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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF ATORVASTATIN AND EZETIMIBE IN BULK BY RP-HPLC

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ABSTRACT

A simple, rapid and selective RP-HPLC method has been developed simultaneously for bulk forms containing HMG-CoA reductase inhibitor, lipid lowering drugs named Atorvastatin and Ezetimibe. The present study describes the development of a comprehensive science and risk based HPLC method development and subsequent validation for the analysis of lipid lowering drugs. The separations were carried out on C-18 reversed phase column (Inertsil ODS C-18(250 x 4.6mm, 5µ) using a mass compatible mobile phase i.e, Methanol : Acetonitrile (80:20)v/v in a isocratic elution mode at a flow rate of 1.0ml/min and column oven temperature of 25˚C. The wavelength of detection was 248nm for Atorvastatin and Ezetimibe. Detection limit was found to be 2.762 for Atorvastatin and 3.161 for Ezetimibe. Analytical validation parameters such as selectivity, linearity, system suitability, accuracy, precision, robustness, ruggedness, limit of detection and quantification (LOD & LOQ) were evaluated as per ICH Q2 (R1). The proposed method can be used for routine analysis of bulk forms containing HMG-CoA reductase inhibitor and lipid lowering drugs such as, Atorvastatin and Ezetimibe.

***KEYWORDS**: Atorvastatin and Ezetimibe, RP-HPLC, Method development and Validation.*

INTRODUCTION

Atorvastatin is chemically named as (3R,5R)-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 is a HMG-CoA reductase inhibitor. This is an enzyme which founds in the liver tissue that plays a key role in the body by the production of cholesterol [1].

Ezetimibe is chemically named as (3R,4S)-1-(4-fluorophenyl)-3-([3S]-3-[4-fluorophenyl]-3-hydroxypropyl)-4-(4-hydroxyphenyl) azetidin-2-one (Fig. 2). It is a drug that lowers plasma cholesterol levels. It decreases the absorption of cholesterol in small intestine. If other cholesterol lowering medications are not tolerated in the combination, in that condition it may be used alone or may be used in combination with statins, when statins alone do not work. 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 and high performance liquid chromatography (HPLC) methods have been reported for the estimation of Atorvastatin and Ezetimibe. The aim of this study is to develop a simple, precise and accurate reverse phase HPLC (RP-HPLC) method for the estimation of Atorvastatin and Ezetimibe in bulk forms as per ICH guidelines. The validation procedure done according to USP 30 guidelines [3-11].

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MATERIALS AND 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 inertsil ODS C18 column (250 mm ×4.6 mm, 5 µ particle size) and detection was performed at 248 nm. The injection volume of sample was 20 µl and the run time was 12 minutes. An isocratic mobile phase consisted of methanol and acetonitrile in the ratio 80:20v/v was carried out with the flow rate at 1 ml/minute.

Reagents and Chemicals:

Atorvastatin and Ezetimibe were obtained as gift samples from Dr. Reddy's laboratories.

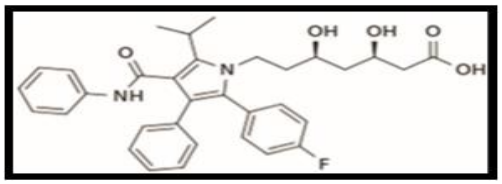

Fig. 1: Structure of Atorvastatin

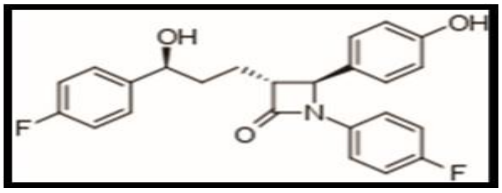

Fig. 2: Structure of Ezetimibe

Ultra pure water was obtained from a Millipore system. HPLC grade acetonitrile and methanol were obtained from standard reagents solutions private limited.

Preparation of stock solution:

Standard stock solutions were prepared by taking 10 mg of Atorvastatin and 10 mg of Ezetimibe into 10 ml volumetric flasks separately with diluents and were dissolved. Sonicated for 20 minutes and volume was adjusted with diluent.

Preparation of working standard:

Working standards were prepared by taking 0.4 ml of Atorvastatin and 0.4 ml of Ezetimibe from the stock solutions into a 10 ml valumetric flask and adjusted the volume with diluent.

Methods:

Initially various combinations of mobile phases were tried systematically to separate Atorvastatin and Ezetimibe on ODS C18 column with a suitable run time. In order to get acceptable peak shapes. Mobile phase composed of methanol and acetonitrile showed the increased resolution between Atorvastatin and Ezetimibe. Therefore this mobile phase combination was selected as optimized mobile phase in the ratio of 80:20v/v respectively. To improve resolution the stationary phase used was inertsil ODS C18 column (250mm×4.6mm, 5µ particle size). Various wavelength detections were carried out to analyze both the drugs but maximum absorption showed at 248nm, so 248 nm was selected as detection wavelength for both drugs. The retention times of Atorvastatin and Ezetimibe were found to be 4.97 and 7.07 respectively. The obtained chromatogram was presented in Fig. 3. The system suitability parameters were given in Table 1.

Table No. 1: System suitability parameters

Parameters	Atorvastatin	Ezetimibe
Retention Time	4.97	7.07
USP Plate count	5395	6683
USP Tailing	1.48	1.14
Resolution		4.3

USP: United States of Pharmacopoeia

Table No. 2: Precision results

Injections	Atorvastatin	Ezetimibe
1	4219201	2278639
2	4237216	2247320
3	4235847	2267407
4	4195611	2254490
5	4226557	2231236
6	4268847	2245756
Mean	4225275	2260652
SD	16219.94	16338.36
%RSD	0.38	0.72

SD: Standard Deviation; RSD: Relative Standard Deviation

Method validation:

The purpose of the method validation is to demonstrate that the method is suitable for its intended use as per ICH guidelines. To establish the performance characteristics, the above method was validated according to ICH guidelines to meet the essentials for the intended purpose. The analytical parameters (performance characteristics) were tested by using the optimized conditions.

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. Six concentrations were prepared by taking serial dilutions of 20, 30, 40, 50, 60, 70 and 80µg/ml from working standard and were analyzed for detector of linearity. The linearity graphs for both the drugs were plotted by taking concentration (µg/ml) on X-axis and peak area (abosorbing units) on Y-axis. They were shown in Figs. 4 and 5.

Precision:

Precision is a description of random errors, a measure of statistical variability it refers to the closeness of measurements to each other from multiple sampling of the same homogenous sample under prescribed conditions. Repeatability was tested by injecting the 6 replicates of 40µg/ml solution on the same day as intra-day precision. The chromatogram was recorded and the results were given in Table 2.

Accuracy:

The accuracy is a closeness of measured value to the known value. It was tested by taking triplicates that are 50%, 100% and 150% concentrations and the percent recovery was calculated. The results were given in Table 3.

Specificity:

Atorvastatin and Ezetimibe chromatographic peaks were evaluated by testing the sample solution for the interference of any degradation components or the impurities due to the methodology, where any other peaks were not found in the chromatogram.

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 tested for the flow rate variations, it did not show any effect on method performance. Which indicates that the method was robust. The results were given in Table 4.

Ruggedness:

Different analysts on three different days performed the inter day variations by injecting 6 replicates of test solution. The percent RSD was calculated and the statistical analysis did not show any significant difference between the results acquired by different analysts.

Detection and quantitation limits:

According to the ICH guidelines by using the signal to noise ratio approach The limit of detection (LOD) and limit of quantification (LOQ) were tested. According to this, the LOD and LOQ of A Atorvastatin were 0.49µg/ml and 0.48µg/ml respectively. The LOD and LOQ of Ezetimibe were 0.52µg/ml and 1.58µg/ml respectively.

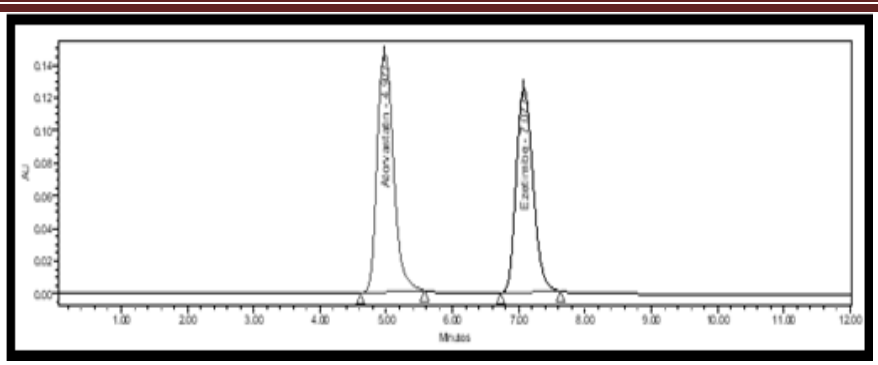

Fig. 3: Standard chromatogram

Table No. 3: Accuracy results for Atorvastatin and Ezetimibe

Analyte	%Concentration	Amount added(µg)	Amount found (µg)	% Recovery	Mean % recovery
Atorvastatin	50	20	19.93	99.69	
	100	40	39.93	99.83	99.83
	150	60	59.98	99.978	
Ezetimibe	50	20	20.01	100.06	
	100	40	40.01	100.04	100.04
	150	60	60.01	100.02	

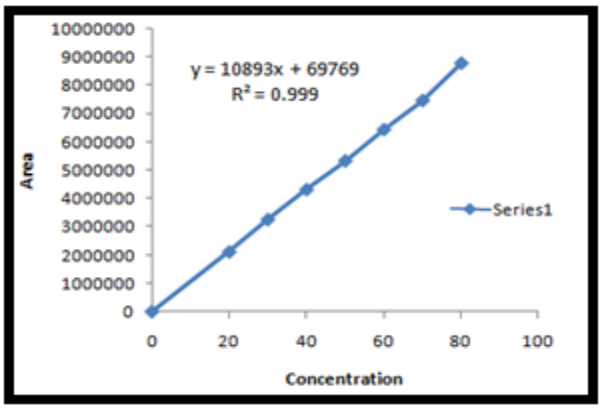

Fig. 4: Linearity graph of Atorvastatin

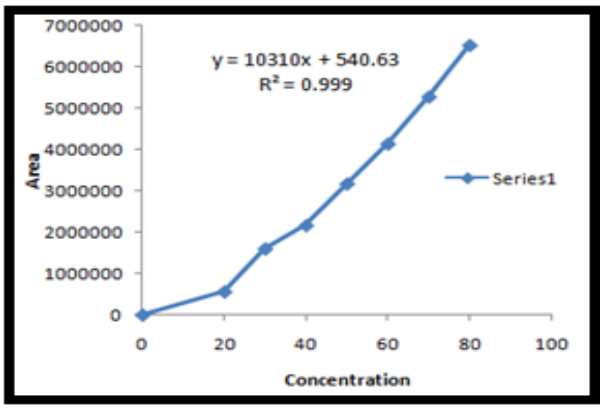

Fig. 5: Linearity graph of Ezetimibe

Table No. 4: Robustness results of Atorvastatin and Ezetimibe

Parameters	Atorvastatin		Ezetimibe	
	Area	%RSD	Area	%RSD
Flow rate 0.8 ml/min	1.49	0.99	0.64	1.53
Flow rate 1.0 ml/min	0.64	1.86	0.90	0.24
Flow rate 1.2 ml/min	0.38	0.37	1.34	0.23

CONCLUSION

In this present work a new simple, selective, linear, precise, accurate and robust HPLC method was developed and validated for the simultaneous estimation of Atorvastatin and Ezetimibe in bulk form in accordance with the ICH guidelines. This method gives good resolution between both the drugs. Linearity was observed in the concentration range of 20 - 80µg/ml for both the drugs. The wavelength detection at 248nm. The system suitability tests revealed that numbers of theoretical plates were above 2000 and the tailing factor is >2. The percentage recoveries of Atorvastatin and Ezetimibe were 99.83% and 100.04% 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 degradation components 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. Thus, this method can be useful for the routine analysis of Atorvastatin and Ezetimibe in combined bulk form.

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