, precise and economical reverse phase HPLC method was described for the estimation of Simvastatin.

MATERIALS AND METHODS

Equipment used

The chromatographic separation was performed on Agilent 1120 Compact Liquid Chromatographic system integrated with a variable wavelength programmable UV detector and a Rheodyne injector equipped with 20µL fixed loop. A reverse phase Agilent 100-5C_{18} Column (250mmx 4.5mm) was used.

Reagents and chemicals
Simvastatin was obtained as a gift sample from NATCO Pharma Ltd. AR grade potassium dihydrogen phosphate (KH_2PO_4) and ortho phosphoric acid were procured from RANKEM Chemicals Pvt. Ltd. HPLC grade Acetonitrile, Methanol and double distilled water were procured from Merk India. For the estimation of commercial formulations Simvastatin tablets (Simvas 10, batch no: SVTY0032) manufactured by Micro Labs Ltd, Unit III, Pondicherry, were procured from the local market.

Chromatographic conditions
Agilent 1120 compact LC chromatographic system, with variable wavelength UV detector and Rheodyne injector with 20µL fixed loop was used for the chromatographic separation. EZ Chrome Elite software was used for data analysis. Chromatographic separation was carried out on a C_{18} Column (250mm x 4.5mm) was used for the chromatographic separation at a detection wavelength of 238nm. Mobile phase composition of Acetonitrile: Methanol: Phosphate buffer pH 3.0 in the ratio of (50:40:10 v/v) was selected for elution and same mixture was used in the preparation of standard and sample solutions. Flow rate was adjusted to 1mL/min and the injection volume was 20µL.

MEHTOD DEVELOPMENT
Spectroscopic determination of Simvastatin indicated that the drug absorbs appreciable at 238nm, hence 238nm was selected as the detection wavelength. Several different mobile phases were used for the initial trials, but optimum results were attained with Acetonitrile: Methanol: Phosphate buffer pH3.0 in the ratio 50:40:10v/v. The peak was symmetric shown in figure 2.

Preparation of mobile phase
Mobile phase was prepared by mixing Acetonitrile, Methanol and Phosphate Buffer Ph 3.0 in the ratio of (50:40:10v/v) and was initially filtered through 0.45µm Millipore membrane filter and sonicated and degassed for 15 min.

Phosphate Buffer pH 3.0:
Dissolve 0.136gm of potassium dihydrogen phosphate and 2 mL of triethylamine in 80 mL 0f water, the pH is adjusted to 3.0 with orthophosphoric acid and sufficient quantity of water was added to produce 100mL.

Preparation of Standard Stock Solution
10 mg of standard Simvastatin was weighed accurately and transferred in to a 10 mL volumetric flask and dissolved in 5mL of Acetonitrile and made up to the mark with the solvent to obtain a final concentration of 1000µg/mL of Simvastatin (Standard sock solution A). From the above stock solution A, 2.5mL aliquot was pipette in to a 25mL volumetric flask and dissolved in the solvent and made up to the mark with the mobile phase to obtain a final concentration of 10025µg/mL of Simvastatin (Working stock solution B).

Preparation of sample stock solution
The contents of twenty marketed Simvas tablets were taken and finely powdered. A mass equivalent to 10mg of Simvastatin was transferred to a 10mL volumetric flask and dissolved in 5mL of the Acetonitrile. The solution was kept for sonication for 15min. The solution was made up to the mark with the solvent and filtered through a 0.4525µ membrane filter (Sample stock solution A). 1mL aliquot of the above solution was transferred to a 10mL volumetric flask and diluted to the mark with the mobile phase to obtain a concentration of 10025µg/mL (Working sample stock solution B).

Optimization of HPLC method
The HPLC method was optimized with an aim to develop an accurate and precise method for the estimation of Simvastatin in pharmaceutical dosage forms. For the method optimization, different mobile phases were tried but acceptable retention times, theoretical plates and good resolution were observed with Acetonitrile, Methanol and Phosphate Buffer pH 3.0 (50:40:10 v/v) using Agilent 100-5C_{18} Column (250mmx 4.5mm).

VALIDATION OF RP-HPLC METHOD
Validation of the optimized method was performed according to the ICH Q2 (B) guidelines.

System suitability
System suitability was carried out with six injections of solutions of 100% concentration having 25µg/ml of Simvastatin into the chromatographic system. Number of theoretical plates (N) obtained and calculated tailing factor (T), were reported in table 1.

Linearity
To establish the linearity of proposed method, appropriate aliquots were pipette out from working stock solution 'A' were to a series of 10ml volumetric flasks and the volume was made up to the mark with mobile phase to obtain final concentrations ranging from 5-25µg/ml of Simvastatin. Three replicates per each concentration were injected and Peak areas of the above solutions were reported. Calibration curves were plotted with observed peak areas against concentration followed by the determination of regression equations and calculation of the correlation coefficients. The calibration curve was shown in Figure 3 and their corresponding linearity parameters were given in table 2.

Limit of Detection (LOD) and limit of Quantitation (LOQ)
The LOD and LOQ were calculated from the slope (s) of the calibration plot and the standard deviation (SD) of the peak areas using the formulae LOD = 3.3 σ/s and LOQ = 10σ/s. The results were given in table 2.

Accuracy
To ensure the reliability and accuracy of the method recovery studies were carried out by standard addition method. A known quantity of pure drug was added to pre-analysed sample and contents were reanalysed by the proposed method and the percent recovery was reported. The results were given in table 3.

Precision
The repeatability of the method was verified by calculating the %RSD of six replicate injections of 100% concentration (25µg/ml) on the same day. The results were given in table 4.

Specificity
Specificity of a method was determined by testing standard substances against potential interferences. The method was found to be specific when the test solution was injected and no interferences were found because of the presence of excipients.

Robustness
Robustness of the method was verified by altering the chromatographic conditions like mobile phase composition, flow rate, detection wavelength, etc and the % RSD should be reported.

Small changes in the operational conditions were allowed and the extent to which the method was robust was determined. A deviation of ±2 nm in the detection wavelength and ±0.1 ml/min in the flow rate, were tried individually. A solution of 100% test concentration with the specified changes in the operational conditions was injected to the instrument in triplicate. %RSD was reported in table 5.

Assay of marketed formulation
20µl of sample solution of concentration 25µg/ml was injected into the chromatographic system and the peak response was measured. The solution was injected three times into the column. The amount present in each tablet was calculated by comparing the areas of standards with the test sample. A typical chromatogram of test solution containing 25µg/ml of Simvastatin was shown in figure 4. The results were shown in table 6.

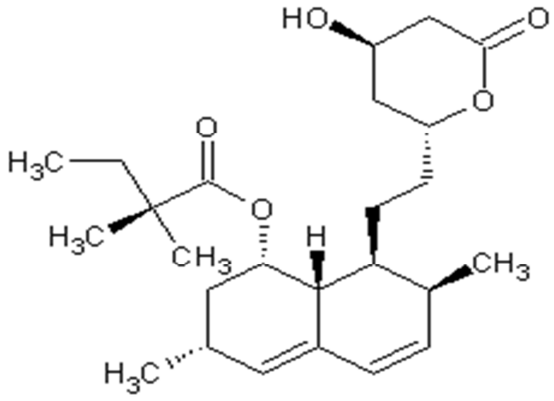

Fig 1: Chemical Structure of Simvastatin

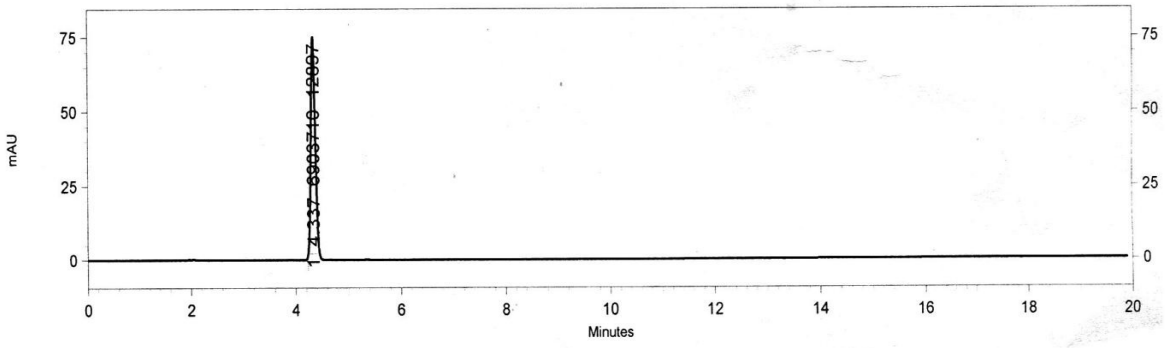

Fig 2: A typical chromatogram of standard solution containing 25µg/mL of Simvastatin

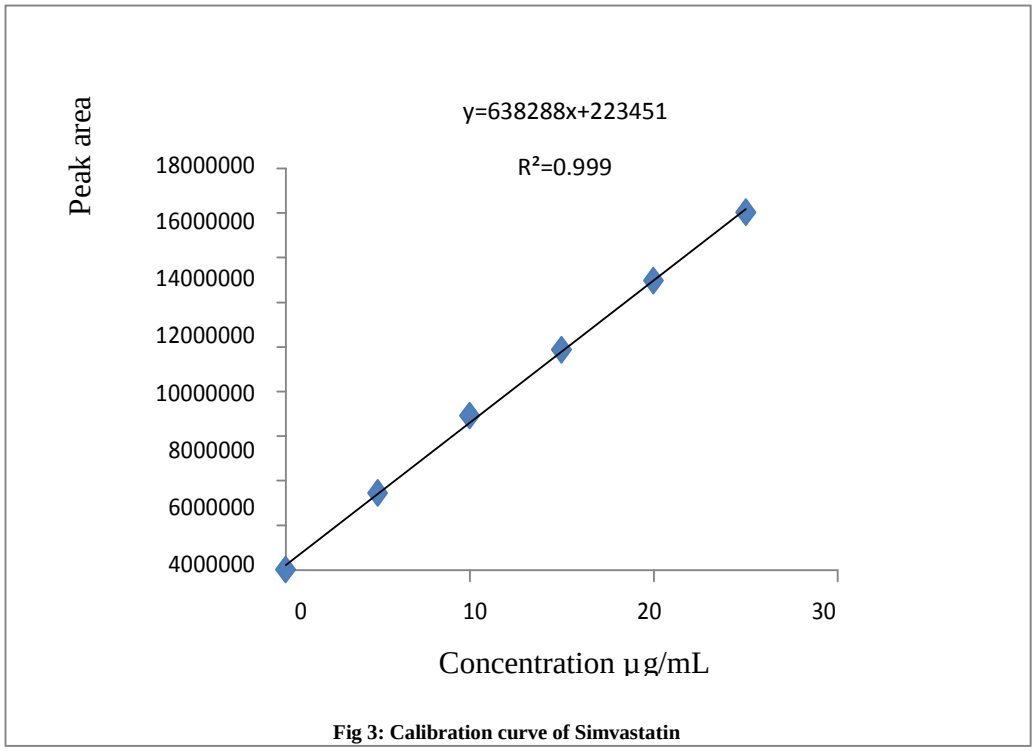

Fig 3: Calibration curve of Simvastatin

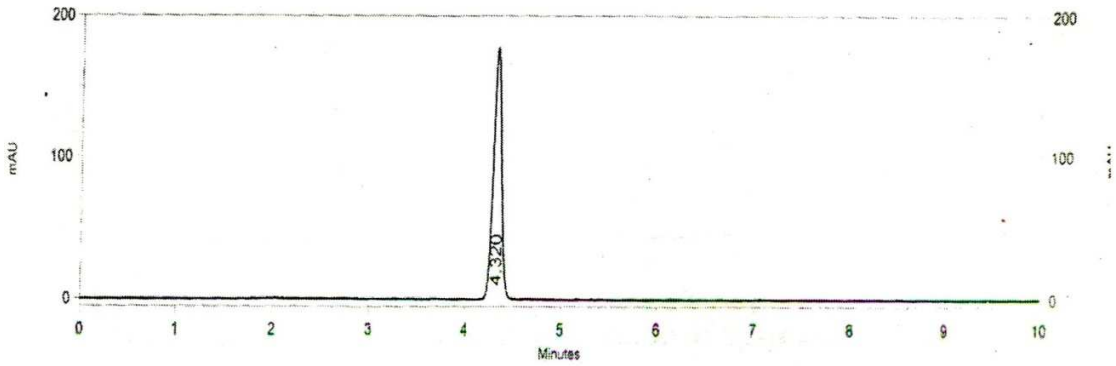

Fig 4: A Typical chromatogram for assay of marketed formulation containing 25 µg/mL of Simvastatin

Table 1: system suitability parameters for Simvastatin (n=5)

S.No	Parameters	Results
1	No. of theoretical plates	12412
2	Tailing factor	0.45
3	Retention time	4.3+0.01
4	%RSD	0.556

**n= No. of determinants*

Table 2: Results for linearity of Simvastatin (n=3)

Parameters	Values
Linearity range	5-25µg/mL
Slope	638288
Intercept	223451
Correlation coefficient	0.999
Regression equation	y=638228x+223451
Limit of Detection	0.21 µg/mL
Limit of Quantitation	0.63µg/ml

**n= No. of determinants*

Table 3: Results for accuracy of Simvastatin (n=3)

Recovery Level (%w/w)	Amount added (µg/mL)		Mean peak area	Amount found (µg/mL)	% Recovery (%w/w)	%RSD
	Standard	test				
80	4	5	6070496	9.15	101.77	1.64
100	5	5	6589778	9.974	99.74	0.75
120	6	5	7298697	11.08	100.77	0.685
Mean recovery(%w/w)	99.74-101.77					

**n= No. of determinants*

Table 4: Results for Precision of Simvastatin (n=6)

Parameters	Precision			
		Inter-day		
	Intra-day	Day1	Day2	Day3
%RSD	0.562	0.48	1.87	1.89

**n= No. of determinants*

Table 5: Results for Robustness of Simvastatin (n=3)

Parameters		Rt(min)	%RSD
Wavelength ± 2nm	236nm	4.36	0.69
	240nm	4.33	0.324
Flow rate ±0.2ml/min	0.8mL/min	4.9	1.35
	1.2mL/min	4.1	1.19

**n= No. of determinants*

Table 6: Results for assay (n=3) of marketed formulation (SIMVAS)

Drug	Labeled claim(mg)	Amount found(mg)	Percentage purity
Simvastatin	10	9.81	98.1

**n= No. of determinants*

RESULTS AND DISCUSSION

After a number of trials with mobile phases of different composition, Acetonitrile, Methanol and phosphate buffer pH 3.0, in the ratio of 50:40:10v/v was selected as mobile phase because of better resolution and symmetrical peaks. Simvastatin showed appreciable absorbance at 238 nm when determined spectrophotometrically and hence it was selected as the detection wavelength. The optimized chromatogram of Simvastatin was shown in Figure 2.

System suitability was carried out by injecting 5 replicate injections of 100% test concentration, number of theoretical plates, HETP and resolution were satisfactory. The chromatogram confirms the presence of Simvastatin at 4.3.0min without any interferences. The parameters were given in table 1.
Concentration range of 5-25µg/mL was found to be linear with correlation coefficient of 0.999. The results were given in table 2.

The limit of detection was found to be 0.21µg/ml and limit of quantitation was found to be 0.63µg/ml. The values were represented in table 2.

Accuracy of the method was verified by performing recovery studies by standard addition method. The percent recovery of the standard added to the pre-analysed sample was calculated and it was found to be 99.74-101.77%w/w. The values obtained were given in table 3.

The proposed method was found to be precise and reproducible with %RSD of 1.89.and it was reported in table 4.

The method was found to be robust after changing the conditions like detection wavelength (±2nm) and flow rate (±0.2ml). %RSD was calculated for each variation and reported. Values obtained were given in table 6 and 5.

The method was found to be specific after verifying the chromatograms showing no interference of the excipients present. Hence, the method was well suitable for the estimation of commercial formulation. Values obtained were given in table 6.

CONCLUSION

The proposed RP-HPLC method was validated as per the International Conference on Harmonisation (ICH) Q2B Guidelines, and was found to be applicable for routine quantitative analysis of Simvastatin by RP-HPLC using UV detector in Pharmaceutical dosage forms. The results of linearity, precision, accuracy and specificity, were proved to be within the limits. The method provides selective quantification of Simvastatin with no interference from other formulation excipients. The proposed method was highly sensitive, reproducible, reliable, rapid, robust and specific. Therefore, this method can be employed in quality control to estimate the amount of Simvastatin in bulk and in pharmaceutical dosage forms.

Acknowledgement
The authors are thankful to the NATCO Pharma Ltd. for providing gift sample of Simvastatin and also to the management of Chebrolu Hanumaiah Institute of Pharmaceutical Sciences, Chowdavaram, Guntur for providing facilities and great support to carry out the research work.

REFERENCES

[1] Indian Pharmacopoeia, Vol.III, **2010**, 2103-2105.
[2] Lacy Charles.F, LoraL. Armstrong, MortonP. Goldman, Leonard L.Lance. Lexi-Comp's Drug Information Hand book International., 13th Edn. **2005**, 905-907.
[3] ICH Harmonized Tripartite Guideline, Validation of Analytical Procedure Methodology, Q2B, **1996**, 1-8.

[4] Alihazeem, Sami Nazzal, *Journalof Pharmaceutical and Biomedical Analysis*, **2009**, 4(2), 137 -141.
[5] Jat R. K, Sharma S, Chipa RC, Singh Rambir and Alam Imran, *Journal of Drug Delivery and Therapeutics*, **2012**, 2(3), 121-124.
[6] Rahman Mujeeb U.R, Gazala Praveen, *International Journal of Pharmaceutical Research and Development*, **2010**, 2(9), 56 -62.
[7] Srinivas. C, Abdul Bari, Padmanabha Rao, *International Journal of Pharmacy and Pharmaceutical Sciences*, **2012**, 4(1), 398-403.
[8] Joshi H.V., J.K.Patel and Lata Kothapalli, *Der Pharma Chemica*, **2010**, 2(2), 152-156.
[9] A.Ravi Varma, J.V.Shanmukha Kumar and S.Mutta Reddy, *Der Pharmacia Lettre*, **2015**, 7(8), 204-212.
[10] Praveen Kumar S.N, Bhadre Gowda D.G., *Journal of Chemical and Pharmaceutical Research*, **2012**, 4(5), 2404-408.
[11] Lucie Novakova, Dalibar Sakinsky, Petr Solich, Tr AC *Trends in Analytical Chemistry*, **2008**, 27(4), 352-367.
[12] L. Guzik, W.Mrozik, W.Kamysz, *Croatica Chemica Acta*, **20101**, 83(4), 371-377.
[13] J.R.Jain, D.R. Shah, S.A.Shah, R.S.Chanhan, *Der Pharma Chemica*, **2011**, 3(4), 245-252.
[14] Srelakshmi V, Uma Maheswara Rao. V, Pugazhendly.S, Sushant. K, Srinivastava, Sunitha.M, *American Journal of Pharmatech Research*, **2013**, 3(5), 370-377.