Sudheer Kumar N .et al. / Vol 6 / Issue 1 / 2016 / 47-52.

International Journal of Pharmaceutical Research & Analysis

e-ISSN: 2249 – 7781
Print ISSN: 2249 – 779X

www.ijpra.com

METHOD DEVELOPMENT AND VALIDATION OF SIMULTANEOUS ESTIMATION OF EZETIMIBE AND GLIMEPIRIDE BY RP-HPLC

Sudheer Kumar N*, Shilpa K, Ajitha A , Uma Maheswara Rao V

Department of Pharmaceutical Analysis & Quality Assurance, CMR College of Pharmacy, JNTU (H) University, Hyderabad, Andhra Pradesh, India.

ABSTRACT

A sensitive, selective and precise high performance liquid chromatographic method has been developed and validated for the simultaneous determination of Ezetimibe and Glimepiride in tablet dosage form. The method employed like C18 column, Symmetry C18 (4.6 x 250mm, 5µm, Make: Waters) as the stationary phase while Phosphate buffer (pH 3.6), Acetonitrile in proportion 45:55 v/v respectively. was used as mobile phase. The Retention time of Ezetimibe and Glimepiride were observed to be 2.273 and 3.630 minutes, respectively. The flow rate was found to be 1ml/min and effluents were monitored at 228 nm. The linear regression analysis data for the calibration plots showed a good linear relationship for both Ezetimibe and Glimepiride and over a concentration range of 10-50 µg/ml. with correlation co-efficient of 0.9989 for Ezetimibe and and 0.9999 for Glimepiride. The LOQ was found to be 4.52 and 3.67µg/ml respectively for Ezetimibe and Glimepiride. The method was validated as per ICH guideline and it was found to be accurate, precise and robust. Marketed formulation was analyzed successfully.

Keywords: Ezetimibe, Glimepiride, HPLC, Validation etc.

INTRODUCTION

Ezetimibelocalises at the brush border of the small intestine, where it inhibits the absorption of cholesterol from the intestine. Specifically, it appears to bind to a critical mediator of cholesterol absorption, the Niemann-Pick C1-Like 1 (NPC1L1) protein on the gastrointestinal tractepithelial cells as well as in hepatocytes [1].

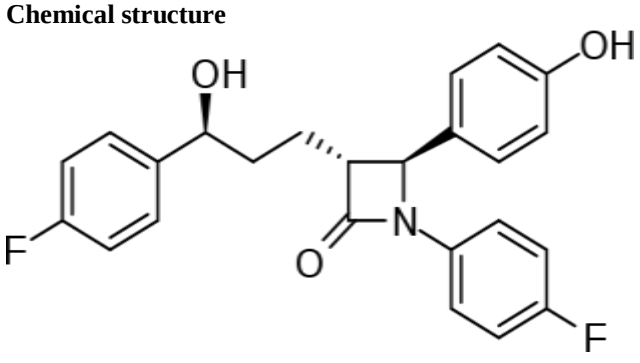
In addition to this direct effect, decreased cholesterol absorption leads to an upregulation of LDL-receptors on the surface of cells and an increased LDL-cholesterol uptake into cells, thus decreasing levels of LDL in the blood plasma which contribute to atherosclerosis and cardiovascular events [2].

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. and also to establish a simple, sensitive, precise, accurate, less time consuming and cost effective, RP-HPLC method for estimation of Ezetimibe and Glimepiride in bulk drug and dosage form [3].

DRUG PROFILE

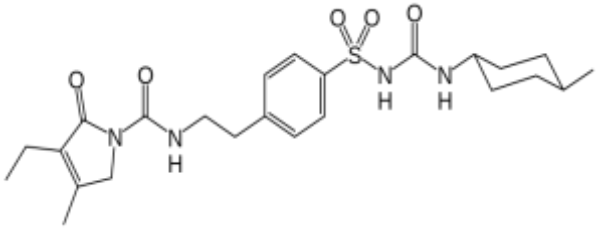
EZETIMIBE

Chemical structure



Corresponding Author:-N. Sudheer Kumar **Email:-**sudheer.noothpally@gmail.com

Chemical name : (3*R*,4*S*)-1-(4-fluorophenyl)-3-[(3*S*)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl)azetidin-2-one.
Molecular formulae:C24H21F2NO3
Molecular Weight : 409.4 g·mol^{-1}
Category : **:** AAntilipidemic
GLIMEPIRIDE
Structure :



Chemical name: 3-ethyl-4-methyl-*N*-(4-[*N*-((1*r*,4*r*)-4-methylcyclohexylcarbamoyl) sulfamoyl] phenethyl)-2-oxo-2,5-dihydro-1*H*-pyrrole-1-carboxamide
Molecular formulae:C24H34N4O4S
Molecular Weight : 490.617 g·mol^{-1}
Category : Antidiabetic

MATERIALS AND METHODS

Instrumentation

The separation was carried out on HPLC system with WATERS, software: Empower 2, 2695 separation module. 996 PDA detector.with binary HPLC pump, andC18 column, Symmetry C18 (4.6 x 250mm, 5µm, Make: X-terra)

Chemicals

Eziwa (10mg Glimepiride and 1mg Ezitimibe) manufactured by Dr. Reddy's Laboratories Ltd. All chemicals and reagents used were of AR grade. Standard sample was taken from Spectrum Pharma training lab.

HPLC Conditions

The mobile phase consisting of Phosphate buffer and acetonitrile (HPLC grade)were filtered through 0.45µ membrane filter before use, degassed and were pumped from the solvent reservoir in the ratio of 45:55 v/v was pumped into the column at a flow rate of 1.0ml/min. The column temperature was 30°C. The detection was monitored at 228 nm and the run time was 7 min. The volume of injection loop was 10µl prior to injection of the drug solution the column was equilibrated for at least 30 min. with the mobile phase flowing through the system [4-7].

Preparation of standard solution

Accurately weigh 10 mg of Ezetimibe and 10mg of Glimepiride into a 10ml of volumetric flask and dissolve the sample using diluent and sonicate it for 15min then finally make up the volume to 10 ml. Now pipette out 0.3ml of this solution into 10 ml of volumetric flask and make up the volume upto mark using same diluents [8].

Preparation of sample solution

Accurately weighed 10 tablets and calculated average weight of those tablets and crushed. Transfer the tablet powder weigh about 10mg of sample into 10ml of volumetric flask added with diluent and sonicated for 30 mins and make up the volume with diluent and filtered through the0.45µm millipore filter paper Transfer above solution 0.3ml into 10ml volumetric flask and make up the volume with diluent [9].

METHOD VALIDATION

System Suitability Studies

The column efficiency, resolution and peak asymmetry were calculated for the standard solutions The values obtained demonstrated the suitability of the system for the analysis of this drug combinations, system suitability parameters may fall within ± 3 % standard deviation range during routine performance of the method [10].

Specificity

Specificity was checked for the interference of impurities in the analysis of blank solution and injecting sample solution under optimized chromatographic conditions to demonstrate separation of both Ezetimibe and Glimepiride from impurities [11].

Accuracy

Accuracy was determined by the recovery studies at three different concentrations (corresponding to 50, 100 and 150% of the test solution concentration) by addition of known amounts of standard to pre-analysed sample preparation. For each concentration, three sets were prepared and injected. The recovery studies were carried out six times and the percentage recovery and standard deviation of the percentage recovery were calculated. From the data obtained in added recoveries of standard drugs were found to be accurate as shown in table 2(a) & 2(b) [12].

Precision

Method Precision was determined by injecting six replicates of drug sample solution. The retention times and peak areas of six replicates are recorded. The precision is expressed as the % RSD of Peak areas and it should not be more than 2% shown in table 3 [13].

Linearity

Linearity of the method was determined by constructing calibration. curves. Standard solutions of Ezetimibe and Glimepiride different concentration level (10ppm, 20ppm, 30ppm, 40ppm, 50ppm)were used for this purpose. Each measurement was carried out in six

replicates to verify the reproducibility of the detector response at each concentration level. The peak areas of the chromatograms were plotted against the concentration of Ezetimibe and Glimepiride to obtain the calibration curves. The five concentrations of the standard were subjected to regression analysis to calculate equation and correlation coefficients as shown in Fig4(a),(b)

Limit of detection and limit of quantitation

Limit of detection and limit of quantitation represent the concentration of analyte that would yield signal to noise ratio of 3 for LOD and 10 for LOQ respectively. To determine LOQ and LOD serial dilutions of mixed standard solution of Ezetimibe and Glimepiride was made from standard solution. The samples were injected in the system and measured signal from the samples was compared with those of blank samples. LOD and LOQ was calculated from linear curve using formulae LOD= 3.3 * σ / slope, LOQ= 10 * σ / slope (Where σ = the standard deviation of the response and S = Slope of calibration curve) shown in table 5,6

Robustness

Robustness of the method was determined by making slight changes in the chromatographic conditions. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP HPLC method developed are rugged and robust shown in table 7(a) and 7(b).

Table 1. System Suitability parameters

S. No	Parameter	Ezetimibe	Glimepiride
1	Retention time	2.273	3.630
2	Theoretical plates	2702	4169
3	Tailing factor	1.28	1.10
4	Resolution	6.71	-
5	Regression factor	0.9989	0.9999

Table 2 (a). Accuracy Observation ofEzetimibe

SPIKE LEVEL	SAMPLE WEIGHT	SAMPLE AREA	µg/ml ADDED	µg/ml FOUND	%RECOVERY	%MEAN
50%	639.68	3054339	148.501	149.23	100.49	
50%	639.68	3032660	148.501	148.63	100.09	
50%	639.68	3049927	148.501	149.31	100.54	100.37
100%	1279.36	3887775	297.002	297.69	100.23	
100%	1279.36	3888059	297.002	297.71	100.24	100.23
100%	1279.36	3887192	297.002	297.65	100.22	
150%	1919	5826194	445.494	446.12	100.14	
150%	1919	5828611	445.494	446.30	100.18	100.12
150%	1919	5822928	445.494	445.87	100.08	

Table 2(b). Accuracy Observation of Glimepiride

SPIKE LEVEL	SAMPLE WEIGHT	SAMPLE AREA	µg/ml ADDED	µg/ml FOUND	%RECOVERY	%MEAN
50%	639.68	308954	3	2.99	99.340	
50%	639.68	621388	3	2.99	99.358	
50%	639.68	309010	3	2.98	99.042	99.25
100%	1279.36	621204	6	5.98	99.87	
100%	1279.36	625087	6	5.99	100.494	100.224
100%	1279.36	621388	6	5.99	100.305	
150%	1919	6627390	9	8.98	101.78	
150%	1919	943015	9	8.98	100.21	100.841
150%	1919	621388	9	8.98	101.203	

Table 3(a). Results of precision for Ezetimibe

S. No	Retention Time	Peak area	USP Resolution	USP Tailing
1	2.264	1010585	3802	1.37
2	2.246	1011075	3546	1.38
3	2.264	1011924	4633	1.39
4	2.246	1014299	4812	1.33
5	2.280	1022159	3802	1.39
Mean		1014008.4		
Std.dev		477460.5		
%RSD		0.5		

Table 3(b). Results of precision for Ezetimibe

S. No	Retention Time	Peak area	USP Resolution	USP Tailing
1	3.132	1496209	4759	1.37
2	3.132	1507963	3695	1.38
3	3.129	1521163	4741	1.39
4	3.113	1522810	3793	1.33
5	3.113	1528916	4741	1.39
Mean		1515412.0		
Std.Dev.		13175.7		
%RSD		0.9		

Table 5. LOD results of the method

Drug	Amount (µg/mL)
Ezetimibe	1.46
Glimepiride	1.22

Table 6. LOQ results of the method

Drug	Amount (µg/mL)
Ezetimibe	4.52
Glimepiride	3.67

Table 7(a). Flow Rate Observation of Ezetimibe

Flow Rate(ml/min)		System Suitability Results		
		USP Plate Count	USP Tailing	Area
Low	0.8	4348	1.10	4104921
Actual*	1.2	4425	1.10	3517199
High	1.00	4400	1.10	3408920

Table 7(b). Flow Rate Observation of Glimepiride

Change in M.P organic composition	System Suitability Results		
	USP Plate Count	USP Tailing	
5%more	2028	0.9	3012763
Actual*	4759	0.9	3245977
5%less	3002	1.0	912635

Table 8(a). Variation of Mobile phase composition of Glimepiride

Change in M.P organic composition	System Suitability Results		
	USP Plate Count	USP Tailing	Area
5%more	3035	1.0	3501336
Actual*	3695	0.9	3517199
5%less	3002	1.0	3415632

Fig 1. Chromatogram of standard preparation

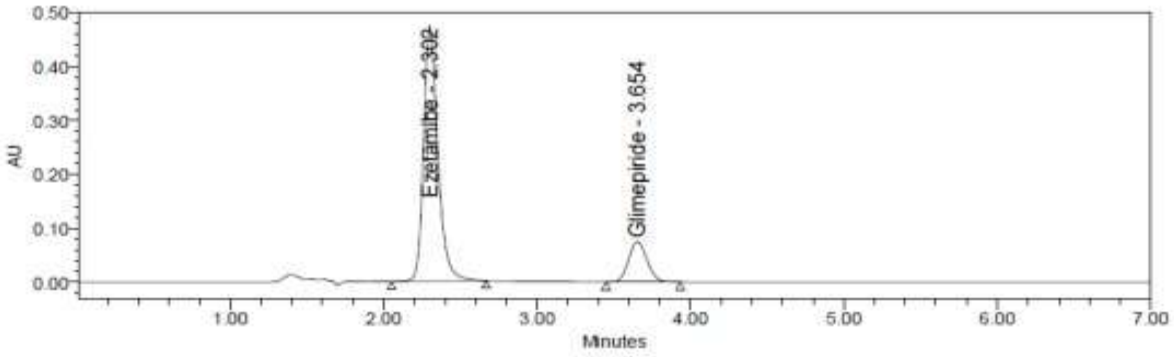


Fig 2. Chromatogram of sample preparation

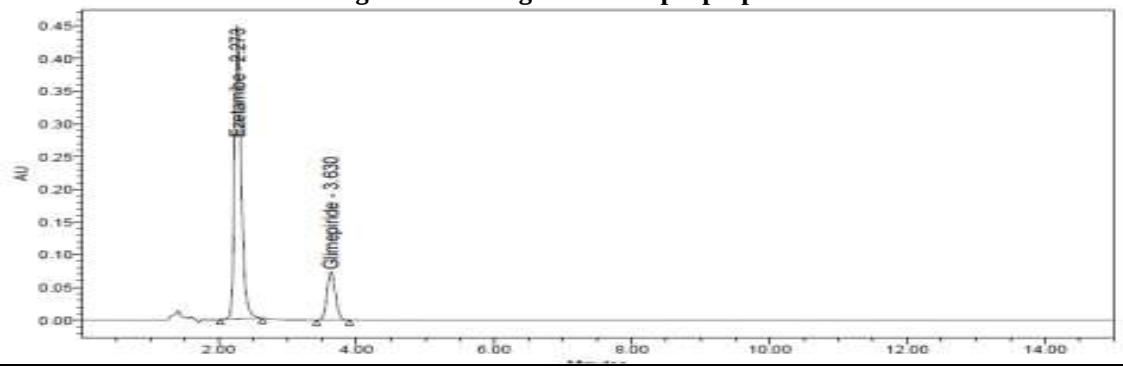


Fig 3. Blank chromatogram

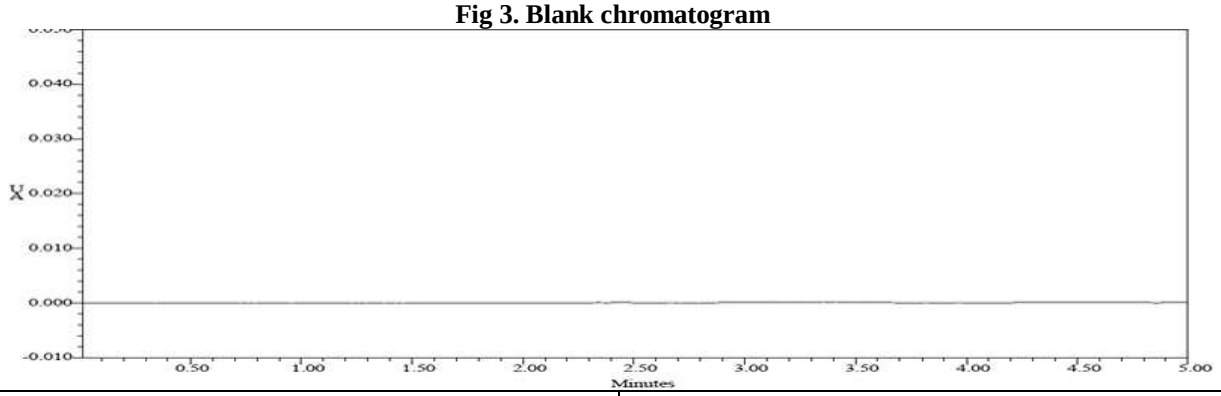


Fig 4(a)

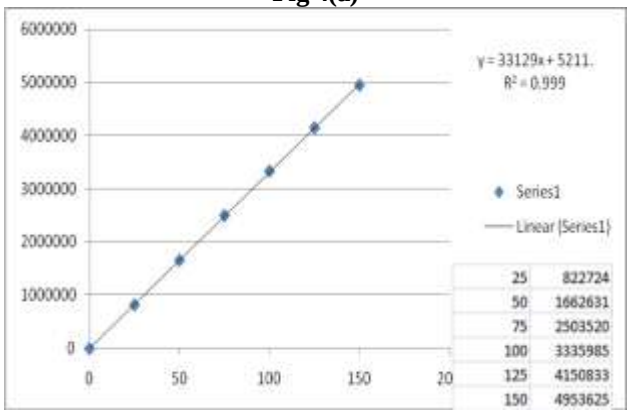
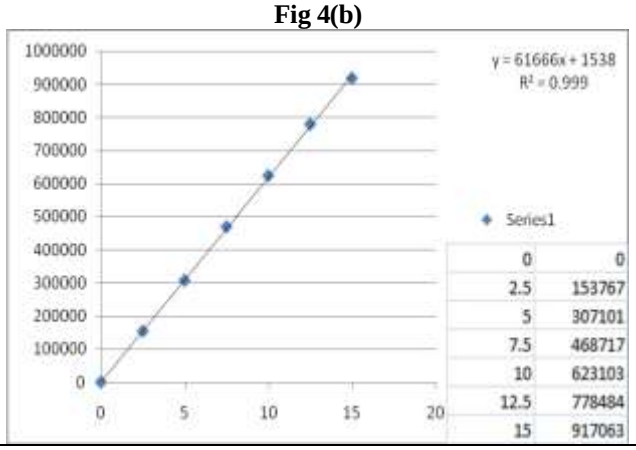


Fig 4(b)



RESULTS AND DISCUSSION

System suitability results were given by table1 and system suitability parameters are retention time, resolution, tailing and plate count were shown uniformity and %RSD was less than 1 so we can say system is suitable for analysis method specificity was concluded by fig-1 are Ezetimibe and Glimepiride standard chromatogram and other one is formulation, they were not observed placebo and excipients peaks interference with standard and analytic peak so it proves method is selective. The result given in table 2 says that the method accuracy passed for both Ezetimibe and Glimepiride evaluated by recovery studies and the percentage mean recovery was found to be 100.47 and 100.31 for Ezetimibe and Glimepiride respectively. The method precision was passed for both the drugs given in table 3. Linearity calibration curve was given below fig: 4the regression co-efficient of Ezetimibe is 0.9989 Glimepiride is 0.9999.The LOD values of Ezetimibe Glimepiride are 1.46 and 1.22 respectively and LOQ values of Ezetimibe Glimepiride are 4.52 and 3.67 respectively.

CONCLUSION

The proposed RP-HPLC method was validated as per International Conference on Harmonization (ICH) guidelines, and found to be applicable for routine quality control analysis for the simultaneous estimation of Ezetimibe and Glimepiride using isocratic mode of elution. The results of linearity, precision, accuracy and specificity, proved to be within the limits. The proposed method is highly sensitive, reproducible, reliable, rapid and specific. Hence, this method can easily and conveniently adopt for routine quality control analysis of Ezetimibe and Glimepiride in its pharmaceutical dosage forms.

ACKNOWLEDGEMENT

The authors are thankful to Spectrum Pharma Training Lab, Hyderabad.

REFERENCES

1. Sharma BK. Instrumental Methods of Chemical Analysis, GOEL Publication House, Meerut, 1999, 133-161, 68-80, 114-165, 286-320.
2. Sethi PD. HPLC-Quantitative analysis of pharmaceutical formulations; 3rdEdn, CBS publishers & distributors, 1977, 182-184.
3. Green C. A step-by-step approach to establishing a method validation program. *Validation Technol*, 6, 2000, 622-633.
4. ICH harmonised tripartite guideline validation of analytical procedures Q2(R1)http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf.
5. Rang & Dale's Pharmacology, 6th Edition, Churchill Livingstone Publishers, 303-318.
6. Indian Pharmacopoeia. Indian Pharmacopoeia commission, Ghaziabad, volume- II, 2010, 1250.
7. British Pharmacopoeia. British Pharmacopoeia Commission Office, London, volume- I, 2010, 758- 759.
8. Martindale. The Extra Pharmacopoeia; 31st Edn; The Royal Pharmaceutical Society, London, 1996, 310.
9. The Merck Index. An Encyclopedia of Chemicals, Drugs and Biological; 14th ed., Merck Co. Inc, 2001, 3429.
10. Skoog DA. Introduction of analytical chemistry. Sounder college of publishing, Harcourt Brace college publishers, 1994, 1-5.
11. Introduction to analytical method validation (online), available from: URL: http://www.standardbase.hu/tech/HPLC%20validation%20PE.pdf.
12. Data elements required for assay validation, (online) available from: URL: http://www.labcompliance.com/tutorial/methods/default.aspx.
13. Introduction to Column. (Online),URL:http://amitpatel745.topcities.com/index_files/study/column care.pdf