

ORIGINAL RESEARCH ARTICLE

Simultaneous Method Development and Validation for Assay of Atorvastatin Calcium and Ezetimibe Drugs in Solid Dosage Form by RP-HPLC

Khan M. Rizwan^{*1}, Jain Deepti², Jain Neetesh¹, Singh Shristi¹, Sharma Vijendra¹ and Agarwal Ashish³

¹*Oriental College of Pharmacy and Research, Oriental University, Indore, India*

²*Schools of Pharmacy, Rajiv Gandhi Technical University, Bhopal, India*

³*Mandsaur Institute of Pharmacy, Rewas-Dewda Road, Mandsaur (M.P), India*

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ABSTRACT

A simple, specific, accurate and precise RP HPLC method has been developed and validated for the simultaneous determination of Atorvastatin calcium (AC) and Ezetimibe (EZ) from combined dosage form by reverse phase C18 column (Shimadzu- LC-10Atp (250mm x 4.6mm) 5 μ). The sample was analysed using Methnol: Acetate Buffer I.P. in the ratio of 70:30(pH adjusted to 3.7 with acetic acid) as a mobile phase at a flow rate of 1.0ml/min and detection at 248nm. The retention time for Atorvastatin calcium (AC) and Ezetimibe (EZ) was found to be 6.96 min and 5.82min respectively. The stability assay was performed for this combination and was validated for accuracy, precision, linearity, specificity and sensitivity in accordance with ICH guidelines. Validation revealed the method is specific, rapid, accurate, precise, reliable, and reproducible. Calibration plots were linear over the 70%-130% concentration ranges for both the drugs of AC and EZ respectively, and recoveries from combined dosage form were between 99% and 101%. The method can be used for estimation of combination of these drugs in combined dosage form.

Keywords: Atorvastatin calcium, Ezetimibe, RP-HPLC.

INTRODUCTION

Atorvastatin ^[1-3] is a selective, competitive inhibitor of HMG-CoA reductase. In animal models, atorvastatin lowers plasma cholesterol and lipoprotein levels by inhibiting HMG-CoA reductase and cholesterol synthesis in the liver, and by increasing the number of hepatic LDL (low-density lipoprotein) receptors on the cell surface to enhance uptake and catabolism of LDL. Atorvastatin also reduces LDL production and the number of LDL particles. Atorvastatin reduces total-C (cholesterol), LDL-C, and apolipoprotein B (apo B) in patients with homozygous and heterozygous familial hypercholesterolemia (FH), nonfamilial forms of hypercholesterolemia, and mixed dyslipidemia. Atorvastatin reduces total-C, LDL-C, very low-density lipoprotein cholesterol (VLDL-C), apo B, triglycerides (TG), and non-high-density lipoprotein cholesterol (non-HDL-C) and increases HDL-C in patients with isolated hypertriglyceridemia. Atorvastatin reduces

intermediate density lipoprotein cholesterol in patients with dysbetalipoproteinemia.

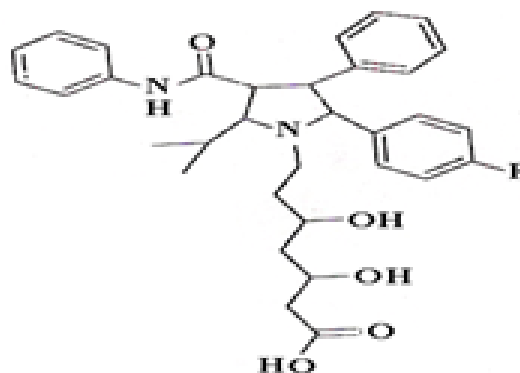


Fig 1(a): Structure of Atorvastatin Calcium

Ezetimibe ^[4-7] is a therapeutically beneficial drug that works by a unique mechanism and differs from traditional lipid lowering agents. Cholesterol absorption is an active process and ezetimibe inhibits the protein transporters on small intestinal brush border which brings about this active

transport. In addition to prevent cholesterol ezetimibe also inhibits phytosterol absorption. Chemically ezetimibe is 1-(4-Fluorophenyl) – 3(R)-[3-(4-fluorophenyl) – 3(S) hydroxy propyl]-4 (S) – (4 – hydroxy phenyl) – 2 azetidinones.

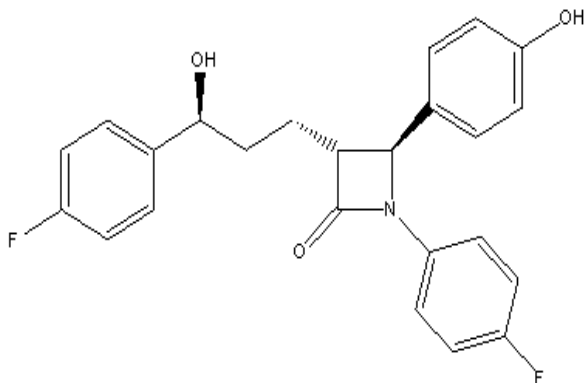


Fig 1(b): Structure of Ezetimibe

Experimental

A High Performance Liquid Chromatograph system, the purity determination performed on a stainless steel column 250mm long, 4.6mm internal diameter filled with Octadecyl silane chemically bonded to porous silica particles of 5µm diameter reverse phase C18 column (Luna CN (250mm x 4.6mm) 5µ). Optimized chromatographic conditions are listed in (Table 1).

Table 1: Optimized Chromatographic conditions

Parameter	Optimized condition
Instrument	Shimadzu- LC-10Atvp/Empower software/PDA detector
Column	Luna C18 (250mm x 4.6mm) 5µ
Mobile phase*	Methanol : Acetate Buffer I.P. in the ratio of 70:30(pH adjusted to 3.7 with acetic acid)
Flow rate	1.0ml/min
Detection	248nm
Injection volume	20µl
Temperature	Ambient

*Filtered through a 0.45µ membrane filter (Millipore), degassed and sonicated.

Materials and Chemicals:

Pure samples of Atorvastatin Calcium and Ezetimibe were obtained from Ranbaxy Pvt. Ltd. Retro Lab and for the estimation of Atorvastatin Calcium and Ezetimibe in commercial formulations. HPLC grade Acetic Acid and Methanol were procured from institute and of Rankem Ltd. High pure water prepared by using Millipore Milli Q plus purification system.

Preparation of Standard Stock Solution:

10 mg of EZ and AC WS (working standards) was accurately weighed and transferred to a 50 ml volumetric flask and dissolution was carried out in diluent (70% methanol) to get a solution of concentration 200 µg/ml (stock A). 12.5 ml of

stock A was taken and diluted with diluent to 25ml to get the solution of concentration 100 µg/ml (stock B). Further dilutions were made from stock B to get a series of dilutions ranging in concentration from 0 – 20 µg/ml.

Mixtures Standard Preparation:

Preparation of mixed standards:

The commercial tablet and capsule formulation of AC and EZ are in the ratio of 1:1. Based on this fact five mixed standards were selected for quantitative analysis, which gave satisfactory results.

Preparation of test sample:-

Sample Stock Preparation:

Twenty tablets were taken and their average weight was determined. They were crushed to fine powder; amount equivalent to 10mg of AC was taken in 10ml volumetric flask. This was then dissolved in 7ml methanol by sonication for about 2 minutes. The volume was made up to the mark with water and was filtered through Whatmann filter paper (No.41). The filtrate was further diluted to get final concentrations of both the drugs in the working range.

Sample Preparation:

Take 5ml of the sample stock solution in 200ml flask and make up the volume with diluent. Filter the solution through 0.45 nylon membrane filter paper.

Validation of the Method [8-11]

The method was validated in terms of linearity, accuracy, precision, specificity and Robustness of the sample applications as per the ICH Guidelines.

Linearity:

The linearity of the method was investigated by serially diluting the stock solutions of Atorvastatin Calcium, Ezetimibe and measured the absorbance at 248nm. Calibration curves were constructed by plotting the area against the concentration. Atorvastatin Calcium shows the linearity in the concentration range from 0-20µg/ml with correlation coefficient of 0.9999 and Ezetimibe shows the linearity in the concentration range from 0-20µg/ml with correlation coefficient of 0.9998.

Accuracy:

Recovery studies were carried out to study the accuracy of the proposed method and ascertained by standard addition method. A known amount of drug was added to reanalyzed tablet powder, at

three level and the percentage recoveries were calculated.

Precision:

Precision was found to be lower than 1%. Ruggedness of the proposed method was determined by analysis of aliquots from homogenous slot by different analysts using similar operational and environmental conditions.

Robustness

As per the ICH norms, small, but deliberate variations by altering the pH and / or concentration of the solvent were made to check the method's capacity to remain unaccepted. The change was made in the strength of methanol. Instead of 50% methanol, 75 % methanol was used as solvent.

Specificity:

Commonly use excipients (starch, lactose, magnesium stearate) were spiked into a preweighed quantity of drug mixture. The chromatogram was taken by appropriate dilutions and the amount of each drug present in the sample mixture was determined.

Stability:

In order to demonstrate the stability of both the standard and sample solutions during analysis, both the solutions were analyzed over a period of 5 hours at room temperature. The peak areas and retention time of both the drugs remained almost unchanged and no significant degradation within the indicated period.

RESULTS AND DISCUSSION

Table 2: Analysis of Formulation and Recovery studies

Assay No.	Standard Deviation of AC	Atorvastatin calcium	Assay (%)	Standard Deviation of EZ	Ezetimibe Assay (%)
1	0.02301	100.691		0.1154	100.36
2	0.08299	99.84		0.1222	100.25
3	0.06394	100.128		0.1081	99.95
4	0.11368	100.115		0.1463	99.98
5	0.13043	99.902		0.0991	100.15
Mean	0.08281	100.135		0.1182	100.138
% RSD	0.78	0.78		0.84	0.84

The precision of the method was determined by studying repeatability and reproducibility. The area of drug peaks and percentage relative standard deviation were calculated. The results revealed that the developed method was found to be reproducible in nature.

The standard drug solutions in varying concentrations ranging from 70% to 130 % of the targeted level of the assay concentration were examined by the assay procedure. Atorvastatin Calcium and Ezetimibe were found to be linear in

1. Estimation

A RP-HPLC method was developed for the simultaneous estimation of Atorvastatin Calcium and Ezetimibe in combined dosage forms, which can be conveniently employed for routine quality control in pharmaceutical dosage forms. The chromatographic conditions were optimized in order to provide a good performance of the assay. The standard and sample solutions were prepared and chromatograms were recorded.

The peak area ratios of standard and sample solutions were calculated. The assay procedure was repeated for 6 times and mean peak area, mean peak area ratio, mean weight of standard drugs, mean weight of sample taken for assay were calculated. The percentages of individual drugs found in formulations, mean and relative standard deviations in formulation were calculated. The result of analysis shows that the amount of drugs present in the formulation has a very good correlation with the label claim of the formulation.

2. Validation of the method

The accuracy of the method was determined by recovery experiments. A known quantity of the pure drug was added to the pre-analyzed sample formulation at 70%-130% levels. The recovery studies were carried out 5 times of each level and the percentage recovery and mean of the percentage recovery were calculated and given in (Table 2). From the data obtained, it was observed that the recoveries of standard drugs were found to be accurate and within the specified limits.

the range of 0 to 20µg/ml and 0 to 20µg/ml respectively.

The slope, intercept and correlation coefficient values were also calculated. The correlation coefficient of Atorvastatin Calcium and Ezetimibe were found to be 0.9999 and 0.9998 respectively. The calibration curves were plotted as peak area Vs concentration of the standard solutions. The calibration graph shows that linear response was obtained over the range of concentrations used in the assay procedure. These data demonstrates that the methods have adequate sensitivity to the

concentration of the analytes. The range demonstrates that the method is linear outside the limits of expected use. The additional peaks were observed in the chromatogram of the formulation, which may be due to excipients present in the formulation. These peaks do not interfere with the

standard peaks, which clearly confirm the assay method was found to be highly specific.

The system suitability studies were performed for the standard solutions and were presented in (Table 3). The values obtained demonstrated the suitability of the system for the analysis of the above drug combination.

Table 3 (a) & (b): System Suitability Parameters Atorvastatin calcium (a) and Ezetimibe(b)

System suitability parameters	Rep-1	Rep-2	Rep-3	Rep-4	Rep-5	Mean	R.S.D
AUC X 10 ⁶	0.766733	0.768127	0.765421	0.767681	0.764923	0.766577	0.181
Retention Time	6.967	6.986	6.989	6.974	6.961	6.975	–
Tailing Factor	1.21	1.24	1.25	1.23	1.22	1.23	–
No. of Theoretical plates	4473	4475	4479	4478	4475	4476	–
System suitability parameters	Rep-1	Rep-2	Rep-3	Rep-4	Rep-5	Mean	R.S.D
AUC X 10 ⁶	0.658871	0.654181	0.662147	0.664571	0.671253	0.662205	0.964
Retention Time	5.821	5.828	5.836	5.825	5.820	5.826	–
Tailing Factor	1.27	1.29	1.25	1.28	1.24	1.266	–
No. of Theoretical plates	4679	4675	4682	4683	4681	4680	–

From the above experimental data results and parameters it was concluded that the developed RP-HPLC method has the following advantages.

- Ø The standard and sample preparation requires less time.
- Ø No tedious extraction procedure was involved in the analytical process.
- Ø Suitable for the analysis of raw materials. Run time required for recording chromatograms were less than 15 times.

laboratories, bio-pharmaceutical and bio-equivalence studies and in clinical pharmacokinetic studies in near future.

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Chromatogram of Atorvastatin Calcium and Ezetimibe

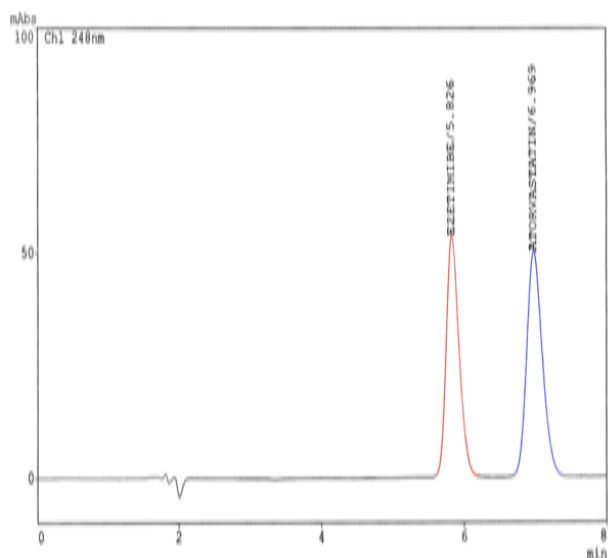


Figure 2: Typical chromatogram of Atorvastatin Calcium and Ezetimibe

Hence, the chromatographic method developed for Atorvastatin Calcium and Ezetimibe were found to be simple, precise, accurate and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing

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