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Research Article

RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF ATORVASTATIN AND EZETIMIBE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Objective: The aim was to develop a simple, selective, linear, precise, and accurate reverse phase high performance liquid chromatography method for simultaneous estimation of atorvastatin and ezetimibe in tablet dosage form.

Methods: The chromatographic separation was performed using hypersil BDS C_{18} coloumn (250 mm × 4.6 mm, 5 mm particle size). Mobile phase composed of phosphate buffer pH-4.5 and acetonitrile (35:65 v/v) at a flow rate of 1 ml/min. Detection was carried out using photodiode array detector at 228 nm. The method was validated as per ICH guidelines.

Results: The retention time for atorvastatin and ezetimibe are observed as 2.36 and 3.43 minutes respectively. Linearity range was observed in concentration of 12.5-75 µg/ml for both atorvastatin and ezetimibe. The percentage recoveries of atorvastatin and ezetimibe are 100.21% and 100.22% respectively. The correlation coefficients for both the components are close to 1.

Conclusion: This method is simple, selective, linear, precise, accurate and sensitive hence can be successfully employed for the routine quality control of dosage forms containing both the drugs in pharmaceutical industries.

Keywords: Reverse transcription polymerase chain reaction, Method development, Atorvastatin, Ezetimibe, Validation.

INTRODUCTION

Atorvastatin is chemically named as (3*R*, 5*R*)-7-(2-[4-fluorophenyl]-3-phenyl-4-[phenylcarbamoyl]-5-propan-2-ylpyrrol-1-yl)-3, 5-dihydroxyheptanoic acid (Fig. 1). It is a member of the drug class known as statins, which are used primarily for lowering blood cholesterol and for prevention of events associated with cardiovascular disease. Like all statins, atorvastatin works by inhibiting HMG-CoA reductase, an enzyme found in liver tissue that plays a key role in the production of cholesterol in the body [1].

Ezetimibe is chemically named as (3*R*, 4*S*)-1-(4-fluorophenyl)-3-([3*S*]-3-[4-fluorophenyl]-3-hydroxypropyl)-4-(4-hydroxyphenyl) azetidin-2-one (Fig. 2). It is a drug that lowers plasma cholesterol levels. It acts by decreasing cholesterol absorption in the small intestine. It may be used alone when other cholesterol-lowering medications are not tolerated, or together with statins, when statins alone do not control cholesterol. It decreases in hepatic cholesterol storage and an increase in the clearance of cholesterol from the blood [2].

Literature survey reveals that few spectrophotometric methods [3-8] and high performance liquid chromatography (HPLC) methods [9-15] have been reported for the estimation of atorvastatin and ezetimibe. The aim of this study is to develop a simple, precise and accurate reversed-phase HPLC (RP-HPLC) method for the estimation of atorvastatin and ezetimibe in pharmaceutical dosage forms as per ICH guidelines [16]. The validation procedure followed the guidelines of USP 30 [17].

METHODS

Instrumental and analytical conditions

The HPLC analysis was carried out on Waters HPLC (2695) equipped with photodiode array (PDA) detector (2996) and auto sampler integrated with empower2 software. The column used is hypersil BDS C_{18} coloumn (250 mm ×4.6 mm, 5 mm particle size) and detection was performed at 228 nm. The injection volume of sample was 10 µl and the run time was 6 minutes. An isocratic mobile phase consisted of phosphate buffer and acetonitrile in the ratio 35:65 v/v at pH 4.5 was carried out with the flow rate at 1 ml/minutes. The mobile phase was filtered through 0.45 µm membrane filter and degassed before use.

Reagents and chemicals

Atorvastatin was obtained as gift sample form Auspi Life Pharma Private Limited, Kukatpally, Hyderabad and ezetimibe was obtained as gift

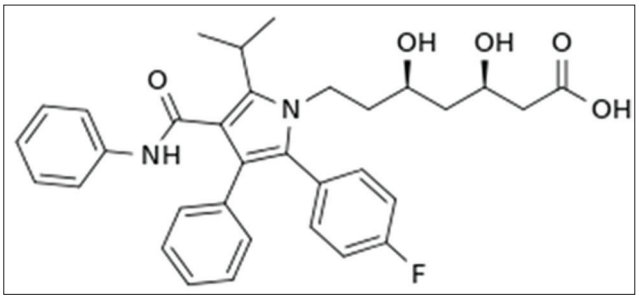

Fig.1: Structure of atorvastatin

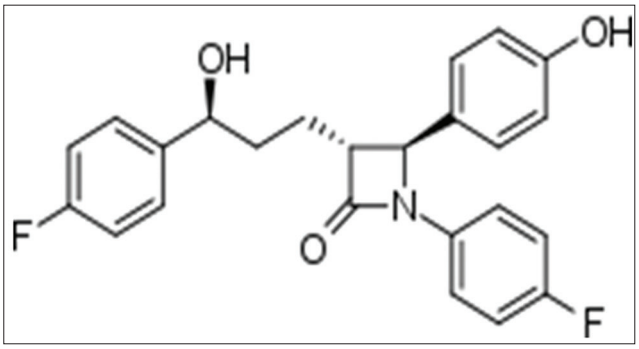

Fig. 2: Structure of ezetimibe

sample from Dr. Jcr Bio-Sciences Private Limited, Hyderabad. Tablets were procured from local pharmacy containing 10 mg of atorvastatin and 10 mg of ezetimibe (atocor E). Ultra pure water was obtained from a Millipore system. HPLC grade acetonitrile was obtained from Merck (India) limited. All other chemicals used were AR grade.

Preparation of mobile phase

Accurately weighed 2.72 g of sodium dihydrogen orthophosphate was transferred into a 1000 ml volumetric flask and about 900 ml of milli-Q water was added and sonicated to degas and finally volume adjusted with water. Then pH adjusted to 4.5 with dilute ortho phosphoric acid solution. Buffer and acetonitrile taken in the ratio of 35:65 in to a mobile phase bottle and mixed. Then filtered through 0.45 µ nylon membrane filter and degassed. The mobile phase was used as diluent.

Preparation of standard stock solution

Standard stock solutions were prepared by dissolving 10 mg of atorvastatin and 10 mg of ezetimibe into 100 ml volumetric flasks separately with diluents. Sonicated for 30 minutes and volume was adjusted with diluents. The working standard solution of atorvastatin (50 µg/ml) and ezetimibe (50 µg/ml) were prepared by diluting the stock solution in mobile phase.

Preparation of sample solution

Twenty tablets of atocor E containing 10 mg of atorvastatin and 10 mg of ezetimibe were weighed and crushed into a fine powder. The quantity of powder equivalent to the weight of one tablet was accurately weighed and dissolved in sufficient amount of mobile phase in a 100 ml volumetric flask. The solution was sonicated for 15 minutes filtered through 0.45 µ nylon membrane filter, and diluted to 100 ml with mobile phase. Further dilution was made with mobile phase to give a final concentration of atorvastatin (50 µg/ml) and ezetimibe (50 µg/ml).

METHODS

Various mobile phase combinations were tried initially to separate atorvastatin and ezetimibe on C_{18} column. In order to achieve acceptable peak shapes and perform the separation on a suitable run time various buffer systems are also tried systematically. Mobile phase composed of phosphate buffer and acetonitrile indicated that the resolution between atorvastatin and ezetimibe increased. Therefore phosphate buffer (pH4.5) and acetonitrile in the ratio 35:65 v/v at a flow rate of 1 ml/minute was selected as optimised mobile phase. hypersil BDS C_{18} coloumn (250 mm × 4.6 mm, 5 mm particle size) was used as the stationary phase to improve resolution. To analyze both drugs, detection was tried at various wavelengths but 228 nm was selected as the detection wavelength as both the drugs showed maximum absorption. The retention time was found to be 2.36 and 3.43 minutes for atorvastatin and ezetimibe respectively. The chromatogram obtained was shown in Fig. 3. The system suitability parameters were shown in Table 1.

Method validation

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The above method was validated according to ICH guidelines to establish the performance characteristics of a method (expressed in terms of analytical parameters) to meet the requirements for the intended application of the method. They were tested using the optimized chromatographic conditions and instruments.

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration of analyte in the sample. Linearity of detector response for atorvastatin and ezetimibe was established by analyzing serial dilutions of a stock solution of the working standard. Six concentrations such as 12.5, 25, 37.5, 50, 62.5 and 75 µg/ml for atorvastatin and ezetimibe are prepared and analyzed. The linearity graph was plotted using concentration verses peak area and shown in Figs. 4 and 5.

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurement obtained from multiple sampling of the same homogenous sample under prescribed conditions. Repeatability of the method was checked by injecting replicate injections of 50 µg/ml of the solution for 6 times on the same day as intra-day precision study of atorvastatin and ezetimibe and the chromatogram was recorded. The mean area and % relative standard deviation (RSD) was calculated. From the data obtained, the developed RP-HPLC method was found to be precise. The result was shown in Table 2.

Accuracy

The accuracy of the method was determined by recovery experiments known concentrations of working standard was added to the fixed

Table 1: System suitability parameters

Parameter	Atorvastatin	Ezetimibe
Retention time	2.367	3.436
USP plate count	5661	9490
USP tailing	0.99	1.09
USP resolution		7.51

USP: United States Pharmacopoeia

Table 2: Precision result for atorvastatin and ezetimibe

Injections	Atorvastatin	Ezetimibe
1	876396	939613
2	872261	924638
3	862879	926401
4	862236	941016
5	871646	933448
6	875646	937785
Mean	870177.3	933816.8
Standard deviation	6187.2	6936.16
%RSD	0.711	0.742

RSD: Relative standard deviation

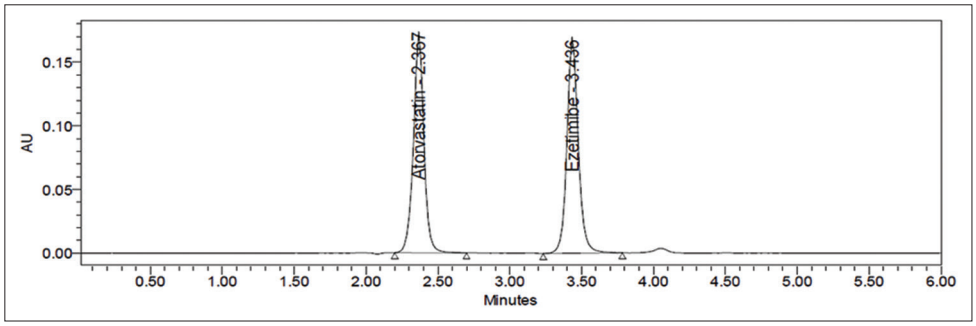

Fig. 3: Standard chromatogram

Table 3: Accuracy result for atorvastatin and ezetimibe

Analyte	% Concentration	Amount added (µg)	Amount found (µg)	% Recovery	Mean % recovery
Atorvastatin	50	25	24.96	99.84	99.99
	100	50	49.98	99.96	
	150	75	75.12	100.16	
Ezetimibe	50	25	25.22	100.88	100.22
	100	50	49.96	99.92	
	150	75	74.90	99.86	

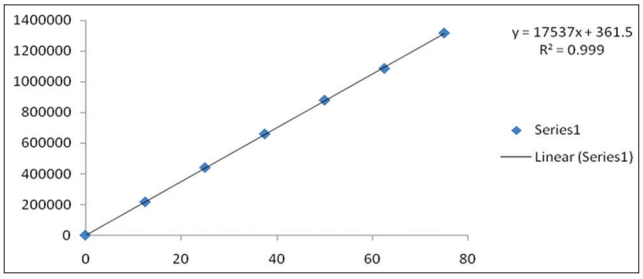

Fig. 4: Linearity graph of atorvastatin

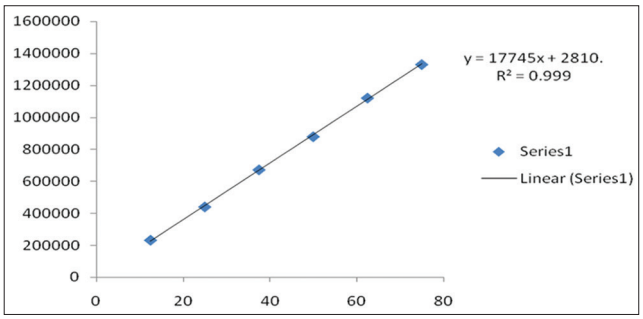

Fig. 5: Linearity graph of ezetimibe

concentration of the pre-analyzed Tablet sample. Percent recovery was calculated by comparing the area with preanalyzed sample. For both the drugs, recovery was performed in the same way. The recovery studies were performed in triplicate. This standard addition method was performed at 50%, 100%, 150% level and the percentage recovery was calculated by substracting the total area from pre analyzed sample area. The results were shown Table 3.

Specificity

Spectral purities of atorvastatin and ezetimibe chromatographic peaks were evaluated for the interference of the tablet excipients, degradation components or due to the presence of impurities as per the methodology. In the work, a solution containing a mixture of the tablet excipients were prepared using the sample preparation procedure to evaluate possible interfering peaks. The representative chromatogram did not show any other peaks, which confirmed the specificity of the method.

Robustness

The robustness of an 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. It was observed that the variations like flow rate of mobile phase, column temperature, ratio of organic content in the mobile phase etc. does not have any significant effect on the method performance, which demonstrated that the developed RP-HPLC method is robust. The results were shown in Table 4.

Ruggedness

Inter day variations were performed by using six replicate injections of standard and sample solutions of concentrations, which were prepared and analyzed by different analysts on three different days over a period of 1 week. Ruggedness also expressed in terms of percentage RSD and statistical analysis showed no significant difference between results obtained employing different analyst.

Table 4: Robustness results for atorvastatin and ezetimibe

Parameters	Atorvastatin		Ezetimibe	
	Rt	USP tailing	Rt	USP tailing
Flow rate 1 (0.9mL/min)	2.385	1.00	3.556	1.06
Flow rate 2 (1.1mL/min)	2.370	0.98	3.442	1.09
Mobile phase 1 (32:68 v/v)	2.376	0.94	3.447	1.08
Mobile phase 2 (38:62 v/v)	2.334	1.02	3.412	1.07
Temaparature 1 (25°C)	2.366	1.08	3.433	1.2
Temperature 2 (35°C)	2.361	1.1	3.435	1.3

Rt: Retention time

Table 5: Assay results

Sample	Batch no	Label claim (mg)	% Amount found	Average
Atorvastatin	1	10	99.6	99.66
	2	10	99.8	
	3	10	99.6	
Ezetimibe	1	10	98.9	99.3
	2	10	99.4	
	3	10	99.6	

Detection and quantitation limits

The limit of detection (LOD) and limit of quantitation (LOQ) of atorvastatin and ezetimibe were determined by using the signal to noise ratio approach as defined in ICH guidelines. According to the determined signal to noise ratio, the LOD and LOQ for atorvastatin were 0.07 µg/ml and 0.21 µg/ml, respectively. For ezetimibe LOD and LOQ were 0.08 µg/ml and 0.25 µg/ml, respectively.

Assay of pharmaceutical formulation

Three different batches of atocor E were analyzed using the validated method. For the analysis, six replicate of each batch were assayed. The mean peak area of the drug was calculated, and the drug content in the tablet was quantified. The result found was comparable with the corresponding labelled amounts, and they were shown in Table 5.

CONCLUSION

In this present work a new simple, selective, linear, precise, accurate and robust HPLC method was developed and validated for the estimation of atorvastatin and ezetimibe in pharmaceutical dosage form in accordance with the ICH guidelines. This method gives good resolution between both the compounds with a short analysis time. Linearity was observed in the concentration range of 12.5-75 µg/ml for both the drugs at 228 nm. The system suitability tests revealed that numbers of theoretical plate were above 2000 and the tailing factor is >2. The percentage recoveries of atorvastatin and ezetimibe were 99.99% and 100.22% respectively, which shows the accuracy of the

method. Precision values were within the acceptability limit, which indicates that the method is precise. Specificity experiment shows that there is no interference of excipients with the main peaks, which confirmed the specificity of the method. The lowest values of LOD and LOQ, as obtained by the method, indicate the sensitivity of the method. The assay results of the pharmaceutical formulation by this method are highly reproducible, reliable and are in good agreement with the label claim of the drug. Thus, this method can be useful for the routine analysis of atorvastatin and ezetimibe in combined dosage form.

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