World Journal of Pharmaceutical and Life Sciences
WJPLS

www.wjpls.org

SJIF Impact Factor: 4.223

METHOD DEVELOPMENT AND VALIDATION OF EZETIMIBE BY RP-HPLC IN BULK AND THE PHARMACEUTICAL DOSAGE FORM

Elkaturi Sanjeeth*[1], Gajavelli Santhosh[1], Kampati Ravi[1] and Duddagi Suchitra[2]

[1]Vision College of Pharmaceutical Sciences & Research, Hyderabad, Telangana, India.
[2]Assistant Professor, Department of Pharmaceutical Analysis and Quality Assurance, NNRG School of Pharmacy, Hyderabad, Telangana, India.

*Corresponding Author: Elkaturi Sanjeeth

Vision College of Pharmaceutical Sciences & Research, Hyderabad, Telangana, India.

ABSTRACT

A simple, rapid, precise, accurate and highly sensitive High Performance Liquid Chromatography method has been developed and validated for the determination of Ezetimibe in bulk and pharmaceutical dosage form. Chromatographic separation was achieved on INERTSIL, C18 (150×4.6 ID) 5μm column using mobile phase consisting of a mixture of Acetonitrile and H_2O in the ratio of 80:20 and the flow rate was adjusted to 1ml/min with UV detection at 232nm. The retention time of Ezetimibe was found to be 3.560 min. Linearity was observed in the range of 20-60 μg/ml for Ezetimibe. In the linearity study, the regression equation and coefficient of correlation for Ezetimibe was found to be (y=6.416x+16.91, r^2=0.998). The proposed method was validated according to ICH Q2B guidelines. The accuracy of the method was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the method and can be used for routine analysis of pharmaceutical dosage form.

KEYWORDS: Methanol, Acetonitrile, Ezetimibe, RP-HPLC, Method Development and Validation.

INTRODUCTION

EZETIMIBE is an anti-hyperlipidemic medication which is used to lower cholesterol levels. Specifically, it appears to bind to a critical mediator of cholesterol absorption, the Niemann-Pick C1-Like 1 (NPC1L1) protein on the gastrointestinal tract epithelial cells as well as in hepatocytes. It is chemically (3R, 4S)-1-(4-fluorophenyl)-3-[(3S)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl) azetidine-2-one. It localizes and appears to act at the brush border of the small intestine and inhibits the absorption of cholesterol. This leads to decrease in the delivery of intestinal cholesterol to the liver.[1,2,3]
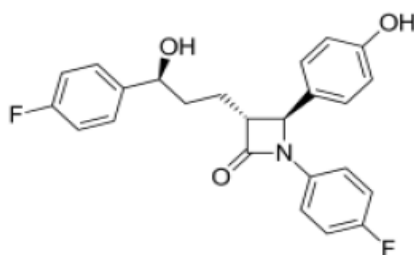
EZETIMIBE

Literature survey revealed that there are many methods like HPLC,[4] UV-Spectrophometric[7] and HPTLC[5] for individual and combined dosage form. The Aim of the present work is to develop a new analytical RP-HPLC method with simple, precise, and shorter retention time for estimation of Ezetimibe in pharmaceutical dosage form.

MATERIALS AND METHODS
Chemicals and reagents
Pure sample of Ezetimibe was gifted from Chandra labs, Hyderabad. The commercial pharmaceutical preparation ZETIA 10mg (containing 10tablets) was procured from local pharmacy. Methanol (HPLC Grade), Acetonitrile (HPLC Grade) and Water (HPLC Grade) was a product of Sigma Aidrich limited.

Instruments used
Chromatography was performed using a HPLC pump Shimadzu(LC 20 AT VP) solvent delivery system and detection was achieved UV/Visible (Nicolet evolution 100) detector using a column INERTSIL column, C18(150×4.6 ID) 5μm. Data acquisition and processing was performed using Spinchrome software. Dissolution of the compound was enhanced by Ultra sonicator-

Citizen, Digital ultrasonic cleaner. The pH of the solution was adjusted by using pH meter- Global digital, and single pan balance-Shimadzu.

Chromatographic conditions

Chromatographic conditions was achieved by using Shimadzu (LC 20 AT VP) RP INERTSIL C18 (150×4.6 ID) 5μm analytical column. The mobile phase used in this study was a mixture in the ratio Acetonitrile:Water (80:20). The mobile phase was filtered through a 0.45μ membrane filter. The mobile phase pumped from the solvent reservoir to column at flow rate of 1ml/min with injection volume of 20μl and the retention time observed for Ezetimibe was 3.56 min at UV detection point 232nm. Optimized chromatographic conditions given in Table: 1 and chromatogram in Figure: 1.

Table 1: Optimized chromatographic conditions.

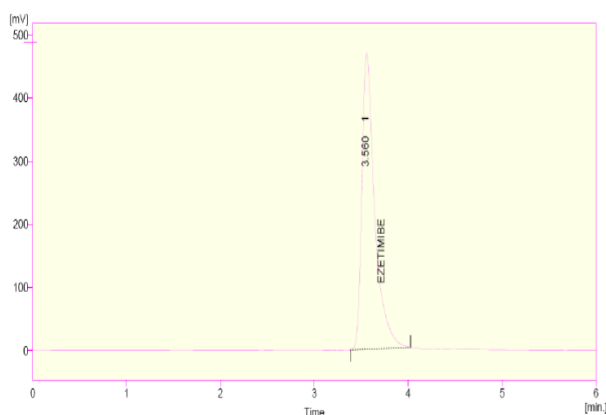| Mobile phase	Acetonitrile:H_2O(80:20)
Column	INERTSIL column,C18(150x4.6 ID) 5μm
Flow rate	1.0 ml/min
Column temperature	Room temperature(20-25oC)
Sample temperature	Room temperature(20-25oC)
Wavelength	232nm
Injection volume	20 μl
Run time	6 min
Retention time	About 3.560

Fig 1: Chromatogram of Ezetimibe (3.56 min).

Preparation of Mobile Phase

A mixture of 80 volumes of Acetonitrile and 20 volumes of water was prepared. The mobile phase was sonicated for 10min to remove gases finally filtered through 0.45μ membrane filter. This prepared solution was used as mobile phase.

Preparation of Standard Stock and working Solutions

A primary stock solution of Ezetimibe of 1000μg/ml was prepared in Methanol. From this stock solution a working standard solution of 40μg/ml was prepared by diluting 0.4ml to 10ml with Methanol. This solution is used for recording chromatogram.

Preparation of Sample Solution

5 Tablets of ZETIA (each tablets contains 10mg of Ezetimibe) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablets stock solutions of 400μgml were prepared by dissolving weight equivalent to 10mg of EZETIMIBE in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and sonicated for 5min and diluted to 25ml with mobile phase. Further dilutions are prepared in 5 replicates of 40μg/ml of EZETIMIBE by adding 1ml of stock solution to 10mlof mobile phase.

Selection of Chromatographic Methods

The proper selection of methods depends upon the nature of the sample its molecular weight and stability. The drugs are selected are polar, ionic, and Reversed phase chromatography can be used because its simplicity and suitability.

METHOD VALIDATION

Validation is an integral part of quality assurance; it involves the systematic study of systems, facilities and processes aimed at determining whether they perform their intended functions adequately and consistently as specified [6,8].

Accuracy

Accuracy of the method was determined by recovery studies. To the formulation, the reference standards of the drugs were added at the level of 50%, 100%, & 150%. Accuracy data is given in the Table: 2

Table 2: Recovery results of Ezetimibe.

Recovery level	Amount taken (mcg/ml)	Area	Average area	% Recovery
50%	20	3928.222	3743.635	101.73
	20	3295.484		
	20	3197.199		
100%	40	4750.032	4497.522	101.60
	40	4566.267		
	40	4176.267		
150%	60	5495.931	5287.917	98.75
	60	5184.41		
	60	5183.410		

Acceptance Criteria

The % recovery of EZETIMIBE should lie between 98% and 102%.

Precision

Prepared sample preparations of EZETIMIBE as per test method and injected 5 times in to the column.Method precision data is given in the Table: 3.

Table 3: Method precision of Ezetimibe.

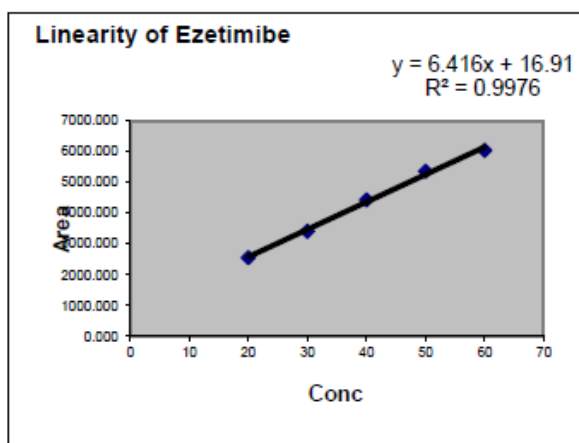| EZETIMIBE		
S.No.	Retention time	Area
1	3.560	4426.890
2	3.530	4417.702
3	3.533	4410.326
4	3.543	4411.818
5	3.533	4442.974
6	3.537	4413.064
avg	3.5393	4420.462
SD	0.0111	12.553
%RSD	**0.31**	**0.28**

Acceptance Criteria

The % Relative standard deviation of Assay preparation of EZETIMIBE should be not more than 2.0%.

Linearity

Linearity is the ability of the method to elicit test results that are directly proportional to analyte concentration within a given range. The linearity was performed by preparing and injecting concentrations ranging from 20-60μg/ml. The Linearity of the method was evaluated by linear regression analysis and least square method. Linearity data is given in the Table: 4 and Fig: 2.

Table 4: Linearity of Ezetimibe.

S.No.	Conc.(μg/ml)	Area
1	20	2559.483
2	30	3414.608
3	40	4426.89
4	50	5354.686
5	60	6043.075

Fig 2: Linearity Calibration graph.

Acceptance Criteria

The correlation coefficient for linear curve obtained between concentrations vs. Area for standard preparations of EZETIMIBE was 0.999.

Specificity

Specificity is the ability to assess accurately the analyte in the presence of components which may be expected to be present in the sample matrix. Typically these might include impurities, degradants, matrix, etc.

Observation

It is observed from the above data, diluents or excipient peaks are not interfering with the EZETIMIBE peaks.

Robustness

The Robustness was performed at different flow rates and different wavelengths by using working standard solution of Ezetimibe. Results remained unaffected by small variations in the parameters. Robustness data is given in the table: 5.

Table 5: Results of Robustness.

Parameter	EZETIMIBE		
	Retention time(min)	Tailing factor	Efficiency
Flow Rate			
0.8ml/min	4.383	2.207	4015
1.2ml/min	2.943	2.241	3898
Wavelength			
230nm	3.510	2.172	4039
234nm	3.517	2.151	4153

Acceptance criteria

The system suitability should pass as per the test method at variable conditions.

Ruggedness

The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the Assay by two different analysts.

Table 6: Results of Ruggedness.

EZETIMIBE	%Assay
Analyst 01	98.72
Analyst 02	97.23

Acceptance criteria

The % Relative standard deviation of Assay values between two analysts should be not more than 2.0%.

Assay

Standard and Sample was injected into the chromatographic system and measured the areas and peaks calculated the % Assay by using the formulae.

Table 7: Assay results.

EZETIMIBE		
	Standard Area	Sample Area
Injection-1	4430.026	4426.890
Injection-2	4447.469	4417.702
Injection-3	4387.764	4410.326
Injection-4	4376.391	4411.818
Injection-5	4347.941	4442.974
Assay(%purity)	98.81	

RESULTS AND DISCUSSION

A simple and selective LC method is described for the determination of EZETIMIBE dosage forms. Chromatographic separation was achieved on C18 column using mobile phase consisting of a mixture of Acetonitrile: H_2O (80:20) with detection at 232nm. Linearity was observed in the range 20-60 µg/ml for EZETIMIBE (r^2 =0.998) for the amount of drug estimated by the proposed methods was in good agreement with the label claim.

The proposed method was validated. The accuracy of the method was assessed by the recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

CONCLUSION

From the above experimental results and parameters it was concluded that, this newly developed method for the estimation of EZETIMIBE was found to be simple, precise, accurate, and highly selective. Moreover shorter duration of analysis for Ezetimibe made this reported method suitable for routine analysis in research institutions, quality control department in industries, approved testing laboratories, bio-pharmaceutical and bio-equivalence studies and in clinical pharmacokinetic studies in near future.

ACKNOWLEDGEMENT

BIBLIOGRAPHY

1. Langreth, Robert (June 29, 2016) "Decoding Big Pharmas secret Drug Pricing Practice''. Bloomberg. Retrived, 15 July, 2016.
2. Jump up stone NJ, Robinson JG, Lichtenstein,AH, et al. (June2014). "2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American College of cardiology/American Heart Association Task Force on Practice Guideline''. Circulation. 129(25suppl2): S1-45. Doi:10.1161/01.cir0000437738.63853.7a. PMID 24222016.
3. Jump up U.S. National Library of Medicine, National Institute Health, U.S. Department of Health and Human Services. Page last updated: Medline Plus: Ezetimibe, 27 October 2014.
4. R.Sistla, V.S.S.K.Tata, Y.V.Kashyap, D. Chandrasekar, P.V.Diwan, Development and Validation of a RP-HPLC method for the determination of Ezetimibe in pharmaceutical dosage forms, Journal of Pharmaceutical Analysis and Biomedical Analysis, 2005; 39(3-4): 517-522.
5. BG Chaudhari, NM Patel, P.B.Shah, K.P.Modi, Development and Validation of a HPTLC Method for Simultaneous Estimation of Atorvastatin and Ezetimibe, Indian Journal of Pharmaceutical sciences, 2006; 68(6): 793-796.
6. D.Suchitra, A.B.N Nageshwara Rao N.Ravindernath, S.Shakunthala Madhavendra, V. Jayathirth Rao, A Validated method for simultaneous estimation of Ramipril,Telmisartan and Hydrochlorothiazide by RP-HPLC method in pharmaceutical dosage form". IJPPS, 2011; 3(4): 185-189.
7. Patel Vandana.B.et al, Simultaneous Spectrophotometric Determination of Atorvastatin calcium and Ezetimibe in tablet dosage form, International Journal of ChemTech Research, 1(2): 233-236.
8. B.K. Sharma, "Instrumental Methods of Chemical Analysis", 23th edition, Goal Publishing House, Merut, 2000; 1-4.