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RP-HPLC ESTIMATION OF EZETIMIBE IN TABLET DOSAGE FORMS

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ABSTRACT

A simple, specific, accurate and precise reverse phase high performance liquid chromatographic method was developed for the estimation of ezetimibe in tablet dosage forms. A Phenomenex Luna C_{18}, 5 µm column having 250 × 4.6 mm i.d. in isocratic mode, with mobile phase containing Acetonitrile: 0.02M Phosphate buffer: methanol (70:20:10v/v) was used. The flow rate was 1.0 ml/min and effluents were monitored at 235 nm. The retention time of ezetimibe was 3.537 min. The method was validated for specificity, linearity, accuracy, precision, limit of quantification, limit of detection, robustness and solution stability. Limit of detection and limit of quantification for estimation of ezetimibe were found to be 1 µg/ml and 3.2 µg/ml respectively. Recoveries of ezetimibe in tablet formulations were found to be in the range of 99.6-101%. Proposed method was successfully applied for the quantitative determination of ezetimibe in tablet dosage forms.

Keywords: Ezetimibe, Reverse phase, HPLC, validation

INTRODUCTION

Ezetimibe is an Antihyperlipidemic and is usually categorized as HMG-CoA reductase inhibitor. Ezetimibe is chemically (3R, 4S)-1-(4-fluorophenyl)-3-[(3S)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxy phenyl) azetidin-2-one[1-2] and has the empirical formula $C_{24}H_{21}F_2NO_3$. Various methods have been reported for estimation of ezetimibe in pharmaceutical formulations and biological matrices such as plasma, which includes the use of spectrophotometry[3-10], HPLC[11-14], HPTLC[15-17] and LC – MS[18-19] methods.

Present studies involves development of RP-HPLC method using simple mobile phase containing acetonitrile, methanol and buffer for quantitative estimation of ezetimibe in tablet dosage forms which is sensitive and requires shorter analysis time. The developed method was validated as per ICH guidelines[20-21].

EXPERIMENTAL

Reagents and chemicals

Pure sample of ezetimibe was obtained as gift sample from M/s Dr. Reddy's Research Laboratories, Hyderabad. Acetonitrile, Methanol and water (HPLC grade) were procured from E. Merck (India) Ltd. Potassim dihydrogen orthophosphate (AR grade, purity 99.5%) was procured from Qualigens. Tablet formulation A (Ezedoc, Lupin Pharma Ltd., India) and B (Ezentia, Sun pharma Ltd., India) containing labeled amount 10mg ezetimibe were purchased from local market.

Instruments used

The liquid chromatographic system consisted of following components: Shimadzu HPLC prominence equipped with LC-20AT pump, variable wavelength programmable UV/VIS detector (SPD-20A) and Rheodyne injector (7725i) with 20 µl fixed loop. Chromatographic analysis was performed using Spinchrom software on a Phenomenex Luna C_{18} column with 250 × 4.6 mm i.d. and 5 µm particle size. The Shimadzu electronic balance (AX 200) was used for weighing purpose.

Chromatographic conditions

A reverse phase C-18 column equilibrated with mobile phase Acetonitrile: 0.02M Phosphate buffer:

methanol (70:20:10v/v) was used. Mobile phase was filtered through 0.45µm membrane filter and degassed. Mobile phase flow rate was maintained at 1.0 ml/min and effluents were monitored at 235 nm. The sample was injected using a 20 µl fixed loop and the total run time was 5 min.

Method development

Acetonitrile, Methanol and water in different proportions were tried and finally Acetonitrile: 0.02M Phosphate buffer: methanol (70:20:10v/v) was selected as an appropriate mobile phase which gave good resolution and acceptable system suitability parameters. The chromatogram of working standard solution is shown in Fig.1.

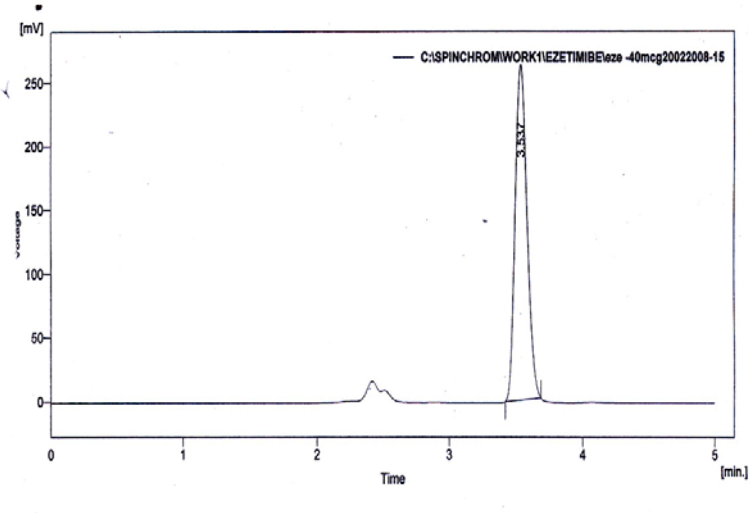

Fig. 1: HPLC chromatogram of ezetimibe (RT is 3.537 min)

Procedure

Preparation of working standard solution of ezetimibe

A stock solution of ezetimibe was prepared by accurately weighing 25 mg of drug, transferring to 25 ml volumetric flask, dissolving in 5 ml of methanol and diluting it upto mark with methanol. Appropriate aliquot of this solution was further diluted to 100 ml with mobile phase to obtain final standard solution of 100µg/ml of ezetimibe. Resultant solution was filtered through 0.22µm membrane filter and then used.

Calibration curve

Accurately measured volumes of working standard solution of Ezetimibe was transferred into a series of 10 ml volumetric flasks and diluted appropriately with mobile phase. 20µl of each solution was injected under operating chromatographic conditions described above. Calibration curve was obtained by plotting the response (area of drug peak) versus concentration of drug. Regression equation was calculated. The method was found linear over a concentration range 10 µg/ml to 100 µg/ml.

Procedure for analysis of tablets

Twenty tablets, each containing 10mg of Ezetimibe was weighed and finely powdered. A quantity of powder equivalent to 25 mg of Ezetimibe was weighed and transferred to a 25ml volumetric flask; methanol was added to the same flask and sonicated for 15min. The volume was made up to 25ml with methanol. The solution was filtered using whatmann filter paper no.41. From this solution appropriate dilutions were made with mobile phase to obtain

concentration in the calibration range and this solution was used for the estimation.

With the optimized chromatographic conditions, a steady baseline was recorded, the working standard solution was injected and the chromatogram was recorded. The retention time of Ezetimibe was found to be 3.537 minutes. The proposed method was found to be specific and no interference from common tablet excipients like lactose, starch, etc has observed. The response factors of the standard solutions and sample solutions were calculated. The assay was calculated from the equation of regression line. The assay procedures were repeated 6 times; the percentage of drug in formulation was calculated. The results of analysis shows that the amount of drug was in good agreement with the label claim of the formulation.

Method validation

Linearity

The method was linear in the range of 10 μg/ml to 100 μg/ml for Ezetimibe standard.

Precision

The precision of the method was demonstrated by interday and intraday variation studies. In the intraday studies, solutions of standard and sample were repeated thrice in a day and percentage relative standard deviation (% RSD) for response factor was calculated. The intraday %RSD of Ezetimibe was found to be 0.28. In the interday variation studies, injections of standard and sample solutions were made on 3 consecutive days and %RSD was calculated. The interday %RSD for Ezetimibe was found to be 0.84. From the data obtained, the developed RP-HPLC method was found to be precise.

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 solution. Percent recovery was calculated by comparing the area before and after the addition of the working standard. 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. Recovery was with in the range of 99.93 ± 1.02% which indicates that the method was accurate (Table. 1).

Table 1: Assay results of tablet formulations using proposed method

Formulations	Labeled amount (mg)	Amount obtained (mg)*	% Recovery**
A	10	9.89 ± 0.94	99.93±0.85
B	10	10.1 ± 0.91	100.05± 0.85

*mean value ± standard deviation of six determinations. **mean value ± standard deviation of three determinations

Limit of detection and Limit of quantification

Limit of detection and limit of quantification were calculated using standard deviation of the response and slope of calibration curve. The LOD for Ezetimibe was found to be 1.0μg/ml. The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified. The LOQ was 3.2 μg/ml.

Robustness

Robustness of the method was checked by making slight deliberate changes in chromatographic conditions like mobile phase ratio and flow rate. It was observed that there were no marked changes in chromatograms, which demonstrated that the developed RP-HPLC method is robust.

Solution stability

In order to demonstrate the stability of both standard and sample solutions, the solutions were analyzed over a period of 12 hrs at a room temperature. The results show that, the retention time and peak area of Ezetimibe remain unchanged (%RSD less than 1) and no significant degradation within the indicated period was observed. This indicates that both solutions were stable for at least 12 hrs, which was sufficient to complete the analytical procedure.

RESULTS AND DISCUSSION

The validation parameters are summarized in Table 2.

The proposed method was found to be linear in the concentration range of 10 to 100 μg/ml and the data of regression analysis of the calibration curves are shown in Table 3.

Table 2: Summary of validation parameters

Parameter	Values
Detection limit(μg/ml)	1
Quantitation limit (μg/ml)	3.2
Accuracy (%)	99.93± 1.02%.
Precision (%RSD)	
Intraday (n=3)	0.28-0.36
Interday (n=3)	0.84-0.94
Repeatability (%RSD, n=3)	0.44-0.92

Table 3: Regression analysis of the calibration curve for the proposed method

Parameter	Values
Calibration range	10-100μg/ml
Slope	39.35266
Standard deviation of slope	0.20153
Intercept	42.73870
Standard deviation of intercept	13.60707
Correlation coefficient (*r*)	0.9999

The method was specific since excipients in the formulation did not interfere in the estimation of Ezetimibe. Accuracy of the method was indicated by recovery values 99.93± 1.02%. Precision is reflected by %RSD values less than 2. The LOQ for Ezetimibe was found to be 3.2μg/ml. These low values

suggest sensitivity of the developed method. System suitability test parameters are shown in Table 4.

Table 4: System suitability test parameters for ezetimibe by the proposed method

System suitability parameter	Values
Retention time (min)	3.537
Resolution	5.06
Tailing factor (asymmetric factor)	1.23

CONCLUSION

The developed RP-HPLC method was simple, sensitive, precise and accurate and hence can be used in routine for the determination of Ezetimibe in bulk as well as pharmaceutical preparations.

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