Research Article

A RP-HPLC Method Development and Validation for the Estimation of Atorvastatin in Bulk and Pharmaceutical Dosage Forms

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ABSTRACT

An easy RP-HPLC method was developed and validated considering the parameters like selectivity, linearity, precision and accuracy for the rapid assay of atorvastatin in tablet dosage form. Flow rate of 1 mL/ min was engaged on an Eclipse XDB plus column (C_{18}) at a temperature of 40 ºC. Mobile phase was comprised of Acetonitrile: Potassium dihydrogen orthophosphate buffer at a ratio of 70:30 (v/v) and detection wavelength was chosen at 232nm. Linearity was detected in between the concentration range of 5-60 µg/ mL Retention time of atorvastatin was observed at 2.273 min. ICH guidelines was followed for method validation. The projected process can be effectively useful for the approximation of atorvastatin in therapeutic dosage forms.

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INTRODUCTION

Atorvastatin (**Figure 1**) is a drug with anti-hyperlipidemic nature. Chemically it is described as (3R,5R)-7-[2-(4-Fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-(propan -2-yl)1H-pyrrol-1-y1]-3,5-dihydroxyheptanoic acid, with a molecular weight of 558.6g/mol and empirical formula of $C_{33}H_{35}FN_2O_5$[1]. Atorvastatin is a synthetic lipid lowering agent, competitively blocks the enzyme hepatic hydroxy methyl-glutaryl coenzyme A (HMG-COA) reductase, accountable for the transformation of HMG-COA to mevalonic acid, a significant step in the cholesterol biosynthesis [2]. A comprehensive literature survey in google scholar reveals that very less analytical methods has been developed for the assessment of atorvastatin [3-7]. The intention of the work is to develop an economic, simple, accurate and precise HPLC method for the assessment of atorvastatin in the tablet dosage form by following the ICH guidelines [8].

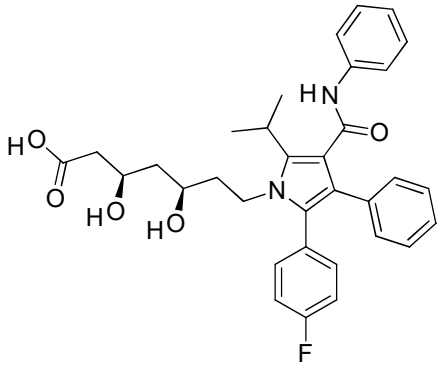

Figure1: Structure of atorvastatin

MATERIALS AND METHOD

Instrument and analytical settings: The analysis was performed on Agilent HPLC system (1220 infinity LC series) equipped with PDA detector and using OPENLAB software. The column employed was Eclipse XDB plus C_{18} column (150x4.6mm, packed with 5µm). Accurately 20µL of the sample was injected and detection was carried out at 232nm. The sample was run for 5 minutes isocratic mobile phase comprising of acetonitrile: potassium dihydrogen orthophosphate buffer 70:30 (v/v) was used with a flow rate of 1mL/ min. The mobile phases were degassed before use.

Chemicals and reagents: Standard drug atorvastatin was a kind gifted by Yarrow pharmaceuticals private Limited, Hyderabad. Tablets were procured from central pharmacy store, factory-made by Aurobindo Pharma Ltd. Ultra-pure water was collected by using a Millipore system. Acetonitrile (HPLC grade) was procured from Merck India limited. Additional chemicals employed were of AR grade.

Composition of mobile phase: 1.36gm of potassium dihydrogen orthophosphate was dissolved in1000 mL of water and mixed; pH was adjusted to 4 with ortho phosphoric acid and sonicated to degas the buffer. The mobile phase was prepared taking acetonitrile and buffer in the ratio of 70:30 (v/v). It was mixed well and

sonicated for 15 minutes and filtered through 0.45 µm filter under vacuum.

Composition of standard solution: About 10 mg of atorvastatin was correctly weighed and transported into a 10 ml volumetric flask and volume was calibrated with the diluents and sonicated to get stock solution of 1000µg/ mL. About 1 mL of stock solution was transported into 10 ml volumetric flask and volume was adjusted with diluents and filtered through 0.45 µm filters, to give a solution of strength 100 µg/ mL.

Composition of sample solution: 20 tablets containing atorvastatin were weighed and the average weight was calculated. Powder equivalent to 10mg of atorvastatin was weighed accurately and transferred into a 10mL volumetric flask, dissolved thoroughly and filtered through 0.45µm filter. Further 1mL of the stock solution was pipetted into a 10mL volumetric flask and diluted up to the mark, mixed well and filtered over 0.45µm filter.

METHOD VALIDATION

To demonstrate the suitability of the method for its intended purpose it was validated as per the ICH guidelines [9, 10].

Linearity: Various dilutions of atorvastatin at a concentration of 5, 10, 20, 30, 40, 50 and 60µg/mL were obtained from the standard solution. 20µL of the solutions were injected in to the chromatographic system. The flow rate was maintained at 1mL/min, the effluents were observed at 232 nm and the chromatograms were noted. The calibration curve of atorvastatin was determined from the peak area and concentrations as shown in **Table 1**. Correlation coefficient was observed to be 0.999 as revealed in F**igure 2**.

Table 1: Linearity of atorvastatin

Conc. (µg/mL)	Average area
5	26421
10	51266
20	110532
30	171738
40	229064
50	286330
60	337524

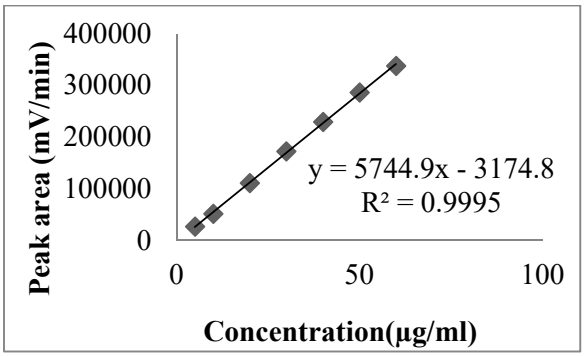

Figure 2: Linearity of atorvastatin

Precision: For precision study, repeatability of the process was tested by injecting replicate injection of 10µg/mL for six times on the same day and the % RSD was obtained to be 0.029 as mentioned in **Table 2**.

Table 2: Precision of atorvastatin

Injections	Area
1	163428
2	163420
3	163326
4	163420
5	163320
6	163452
Mean	163394.3
SD	47.7663
%RSD	0.029

Accuracy: Atorvastatin standard drug was accurately weighed and added to tablet excipients at three different concentration levels (80%, 100% and 120%). The percentage recovery was calculated and shown in **table 3**.

Specificity: The possibility of any Interference of the tablet excipients with atorvastatin were evaluated by inspecting the chromatographic peak as per the standard methodology. In the work, no interference peaks were observed.

Robustness: Temperature and mobile phase composition were varied to determine the robustness. There was no major difference between results, under the employed conditions. This shows robustness of the method. The data is represented in **Table 4**.

Table 3: Accuracy of atorvastatin

%Conc.	Amount added (mg)	Amount found (mg)	% Recovery	Mean Recovery
80%	8	7.95	99.4 %	99.5%
100%	10	9.96	99.6%	
120%	12	11.94	99.5%	

Table 4: Robustness of atorvastatin

Parameters	Adjusted to	Average area	Rt	SD	% RSD
Flow rate as per method 1.0 mL/min	0.9	11480	2.24	66.74704	0.58
	As it is	14347	2.27	35.13545	0.24
	1.1	214031	2.33	1074.246	0.5
Wavelength 232nm	230	29420	2.26	91.31356	0.31
	As it is	28663	2.28	198.5955	0.69
	235	21474	2.30	147.2419	0.68

Ruggedness: It was determined by different analyst over a period of one week by using six replicate injections. Ruggedness was conveyed in terms of % RSD and the statistical analysis showed no significant difference.

Detection and quantitation limits: LOQ and LOD of atorvastatin were determined from signal-to-noise ratio; it gave the limits of detection of 0.16µg/mL and limits of quantitation of 0.52µg/mL

System suitability: It was carried out by injecting the standard drug in six replicates at an interval of 6 minutes. The standard deviation values were logged and the parameters are represented in **table 5**.

Table 5: System Suitability of atorvastatin

Concentration	Injection	Area	R_t
10 µg/mL	1	28952	2.275
	2	28429	2.268
	3	28612	2.273
	4	28330	2.269
	5	28573	2.267
	6	28619	2.272
Statistical Analysis	Mean	2858.5	2.2706
	SD	179.712	0.002655
	% RSD	0.62	0.11
	Tailing Factor	1.05	
	Plate Count	2409	

Assay of Atorvastatin tablet:

Different batches of atorvastatin tablets were examined by the validated method. Twenty tablets were weighed and finely powdered. Powder equivalent to about 10mg of atorvastatin was transported to a 10mL of volumetric flask and volume was adjusted with diluent then filtered through 0.45µm membrane filter. Additional dilutions were made to get 10µg/mL of atorvastatin. The amount of drug present in the tablets was calculated from the mean peak area and the results were mentioned in **table 6**. The examined atorvastatin concentration was found to be very close to the label claim amount. The atorvastatin content in the tablet samples varied from 99.8 to 98.9 %.

Table 6: Contents of atorvastatin in tablets (n=6)

Sample tablet	Batch	Label Claim(mg)	Amount found(mg)±SD	%Amount found
Atoril (10mg)	1	10	9.96±0.11	99.6
	2	10	9.87±0.09	98.7
	3	10	9.96±0.13	99.6

S.D=Standard Deviation

RESULTS AND DISCUSSION

Elution of the atorvastatin was governed by the polar mobile phase. Symmetric peaks were obtained, with short run time by optimizing the ratio of the acetonitrile and buffer. Reasonable separation, fine resolved and well symmetrical peaks were attained through the mobile phase of acetonitrile: potassium dihydrogen orthophosphate buffer at the ratio of 70:30(v/v). The retention time of atorvastatin was observed at 2.273min. The RSD values for precision and accuracy readings attained were less than 2% which shown that the developed method was accurate and precise. The established chromatographic method was also used for the determination of atorvastatin in tablet formulation. A chromatogram of atorvastatin is shown in **Figure 3**.

CONCLUSIONS

A validated RP-HPLC method has been established for the estimation of atorvastatin in tablet dosage form. The developed method was found to be rapid, accurate, simple, specific and precise. Hence, it is appropriate for the monotonous study of atorvastatin in therapeutic dosage form.

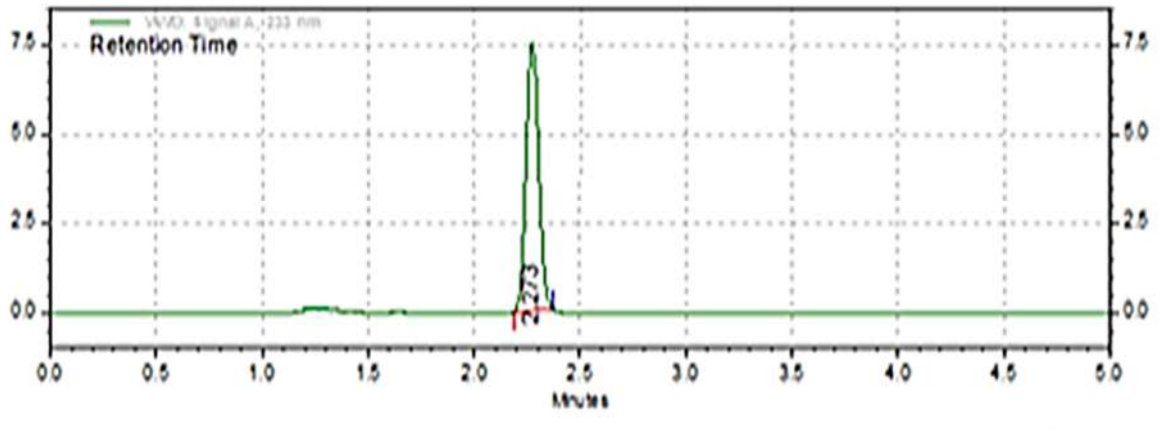

Figure 3: HPLC chromatogram of atorvastatin

Table7: Chromatographic conditions

Parameters	Method
Stationary phase (column)	Eclipse XDB plus C_{18} column(150 × 4.6 mm, packed with 5 µm)
Mobile Phase	Acetonitrile : KH_2PO_4 Buffer (70:30 v/v)
Flow rate (ml/min)	1.0
Run time in minutes	5.0
Column temperature in °C	40
Volume of injection (ml)	20
wavelength of detection (nm)	232
Retention time(min)	2.27

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