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Research Article

REVERSED PHASE-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD DEVELOPMENT AND VALIDATION OF ATORVASTATIN IN BULK DRUG AND FORMULATION

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ABSTRACT

Objective: To develop and validate a simple, selective, rapid, precise, and accurate high performance liquid chromatographic (HPLC) method for determination of atorvastatin in bulk and its pharmaceutical formulation product.

Method: Reversed phase-HPLC (RP-HPLC) method was performed by a mobile phase consisting mixture of methanol and ammonium acetate buffer (pH 4.5) in the proportion 60:40 v/v. A ZORBAX Eclipse plus C_{18} (4.6 mm × 100 mm, 3.5 µ) column was used as a stationary phase. HPLC analysis of atorvastatin was carried out at a wavelength of 241 nm with a flow rate of 1 ml/minute.

Results: The linear regression analysis data for the calibration curve showed a good linear relationship with a correlation coefficient 0.9984. The linear regression equation was y=3726540.2x+27390388.1. This was found to give a sharp peak of atorvastatin at a retention time of 2.77 minutes. Validation parameters were evaluated for the method according to the ICH (Q2R1) guidelines. The limit of detection and limit of quantification for the method were 0.6721 µg/mL and 1.9989 µg/mL, respectively. The % relative standard deviation values for intra-day precision and inter-day precision were found to be 0.31% and 0.30%, respectively. An accuracy of the method was determined through recovery studies which were found to be within 97.57-102.22%.

Conclusion: The method was validated for system suitability, accuracy, precision, robustness, and ruggedness. The precision, accuracy, sensitivity, short retention time and composition of the mobile phase indicated that this method is better than the earlier methods developed for the quantification of atorvastatin.

Keywords: Atorvastatin, Reversed phase-high performance liquid chromatographic method development, Validation.

INTRODUCTION

This compound belongs to the class of organic compounds known as diphenylpyrroles. It is aromatic heterocyclic compounds with a structure based on a pyrrole ring linked to exactly two phenyl groups. It is chemically known as 7-[2-(4-fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-(propan-2-yl)-1H-pyrrol-1-yl]-3,5-dihydroxyheptanoate. The chemical structure is given in Fig. 1. It may be used to reduce the risk of cardiovascular events in patients with acute coronary syndrome and may be used in the treatment of primary hypercholesterolemia and mixed dyslipidemia, homozygous familial hypercholesterolemia, primary dysbetalipoproteinemia, and/or hypertriglyceridemia as an adjunct to dietary therapy to decrease serum total and low-density lipoprotein cholesterol (LDL-C), apolipoprotein B, and triglyceride concentrations, while increasing high-density lipoprotein cholesterol levels. Atorvastatin selectively and competitively inhibits the hepatic enzyme 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase [1,2].

As HMG-CoA reductase is responsible for converting HMG-CoA to mevalonate in the cholesterol biosynthesis pathway, these results in a subsequent decrease in hepatic cholesterol levels. Decreased hepatic cholesterol levels stimulate up regulation of hepatic LDL-C receptors which increases hepatic uptake of LDL-C and reduces serum LDL-C concentrations. Atorvastatin is rapidly absorbed after oral administration with maximum plasma concentrations achieved in 1-2 hrs. Atorvastatin is extensively metabolized to ortho-and para-hydroxylated derivatives and various beta-oxidation products. Literature survey revealed that several high performance liquid chromatographic (HPLC) methods for determination of atorvastatin were reported but with longer retention time. Since the retention time is one of the important factors for the separation of drugs using HPLC, the present work reports the development and validation of HPLC method for the estimation of atorvastatin in bulk and formulation with shorter retention time, and the method has been highly used for its sensitivity and reproducibility.

METHODS

Instruments

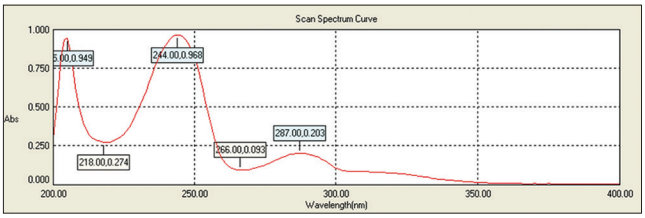
HPLC Agilent 1260 with PDA detector and auto sampler, Zorbax eclipse Plus C_{18} (4.6 × 100 mm, 3.5 µ) equipped with Open lab EZ Chrome software, Lab India – T60 UV/Vis double beam spectrophotometer, Mettler Toledo ME 204 balance, Eutech pH 700 pH meter.

Chemicals and reagents

The sample of atorvastatin standard was procured as gift sample from MSN Laboratories Ltd, Hyderabad, India. HPLC-grade methanol, water, and ammonium acetate were procured from Merck Pharmaceuticals Private Ltd., Mumbai, India. Glacial acetic acid was also used.

Selection of detection wavelength

The UV spectrum of diluted solutions of various concentrations of atorvastatin in mobile phase was recorded using UV spectrophotometer. The wavelength of maximum absorbance was observed at 241 nm. This wavelength was used for detection of atorvastatin.



Preparation of mobile phase

The content of the mobile phase was prepared from filtered and degassed mixture in the ratio 60:40 v/v of methanol and ammonium acetate buffer (1.54 g of ammonium acetate in 1.0 liter distilled Water) and pH was adjusted to 4.5 with 0.2 M glacial acetic acid.

Preparation of atorvastatin standard stock solution

100 mg of Atorvastatin powder was weighed and transferred into 100 ml volumetric flask, and 30 ml of diluent was added to it, sonicated and was further filtered through 0.45 µ filter paper and the volume was made up with diluent. Then 100 µg/mL working standard solution was prepared by pipetting out 10 ml of 1000 µg/mL solution into the 100 ml volumetric flask, and the remaining volume was made up with diluent.

Preparation of sample solution

10 tablets, each containing 500 mg of atorvastatin, were weighed and crushed into fine powder and amount of powder equivalent to 100 mg of atorvastatin was weighed and transferred into 100 ml dried volumetric flask. The content was dissolved by adding 30 ml of diluent and rapidly shaking for few minutes. The volume was made up with diluent, mixed well, and injected immediately.

Optimized method for atorvastatin

Chromatographic conditions

Column: Zorbax Eclipse Plus C_{18} (4.6 mm × 100 mm, 3.5 µ)
Mobile phase: Methanol:ammonium acetate buffer - pH 4.5 (60:40)
Flow rate: 1 ml/minutes
Wavelength: 241 nm
Column temperature: 25°C
Injection volume: 15 µL
Run time: 5 minutes
Diluent: Methanol:water (60:40)
Elution: Isocratic
Needle wash: Methanol

METHOD VALIDATION [5-11]

Linearity

The linearity of an analytical procedure is its ability to obtain test results, which are directly proportional to the concentration of an analyte in the sample. A linear relationship should be evaluated across the range of the analytical procedure. It is demonstrated directly on the drug substance by dilution of a standard stock solution of the drug product components, using the proposed procedure. For the establishment of linearity, minimum of five concentrations are recommended by ICH guideline. The value of correlation coefficient should fall around 0.99. The regression equation and correlation coefficient was calculated and found to be within the required limits as shown in Tables 1 and 2, respectively.

Precision

The precision of the analytical procedure expresses the closeness of agreement among series of measurements obtained from multiple sampling of the same homogeneous sample. The precision of the analytical procedure is usually expressed as the variance, standard deviation, or coefficient of variation of series of measurements. The intra-day and inter-day precision results were shown in Tables 3 and 4, respectively.

Accuracy/recovery

The accuracy of the analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. The evaluation of accuracy has got very prime importance as it deliberately forces the method to extract the drug and impurities at higher and lower level. The recovery results for accuracy study of atorvastatin were represented in Table 5.

Limit of detection (LOD) and limit of quantification (LOQ)

LOD is the lowest concentration in a sample that can be detected but not necessarily quantified under the stated experimental conditions.

Table 1: Linearity results

S. No	Concentration (µg/ml)	Peak area
1	20	979004765
2	40	179504390
3	60	257038920
4	80	320327053
5	100	400143166

Table 2: Linearity parameters and their values

S. No	Parameters	Values
1	Concentration range	20-100 µg/mL
2	Regression equation (Y)	Y=3726540.2X+27390388.1
3	Correlation coefficient (r^2)	0.998
4	Slope (m)	3726540.2
5	y-intercept (c)	27390388.1

Table 3: Intra-day results for precision

Concentration (µg/mL)	Injection number	Area	% RSD
60 (µg/ml)	1	257038920	0.31
	2	258212471	
	3	256126510	
	4	257201546	
	5	257003481	
	6	256021567	

Table 4: Inter-day results for precision

Concentration (µg/ml)	Injection number	Area	% RSD
60 (µg/ml)	1	257124852	0.30
	2	258569842	
	3	256521436	
	4	257254216	
	5	257042157	
	6	256367421	

The LOQ is the lowest concentration of the analyte in a sample that can be determined with acceptable precision and accuracy. LOD and LOQ were calculated using following formula:

$$LOD = 3.3\ SD/S \text{ and } LOQ = 10SD/S$$

Where, SD = Standard deviation of response (peak area) and S = Slope of the calibration curve.

Robustness

The robustness of the analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The results for robustness study were shown in Tables 6-8, respectively.

RESULTS AND DISCUSSIONS

Linearity

The standard calibration curve was constructed between concentration V_s peak area and linearity was found in the range from 20 to 100 µg/mL. The regression equation and correlation coefficient were calculated and found to be within the required limits as specified in ICH guidelines. The calibration curve was given in Fig. 2.

Precision

Intra-day precision was investigated by replicate applications and measurements of peak area for atorvastatin for six times on the same

Table 5: Results for accuracy

Sample number	% level	Standard amount	Spiked amount	% recovery	Mean recovery
1	75	80	60	102.20	Mean=102.221
2		80	60	102.22	SD=0.0180
3		80	60	102.24	% RSD=0.02%
1	100	80	80	99.98	Mean=99.99
2		80	80	100	SD=0.0115
3		80	80	100	% RSD=0.01%
1	125	80	100	97.57	Mean=97.57
2		80	100	97.58	SD=0.0057
3		80	100	97.57	% RSD=0.01%

SD: Standard deviation, RSD: Relative standard deviation

Table 6: Results for robustness flow rate variation

S. No	Concentration (µg/mL)	Area	
		0.8 (ml/minute)	1.2 (ml/minute)
1	(60 µg/mL)	257038916	257135915
2		254217540	257217540
3		257982451	256824524
4		25824503	256214500
5		255124901	255524907
6		257412031	257612034
% RSD		0.63	0.30

RSD: Relative standard deviation

Table 7: Results for robustness pH variation

S. No	Concentration (µg/mL)	Area	
		pH 4.3	pH 4.7
1	(60 µg/mL)	257412820	254871230
2		254871245	258746950
3		256485124	257481245
4		251538546	254132549
5		252487120	253157245
6		259124850	259854721
% RSD		1.15	1.06

RSD: Relative standard deviation

Table 8: Results for robustness mobile phase composition variation

S. No	Concentration (µg/mL)	Area (Methanol:Buffer)	
		65:35 v/v	75:25 v/v
1	(60 µg/mL)	254745147	257481247
2		256487120	257421510
3		258694721	256854214
4		257124573	253142658
5		257654801	258451270
6		256124523	256140124
% RSD		0.53	0.72

RSD: Relative standard deviation

day under similar conditions. Inter-day precision was obtained from % relative standard deviation (RSD) values obtained by repeating the assay 6 times on 2 different days. The % RSD was calculated which was within the acceptable limits of not more than 2.0.

Accuracy

The accuracy of the method was tested by the triplicate sample at 3 different concentrations equivalent to 75%, 100%, and 125% of the active ingredient, by adding a known amount of atorvastatin standard to a sample with a pre-determined amount of atorvastatin. The recovered amount of atorvastatin, % RSD of recovery, and % recovery of each concentration were calculated to determine the accuracy.

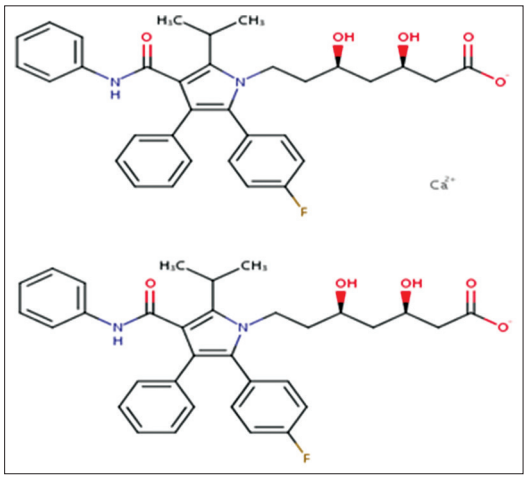


Fig. 1: Structure of atorvastatin [3,4]

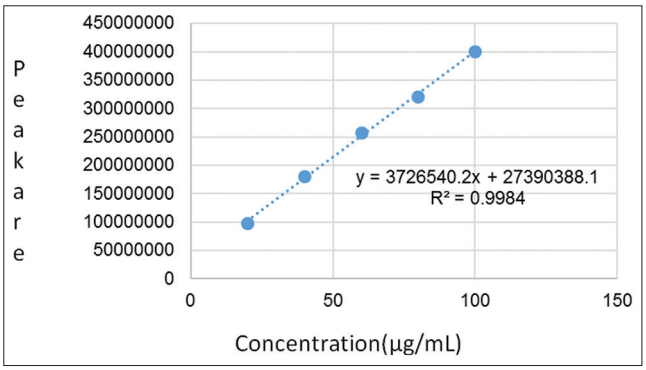


Fig. 2: Calibration curve of atorvastatin

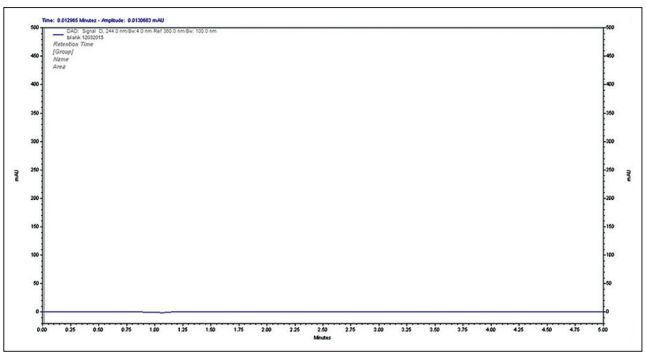


Fig. 3: Blank chromatogram of atorvastatin

Robustness

Robustness is the ability to provide accurate and precise results under a variety of conditions. To measure the extent of method robustness, the

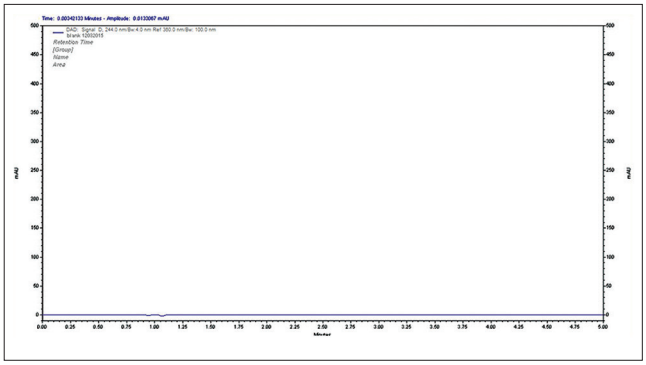


Fig. 4: Chromatogram of placebo

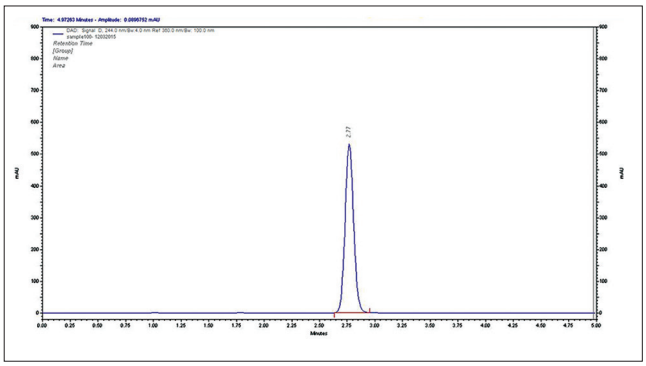


Fig. 5: Standard chromatogram of atorvastatin

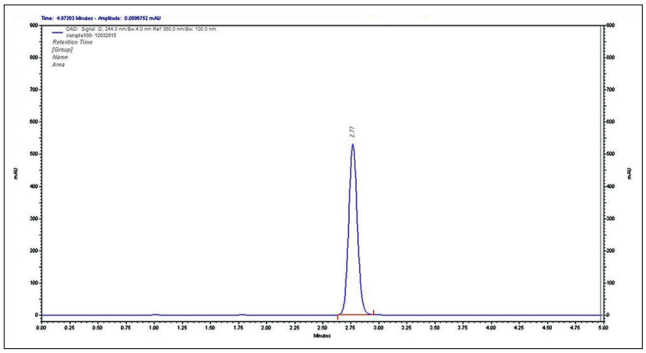


Fig. 6: Formulation chromatogram of atorvastatin

most critical parameters were interchanged while keeping the other parameters unchanged and in parallel, the chromatographic profile was observed and recorded. The studied parameters were the composition of flow rate, pH, and mobile phase. The results for robustness study indicated that the small change in the conditions did not significantly affect the determination of atorvastatin.

LOD and LOQ

The LOD was found to be 0.6721 µg/mL.

The LOQ was found to be 1.9989 µg/mL.

CONCLUSION

The proposed method for the assay of atorvastatin was rapid, accurate, precise, and sensitive for the quantification of atorvastatin from its pharmaceutical dosage forms. The method was validated for linearity, accuracy, precision, LOD, LOQ, and robustness. The method was free from the interference of other active ingredient and excipients. Hence, it can be concluded that this method may be employed for routine quality control analysis of atorvastatin in active pharmaceutical ingredient and formulation product. The chromatograms for blank, placebo, standard and formulation product were given in Figs. 3, 4, 5 and 6.

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