

Reverse phase high performance liquid chromatographic determination of Ezetimibe in bulk and pharmaceutical formulations

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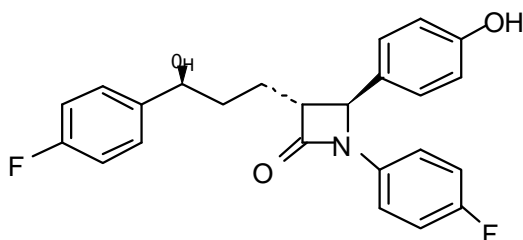
ABSTRACT

This paper describes about validated Reverse phase High Performance Liquid Chromatographic method for determination of Ezetimibe in bulk and pharmaceutical formulations has been developed. Chromatographic separation was carried out using a mobile phase of Potassium dihydrogen phosphate buffer (containing triethylamine) and acetonitrile (55:45v/v) on a C₁₈-ODS-Hypersil (5 μ, 250×4.6 mm) column in isocratic mode at a flow rate of 1.5ml/minute with UV detection at 234nm. Atorvastatin calcium was used as internal standard. The chromatographic method was linear over the concentration range of 50-250μg/ml. The Limit of Detection and Limit of Quantitation were 0.828 and 2.5 μg/ml respectively. The mean percentage purity and recovery were found to be 101.22% and 100.36% respectively. Suitable method was developed to determine Ezetimibe in bulk and pharmaceutical formulations.

Key words: RP -HPLC, Ezetimibe, Tablets.

INTRODUCTION

Ezetimibe is a new class of lipid lowering drug, which differs from other classes of cholesterol reducing compounds. It is chemically as (3R, 4S) - 1 - (4-Fluorophenyl)-3-Hydroxy propyl]-4-(Hydroxy phenyl)-2-azetidinone.¹



James E. Patrick et al studied the Ezetimibe pharmacokinetics². Literature survey reveals the availability of few analytical methods such as HPLC³⁻⁵, LC-MS⁶, derivative spectrophotometry⁷ and voltammetry⁸ for determination of Ezetimibe in pharmaceutical formulations. In the present investigation the authors propose a simple, sensitive and reproducible RP-HPLC method for the determination of Ezetimibe.

EXPERIMENTAL

Instrument

Shimadzu HPLC SPD-10ATVP, Japan equipped with UV visible detector was used for HPLC analysis.

Chemicals and materials

Potassium dihydrogen phosphate, triethylamine and acetonitrile (HPLC grade) were procured from E. Merck India, Limited. Water was purified by double distillation and filtered through 0.2 µm pore sized membrane filter. Ezetimibe was obtained as a gift sample from Hetero Drug House Private Limited, Hyderabad, India. Two marketed brands of Ezetimibe were used for estimation.

The chromatographic separation was carried out in an isocratic mode utilizing Hypersil C₁₈ column with dimensions (5 µm, 250 mm x 4.6 mm) as stationary phase and the mobile phase composed of buffer and acetonitrile in 55:45 ratios at a flow rate of 1.5 ml/minute. The buffer was prepared using 1.3609 g of potassium dihydrogen phosphate and 1.0 ml of triethylamine. Acetonitrile and water in 3:1 ratio was used as diluent.

Preparation of standard solutions

In chromatographic determination, separate stock solutions of reference compound and internal standard were prepared by weighing the appropriate amount into volumetric flasks and made up to volume using the diluent. Specific volume of each solution relating to the required concentration were pipetted into volumetric flask and made to volume to obtain serial dilutions using the diluent.

Sample preparation

Twenty tablets of both brands were weighed and powdered in a mortar. In chromatographic method, a quantity equivalent to 50 mg was weighed accurately and transferred into 100 ml volumetric flask along with the internal standard. About 75 ml of diluent was added and sonicated for 30 minutes and made to volume. This solution was then filtered through membrane filter. First 10 ml of solution was discarded and then diluted suitably to get a concentration of 100 µg/ml.

Method validation

Linearity

Calibration curve for chromatographic method was constructed by plotting ratio of peak areas (standard/internal standard) against concentration of solution. Figure 1 shows HPLC-UV chromatogram of Ezetimibe. Linear ranges and correlation coefficient⁹.

Obtained for chromatographic method were depicted in table 1.

Table 1: Analytical performance parameters of ezetimibe

Parameters	Ezetimibe RP-HPLC Method
Linearity dynamic range (LDR; g/ml)	50-250
Correlation coefficient (r)	0.9993
Slope (m)	0.00862
Intercept (c)	0.05576

Table 2: System suitability parameters (RP-HPLC)

Parameters standard	Ezetimibe	Internal
Retention Time (minutes)	3.91	5.64
Tailing Factor	1.04	1.01
Resolution Factor	4.932	4.932
Number of Theoretical Plates	8298	7817

Table 3: Quantification parameters of ezetimibe

Sample	Label claim	RP-HPLC method Assay	%R.S.D.
Tablet A	10	101.22	0.1281
Tablet B	10	103.30	0.1307

Table 4: Quantification parameters of ezetimibe

Sample concentration µg/ml	Fortified concentration µg/ml	Percentage Recovery Tablet A	Percentage Recovery Tablet B
100	150	100.50	100.81
	200	100.61	100.93
	250	100.71	100.71

RESULTS

The linearity was obeyed in the concentration range of 50-250 µg/ml in Chromatographic method. From the chromatogram, the retention times were found to be 3.91 minutes and 5.64 minutes for Ezetimibe and Atorvastatin respectively. The Limit of Detection and Limit of Quantitation were 0.828 and 2.5 µg/ml respectively. Quantitative estimation of the tablet showed average percentage purity of 101.22% with mean recovery of 100.36%. The system suitability parameters indicate that the developed method has acceptable accuracy and precision. In the above determined method, the relative standard deviation was below 1, which is a good index of accuracy and precision.

CONCLUSION

The developed method was simple, rapid, accurate, and reproducible was more suitable to determine Ezetimibe in bulk and pharmaceutical formulations.

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REFERENCES

1. The Merck index, Thirteenth edition, Merck and co., Inc.U.S.A, 3949 (2002).
2. James E. Patrick, Teddy Kosoglou, Kathe L. Stabber, Kevin B. Alton, Stephen E.Maxwell, Yali Zhu, Paul Statkevich, Robert Iannucci, Swapan Chowdhury, Melton Affrime, and Mitchell N. Cayen, Disposition of the Selective Cholesterol Absorption Inhibitor Ezetimibe in Healthy Male Subjects, *Journal of Drug metabolism and Disposition*, **30**(4): 430-437 (2002).
3. MuhammadAshfaq, IslamUllahkhan, SyedShanazQutab, SyedNaeemrazzaq, HPLC Determination of Ezetimibe and simvastatin in pharmaceutical Formulations, *J. Chil. Chem. Soc*, **52**(3):1220-1223 (2007).
4. G.Carlucci, P.Mazzeco,L.Biordi, M.Bologna, *J. Pharm. Biomed. Anal*, **10**(9): 693 (1992).
5. H.Ochiai, N.Uchiyama, K.Imagaki, S.Hata,T.Kamei., *J. Chromatogr. B. Biomed. Sci. Appl*, **694**(1): 211(1997).
6. B. Barrett, JHuclova. V. Borek Dohalsky, B. Nemeč, I. Jelinek, *J. Pharm. Biomed. Anal*, **41**(2): 517 (2006).
7. L.Wang,M.Asgharnejad, *J. Pharm. Biomed. Anal*, **21**(6):1243(2000).
8. O. Corah. S. AOzkan, *Pharmazie*, **61**(4): 285 (2006).
9. Frederick J Gravette's, Lancy B and Wallnau, *Essential of Statistics for the behavioral Sciences*, 2nd Edition, West Publishing Company, **PP2-4**: 173-175 (1995).
10. ICH Steering Committee, *Validation of analytical procedures/methodology*, ICH Harmonized Tripartite Guidelines, (1996).