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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF EZETIMIBE: A SYSTAMATIC REVIEW

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ABSTRACT

Analytical method development and Validation are the continuous and inter-dependent task associated with the research & development, quality control and quality assurance departments. Analytical procedures play a critical role in equivalence risk assessment and management. It helps in establishment of product-specific acceptance criteria and stability of results. Validations determine that the analytical procedure is suitable for its intended purpose. Literature survey reveals that the analytical methods based on UV spectrometry, RP-HPLC and HPTLC for the determination of Ezetimibe personally and in combination with different drugs. The parameters were validated according to ICH guideline in terms of accuracy, precision, robustness, and other components of analytical validation. The developed methods are simple, sensitive and reproducible and can be used for the analysis of Ezetimibe in bulk and Tablet dosage form.

KEYWORDS: Ezetimibe, UV, HPLC, Validation.

INTRODUCTION

Cholesterol Absorption Inhibitor Ezetimibe is used to lower cholesterol and triglyceride (fatlike substances) levels in the blood. Using Ezetimibe may help prevent medical problems caused by such substances clogging the blood vessels. Ezetimibe is available only with your doctor's prescription.

IUPAC NAME: (3R,4S)-1-(4-fluorophenyl)-3-[(3S)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl) azetidin-2-one.

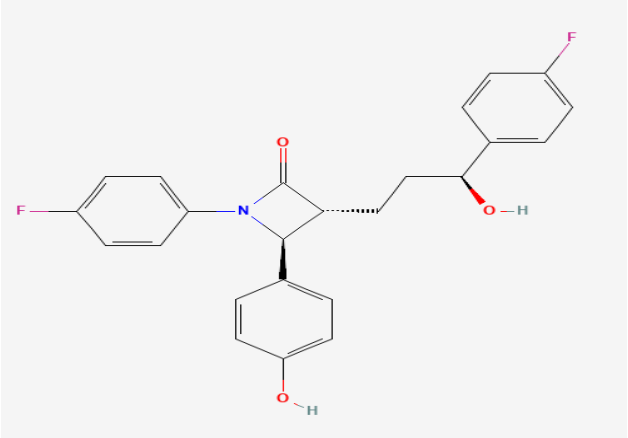

Fig.1: STRUCTURE OF EZETIMIBE.

Ezetimibe is chemically known as (3R,4S)-1-(4-fluorophenyl)-3-[(3S)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl) azetidin-2-one.Ezetimibe is a beta-lactam that is azetidin-2-one which is substituted at 1, 3, and 4 by p-fluorophenyl, 3-(p-fluorophenyl)-3-hydroxypropyl, and 4-hydroxyphenyl groups, respectively (the 3R, 3'S, 4S enantiomer). It has a role as an anti-cholesteremic drug, an anti-lipemic drug and an anti-metabolite, Ezetimibe has a molar mass of 409 gm/mol and having a structural formula: $C_{24}H_{21}F_2NO_3$.

Ezetimibe drug substance is white crystalline solid powder and it shows poor solubility in aqueous solvents and Soluble in organic solvent such as methanol and acetonitrile, sparingly soluble in aqueous buffers.

REVIEW OF LITERATURE

1. **Khemchand gupta[1] *et al.*,** have Simple and sensitive spectrophotometric method has been developed for the quantitative estimation of Ezetimibe from pharmaceutical tablet dosage form. The method was developed are based on the solubility of Ezetimibe in acetate buffer pH 4.5 containing 0.45% SLS. The drug showed maximum absorbance at 232 nm. Linearity was obeyed in concentration range of 5-30 µg/mL. The results of analysis were validated statistically and by recovery studies.

2. **Seema m. Dhole**[2] ***et al.,*** have developed a rapid, precise, accurate and specific first derivative UV spectrophotometric method was developed for the simultaneous estimation of simvastatin and ezetimibe in tablet dosage form. The first derivative spectrum was recorded between 200 and 350 nm and a zero-crossing technique for first-derivative measurement at 235 nm and 266 nm of simvastatin and ezetimibe.Methanol was used as solvent the developed method was validated for linearity, accuracy and precision as per ICH guidelines. The method illustrated excellent linearity (correlation coefficient (r2 > 0.999) in the concentration range of 2-20 µg/mL for simvastatin and ezetimibe. Precision (%R.S.D. < 1.50) and analytical recovery was found in the range of 91-101%, show the suitability of the and analytical recovery was found in the range of 91-101%,
3. **Metreyi sharma**[3] ***et al.,*** UV, first, second and third derivative spectrophotometric methods have been developed for the determination of ezetimibe in pharmaceutical formulation. The solutions of standard and sample were prepared in methanol. For the first method, UV spectrophotometry, the quantitative determination of the drug was carried at 233 nm and the linearity range was found to be 6-16 µg/ml. For the first, second and third derivative spectrophotometric methods the drug was determined at 259.5 nm, 269 nm and 248 nm with the linearity ranges 4-14 µg/ml, 4-14 µg/ml and 4-16 µg/ml. The calibration graphs constructed at their wavelength of determination were found to be linear for UV and derivative spectrophotometric methods. All the proposed methods have been extensively validated.
4. **P.Baby Sudha lakshmi**[4]***et al***., Two simple, sensitive, selective and accurate spectrophotometric methods (Method A and Method B) for the determination of ezetimibe in bulk drug and pharmaceutical formulations (tablets) have been described. Method A and B are based on the redox/complex formation reaction of drug with 1,10-phenanthroline and hexacyanoferrate(III) in presence of ferric chloride to form coloured chromogens exhibiting λmax at 510 and 740 nm respectively. The results of analysis for the two methods have been validated statistically and by recovery studies. The results are compared with those obtained using UV spectrophotometric method in alcohol at 231.7 nm.
5. **Imran M**[5]***et al.,*** a new and rapid stability indicating ultraviolet spectroscopic methods were developed and validated for the estimation of ezetimibe and carvedilol in pure form and in their respective formulations. Since both the drugs are poorly water soluble, 20% v/v acetonitrile in triple distilled water was selected as the solvent system for both the drugs. This ensured adequate drug solubility and maximum assay sensitivity. The linearity range for ezetimibe and carvedilol at their respective wavelength of detection of 232 nm and 238 nm was obtained as 2–50 µg/ml and 2–20 µg/ml respectively. The linear regression equations obtained by least square regression method, were Y = 0.0443 · X + 0.0106 for ezetimibe and Y = 0.1080 · X + 0.034 for carvedilol, where Y is the absorbance and X is the concentration (in µg/ml) of pure drug solution.
6. **Mohammed Ishaq Beludari**[6]***et al.,*** The proposed method is based on the separation of the two drugs in reversed phase mode using Water's C18 250 4.6 mm, 5m column maintained at an ambient temperature. The optimum mobile phase consisted of Acetonitrile: water: 0.02 M phosphate buffer pH 8 (40:10:50 v/v), flow rate of mobile phase was set 1.0 mL min1 and PDA detection was performed at 230 nm. Linearity was obtained in the concentration range of 30–90 mg mL 1 for both RSV and EZE with correlation coefficients of 0.999 and 0.998.
7. **Nilesh Jain**[7] ***et al.,*** This work is concerned with application of simple, accurate, precise and highly selective reverse phase high performance liquid chromatographic (RP-HPLC) method for simultaneous estimation of simvastatin and ezetimibe in combined dosage form. Chromatographic separation was achieved isocratically at 25°C ± 0.5°C on Luna C18 column (250 x 4.6 mm i.d.) with a mobile phase composed of methanol: water: acetonitrile in the ratio of 75: 18.75: 6.25 % v/v/v at flow rate of 1.8 ml/min. Detection is carried out using a UV-PDA detector at 231 nm. The retention time of simvastatin and ezetimibe was found to be 13.5 + 0.5 min and 4.02 + 0.3 min. respectively. The method was found to be linear in the range of 1-50 µg/ml with mean recovery of 99.21% for simvastatin and 99.50% for ezetimibe.
8. **Praveen Kumar**[8] ***et al.,*** A reliable and sensitive isocratic stability indicating RP-HPLC method has been developed and validated for assay of Ezetimibe in tablets An isocratic separation of Ezetimibe was achieved on Zorbax SB C18 (250mm x 4.6mm), 5 µm particle size columns with a flow rate of 1 ml/min and using a UV detector to monitor the eluate at 232nm. The mobile phase consisted of 0.02N ortho phosphoric acid: acetonitrile (20:80 v/v). All degradation products in an overall analytical run time of approximately 6 min with the parent compound Ezetimibe eluting at approximately 3.5 min. Response was a linear function of drug concentration in the range of 1-10 µg/ml (r 2 = 0.9993). Accuracy (recovery) was between 100.80.
9. **Prabhat K. Shrivastava**[9] ***et al.,*** A Simple, accurate, precise and economical HPLC method was developed for estimation of ezetimibe in tablet dosage form. To achieve the separation, the mixture of solvent acetonitrile and methanol in ratio of 50:50 v/v was selected as mobile phase. This mixture was found to be appropriate allowing good separation of the ezetimibe at retention time 4.9 minutes at flow

rate of 1.0 ml/min and detection wavelength 245.0 nm. The linearity was found in the concentration range 0 ñ 50.0 g/ml. All these analytical validation parameters were observed and the RSD was found to be less than one, which indicates the validity of method.

10. **Sandeep S. Sonawane[10] *et al.*,** A Two methods are described for the simultaneous determination of Atorvastatin calcium and Ezetimibe in binary mixture. The first method was based on UV-spectrophotometric determination of two drugs, using simultaneous equation method. It involves absorbance measurement at 232.5 nm (λmax of Ezetimibe) and 246.0 nm (λmax of Atorvastatin calcium) in methanol; linearity was obtained in the range of 5 – 25 µg.mL-1 for both the drugs. The second method was based on HPLC separation of the two drugs in reverse phase mode using Luna C18 column. Linearity was obtained in the concentration range of 8-22 µg.mL-1 for both the drugs. Both these methods have been successively applied to pharmaceutical formulation and were validated according to ICH guidelines.
11. **Mujeeb Ur Rahman[11] *et al.*,** This work is concerned with application of simple, accurate, precise and highly selective reverse phase high performance liquid chromatographic (RP-HPLC) method for simultaneous estimation of simvastatin and ezetimibe in combined dosage form. Chromatographic separation was achieved isocratically phenomenax C18 column (250 x 4.6 mm i.d.) with a mobile phase composed of 75:20:5 of acetonitrile:methanol:orthophosphoric acid (0.1%) % v/v/v at flow rate of 1 ml/min. Detection is carried out using a UV-vis detector at 238 nm. The retention time of simvastatin and ezetimibe was found to be 3.701 min and 5.975 min. respectively. The method was found to be linear in the range of 50-175 µg/ml with mean recovery of 99.78% for simvastatinand98.81%forezetimibe.
12. **Baokar Shrikrishna B[12] *et al.*,** This study aimed at developing and validating an HPLC method for the assay of Ezetimibe in tablets' formulation A chromatographic system comprising ODS-3V 4.6 mm x 250mm column, a mobile phase of Buffer and acetonitrile, a flow rate of 1.5 ml/min and a UV detector set at 230 nm has shown good chromatographic separation for Ezetimibe. The degree of linearity of the calibration curves, the percent recoveries of Ezetimibe and related substances, the limit of detection (LOD), and limit of quantization (LOQ) for the HPLC method have been determined. The HPLC method under study was found to be specific, precise, accurate, reproducible indicating stability and robust.
13. **Jayapal Reddy Sama[13] *et al.*,** A Simvastatin and Ezetimibe are used to treat hyperlipidaemia. A simple, precise cost effective and stability indicating RP-HPLC method has been developed and validated for the simultaneous determination of Simvastatin and Ezetimibe in pharmaceutical formulations.. Chromatographic separation was achieved on a X-terra RP-18 column(50 × 4.6 mm, 5µ) using a mobile phase consisting of 0.05M phosphate buffer pH3.0 and Acetonitrile in the ratio of 45:55 at a flow rate of 0.8ml per minute. The detection was made at 236nm. The retention time of Simvastatin and Ezetimibe were 3.3 and 0.8 minutes respectively. The method was found linear over the range of 5-15 µg per ml for Ezetimibe and 40-120 µg per ml for Simvastatin.
14. **S. Ashutosh Kumar[14] *et al.*,** This paper describes a simple, precise and accurate RP-HPLC method for simultaneous estimation of atorvastatin and ezetimibe in plasma. Methods: The chromatographic separation of the drugs were performed on an X-Terra C8 (4.6 x 150 mm, 3.5 m), with phosphate buffer [pH 3.5 with Ortho Phosphoric Acid] – acetonitrile 40:60 (v/v) as mobile phase. The detection was performed at 235 nm. The flow rate was maintained at 1.2 mL/min. The run time was 8.0 min. accuracy (100.08- 100.84 % for atorvastatin calcium and 100.56- 101.00 % for ezetimibe), The LLOQ obtained by the proposed method were 1.294 and 1.384 µg/mL for atorvastatin and ezetimibe respectively.
15. **Anuradha K. Gajjar[15] *et al.*,** A simple, precise and rapid stability-indicating reversed-phase high performance liquid chromatographic (RP-HPLC) method has been developed and subsequently validated for the simultaneous estimation of Rosuvastatin (RSV) and Ezetimibe (EZE) from their combination drug product. The proposed method is based on the separation of the two drugs in reversed-phase mode using Hypersil C18 150 x 4.6 mm, 5µ column maintained at a temperature of 40°C. The optimum mobile phase consisted of 0.05 M phosphate buffer (pH 2.5)-Methanol (45+55, v/v), mobile phase flow rate of 1.0 mL min-1 and UV detection was set at 242 nm. Linearity was obtained in the concentration range of 5-80 µg mL-1 for both RSV and EZE with correlation coefficients of 0.99999 and 0.99994 respectively. Mean percent recovery of triplicate samples at each level for both drugs were found in the range of 98% to 102% with RSD of less than 2.0%
16. **Mathrusri Annapurna Mukthinuthalapati[16] *et al.*,** A stability-indicating RP-HPLC method was developed and validated for the simultaneous determination of Rosuvastatin and Ezetimibe in tablet dosage forms using C 18 column with mobile phase consisting of tetra butyl ammonium hydrogen sulphate-acetonitrile (32:68, v/v) with a flow rate of 1.0 ml/min (UV detection 254 nm). Linearity was observed over the concentration range 0.1-200 µg/ml for both Rosuvastatin (r2 =0.9998) and Ezetimibe (r2 =0.9998). The LOD and LOQ were found to be 0.0282 µg/ml and 0.0853 µg/m for Rosuvastatin and the LOD and LOQ for Ezetimibe were 0.0297 µg/ml and 0.0901 µg/ml respectively. The percentage RSD

for intra-day precision was found to be 0.41-0.94 and 0.31-0.59 for Rosuvastatin and Ezetimibe respectively whereas the inter-day precision was found to be 0.68-0.95 and 0.68-1.02 for RosuvastatinandEzetimiberespectively.

17. **B. Neelima[17] *et al.*,** A simple, precise and rapid RP-HPLC method was developed for the simultaneous determination of simvastatin and ezetimibe in combined pharmaceutical dosage forms. The method was carried out on a Shim-pack, RP-C18 column using a mixture of acetonitrile: methanol: buffer (triethylamine pH-3) in the ratio 15:45:40 and detection was done at 240 nm using external standard method as quantitation. The linearity range of simvastatin and ezetimibe were 0.5 to 20 µg/ml. The intra-day and inter-day precision were in the range of 1.02-1.43 and 0.53-0.94 for simvastatin, 0.24- 1.29 and 0.93-1.32 for ezetimibe.
18. **Anil Shahaji Khile[18] *et al.*,** A new simple, accurate, rapid and precise isocratic RP-HPLC was developed and validated for the determination of Rosuvastatin and Ezetimibe in Pharmaceutical tablet dosage form by droping method. The Method employs Shimadzu LC system on Hypersil ODS column (4.6 x 250mm, 5µm) and flow rate of 1.5ml/min with an injection volume 20µl. Buffer, Acetonitrile and Methanol was used as mobile phase in the composition of 40:30:30v/v. The Detection was carried out at 230nm. Linearity ranges for Rosuvastatin and Ezetimibe were 11-33µg/ml, 10-30µg/ml respectively for HPLC. RetentionTime of Rosuvastatin and Ezetimibe were found to be 3.7 and 5.7min respectively.
19. **R.Sistla[19]*et al.*,** A Ezetimibe belongs to a group of selective and very effective 2-azetidione cholesterol absorption inhibitors that act on the level of cholesterol entry into enterocytes. A rapid, specific reversed-phase HPLC method has been developed for assaying ezetimibe in pharmaceutical dosage forms. The assay involved an isocratic elution of ezetimibe in a Kromasil 100 C_{18} column using a mobile phase composition of water (pH 6.8, 0.05%, w/v 1-heptane sulfonic acid) and acetonitrile (30:70, v/v). The flow rate was 0.5 ml/min and the analyte monitored at 232 nm. The assay method was found to be linear from 0.5 to 50 µg/ml. All the validation parameters were within the acceptance range.
20. **Rahul P. Dixit.[20] *et al.*,** A simple, selective and stability-indicating HPTLC method has been established for analysis of simvastatin and ezetimibe. The method uses aluminium-backed silica gel $60F_{254}$ TLC plates as stationary phase with *n*-hexane–acetone 6:4 (*v*/*v*) as mobile phase. Densitometric analysis of both drugs was carried out in absorbance mode at 234 nm. This system was found to give compact bands for simvastatin and ezetimibe (R_F 0.39 ± 0.05 and 0.50 ± 0.05, respectively). Linear relationships were obtained between response and amount of drug in the range 200–1,600 ng per band with high correlation coefficients (r^2 = 0.9917 ± 0.0018 for simvastatin and r^2 = 0.9927 ± 0.0021 for ezetimibe). Simvastatin and ezetimibe were subjected degradation by acid, pH 6.8 phosphate buffer, oxidation, dry heat, and wet heat.

CONCLUSION

Literature survey suggested that various UV[1-6], HPLC[7-19], HPTLC[20] and few simultaneous methods were developed and reported. The published methods were validated for various parameters as per ICH guidelines. Statistical analysis proved that the published methods were reproducible and selective. Thus, it can be concluded that the reported and published methods can be successfully applied for the estimation of the Ezetimibe in pure and pharmaceutical dosage form.

REFERENCE

1. Gupta KH, Singhvi IN. Development of UV spectrophotometric method for the estimation of ezetimibe from tablet formulation. *Int.J of Chem Sci*, 2015; 13: 1051-6.
2. Seema MD, Manjusha PY. First derivative UV-spectrophotometric method for simultaneous determination of simvastatin and ezetimibe in tablet dosage form. *Pharmaceutical Leterature*, 2015; 7: 124.
3. Sharma M, Mhaske DV, Mahadik M, Kadam SS, Dhaneshwar SR. UV and three derivative spectrophotometric methods for determination of ezetimibe in tablet formulation. *Ind J.of pharma. sci*, 2008; 70(2): 258.
4. Lakshmi P, Ramchandran D, Rambabu C. Spectrophotometric determination of ezetimibe. *E.J. of chem*, 2010 1; 7(1): 101-4.
5. Imran M, Singh RS, Chandran S. Stability indicating ultraviolet spectroscopic method for the estimation of ezetimibe and carvedilol. Die Pharmazie-An *Int. J. of Pharma. Sciences*, 2006 1; 61(9): 766-9.
6. Beludari MI, Prakash KV, Mohan GK. RP-HPLC method for simultaneous estimation of rosuvastatin and ezetimibe from their combination tablet dosage form*. İnt. J of chemi and analy science*, 2013 1; 4(4): 205-9.
7. Jain N, Jain R, Swami H, Jain DK. RP-HPLC method for simultaneous estimation of simvastatin and ezetimibe in bulk drug and its combined dosage form. *Asian J. Research Chem*, 2008; 1(1): 29-31.
8. Kumar P, Ahmad Y, Ghosh A. A stability indicating RP-HPLC method development for determination of ezetimibe in tablet dosage form. *Der Pharma Chemica*, 2012; 4(3): 1296-304.
9. Shrivastava PK, Basniwal PK, Shrivastava SK, Jain D. Validated RP-HPLC method for estimation of ezetimibe in different tablet dosage form. *Int. J. Ph. Sci*, 2009; 1(1): 174-81.
10. Sonawane SS, Shirkhedkar AA, Fursule RA, Surana SJ. Application of UV-Spectrophotometry and RP-HPLC for Simultaneous Determination of Atorvastatin Calcium and Ezetimibe in

Pharmaceutical Dosage Form. *Eurasian J of A. Chem*, 2006; 1: 1(1).
11. Rahman, Mujeeb Ur, et al. "Simultaneous estimation of simvastatin and ezetimibe in pharmaceutical tablet dosage forms by RP-HPLC: A Review." *Int J Pharma. Res Dev*, 2010; 2: 56-62.
12. Baokar Shrikrishna B, Erande RS, Shaikh SG. Analytical method development and validation for estimation of ezetimibe from tablet dosage form by using RP-HPLC. *Int. J. of Res. Pharma. and Biomed. Sci*, 2011; 2(2): 833-41.
13. Sama JR, Kalakuntla RR, Rao VS, Reddanna P. Simultaneous estimation of Simvastatin and Ezetimibe in pharmaceutical formulations by RP-HPLC method. *J of Pharma Sci and R.*, 2010 1; 2(2): 82.
14. Kumar SA, Debnath M, Rao JS, Sankar DG. New validated stability-indicating RP-HPLC method for simultaneous estimation of atorvastatin and ezetimibe in human plasma by using PDA detector. *Advanced Pharmaceutical Bulletin*, 2015; 5(3): 385.
15. Gajjar AK, Shah VD. Development and validation of a stability-indicating reversed-phase HPLC method for simultaneous estimation of rosuvastatin and ezetimibe from their combination dosage forms. *Eurasian J. A. Chem*, 2010 1; 5(3): 265-83.
16. Mukthinuthalapati MA, Bukkapatnam V, Bandaru SP, Grandhi NS.Simultaneous Determination of Rosuvastatin and Ezetimibe in pharmaceutical formulations by Stability Indicating Liquid Chromatographic Method. *J of BE & BA*, 2014 20; 6(6): 174.
17. Neelima B, Kumar PR, Krishna MM, Bindu VH. Simultaneous estimation of simvastatin and ezetimibe by RP-HPLC in pure and pharmaceutical dosage form. *Oriental J of Chem*, 2008; 24(1): 195.
18. Khile AS, Devi NG, Rao MS, Ramachandran D. Development and Validation of RP-LC Method For Simultaneous Estimation of Rosuvastatin And Ezetimibe In Bulk and Its Pharmaceutical Formulations. *Am J Pharma. Tech. Res*. 2017; 7(2).
19. Sistla R, Tata VS, Kashyap YV, Chandrasekar D, Diwan PV. Development and validation of a reversed-phase HPLC method for the determination of ezetimibe in pharmaceutical dosage forms. *J of pharma and biomedical analysis*, 2005 15; 39(3-4): 517-22.ICH,
20. Dixit RP, Barhate CR, Nagarsenker MS. Stability-indicating HPTLC method for simultaneous determination of ezetimibe and simvastatin. *Chromatographia*, 2008; 67(1): 101-7.
21. Q2A Text on Validation of Analytical Procedures, 1994.
22. ICH, Q2B Validation of Analytical Methodology, 1996.
23. ICH, Q2 (R1) Validation of Analytical Procedures: text and methodology, 2005.