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STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS QUANTIFICATION OF EZETIMIBE AND GLIMEPIRIDE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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Abstract:

A simple, specific, accurate, and precise reverse phase high performance liquid chromatographic (RP-HPLC) method and Stability indicating tests was developed and validated for the simultaneous quantification of Ezetimibe and Glimepiride in Bulk drugs and Pharmaceutical dosage form. The quantification is carried out using Kromosil C18 (150 ×4.6mm, 5µ) column with mobile phase consisted of a mixture of Acetonitrile and Potassium dihydrogen ortho phosphate buffer in the ratio of 65:35 (v/v) delivered at a flow rate of 1.0 ml / min and effluents were monitored at 228 nm.The retention times of Ezetimibe and Glimepiride were found to be 2.789 min and 3.282 min respectively. The linearity for Ezetimibe and Glimepiride were in the range of 25-150 µg/ml and 2.5-15 µg/ml with correlation co-efficient of 0.999 for both drugs. The mean % recoveries of Ezetimibe and Glimepiride were found to be 98.41 to 100.78 % and 98.39 to 100.80 % respectively. The proposed method was validated as per ICH guidelines and it was found to be accurate, precise and robust, and it was applied to the estimation of Ezetimibe and Glimepiride in combined tablet dosage form. Forced degradation studies indicated the suitability of the method for stability studies.

Keywords: *Ezetimibe, Glimepiride, RP-HPLC, Validation and ICH Guidelines.*

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INTRODUCTION:

Diabetes is a metabolic disorder accompanied by insulin insufficiency and by impaired insulin secretion. The symptoms are characterized by hyper glycaemia, impaired insulin secretion, glucosuria, hyper lipaemia (insulin resistance in skeletal muscles, liver and adipose tissue), negative nitrogen balance, Sometimes ketonaemia. Such patients are often obese and generally present in adult life, the incidences rising progressively with β-cell function declines. These defects have been treated by use of oral insulin secretogogues (sulphonyl urea/glinides) or insulin, biguanides, thiazolidinediones and anti-cholesteremic agents [1].

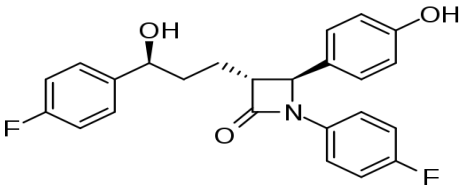

Fig. 1: Chemical Structure of Ezetimibe

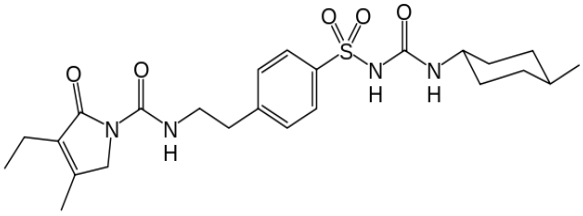

Fig. 2: Chemical Structure of Glimepride

Ezetimibe ($C_{24}H_{21}F_2NO_3$) is chemically (3R, 4S)-1-(4-fluorophenyl)-3-[(3S)-3 - (4-fluorophenyl)-3 - hydroxyl propyl]-4 - (4-hydroxyphenyl) azetidin-2-one (Fig. 1) [2], a new anti-hyper lipidemic agent [3], which inhibits the absorption of cholesterol from intestine, used in the treatment of primary hypercholesterolemia. It inhibits the absorption of biliary and dietary cholesterol from small intestine without affecting absorption of fat soluble vitamins, triglycerides and bile acids. After oral administration, EZE is metabolized into glucuronide in the liver and small intestine, which is also active in prevention of absorption of cholesterol.

Glimepiride ($C_{24}H_{34}N_4O_5S$) is chemically 3-ethyl- 4-methyl-N-(4-[N-((1r,4r)-4-methyl cyclohexyl carbamoyl) sulfamoyl] phenethyl)-2-oxo-2,5-dihydro-1H-pyrrole-1carboxamide (Fig. 2) [4], 3rd generation sulfonylurea derivative used in the treatment of type-II diabetes mellitus and also non insulin dependent diabetes mellitus(NIDDM) [5].The primary mechanism of action of Glimepiride in lowering blood glucose (Secretogogues) appears to be dependent on stimulating the release of insulin from functioning pancreatic β-cells and by inducing increased activity of intracellular insulin receptors [5]. It is official in Indian pharmacopoeia (IP) [6], British pharmacopoeia (BP) [7], United States Pharmacopoeia (USP) [8] and European pharmacopoeia (EU) [9] describe liquid chromatographic method for estimation.

The combination of these two drugs is not official in any pharmacopoeia. Fixed dose combination therapy of Ezetimibe and Glimepiride is indicated for the treatment of type 2 diabetes mellitus. Recent studies reveal that the treatment of` Lipidemia with concomitant administration of Ezetimibe and Glimepiride, shows significantly better symptom relief when compared with each of the treatments alone.

Literature survey revealed that few analytical methods have been reported for estimation of Ezetimibe and Glimepiride individually or in combination with other drugs. The reported methods are Spectrophotometric [10-14], RP-HPLC [15-31]. Simultaneous estimation of Ezetimibe and Glimepride in combined pharmaceutical formulations by RP-HPLC [32-34] and stability indicating RP-HPLC [35] methods were reported. The present study was aimed to develop a simple, sensitive, rapid, precise and accurate stability indicating RP-HPLC method for simultaneous estimation of Ezetimibe and Glimepride. Developed method was validated according to ICH guidelines [36,37].

MATERIAL AND METHODS:

Chemicals and reagents: Pure samples of Ezetimibe and Glimepride were obtained from Spectrum Pharma Research Solutions, Hyderabad, India. The marketed formulation of EZIWA tablets (Ezetimibe 10 mg/tablet and Glimepride 1 mg/tablet) Manufactured by Kaytross Health Care Private Limited in India were procured from local pharmacy Store. Analytical grade of Potassium dihydrogen orthophosphate, Orthophosporic acid and HPLC grade of Acetonitrile were procured from SD Fine Chemicals Ltd., Mumbai, India. HPLC grade water was obtained by double distillation and purified additionally with Milli-Q water purification system.

Instrumentation: The analysis was performed by using a chromatographic system Water 2695 series HPLC comprised of vacuum degas, auto injector, and dual gradient pump with UV-Visible detector. The HPLC system was equipped with Empower 2 software.

Chromatographic conditions: Ezetimibe and Glimepride was analysed with Kromasil C_{18} column (150 x 4.6 mm, 5 µm particle size) for the chromatographic separation. The mobile phase was composed of a mixture of Acetonitrile and Potassium dihyrogen orthophosphate buffer in the ratio of 65:35 v/v and it was delivered at a flow rate of 1.0 mL/min and UV detection was performed at 228 nm. And Acetonitrile and Water (50:50) was used as diluent. Injection volume was 10 µL. The run time was 6 min. The retention time of Ezetimibe 2.789 min and Glimepride was found to be 3.282 min respectively.

Mobile phase preparation: Accurately weighed 1.36 g of Potassium dihydrogen orthophosphate transferred in a 1000 mL clean and dry volumetric flask, add about 500 mL of HPLC grade water purified with Milli-Q purification system and Sonicated for degassing finally make up the volume with water. And pH was adjusted to 4.8 with dilute Orthophosporic acid solution. 650 mL of Acetonitrile and 350 mL of buffer were added in a 1000 mL flask.

Standard stock preparation: Accurately weighed and transferred 10 mg and 1 mg of Ezetimibe and Glimepride working standards into 10 ml clean dry volumetric flask, add 5 ml of Acetonitrile and sonicated for 5 minutes and make up to the final volume with diluent. From the above stock solutions, 1 ml was pipette out in to a 10 ml volumetric flask and then make up to the final volume with diluent. And it gives 100 µg/ml of Ezetimibe & 10 µg/ml of Glimepride.

Sample Solution Preparation: Accurately 20 tablets were weighed individually and the average weight was calculated and powdered. The tablet powder equivalent to 10 mg Ezetimibe and 1 mg Glimepride transferred into a 100 mL volumetric flask, to that 60ml of diluent was added and sonicated for 10 min at controlled temperature to dissolve the powder, further the volume made up with diluent, and filtered through 0.45 µ membrane filter (Stock solution). From this solution 0.5 mL was diluted to 10 mL with diluent to give a concentration of 100 µg/mL and 10 µg/mL solution of Ezetimibe and Glimepiride respectively.

Method Validation

Developed method was validated as per ICH guidelines over the system suitability, linearity, accuracy, precision, limit of detection, limit of quantification, robustness, specificity and solution stability.

System Suitability: System suitability is an integral part of the chromatographic system. It is verification of resolution, capacity factor, tailing factor, theoretical plate count, relative retentions etc are calculated and compared with standard specification of system.

Linearity: Linearity is the ability (within specified range) to obtain test results are directly proportional to the concentration of analyte in the sample. Linearity is evaluated by visual inspection of plot of signal as a function of analyte concentration. If there is a linear relationship test results are calculated by regression line by method of least squares.

Range: The range of analytical procedure is the interval between the upper and lower concentration of analyte in the sample.

Accuracy: Accuracy of analytical method is 'measure of how close the experimental value to the true value' accuracy of the method was determined by standard addition method. A known amount of standard drug is added to the fixed amount of pre-analysed injection solution. Percent recovery is calculated by comparing the area before and after addition of the standard drug. The standard addition method is performed at 50%, 100% and 150% level. The solutions are analysed in triplicate at each level as per the proposed method.

Precision: The closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

Limit of detection and Limit of Quantification: Limit of detection (LOD) is defined as the lowest concentration of analyte that gives a detectable response. Limit of Quantification (LOQ) is defined as the lowest concentration of analyte that can be quantified with a specified level of accuracy and precision. For this study, six replicates of the analyte at lowest concentration are measured and quantified.

Robustness: The robustness of the proposed method is estimated by changing flow rate of the mobile phase, pH of the buffer and composition of the mobile phase.

Specificity: Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. The specificity of method was determined by comparing the chromatograms of blank, standard and sample.

Degradation studies

To determine the stability, the standard and sample solutions are observed at different degradation studies in laboratory conditions, the result and data will compared with standard chromatograms.

Acid degradation studies: To 1 mL of stock solution of Ezetimibe and Glimepride, 1 mL of 2N Hydrochloric acid was added and refluxed for 30 mins at 60^0C.The resultant solution was diluted to obtain 100 µg/ML and 10 µg/mL solutions and 10 µL solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali degradation studies: To 1ml of stock solution of Ezetimibe and Glimepride, 1 mL of 2N Sodium hydroxide was added and refluxed for 30 min at 60^0 C. The resultant solution was diluted to obtain 100µg/mL and 10 µg/mL solutions and 10 µL solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Oxidative degradation studies: To 1mL of Ezetimibe and Glimepride, 1mL of 20% hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 30 min at 60^0 C. The resultant solution was diluted to obtain 100 µg/mL and 10 µg/mL solutions and 10 µL solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Thermal degradation studies: The standard drug was placed in oven at 105^0C for 6 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to get 100 µg/mL and 10 µg/mL solutions and 10 µL were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Photo stability studies: The photo chemical stability of the drug was also studied by exposing the 100 µg/mL and 10 µg/mL solutions to UV Light by keeping the beaker in the UV Chamber for 7 days or 200 Watt hours /m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted to get 100µg/mL and 10 µg/mL solutions and 10 µL were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Neutral degradation studies: Stress testing under neutral conditions was studied by refluxing the drug in water for 6 h at a temperature of 60^0C. For HPLC study, the resultant solution was diluted to get 100 µg/mL and 10 µg/mL solutions and 10 µL were injected into the system and the chromatograms were recorded to assess the stability of the sample.

RESULTS AND DISCUSSION:

The HPLC procedure was optimised with a view to develop an accurate assay method for simultaneous determination of Ezetimibe and Glimepride in bulk and pharmaceutical dosage form by using column Kromasil C18 (150 x 4.6 mm internal diameter; 5 µm particle size) with mobile phase composition of Acetonitrile: Buffer in the ratio of 65:35 v/v. Resulted in peaks with good shape and well resolved. The flow rate was 1.0 mL/min and both the components were measured with UV-Visible detector at 228 nm. The results of optimized HPLC conditions were shown in Table 1. The method was linear in the range of 25-150 µg/mL and 2.5-15 µg/mL for Ezetimibe and Glimepride with correlation coefficient 0.999 for both. Linear regression data for Ezetimibe and Glimepride were given in Table 2, the linearity curves for Ezetimibe and Glimepride were shown in Fig. 3 and Fig. 4. The mean % recoveries of Ezetimibe and Glimepiride were found to be 98.41 to 100.78 % and 98.39 to 100.80 %, which indicate the method is accurate. The accuracy results were shown in Table 3. The % RSD for method precision was found to be 0.6 and 0.8 for Ezetimibe and Glimepride respectively. Which indicate the method is precise. The precision results were shown in Table 4. The retention time of Ezetimibe and Glimepride was 2.789 min and 3. 282 min respectively. The number of theoretical plates calculated was 6137 for Ezetimibe and 6534 for Glimepride and symmetry factor was 1.16 for Ezetimibe and 1.17 for Glimepride, which indicates efficient performance of the column. The LOD for Ezetimibe and Glimepride were found to be 0.552 µg/mL and 1.988µg/mL respectively. The LOQ for the Ezetimibe and Glimepride were found to be 1.675µg/mL and 6.025µg/mL respectively, which indicates the sensitivity of the method. Results of system suitability and validation parameters of Ezetimibe and Glimepride were shown in the Table 5. Validated RP-HPLC method was applied for the determination of Ezetimibe and Glimepride in commercial tablet formulation that was obtained by injected 3 replicates of the sample solutions. The amounts of Ezetimibe and Glimepride estimated were found to 9.96 mg and 0. 99 mg/tablet. The results assay was shown in Table 6. Typical chromatogram of standard Ezetimibe and Glimepride was shown in Fig. 5. No interfering peaks were found in the chromatogram of the formulation within the run time indicating that excipients used in pharmaceutical formulations did not interfere with the estimation of the drugs by the proposed method.

The typical variations studied under this parameter were mobile phase composition and detection wavelength. Overall % RSD was found to be less than 2% for all the variations which indicates that the proposed method is robust. Forced degradation studies was observed that upon treatment of Ezetimibe and Glimepride with different strengths of acid (2N HCl), alkali (2N NaOH), 20% Hydrogen peroxide, thermal (105^0C), photolytic (UV chamber) and water. The results of degradation studies were shown in Table 7. The results of Ezetimibe and Glimepride were not stable under the applied stress conditions like acid, alkaline, oxidative and thermal degradation conditions.

Table 1: Optimized Chromatographic Conditions of Ezetimibe and Glimepride

Parameter	Condition
Mobile phase	Acetonitrile: Potassium dihydrogen orthophosphate buffer (65:35% v/v)
pH	4.8 (Adjusted with dil. Ortho phosphoric acid)
Column	Kromasil, C_{18} Column (150 x 4.6 mm; 5 µm)
Injection volume	10 µL
Column temperature	Ambient
Wave length	228 nm
Flow rate	1.0 mL/min
Run time	6 min
Retention time	Ezetimibe- 2.789 min, Glimepride- 3.282 min

Table 2: Linearity results of Ezetimibe and Glimepride

S.No	Ezetimibe		Glimepiride	
1	Concentration (µg/mL)	Area	Concentration (µg/mL)	Area
2	25	1682329	2.5	561901
3	50	3134453	5	1050008
4	75	4888810	7.5	1678313
5	100	6349796	10	2204858
6	125	7672254	12.5	2684588
7	150	9287896	15	3230837

Table 3: Accuracy result of Ezetimibe and Glimepride

	Ezetimibe				Glimepride			
Spike Level	Amount added (µg/mL)	Amount found (µg/mL)	% recovery	mean % recovery	Amount added (µg/mL)	Amount found (µg/mL)	% recovery	mean % recovery
50%	50	48.49	96.98	100.78	5	5.14	102.8	100.8
50%	50	48.58	97.16		5	5.08	101.6	
50%	50	48.87	97.74		5	5.10	102	
100%	100	98.48	98.48	98.41	10	9.83	98.3	97.75
100%	100	98.27	98.27		10	9.83	98.3	
100%	100	99.78	99.78		10	9.66	96.66	
150%	150	148.76	99.73	98.56	15	14.82	98.83	98.39
150%	150	149.37	99.58		15	14.87	99.13	
150%	150	145.43	96.95		15	14.58	97.21	

Table 4: Method Precision Results of Ezetimibe and Glimepride

S. No.	Ezetimibe		Glimepride	
	RT	Peak Area	RT	Peak Area
1	2.566	5656457	2.963	2105521
2	2.578	5661434	2.974	2119619
3	2.581	5641911	2.978	2149921
4	2.614	5617709	3.031	2131546
5	2.620	5594964	3.039	2145625
6	2.621	5693133	3.040	2146357
Average		5644268		2133098
Std. Dev		34562.5		17665
% RSD		0.6		0.8

Table 5: Results of System Suitability and Validation Parameters

S. No.	Parameter	Results	
		Ezetimibe	Glimepride
1	Linearity range (µg/mL)	25 - 150	2.5 - 25
2	Slope (m)	61513	21561
3	Intercept (c)	10305	12993
4	Correlation coefficient (R^2)	0.999	0.999
5	Retention times (min)	2.409	3.807
6	Theoretical plates (N)	6137	6534
7	Tailing factor	1.16	1.15
8	Repeatability (%RSD)	0.6	0.8
9	LOD (µg/mL)	0.552	1.988
10	LOQ (µg/mL)	1.675	6.026
11	Resolution (Rs)	2.24	

Table 6: Results of marketed formulation (Assay)

S.NO	Brand	Drug	Label claim (mg/tablet)	Amount found (mg/tablet)	% Assay
1	EZIWA	Ezetimibe	10	9.96	99.6
2		Glimepride	1	0.99	99

Table 7: Results of Degradation Studies

Stress condition/ Duration/Solution	Ezetimibe		Glimepride	
	% Assay	% of Degradation	% Assay	% of Degradation
Acid degradation (1ml +1ml 2N HCl, 30 min at 60^0C)	92.224	7.775	92.174	7.825
Alkaline degradation (1ml+1ml 2N NaOH, 30 min at 60^0C)	93.704	6.295	92.685	7.314
Oxidative degradation (1ml +1 ml 20% H_2O_2, 30min at 60^0C)	93.897	6.102	93.162	6.837
Thermal degradation (105^0C /6 h)	95.683	4.316	94.107	5.892
Photolytic degradation (UV light 7days or 200 Watt h/m^2)	96.624	3.375	97.030	2.969
Neutral degradation (60^0C/ 6 h, in water)	98.117	1.882	98.195	1.804

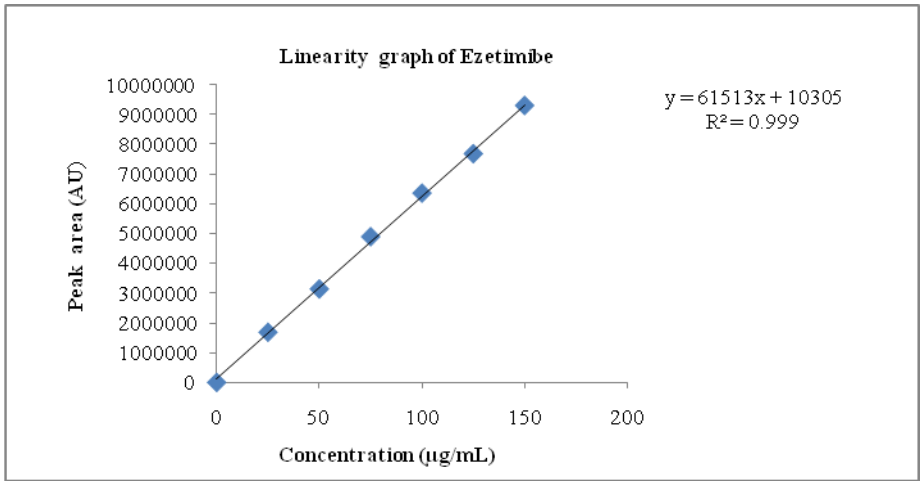

Fig. 3: Linearity Graph of Ezetimibe

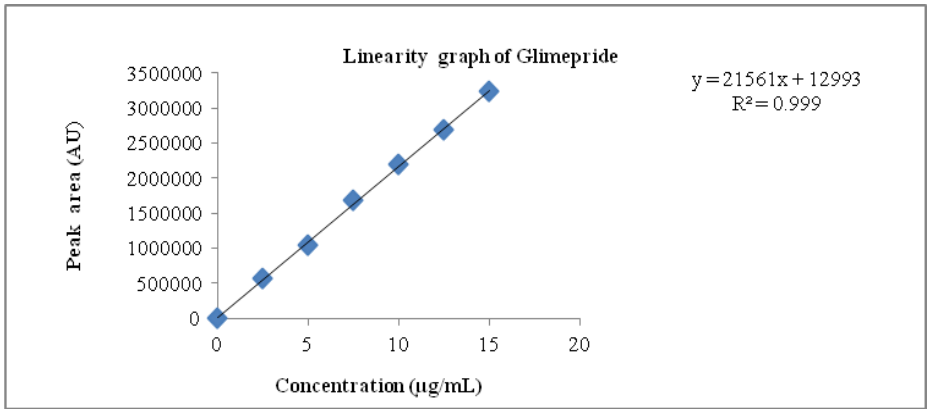

Fig. 4: Linearity Graph of Glimepride

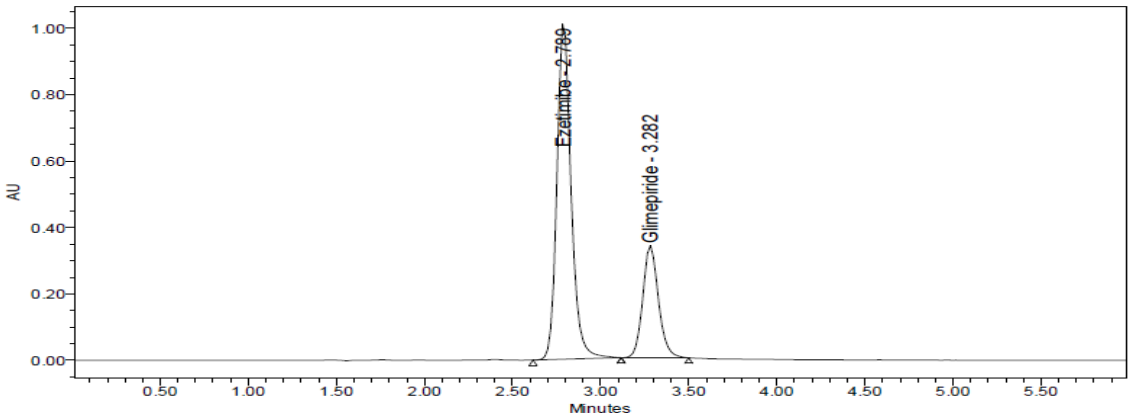

Fig. 5: Chromatogram of Ezetimibe and Glimepride

CONCLUSION:

Validated stability indicating RP-HPLC method was simple, specific, accurate, precise and sensitive. And it can be used for simultaneous estimation of Ezetimibe and Glimepride in bulk samples and its pharmaceutical dosage forms.

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REFERENCES:

1. Rang and Dale's, Pharmacology (6th edition), 2007:403.
2. Martindale, the Complete Drug Reference; Pharmaceutical Press (36th Edition), 1:1284.
3. O'Neil MJ. The Merck Index-An Encyclopaedia of Chemicals, Drugs and Biological, 3949:693.
4. Martindale, the Complete Drug Reference; Pharmaceutical Press (36th Edition), 1: 441, 1284.
5. O'Neil MJ. The Merck Index- An Encyclopaedia of Chemicals, Drugs and Biological, 4453:790.
6. Indian Pharmacopoeia. The controller of publication. 6th edition. Govt. of India. New Delhi, 2010; 2:1418-1420.
7. British Pharmacopoeia 2007; 1:957.
8. United States pharmacopoeia. The United States Pharmacopoeial convention Inc, Rockville, 2009; 2497.
9. European Pharmacopoeia (6th edition) Starboary: Council of Europe, 2008; 2:1156.
10. Hanifa Begum, Sh. Rizwan, Khaled Bin Sayeed, Asiya Fatima, Development and Validation of Stability indicating UV Spectrophotometric Simultaneous equation method for determination of Ezetimibe and Glimepiride in bulk drugs and marketed formulation. International Journal of Research and Development in Pharmacy and Life Sciences, 2015; 4(2):1412-1421.
11. Abdul Aziz Ramadan, Hasna Mandil, Souad Zein. Spectrophotometric Determination And Validation Of Glimepiride In Pure And Tablet Dosage Forms Through Ion-Pair Complex Formation Using Bromocresol Green" Int J Pharm Pharm Sci, 8(6): 216-221.
12. Arun MP, Rahul BP. Development and validation of UV-Spectrophotometric method for simultaneous estimation of Glimepiride and Ezetimibe in tablet dosage form. International Journal of Pharmaceutics and Drug Analysis 2014; 2(3):239-245.
13. Bhargavi S, Suryasagar G, Krishna Sowmya D, Ashok K, Sreekanth N. UV spectroscopic method for determination of Glimepiride in pharmaceutical dosage forms, International Journal of Pharmaceutical Sciences Review and Research 2013; 21(2):131-133.
14. P.H.Sakpal, M.N.Deodhara, V.P.Ghodsea, B.A.Agarwal, H.Khiste, Sonawane A and P.L. Ingale. Simultaneous Spectrophotometric estimation of Ezetimibe and Atorvastatin in pharmaceutical dosage form. Int. J. Chem. Sci. 2012; 10(1): 131-136.
15. M.M. Eswarudu, P. Mounika, D.Venkatesh and B.Naga Lakshmi. Method Development and Validation for Simultaneous Estimation of Rosuvastatin Calcium and Ezetimibe in Pharmaceutical Dosage Form by RP-HPLC. International Research Journal of Pharmaceutical and Applied Sciences.2012; 2(1):24-36.
16. P.S.R.Ch.N.P.Varma D, A. Lakshmana Rao and S. C. Dinda. Development and Validation of Stability Indicating RP-HPLC Method for Simultaneous Estimation of Rosuvastatin and Ezetimibe in Combined Tablet Dosage Form. Rasayan Journal of Chemistry, 2012; 5(3): 269-279.
17. Kumar P, Ghosh A, and Chaudhary M. Simultaneous Estimation of Ezetimibe and Atorvastatin in Pharmaceutical Formulations by RP HPLC Pharmaceutica Analytica. 2012; 3(6):6-6.
18. Suchitra M, Sunitha D, Parthiban C, Siddartha B and Madhavi C. Method development and validation of Metformin, Glimepiride and Pioglitazone in tablet dosage form by RP-HPLC, International Research Journal of Pharmacy 2013; 4(8):250-254.
19. Mujeebur Rahman, Gazala Parveen, N.K.Nyola, Shahroz Khan, Sushma Talegaonkar, M. Shaharyar and R.K.Khar. Simultaneous Estimation of Simvastatin and Ezetamibe in Tablet dosage forms by RP-HPLC. A Review**,** IJPRD 2010; 8(9):56- 62.
20. Sakuntala MSV, Prasad SVUM, Sridevi S, Kishore Yadav S, Srinivas Reddy K. A RP-HPLC method development and validation for simultaneous estimation of Glimepiride and Pioglitazone HCl in tablet dosage form, Journal of Chemical and Pharmaceutical Research 2012; 4(1):154-159.
21. Rajashekar V, Rajeswar Dutt K, Ramathilagam N. RP-HPLC method development and validation for the simultaneous estimation of Rosuvastatin and Ezetimibe in tablet dosage form, International Journal of Pharmacy and Analytical Research 2014; 3(1):13-21.
22. Mohammad Ishaq Beludari, Vanitha Prakash K, Krishna Mohan G. RP-HPLC method for simultaneous estimation of Rosuvastatin and Ezetimibe from their combination tablet dosage form, International Journal of Chemical and Analytical Science 2013;4(4):205-209.
23. Sahu S, Narayan UL, Garnaik B and Patro SK. Stability indicating RP-HPLC method for determination of Ezetimibe in pure and pharmaceutical formulation, International Journal of Research in Pharmaceutical and Biomedical Sciences 2013; 4(4):1249-1255.

24. Praveen Kumar, Yusra Ahmad, Amitav Ghosh. A stability indicating RP-HPLC method development for determination of Ezetimibe in tablet dosage form, Scholars Research Library 2012; 4(3):1296-1304.
25. Nagaraju. P, Krishna Chaithanya. K, Chandra babu.D, Srinivas. V.D.N and Padma S.V.N. RP-HPLC Estimation of Ezetamibe In tablet Dosage Forms. International Journal of Research in Pharmacy and pharmaceutical Sciences, 2010; 2 (1): 33-37.
26. Chetan M.B, Jane J and Subrahmanyam EVS. HPLC and spectroscopic estimation of ezetimibe, International Journal of Research in Pharmaceutical and Biomedical Sciences 2011; 2(1):241-244.
27. Dixit R.P, Barhate CR, Padhye SG, Vishwanathan CL and Nagasenker MS. Stability indicating RP-HPLC method for simultaneous determination of Simvastatin and Ezetimibe from tablet dosage form, Indian Journal of Pharmaceutical Sciences 2010; 72(2):204-210.
28. Shrivastava PK, Basniwal PK, Shrivastava SK, Deepti Jain. Validated RP-HPLC method for estimation of Ezetimibe in different tablet dosage forms, International Journal of Pharmaceutical Sciences 2009; 1(1):176-181.
29. Akmar SK, Lata K, Asha Thomas, Sumitra Jangam, Deshpande AD. Reverse phase high performance liquid chromatography method for estimation of Ezetimibe in bulk and pharmaceutical formulations, Indian Journal of Pharmaceutical Sciences 2007; 69(5): 695-697.
30. Meghana Reddy R, Venkata Ravindranath T. Method development and validation for simultaneous estimation of Metformin, Glimepiride and Atorvastatin in combined tablet dosage form by UPLC, World Journal of Pharmacy and Pharmaceutical Science 2013; 2(6): 5229-5247.
31. Baokar Shrikrishna B., Erande R.S, and Shaikh S.G. Analytical Method development and validation for Estimation of Ezetimibe from Tablet dosage form by using RP-HPLC. International Journal of Pharmaceutical and biomedical sciences.2011; 2(2): 833- 841.
32. Siddhartha. B. and Radhika Malipelli. Simultaneous Estimation And Validation Of Ezetimibe And Glimepride In Bulk And Pharmaceutical Dosage Form By RP-HPLC. International Journal of Research and Development in Pharmacy and Life Sciences.2014; 3(6):1280-128.
33. P. Nagaraju and T. Sowjanya Jyothi. Simultaneous Quantification And Validation of Glimepiride And Ezetimibe By RP-HPLC Bulk And Pharmaceutical Dosage Form Pharmagene; Vol: 2, Issue: 3.
34. Sudheer kumar N, Shilpa K, Ajitha A , Uma Maheswara Rao V. Method development and validation of simultaneous estimation of Ezetimibe and Glimepiride by RP-HPLC. International journal of pharmaceutical research & analysis. 2016; 6(1):47-52.
35. Pavani P, Srilekha A and Sreedhar B: Stability Indicating RP-HPLC Method Development and Validation for Simultaneous Estimation of Glimepiride and Ezetimibe in Bulk and Tablet Dosage Form. Int J Pharm Sci Res 2015; 6(3): 1066-77.
36. ICH Harmonised Tripartite Guideline, Validation of analytical procedures: Text and methodology, Q2 (R1), International Conference on Harmonization, Geneva. 2005; 1-13.
37. ICH Harmonized Tripartite Guideline Stability Testing of New Drug Substances and Products Q1A (R2), International Conference on Harmonization, Geneva. 2003; 1-18.