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Research Article

Development, Optimization and Validation of HPLC Method for Determination of Pravastatin Sodium in Tablets

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ABSTRACT

A simple, accurate, rapid and precise HPLC method was successfully developed and validated for the determination of Pravastatin sodium in tablets. Separation of the drug was achieved on a reverse phase C 18 column using a mobile phase consisting of a methanol : water : triethylamine : glacial acetic acid in the ratio of 455: 545: 2: 1.2 v/v. The flow rate was 2 mL/min and the detection wavelength was 238 nm. The linearity was observed in the range of 25-200 µg/mL with a correlation coefficient of 0.9994. The proposed method was optimized and validated for its linearity, accuracy and precision. This method can be employed for routine quality control analysis of Pravastatin sodium in tablet formulation.

Keywords: Pravastatin, Validation, HPLC, Tablets.

INTRODUCTION

Pravastatin Sodium (PRA) is chemically, Sodium (3*R*,5*R*)-3,5-dihydroxy-7-[(1*S*,2*S*,6*S*,8*S*,8a*R*)- 6 - hydroxy - 2- methyl-8-[[(2*S*)- 2 - methylbutanoyl]oxy]-1, 2, 6, 7, 8, 8a-hexahydronaphthalen-1-yl] heptanoate (Fig. 1). It belongs to a class of drugs called statins, which are employed to lower hypercholesterolemia and related conditions and to prevent cardiovascular diseases [1-3]. The drug is available in the tablet dosage form and if official in BP [4]. Statin drugs are one of the most used pharmaceutical classes of products throughout the world. It has been ranked among the top ten prescription drugs since 1999. Literature survey revealed that few analytical methods such as HPLC [5-9], HPLC with fluorescence detection [10], HPLC-MS [11-12], capillary and gas chromatography [13-16], UV [17], polarographic and electroanalytical [18-19] methods have been reported. The proposed method was developed, optimized and validated according to International conference on Harmonization (ICH) guidelines.

EXPERIMENTAL

Instrumentation

To develop a high pressure liquid chromatographic method for quantitative estimation of Pravastatin using Shimadzu HPLC system on LiChrospher C18 column (125x4 mm, 5 µm) was used. The instrument is equipped with a LC 10 AD pump for solvent delivery and a SPD-10A Diode array detector. A 20 µl Rheodyne injector port was used for injecting the samples. Data was analyzed by using Lab Solutions software. The detection was carried out at 238 nm. The run time was set at 8 min. Under these optimized chromatographic conditions the retention time obtained for the drug was 7.193 min. A typical chromatogram showing the separation of the drug is given in Figure 2.

Materials

All chemicals and reagents were used of HPLC. Pravastatin reference standard was obtained from Sigma Aldrich. Tablet formulation containing

Pravastatin 10 mg was purchased at the local market. HPLC grade glacial acetic acid, triethylamine and methanol were procured from Merck Ltd. All other chemical reagents were of analytical grade.

Preparation of the mobile phase and diluent
455 mL of methanol was mixed with 545 mL of distilled water, 2 mL triethylamine and 1.2 mL glacial acetic acid was used as mobile phase. The solution was degassed in an ultrasonic water bath for 10 minutes and filtered through 0.45 µm filter under vacuum. Mixture of methanol : water (45:55) was used as diluent.

Preparation of standard drug stock solution: 20 mg of Pravastatin was accurately weighed, transferred to 100 mL volumetric falsk and is dissolved in 70 mL of the diluent. Sonicated the solution for few minutes to dissolve the drug completely and the volume is made up to 100 mL with diluent to get a concentration of 0.2 mg/mL stock solution. This solution is further diluted with same solvent to obtain required working standard concentrations.

Preparation of sample solution: 20 commercial tablets of Pravastatin were finely powdered and the powder equivalent to 20 mg of Pravastatin was accurately weighed and transferred to 100 mL volumetric flask and dissolved in 70 mL of diluent. The above solution was subjected to sonication for 5 min and the solution is made up to 100 mL with diluent resulting in preparation of 0.2 mg/mL solution. The solution was filtered through a 0.45 µm filter paper discarding the first few mL of filtrate. From the stock solution by further dilutions were prepared the required working solutions.

Calibration plot
From the stock solution further dilutions were prepared by diluting required volume of diluent. 20 µL of each dilution was injected six times into the column at a flow rate of 2 mL/min and the corresponding chromatograms were obtained. From these chromatograms, the average area under the peak of each dilution was computed. The calibration graph constructed by plotting concentration of the drug against peak area was found to be linear in the concentration range of 25 – 200 µg/mL of the drug. The relevant data are furnished in Table 1. The regression equation of this curve was computed. This regression equation was later used to estimate the amount of Pravastatin in tablet dosage form.

Validation of the proposed method
The specificity, linearity, precision, accuracy, limit of detection, limit of quantification, robustness and system suitability parameters were studied systematically to validate the proposed HPLC method for the determination of Pravastatin. Solution containing 100 µg/mL of Pravastatin was subjected to the proposed HPLC analysis to check intra-day and inter-day variation of the method and the results are furnished in Table 2. The accuracy of the HPLC method was assessed by analyzing solutions of Pravastatin at 50, 100 and 150% concentration levels by the proposed method. The results are furnished in Table 3. The system suitability parameters are given in Table 4.

Estimation of Pravastatin in tablet dosage form
Two commercial brands of tablets were chosen for testing the suitability of the proposed method to estimate Pravastatin in tablet formulation. Twenty tablets were weighed and powdered. An accurately weighed portion of this powder equivalent to 20 mg of Pravastatin was transferred into a 100 mL volumetric flask and dissolved in 70 mL of a 45:55 v/v mixture of methanol and water. The contents of the flask were sonicated for 5 min. The volume was made up with the diluent and the solution was filtered through a 0.45 µm membrane filter. This solution containing 100 µg/mL of Pravastatin was injected into the column six times. The average peak area of the drug was computed from the chromatograms and the amount of the drug present in the tablet dosage form was calculated by using the regression equation obtained for the pure drug. The relevant results are furnished in Table 5.

RESULTS AND DISCUSSION
In the proposed method, the retention time of Pravastatin was found to be 7.193 min. The Table 1 presents the equation of the regression line, correlation coefficient (r^2) values of the slope and intercept between the peak areas and concentrations of 25-200 µg/ml with r^2=0.9994. The regression equation of the linearity plot of concentration of Pravastatin over its peak area was found to be Y = 29670.5X+518, (r^2=0.9994), where X is the concentration of Pravastatin and Y is the corresponding peak area. The calibration curve equation shows a good linearity curve which means that the linearity test is validated. The number of theoretical plates calculated was 2148, which indicates efficient performance of the column. The limit of detection and limit of quantification were

found to be 0.3 µg/mL and 2 µg/mL respectively, which indicate the sensitivity of the method. The use of mobile phase in this ratio resulted in peak with good shape and resolution. The high percentage of recovery indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram of the formulation within the run time indicating that excipients used in tablet formulation did not interfere with the estimation of the drug by the proposed HPLC method.

CONCLUSION

The proposed HPLC method is rapid, sensitive, precise and accurate for the determination of Pravastatin and can be reliably adopted for routine quality control analysis of Pravastatin in its tablet dosage form.

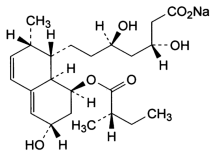

Figure 1: Chemical Structure of Pravastatin Sodium

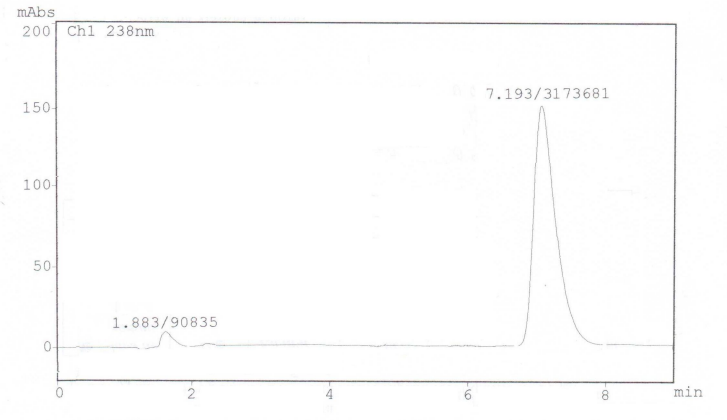

Figure 2: Typical chromatogram of Pravastatin

Table 1
Calibration data of the method

Concentration, µg/mL	Mean peak area (n=5)
25	772030
50	1553774
100	3168419
150	4714033
200	6336838

Table 2
Precision of the proposed HPLC method

Concentration of Pravastatin, 100 mg/mL	Peak Area	
	Intra – Day	Inter – Day
Injection - 1	10.07	10.05
Injection - 2	10.08	10.04
Injection - 3	10.08	10.03
Injection - 4	9.92	9.90
Injection - 5	9.88	9.86
Injection - 6	9.96	9.93
Average	10.0	9.97
Standard Deviation	0.0895	0.0818
% RSD	0.896	0.878

Table 3
Accuracy studies

Concentration	Amount Added, mg	Amount Found, mg	%, Recovery	%, Mean Recovery
		5.18	48.50	97.00
50 %	5.34	5.35	50.09	100.2
		5.21	48.78	97.56
		10.74	100.6	100.6
100 %	10.68	10.47	98.07	98.07
		10.75	100.7	100.7
		15.89	148.9	99.30
150 %	16.01	16.22	151.9	101.3
		15.86	148.6	99.06
Average				99.31
SD				1.514
% RSD				1.525

Table 4
System suitability parameters

Parameters	Results
Linearity, mg/mL	25 – 200
Correlation coefficient	0.999
Theoretical plates (N)	2148
Tailing factor	1.4
LOD, µg/mL	0.3
LOQ, µg/mL	2

Table 5
Assay and recovery studies

Formulation	Label claim, mg	Amount found, mg	% Amount found
Formulation – 1	10.00	10.02	100.2
Formulation – 2	10.00	9.96	99.60

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