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HPLC METHOD DEVELOPMENT, METHOD DETERMINATION AND METHOD VALIDATION OF DOSAGE FORM OF EZETIMIBE

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OBJECTIVE

To develop a method determination and method validation of dosage form of Ezetimibe and stability-indicating liquid chromatographic analytical method for assay of Ezetimibe and for determination of the content uniformity of a tablet formulation. **Methods:** This paper aimed to develop a simple, sensitive, and rapid chromatographic procedure for Ezetimibe. The mobile phase of 0.1% ortophosphoric acid and acetonitrile (50:50) at flow rate of 1 mL/min on reverse phase. The separation was carried out on a 250-mm × 4.6-mm inside-diameter reverse-phase column (Zorbax SB-C_{18}, 5 µm), Water: Acetonitrile: Methanol (40:50:10, v/v) as a diluent. **Results:** Total analytical run time for selecting The assays exhibited good linearity (r^2 = 0.9999) over the studied range of 2 20 µg/ml to 80 µg/ml. the LOD and LOQ values of the analytical method are 0.1 µg/ml and 0.2 µg/ml respectively which correspond to 0.2% and 0.4% of working concentration Overall %RSD of replicate sets of method and intermediate precision study is 0.67, the difference between mean % assay values of method precision and Intermediate precision is 0.4. The performance of the method was validated according to the present ICH guidelines.

KEYWORDS: Ezetimibe, HPLC, Method validation, stability.

INTRODUCTION

Ezetimibe is a drug that lowers plasma cholesterol levels. It acts by decreasing cholesterol absorption in the small intestine. It may be used alone (marketed as Zetia or Ezetrol), when other cholesterol-lowering medications are not tolerated or together with statins (e.g., ezetimibe/simvastatin, marketed as Vytorin or Inegy) when statins alone do not control cholesterol.

Ezetimibe is chemically 1-(4-flurophenyl)-3(R)-[3-(4-flourophenyl)-3(S)-hydroxypropyl]-4(S)-(4-hydroxyphenyl)-2-azetidinone. Its molecular formula is $C_{24}H_{21}F_2NO_3$ and it has a molecular weight of 409.43 g mol^{-1}. Ezetimibe is a white crystalline powder that is practically insoluble in water and freely to very soluble in ethanol, methanol, and acetone.

From the literature survey conducted, it was found that there are analytical methods reported for ezetimibe.[1-8]

MATERIALS AND METHODS

An HPLC system Waters 2695 fitted with a Photodiode Array Detector (PDA) and an Empower Software was used. The mobile phase of 0.1% ortophosphoric acid and acetonitrile (50:50) at flow rate of 1 mL/min on reverse phase The separation was carried out on a 250-mm×4.6-mm inside-diameter reverse-phase column (Zorbax SB-C_{18}, 5µm), Water:Acetonitrile:Methanol (40:50:10, v/v) as a diluents.

Chemicals and reagents

Ezetimibe working standard, Acetonitrile HPLC grade, Water HPLC grade,Orthophosphoric acid –Merck.

METHODOLOGY

Chromatographic conditions

Mobile phase: - 0.1% Orthophosphoric acid (v/v): Acetonitrile (50:50, v/v)

Column: - Zorbax SB-C_{18}, 250 × 4.6-mm, 5 µm

Flow rate: - 1.0 ml/min

Detection: - 232 nm

Injection volume: - 20 µl

Diluent: - Water: Acetonitrile: Methanol (40:50:10, v/v)

Standard preparation

Stock solution: Weigh accurately about 50 mg Ezetimibe reference standard and transfer into 100 ml volumetric flask. Add 70 ml of diluent to dissolve the substance by sonication for one minute and then dilute to volume with diluent. The concentration obtained is 500 µg/ml of Ezetimibe. Pipette out 5 ml of above standard stock solution and transfer into 50 ml volumetric flask and dilute to volume with diluent. The concentration obtained is 50 µg/ml of Ezetimibe.

Mobile phase

The mobile phase of 0.1% ortophosphoric acid and acetonitrile (50:50) at flow rate of 1 mL/min on reverse phase.

Test Preparation

Weigh accurately 1 tablet and transfer into 200 ml volumetric flask. Add 140 ml of diluent into the flask and sonicate of 30 minutes with normal hand-shaking. Cool the flask to room temperature and dilute to volume with diluent. Filter 10 ml of this solution through 0.45 µm nylon syringe filter. The concentration obtained is 50 µg/ml of Ezetimibe.

System suitability

% RSD for replicate standard preparation should be less than 2.0 % for analyte peak. Asymmetry of the analyte peak should be less than 2.0 in standard preparation. Theoretical plates of the analyte peak should be more than 5000 in standard preparation.

Procedure

Inject the blank preparation and record the chromatogram, Inject five replicate of standard preparation and check for the system suitability and record the chromatograms, Inject test preparations in single and record the chromatograms, Calculate the % content uniformity of the sample and also calculate % RSD for the same.

RESULT AND DISCUSSION

The determination of ezetimibe in pharmaceutical products using RP-HPLC method. Several trials were done to obtain good separation using different ratios of mobile phase and flow rates. The mobile phase used was 0.1% Orthophosphoric acid (v/v): Acetonitrile (50:50, v/v) and the flow rate was found to be 1.0ml/min on Zorbax SB-C_{18}, 250 × 4.6-mm, 5 µm.

Estimation

A RP-HPLC method was developed for the estimation of ezetimibe, 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. (Fig.1).

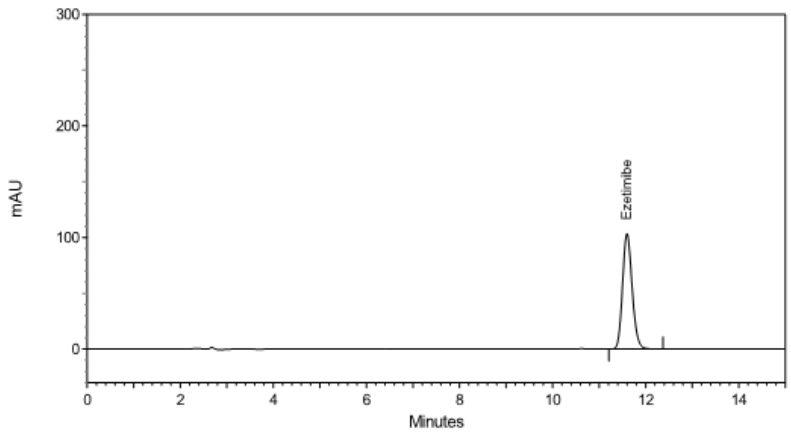

Fig 1: HPLC Chromatogram for Ezetimibe.

Validation procedure for content uniformity determination of Ezetimibe

Precision study was established by evaluating method precision and intermediate precision study. Method precision of the analytical method was determined by analyzing test preparations. Content of all test preparations was determined and mean % content uniformity value, standard deviation, % relative standard deviation and 95% confidence interval for the same was calculated.

Intermediate precision of the analytical method was determined by performing method precision on another day by another analyst using different make of raw materials under same experimental condition. Content of all test preparations was determined and mean % content uniformity value, standard deviation, % relative standard deviation and 95% confidence interval for the same was calculated. Overall content value of method precision and intermediate precision was compared and % difference and overall % relative standard deviation was calculated.

System suitability

A system suitability test for the chromatographic system was performed before each validation parameter. Five replicate injections of standard preparation were injected and asymmetry, theoretical plate and % RSD of peak area were determined for same as shown in Table 1.

Table 1: System suitability.

System suitability parameters (In-house limit)	% RSD[a] (NMT[b] 2.0)	Theoretical plates (NLT[c] 5000)	Asymmetry (NMT 2.0)
Method precision	0.20	15846	1.17
Intermediate precision	0.10	15548	1.12

[a] =Relative standard deviation
[b]= Not more than
[c]= Not less than

LOD and LOQ

The observation and result obtained for each validation parameter including specificity, linearity, LOD (Fig 2) and LOQ (Fig 3), precision, accuracy, robustness, solution stability and system suitability lies well with-in the acceptance criteria. Since, all the results are with-in the limit, the developed analytical method is considered as validated and suitable for intended use.

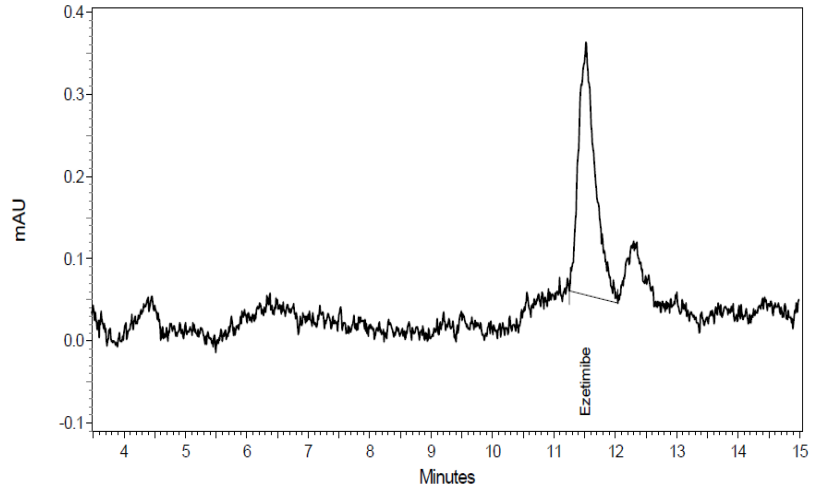

Fig 2: Chromatogram of LOD.

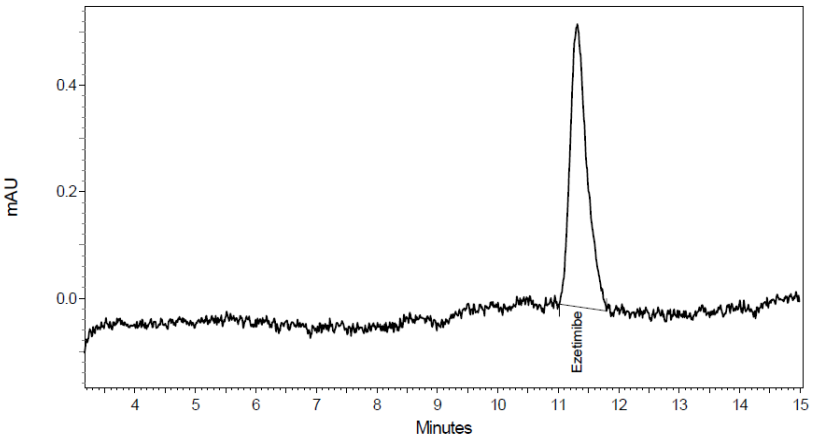

Fig 3: Chromatogram of LOQ.

Linearity and range study

The linearity plot in Fig 4 was prepared with 7 concentration levels (20, 30, 40, 50, 60, 70, and 80 µg/ml of Ezetimibe). These concentration levels were respectively corresponding to 40, 60, 80, 100, 120, 140, and 160% of test solution concentration. The correlation coefficient value should not be less than 0.995 over the working range. The correlation coefficient value of the analytical method is 0.9999 over the working range of 20 µg/ml to 80 µg/ml.

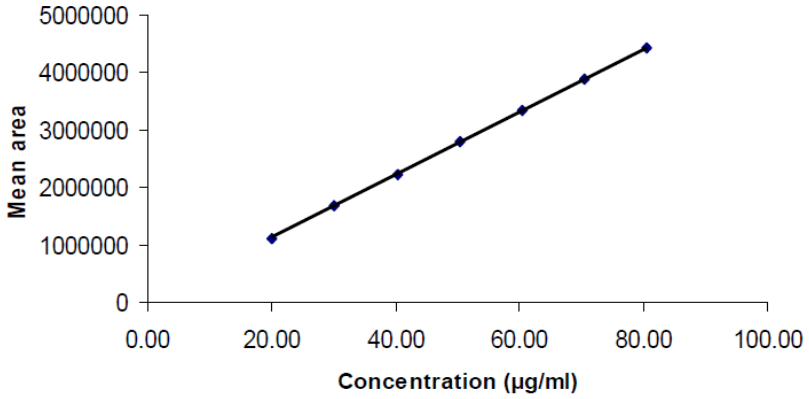

Fig 4: Evaluation of linearity.

Forced Degradation

Stress study was carried out by application of chemical and physical forced degradation. To perform forced degradation study, the drug content equivalent to 50 mg was employed for acidic, alkaline and oxidant media and also for thermal and photolytic conditions. After the degradation treatments were completed, the stress content were allowed to equilibrate to room temperature and diluted with diluent to attain 50 µg/ml concentrations.

Acidic Condition

Acidic degradation study was performed by refluxing the drug content in 1 N HCl at 80° C for 1 hour and then the mixture was neutralized.

Alkaline Condition

Alkaline degradation study was performed by keeping the drug content in 0.1 N NaOH at room temperature for 90 minutes and then the mixture was neutralized.

Oxidative Condition

Oxidative degradation study was performed by refluxing the drug content in 3% *v/v* H2O2 at 80° C for 1 hour.

Thermal Condition

Thermal degradation study was performed by keeping the powdered drug content at 70° C for 72 hours.

Photolytic Condition

Photolytic degradation study was performed by exposing the powdered drug content in sun-light for 72 hour.

There is no any interference of blank peaks, placebo peaks and impurity peaks with the analyte peak in test preparation and stress test preparations. The peak purity of the analyte peak in standard preparation, test preparation and stress test preparations is well with-in the acceptance criteria. All the results are well with-in the acceptance criteria. Hence, the analytical method is found specific summary as given Table 2.

Table 2: Summary of forced degradation profile in specificity study.

Degradation condition	Peak purity	Total Degradation, %	Major impurity, %	RRT of major impurity
Acidic	1.0000	73.89	36.58	1.6
Alkali	1.0000	23.87	20.74	1.6
Oxidative	1.0000	8.75	2.05	1.6
Thermal	1.0000	0.94	0.32	0.3
Photolytic	1.0000	3.90	2.31	0.3

CONCLUSION

The entire validation activity was performed in accordance with the acceptance criteria and no deviation was observed. The observation and result obtained for each validation parameter including specificity, linearity, LOD and LOQ, precision, accuracy, robustness, solution stability and system suitability lies well with-in the acceptance criteria. Since, all the results are with-in the limit, the developed analytical method is considered as validated and suitable for intended use.

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