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Development of validated RP-HPLC method for the estimation of Ezetimibe in bulk drug and formulations

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ABSTRACT

A simple, selective, linear, precise and accurate isocratic RP-HPLC method was developed for the estimation of Ezetimibe in bulk drug and tablet dosage forms. The method showed a linear response for concentrations in the range of 10-100µg/mL using acetonitrile, water and methanol (56:40:4) as the mobile phase with uv-detection at 232nm.The flow rate was kept at 1.0mL/min and the injection volume was 20µL. the separation was performed at ambient temperature. The retention time for Ezetimibe was 6.80 mins. The developed method was statistically validated for the linearity, precision, robustness, LOD and LOQ. The % recovery was within the range between 99% and 100.5%. The percentage RSD for precision and accuracy of the method was found to be less than 2%. The method was validated as per the ICH guidelines. [1]The method was successfully applied for routine analysis of Ezetimibe in bulk samples and formulations.

Keywords: Ezetimibe, RP-HPLC, UV-detection and validation.

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INTRODUCTION

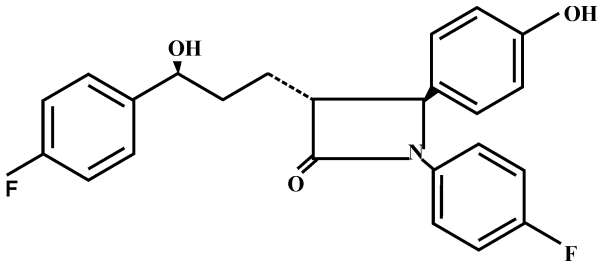

Figure 1: Structure of Ezetimibe

Ezetimibe [Figure-1] is an anti-lipemic drug. It is used to treat hypercholesterolaemia. It is chemically 1-(4-fluorophenyl)-(3R)-[3-(4-fluorophenyl)-(3S)-hydroxy propyl]-4S- (4-hydroxy phenol)-2- azetidinone. Literature survey revealed that a few analytical methods reported for the determination of Ezetimibe in pure drug, pharmaceutical dosage forms using liquid chromatography [2-9], either in single (or) in combined forms. The aim of the present work is to develop and validate a simple, fast, reliable & economic isocratic RP-HPLC method with UV detection for the determination of Ezetimibe in bulk and in tablet dosage forms.

MATERIALS AND METHODS

Instruments

This analytical work performed on shimadzu, LC-10ATVP, series binary gradient pump with SPD-10AVP UV-detector, phenomena C_{18} – column (250×4.6 MM, 5µ particle size) as stationary phase. The samples are weighed on Shimadzu electronic analytical balance (AX-200).

Reagents and Chemicals

Analytical pure Ezetimibe has been obtained as a gift sample from M/S.Micro labs, Hosur, India. Tablets were purchased from the local market. All solvents and reagents used were of HPLC grade.

Preparation of mobile phase and standard stock solution

The mobile phase was prepared by mixing acetonitrile, water and methanol in the ratio of 56:40:4. The obtained solution was sonicated for 10 mins and then it was filtered through a 0.45µ membrane filter paper.

An accurately weighed quantity of 25mg of Ezetimibe was transferred to 25ml volumetric flask, which was then dissolved and made upto volume with mobile phase to give 1mg/mL.

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Optimized chromatographic conditions

Table – 1: System Suitability Parameters

S.No	Parameters	Results
1.	Retention Time	6.80
2.	Peak Area Response	14368745
3.	Theoritical Plates	5628
4.	Tailling Factor	0.74
5.	Assymetry Factor	1.09
6.	Repeatability	0.0619

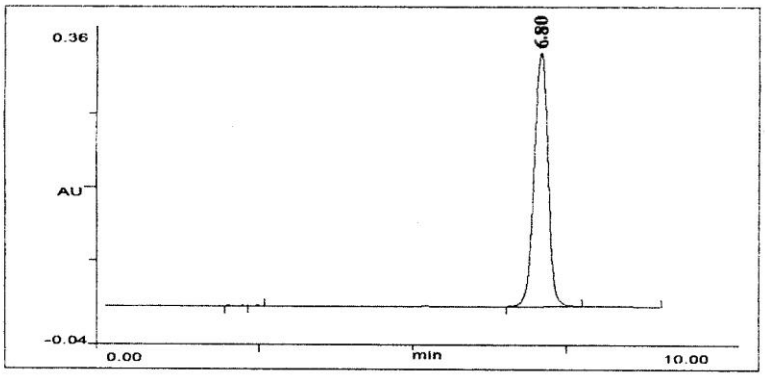

Figure 2: HPLC Chromatogram of Ezetimibe in mobile phase

RP-HPLC analysis was performed by isocratic elution with flow rate of 1mL/min. the mobile phase containing Acetonitrate, water and methanol in the ratio of 56:40:4 (%v/v) to obtain well resolved peak of Ezetimibe (Rt = 6.80 min) as shown in Figure-2. Wavelength of maximum absorption was selected by UV- detector. The drug shows good reponse at 232nm. The results of system suitability parameters were given in the Table-1.

METHOD VALIDATION

Linearity

Appropriate aliquots of the drug were pippetted out from the standard stock solution into a series of 10 ml volumetric flasks. The volume was made upto the mark with mobile phase to obtain a set of solutions for ezetimibe of concentrations 10, 20, 30, 40, 60, 80 and 100 µg/ml. Triplicate dilutions of each concentration of the drug were prepared separately. From these

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triplicate solutions 20 µl injection of each concentration of the drug were injected into the HPLC system three times separately and chromatographed under the optimized conditions. Evaluation of the drug was performed with the UV detector set at 232 nm and the peak areas were recorded. The Beer - Lamberts law was obeyed in the concentration range of 10 -100 µg/mL for Ezetimibe as shown in Figure - 3. The linearity of calibration graphs and adherence of the system to Beer's law was validated by high value of correlation co-efficient (r^2 = 0.9994 and regression equation is Y= 287943x – 26523).

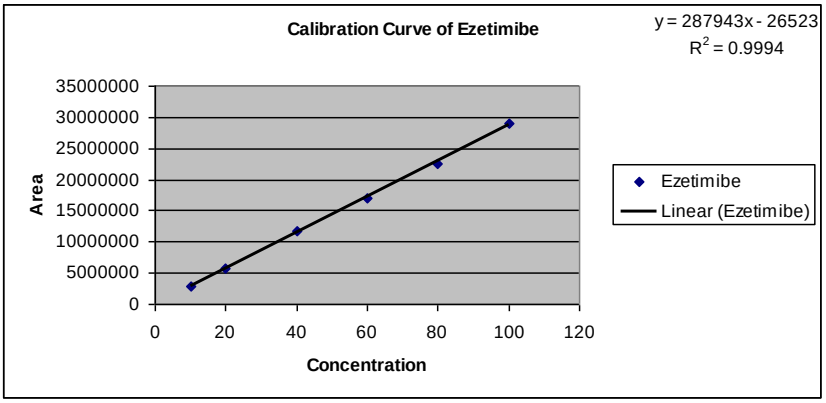

Figure 3: Calibration curve of Ezetimibe

Table – 2: Linearity of Ezetimibe

Concentration (µg/ml)	Area
10	2944735
20	5671355
40	11665946
60	17084476
80	22646435
100	29090175

The linearity table of Ezetimibe is shown in Table-2.

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Analysis of the marketed formulations

Twenty tablets (Ezedoc – 10 mg) were weighed accurately and crushed to form fine powder equivalent to 10mg of Ezetimibe was dissolved in 50 ml of volumetric flask with mobile phase to give 200µg/mL the flask was sonicated for 20 min and then the solution was filtered using whatmann filter paper.

Table -3: Analysis of Commercial tablets (Ezedoc – 10mg) (nº = 6)

Analyte	Label Claim	Amount found mg / tablet	Mean	% RSD
Ezetimibe	10	9.96	10.10	0.2291

º = Mean of 6 determinations

10ml of above solution were transferred into six different 50ml volumetric flasks and then volume was made upto the mark with mobile phase to obtain 40µg/mL of Ezetimibe. The chromatographic conditions and peak area were measured. The results are shown in Table-3

Precision

Table – 4: Precision data of the proposed method (nº = 6)

Analyte	Intraday			Interday		
	Conc. (µg/ml)	Area	% RSD	Conc. (µg/mL)	Area	% RSD
Ezetimibe	50	14368745	0.2088	50	14374153	0.3025

Intraday and inter-day precision of the assay samples containing Ezetimibe (50 µg/ml) were analyzed six times in the same day (intraday) and for three consecutive days by different analysts. The results are shown in Table - 4.

Accuracy

Table – 5: Recovery Studies of Ezetimibe

Analyte	% Type of level	Formulation	Amount of Drug added	Amount Recovery	% Recovered	% RSD
Ezitimibe	80	40	32.02	32.05	100.05	0.35
	100	40	40.32	40.01	99.2	0.25
	120	40	48.05	48.02	99.9	0.26

It was found out by recovery study[10] using standard addition method. Known amount of standard Ezetimibe was added to pre- analyzed (40 µg/mL) samples at a level from 80% upto 120% and then subjected to the proposed HPLC method. Results of recovery studies are shown in Table - 5.

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Robustness

The robustness of the developed method was studied by making small deliberate variations in the method parameters such as the flow rate, percentage of methanol and acetonitrile in the mobile phase, and the column temperature. The solution containing 50 µg/mL of Ezetimibe was injected into sample injector of HPLC under the different conditions. The results of the robustness study are given in Table - 6.

Table – 6: Robustness Studies of Ezitimibe

Chromatographic Conditions:-		
Factor	Level	r_t
Flow Rate (1ml/min)		
0.90	-1	6.81
1.00	0	6.80
1.10	1	6.79
Mean ± SD (n=3)		6.80 ±0.10
% of Methanol in the Mobile Phase		
38	-1	6.82
40	0	6.80
42	1	6.81
Mean ± SD (n=3)		6.81 ±0.06
% of Acetonitrile in the Mobile Phase		
54	-1	6.79
56	0	6.80
58	1	6.78
Mean ± SD (n=3)		6.79 ±0.10
Temperature (º C)		
24	-1	6.84
25	0	6.80
26	1	6.78
Mean ± SD (n=3)		6.80 ±0.08

Sensitivity

The LOD and LOQ were separately determined based on the standard deviation of response of the calibration curve. The standard deviation of Y-intercept of regression lines and slope of the calibration curves were used to calculate the LOD and LOQ.

RESULTS & DISCUSSIONS

In this present study an attempt has been made to develop RP-HPLC method for the determination of Ezetimibe in pure and tablet dosage form. The results obtained were reproducible and reliable. The validity and precision of the methods were evident from the statistical and analytical parameters obtained. Therefore, it is included that the method

developed is simple, rapid, selective, economical, accurate and precise. Hence this method is suitable for application in routine quality control analysis of pharmaceutical preparations.

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