World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.org/
Original Article

Method Development and Validation for Simultaneous Estimation of Atorvastatin and Ezetimibe in Pharmaceutical Dosage Form by HPLC

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Received: 12-07-2014 / Revised: 20-07-2014 / Accepted: 22-07-2014

ABSTRACT

A new HPLC method was developed and validated for the determination of Atorvastatin and Ezetimibe in tablet dosage form. The chromatographic separation was achieved on a Inspire C_{18}(4.6 x 250mm, 5µm) with a mobile phase combination of phosphate buffer, methanol and Acetonitrile (40:10:50) at a flow rate of 1.2 ml/min, and the detection was carried out by using UV detector at 233 nm. The total run time was 8minutes.The retention time of phosphate buffer, methanol and Acetonitrile were found to be 2.237 min. and 3.164 min. respectively. The performance of the method was validated according to the present ICH guidelines.

Key words: Atorvastatin, Ezetimibe, HPLC

INTRODUCTION

Atorvastatin, is a member of the drug class known as statins. Chemical name of Atorvastatin is (3R,5R)-7-[2-(4-fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-(propan-2-yl)-1H-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid. Atorvastatin is a competitive inhibitor of hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-determining enzyme in cholesterol biosynthesis via the mevalonate pathway. HMG-CoA reductase catalyzes the conversion of HMG-CoA to mevalonate. Atorvastatin acts primarily in the liver. Decreased hepatic cholesterol levels increases hepatic uptake of cholesterol and reduces plasma cholesterol levels [1-2]. Ezetimibe is an anti-hyperlipidemic medication which is used to lower cholesterol levels. Chemical name of Ezetimibe is (3R,4S)-1-(4-fluorophenyl)-3-[(3S)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl)azetidin-2-one. Ezetimibe localizes and appears to act at the brush border of the small intestine and inhibits the absorption of cholesterol. This leads to a decrease in the delivery of intestinal cholesterol to the liver [3]. The literature survey revealed that there are few HPLC and spectroscopic methods available for the determination of Atorvastatin and ezetimibe in pure and combined dosage forms. The present study was aimed to develop a new HPLC method for simultaneous estimation of Atorvastatin and Ezetimibe in combined pharmaceutical dosage form.

EXPERIMENTAL

Chemicals and reagents: Atorvastatin and Ezetimibe bulk drugs were made available from Pharmatrain, Hyderabad. Orthophosphoric acid,methanol were obtained from Merk. Commercially available Atocor E (Dr.Reddy's) was used for the dosage form analysis. All chemicals and reagent used were of HPLC grade, Milli-Q-water was used throughout the experiment.

Equipments: The Waters HPLC system with a UV or photo diode array detector was used for method

Chromatographic condition: The mobile phase used was mixture of phosphate buffer, methanol and Acetonitrile in the ratio of 40:10:50 employing isocratic elution at a flow rate of 1.2 ml/min. The analytical column used was Inspire C_{18}(4.6 x 250mm, 5µm).The detection was carried out at a wavelength of 233nm for a run time of 8 min.

Preparation of standard solution: Accurately weigh and transfer 10 mg of Atorvastatin & 10mg of Ezetimibe working standard into two separate 100ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock

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solution). Further pipette 1.5 ml of Atorvastatin & 1.5 ml Ezetimibe of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Assay of Pharmaceutical Dosage form: (Sample Preparation): Twenty tablets of Atocor E were weighed to get the average weight and then ground. An amount of powder equivalent to 10 mg was transferred to a 100 mL volumetric flask, added 70 mL of methanol and sonicated for 10 min with intermediate shaking. Followed by makeup the volume with methanol to obtain a solution containing 100µg/ml Atorvastatin and 100µg/ml Ezetimibe. Further pipette 1.5 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

RESULTS AND DISCUSSION

Method development: Chromatographic parameters were preliminary optimized to develop HPLC method for simultaneous estimation of Atorvastatin and Ezetimibe with short analyses time (8 min), and acceptable resolution (> 2). The isoabsortive point of Atorvastatin and Ezetimibe selected was 233 nm. In order to identify a suitable organic modifier, various compositions of acetonitrile and methanol were tested along with phosphate buffer. Different columns like Waters, Inertsil, Inspire columns were tried. Resolution was the major problem while we are developing method. Resolution was less very less when we are using one organic phase, to increase resolution phosphate buffer, methanol and acetonitrile were used in the ratio of 40:10:50. Finally separation for simultaneous determination of Atorvastatin and Ezetimibe was carried out by isocratic elution with a flow rate of 1.2 mL/min Inspire C_{18}(4.6 x 250mm, 5µm).The standard chromatogram was shown in figure1 .The system suitability parameters were shown in Table-1.

Method Validation:

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[4].

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 (amount) 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. Five concentrations ranging from 5-25 µg/ml for Atorvastatin and Ezetimibe were prepared and analyzed. The linearity graph was plotted using concentration Vs peak area and they were shown in Fig-1,2. Slope, correlation coefficient (R) and intercept were calculated and the results were shown in Table-2.

Precision: For the precision study, repeatability study was carried out for short time interval under the same chromatographic conditions. For the intermediate precision study, repeatability study was carried out in different day under the same chromatographic conditions. The sample was injected in six replicate. The peak area for all the six replicate was recorded. The mean and % relative standard deviation (%RSD) was calculated. From the data obtained the developed RP-HPLC method was found to be precise. The results were shown in Table-3.

Accuracy: The accuracy of the method was determined by recovery experiments. Known concentration of working standard was added to the fixed concentration of the pre-analyzed tablet sample. Percent recovery was calculated by comparing the area with preanalyed 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 subtracting the total area from preanalyed sample area. The results were shown in Table-5.

Robustness: Robustness of the method was checked by making slight deliberate changes in chromatographic conditions like mobile phase ratio(±10%), flow rate (0.2ml/min). It was observed that there were no marked changes in system suitability parameters, which demonstrated that the developed RP-HPLC method is robust.

LOD and LOQ: The LOD and LOQ of Atorvastatin and Ezetimibe were determined by using the signal to noise approach as defined in ICH guidelines .The concentration with signal to noise ratio of at least 3 was taken as LOD and concentration with signal to noise ratio of at least 10 was taken as LOQ. The results were shown in Table-7.

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine Atorvastatin and Ezetimibe in their tablet dosage form. The result obtained for Atorvastatin and Ezetimibe was comparable with the corresponding labeled amounts and they were shown in Table-8.

CONCLUSION

In the present work a new, accurate, precise and robust HPLC method was developed and validated for estimation of Atorvastatin and Ezetimibe in pharmaceutical dosage form in accordance with the ICH parameters. The method gives good resolution between both the compounds with a short analysis time (8 min). Linearity is observed in the concentration range of 5-25 µg/ml for both the drugs at 235 nm. The results of the analysis of pharmaceutical formulation by the proposed method are highly reproducible and reliable and it is in good agreement with the label claim of the drug. The method can be useful for the routine analysis of the Atorvastatin and Ezetimibe in combined dosage form without any interference of excipients.

Fig.1: Standard chromatogram

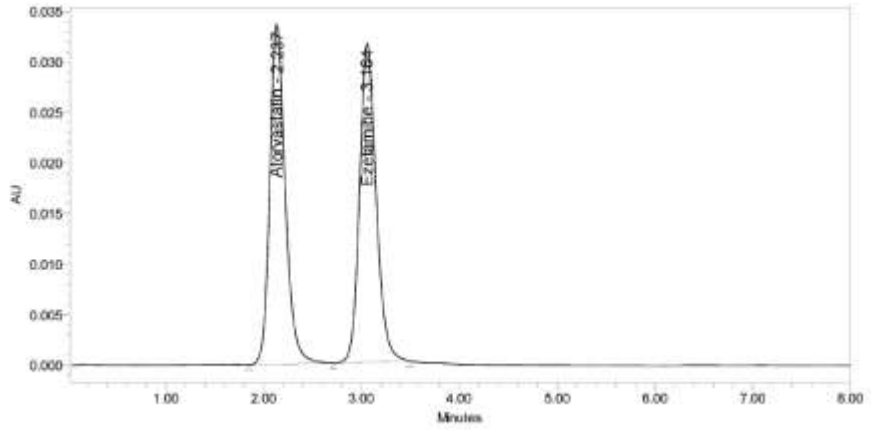

Table 1:system suitability parameters

Parameter	Atorvastatin	Ezetimibe
Retention time	3.533	4.462
USP Plate count	2566	2487
USP Tailing	1.21	1.15
USP Resolution		3.11

Table 2:Regression and statistical parameters

Parameter	Atorvastatin	Ezetimibe
Concentration Range (µg/ml)	5-25	5-25
Correlation coefficient	0.999	0.999
Intercept	2226	1631
Slope	24561	25107

Table 3: Precision results for Atorvastatin and Ezetimibe

S.No.	Atorvastatin	Ezetimibe
1.	369499	373294
2.	371265	375093
3.	370011	373793
4.	368216	372172
5.	366657	369875
6.	365666	370032
Average	368552.3	372376.5
Standard deviation	2117.49	2098.75
% RSD	0.57	0.56

Fig.1.2:Linearity plot for Atorvastatin and Ezetimibe

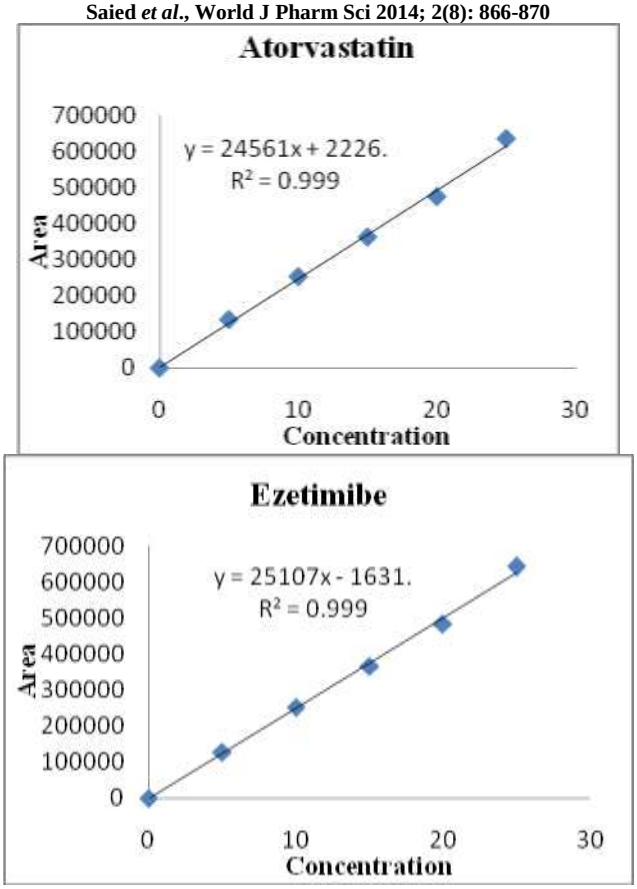

Table 4: ID Precision results for Atorvastatin and Ezetimibe

S.No.	Atorvastatin	Ezetimibe
1.	367293	370441
2.	367194	370135
3.	367340	369973
4.	367655	370394
5.	368223	370733
6.	368313	370817
Average	367669.7	370415.5
Standard deviation	489.30	327.90
% RSD	0.13	0.09

Table 5: Accuracy Results For Atorvastatin And Ezetimibe

Analyte	% Level	Nominal Value (mg)	Found (mg)	% Recovery	Mean % Recovery
Atorvastatin	50%	5	5.04	100.74	99.59
	100%	10	9.97	99.72	
	150%	15	14.65	98.30	
Ezetimibe	50%	5	4.92	98.58	98.98
	100%	10	9.80	99.56	
	150%	15	14.72	98.80	

Table 6: Robustness Results for Atorvastatin And Ezetimibe

	Atorvastatin			Ezetimibe		
	Retention time	USP Plate count	USP Tailing	Retention time	USP Plate count	USP Tailing
Flow 1	4.172	2731	1.24	5.290	3724	1.15
MP1	4.086	2547	1.25	5.124	3628	1.32
Flow 2	3.102	2426	1.22	3.921	3322	1.15
MP2	3.118	2639	1.39	3.637	3346	1.34

Flow 1- 1.0ml/min, Flow 2- 1.4ml/min, MP 1- 50:5:45 (phosphate buffer: Methanol:Acetonitrile), MP 2- 30:15:55 (phosphate buffer : Methanol:Acetonitrile).

Table 7: LOD and LOQ Results For Atorvastatin And Ezetimibe

	LOD (µg/ml)	LOQ (µg/ml)	LOD S/N Ratio	LOQ S/N Ratio
Atorvastatin	0.06	0.2	3.02	9.95
Ezetimibe	0.062	0.211	2.93	9.98

Table 8: Assay Results

	Label claim	% Assay
Atorvastatin	10 mg	100.39
Ezetimibe	10 mg	100.18

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