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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF EZETIMIBE AND GLIMEPIRIDE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, precise, specific and accurate reverse phase high performance liquid chromatography (RP-HPLC) method was developed and validated for determination of ezetimibe and glimepiride in pharmaceutical tablet dosage form. The different analytical performance parameters such as linearity, accuracy, precision, range, LOD and LOQ were determined according to ICH guideliness. RP-HPLC was conducted on Hypersil BDS C18 (250 mm length x 4.6 mm ID, 5µm) column. The mobile phase was consisting of buffer (0.01M potassium di hydrogen phosphate at pH 4.8) and acetonitrile in the ratio (30:70% v/v) and the flow rate was 1ml/min. Ezetimibe and Glimepiride were monitored using WATERS HPLC 2695 SYSTEM with auto injector and PDA detector. Linearity was observed in concentration ranges of 2.5-15 µg/ ml and 25-125 µg/ ml for Glimepiride and Ezetimibe respectively. Regression equation of Ezetimibe is y = 19217x + 1355, and of Glimepiride is y = 11306x + 2315. Correlation coefficient was found to be 0.999, 0.999 for Ezetimibe and Glimepiride respectively. The %RSD of repeatability was found to be 0.3730 and 0.3577 for Ezetimibe and Glimepiride, respectively. The %RSD of inter day precision was found to be 0.3660and 0.3501for Ezetimibe and Glimepiride, respectively. The %recovery was found to be 99.78% for Ezetimibe, 99.98% for Glimepiride. LOD value of Ezetimibe and Glimepiride was found to be 0.2, 0.7, respectively. LOQ value of Ezetimibe and Glimepiride was found to be 0.7, 2 respectively. All the system suitability parameters were found within range.

Key Words:-Ezetimibe, Glimepiride, RP-HPLC, Linearity, Accuracy, Precision.

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INTRODUCTION

Ezetimibe is an anti-hyperlipidemic medicine which is mainly used to lower cholesterol levels. Specifically, it appears to bind with a critical mediator of cholesterol for absorption, the Niemann-Pick C1-Like 1 (NPC1L1) protein which is located on the gastrointestinal tract epithelial cells as well as in hepatocytes. Ezetimibe has been reported to be a cholesterol transport inhibitor. The chemical name of Ezetimibe is 1-(4-fluorophenyl)-3(R)-[3-(4-fluorophenyl)-3(S)-hydroxypropyl]-4(S)-(4-hydroxyphenyl)-2-azetidinone. Structure of Ezetimibe was shown in figure 1 (Abdul et al., 2014; Beckett AH and Stenlake JB, 2002; Chatwal and Anand, 2000).

Glimepiride

Glimepiride is a 3rd generation sulfonylurea compound. It increases insulin release from pancreatic beta cells and also increases the activity of intracellular insulin receptors. Studies on adipocytes and skeletal muscle reports that Glimepiride induces the PI3 kinase and Akt pathway, along with endothelial nitric oxide synthase and insulin receptor substrate-1/2. Glimepiride also increases osteoblast differentiation and proliferation, which is thought to be related to its ability to activate the Akt and PI3K pathway. Glimepiride also enhances intrinsic

peroxisome proliferator-activated-receptor γ activity. Glimepiride also increases the protein expression of glucose transports 1&4, and is a potent KIR channel blocker. Chemically, glimepiride is identified as 1-[[p-[2-(3-ethyl-4-methyl-2-oxo-3-pyrroline-1carboxamido) ethyl] phenyl] sulfonyl]-3-(trans-4-methylcyclohexyl) urea. Structure of Glimepiride was shown in figure 2 (Chetan *et al*., 2011; Christian GD, 2004).

MATERIALS AND METHODS

Drug Samples (Raw material)

Ezetimibe and Glimepiride were obtained from Spectrum Pharma Research Solutions, Hyderabad and these are identified by IR spectroscopy and melting point.

Formulations Used

Eziwa (Kaytross Health Limited) 25 mg tablets (containing 10 mg of Ezetimibe and 1 mg of Glimepiride) were purchased from local pharmacy.

Chemicals and Solvents used

Distilled water, methanol (HPLC grade), water (HPLC grade), acetonitrile, potassium dihydrogen phosphate, ortho phosphoric acid solution.

Instruments used

HPLC instrument used WATERS HPLC 2965 SYSTEM with Auto Injector and PDA Detector. Software used is Empower 2.UV-VIS spectrophotometer PG Instruments T60.

Preparation of Standard solutions

Accurately weighed 10 mg and 5 mg of Ezetimibe and Glimepiride working standards into 10 ml and 50 ml clean dry volumetric flasks separately add 3/4th of methanol, sonicated for 30 minutes and make up to the final volume with methanol. From the above stock solutions 1 ml was pipette out into 10 ml volumetric flask and then make up to the final volume with diluents (Douglas AS).

Accuracy

Three concentrations 50%, 100%, 150%, were injected in a triplicate manner and amount recovered and percentage recovery were calculated.

Sample preparation

50%: Twenty tablets were weighed and calculate the average weight of each tablet then tablet powder equivalent to 125mg was transferred into a 50mL volumetric flask, 30ml of methanol added and sonicated for 25 min, further the volume made up with methanol and filtered. From the filtered solution 1ml was pipette out into a 10 ml volumetric flask and made up to 10ml with diluents.

100%: Twenty tablets were weighed and calculate the average weight of each tablet then tablet powder equivalent to 250mg was transferred into a 50mL volumetric flask, 30ml of methanol added and sonicated for 25 min, further the volume made up with methanol and filtered. From the filtered solution 1ml was pipette out into a 10 ml volumetric flask and made up to 10ml with diluents.

150%: Twenty tablets were weighed and calculate the average weight of each tablet then tablet powder equivalent to 375mg was transferred into a 50mL volumetric flask, 30ml of methanol added and sonicated for 25 min, further the volume made up with methanol and filtered. From the filtered solution 1ml was pipette out into a 10 ml volumetric flask and made up to 10ml with diluents.

Precision

Procedure

Twenty tablets of formulation (Eziwa containing 10mg of Ezetimibe and 1 mg of Glimepiride) were weighed accurately. The average weight of tablets was found and powdered. The tablet powder equivalent to 50 mg of Ezetimibe was weighed and transferred into 50 ml volumetric flask and added a minimum quantity of methanol to dissolve the substance and sonicated for 25 min and made up to the final volume with same methanol then filtered (1000 µg/ml of Ezetimibe and 100 µg/ml of Glimepiride). From this solution further dilutions were made by diluting with diluents.

Repeatability

Six homogenous samples were prepared by taking 1ml of sample solution into six 10 ml volumetric flasks and these samples were analyzed.

Intermediate precision

Two analysts as per test method conducted the study. For Analyst-1 Refer Precision (Repeatability) results and the results for Analyst-2 were discussed.

Robustness

Robustness was performed by changing the parameters flow rate (±10%), mobile phase composition and temperature for samples as per the test concentrations.
LOD and **LOQ** values were calculated from linearity studies.

System suitability studies

The system suitability studies conceded as per ICH guidelines. The parameters like tailing factor, resolution and number of theoretical plates were calculated (ICH, 1994).

Optimization of chromatographic conditions

Initial separation conditions

The following chromatographic conditions were present initially to get better resolution of Ezetimibe and Glimepiride.

Mode of separation : Gradient
Detector wavelength : 225nm
Flow rate : 1ml/min
Temperature : 30°c
Sample load : 10µl
Diluents : Methanol

Table 1. System suitability parameters for the optimized chromatogram by RP – HPLC

Parameters	Ezetimibe	Glimepiride
Tailing factor	1.08	1.28
Retentiontime(min)	2.3	3.6
Theoretical plates	2571	2913

Table 2. Optical characteristics of Ezetimibe and Glimepiride

Parameters	Ezetimibe	Glimepiride
Calibration range(µg/ml)	25-150	2.5-15
Optimized wavelength	225 nm	225 nm
Regression equation	y = 19217x + 1355	y = 11306x + 2315
Correlation coefficient(r^2)	0.999	0.999
Repeatability (%RSD)	0.3730	0.3577
Inter day precision (%RSD)	0.3660	0.3501
% Recovery	99.78	99.98
Limit of Detection (µg/ml)	0.23	0.7
Limit of Quantitation (µg/ml)	0.67	2.04

Table 4. Linearity data of ezetimibe

S.No	Concentration(µg/ml)	Area
1	0	0
2	25	481170
3	50	983941
4	75	1413042
5	100	1927357
6	125	2407909
7	150	2885009

Table 5. Linearity data of glimepiride

S.No	Concentration(µg/ml)	Area
1	0	0
2	2.5	285521
3	5	575091
4	7.5	842503
5	10	1134612
6	12.5	1416835
7	15	1697391

Table 6. Analysis data of tablet formulation

Sample	Sample No.	Labelled amount (mg/tab)	Amoun found (mg/tab)	%Obtained	Average	SD	%RSD
Ezetimibe	1	10	9.943	99.43			
	2	10	9.902	99.02			
	3	10	10.004	100.04			
	4	10	9.916	99.16			
	5	10	9.911	99.11			
	6	10	9.929	99.29	99.34	0.3706	0.372
Glimepiride	1	1	0.9989	99.89			
	2	1	0.9995	99.95			
	3	1	0.9990	99.90			
	4	1	0.9930	99.30			
	5	1	1.0041	100.41			
	6	1	1.0003	100.03	99.91	0.3574	0.36

Table 7. Accuracy data of ezetimibe

Recovery level	Amount taken(µg/ml)	Area	Amount recovered	%Recovery	Mean %Recovery	Avg %Recovery
50%	50	901656	50.269	100.5681		
	50	894332	49.875	99.7518		
	50	893593	49.834	99.6693	99.9964	
100%	100	1803324	99.5717	99.7517		
	100	1775322	99.6693	99.6693	99.9367	
	100	1775425	100.5693	100.5693		99.78
150%	150	2666330	148.6982	99.1321		
	150	2665096	148.624	99.0862		
	150	2689874	150.0112	100.0075	99.4086	

Table 8. Accuracy data of glimepiride

Recovery level	Amount taken(µg/ml)	Area	Amount recovered	%Recovery	Mean %Recovery	Avg %Recovery
50%	5	537780	4.9767	99.5356		
	5	540289	5.000	100		
	5	538542	4.9838	99.6766	99.7374	
100%	10	1078112	9.9845	99.8458		
	10	1076762	9.9647	99.6468	100.0133	
	10	1086493	10.0547	100.5474		
150%	15	1620124	14.9931	99.5418		
	15	1632203	15.1049	100.6994	100.2089	
	15	1627086	15.0575	100.3837		99.98

Table 9. Repeatability data of tablet formulation

Sample	Sample No.	Labelled amount (mg/tab)	%Obtained	Mean (%)	SD	%RSD
Ezetimibe	1	10	99.43	99.34	0.3706	0.3730
	2	10	99.02			
	3	10	100.04			
	4	10	99.16			
	5	10	99.11			
	6	10	99.29			

Glimepiride	1 2 3 4 5 6	1 1 1 1 1 1	99.89 99.95 99.90 99.30 100.41 100.03	99.91	0.3574	0.3577

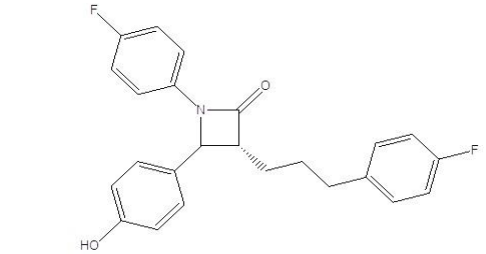
Table 10. Inter day precision data of tablet formulation

Sample	Sample No.	Labelled amount (mg/tab)	%Obtained	Mean	SD	%RSD
Ezetimibe	1 2 3 4 5 6	10 10 10 10 10 10	102.4 103.3 102.5 102.9 102.6 103.2	102.8	0.3763	0.3660
Glimepiride	1 2 3 4 5 6	1 1 1 1 1 1	102.12 101.27 101.14 101.74 101.56 101.25	101.51	0.3554	0.3501

Table 11. Robustness data of tablet formulation

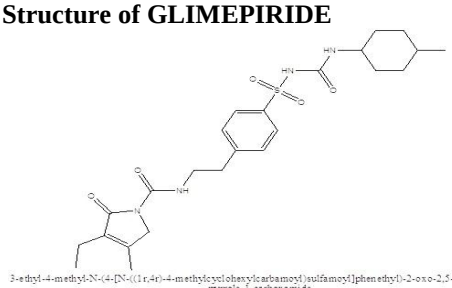
S.No	Robustness condition	Ezetimibe%RSD	Glimepiride%RSD
1	Flow minus	0.0	0.0
2	Flow Plus	0.2	0.3
3	Mobile phase minus	0.2	0.1
4	Mobile phase Plus	0.1	0.1
5	Temperature minus	0.0	0.3
6	Temperature Plus	0.7	0.7

Fig 1. Structure of Ezetimibe



(3R)-1-(4-fluorophenyl)-3-(3-(4-fluorophenyl)propyl)-4-(4-hydroxyphenyl)azetidin-2-one

Fig 2. Structure of GLIMEPIRIDE



3-ethyl-4-methyl-N-(4-[N-((1r,4r)-4-methylcyclohexylcarbamoyl)sulfamoyl]phenethyl)-2-oxo-2,5-dihydro-1H-pyrrole-1-carboxamide

Caution: Stereochemical terms discarded: 1r, 4r

Fig 3.Overlain spectrum of ezetimibe and glimepiride in methanol

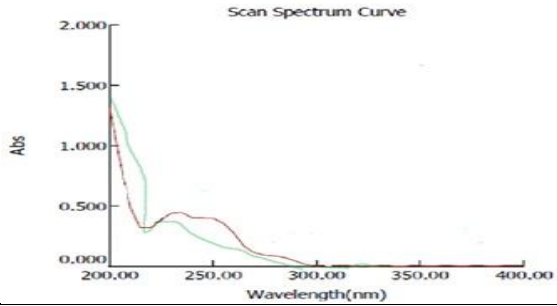
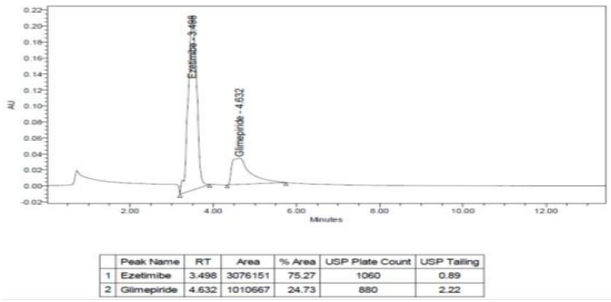
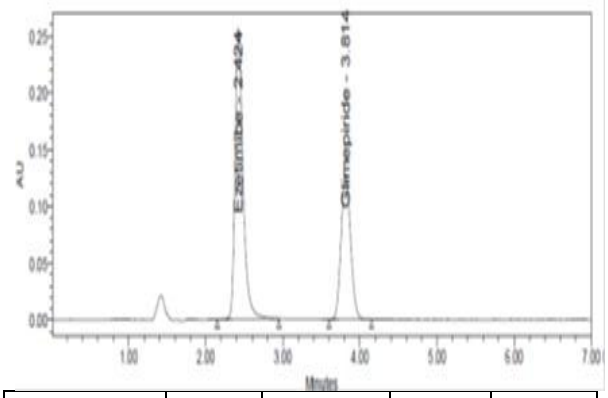


Fig 4. Linearity chromatogram of ezetimibe and glimepiride (25, 2.5µg/ml)



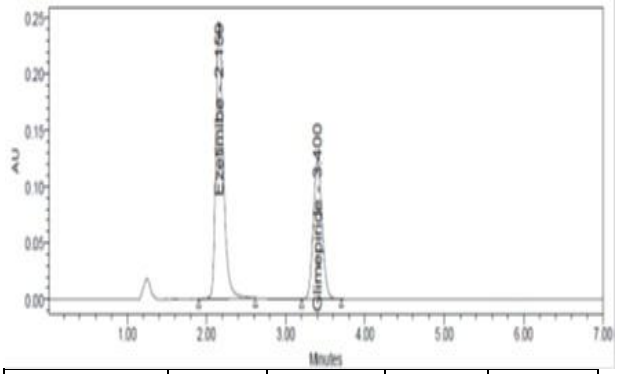
	Peak Name	RT	Area	% Area	USP Plate Count	USP Tailing
1	Ezetimibe	3.498	3076151	75.27	1060	0.89
2	Glimepiride	4.632	1010667	24.73	880	2.22

Fig 5. Chromatogram of interday precision -1



Peak name	RT	Area	USP plate count	USP tailing
Ezetimibe	2.424	1837733	2810	1.40
Glimepiride	3.814	1093299	4425	1.10

Fig 6. Chromatogram of robustness-1 (flow rate plus-1)



Peak name	RT	Area	USP plate count	USP tailing
Ezetimibe	2.159	161256	2719	1.34
Glimepiride	3.400	958847	4233	1.11

RESULTS AND DISCUSSION

Simple, rapid, precise, accurate and gradient RP-HPLC method was developed and validated for the estimation of Ezetimibe and Glimepiride in pure and in combined tablet dosage forms.

An exertion has been made for a simple, rapid, accurate and precise method for the estimation of Ezetimibe and Glimepiride in pure and formulation by an RP-HPLC method.The solutions of 10 µg/ml of Ezetimibe and Glimepiride in methanol were prepared and scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. It was found that two drugs have marked absorbance at 225 nm and can be effectively used for estimation of two drugs. The spectrums were shown in Figures 1-3, respectively. The optimization was done by changing the composition of mobile phase, ratio and column. The mobile phase consists of Potassium dihydrogen phosphate (pH 4.8): Acetonitrile (80:20%v/v) was initially employed and chromatogram was recorded as shown in Figure 4. Different mobile phases were tried for optimization and the chromatograms were shown in Figures 5-10. Finally optimized chromatogram obtained by taking mobile phase Potassium dihydrogen phosphate: Acetonitrile (30:70%v/v) with hypersil BDS 250 mm column at 1ml/min flow rate. The optimized chromatogram was shown in Figure 3. The retention time of Ezetimibe and Glimepiride was found to be 2.273, 3.630 respectively. The optical characteristics of Ezetimibe and Glimepiride were shown in Table 2 & 3, respectively.

With optimized chromatographic conditions, stock solutions of Ezetimibe and Glimepiride were prepared and also prepared the mixture of Ezetimibe concentration range was 25-150 µg/ml and Glimepiride concentration range was 2.5-15 µg/ml. Each solution was injected and recorded the chromatogram at 225 nm. The chromatograms are shown in Figures 3-4, respectively.The linearity data of Ezetimibe and Glimepiride were shown in Tables 4 & 5, respectively. The correlation co–efficient was found to be 0.999 for two drugs.

The tablet dosage form (EZIWA) was selected for the analysis. Standard preparations were made from the API and Sample Preparations are from Formulation. Both samples and standards were injected as six homogeneous samples. Drug in the formulation was estimated by taking the standard as reference. The Average %Assay was calculated and found to be 99.34% and 99.91% for Ezetimibe and Glimepiride respectively. Chromatograms are shown in Figures 3, respectively. The analysis data was shown in Table 3.

The accuracy of the method was performed by recovery studies. The chromatograms were recorded. The %recovery was found to be 99.78% for Ezetimibe and 99.98% for Glimepiride. The values are given in the Tables 7 & 8, respectively. The %recovery study revealed that no interference produced due to excipients used in formulation. Therefore, the developed method was found to be accurate.

The precision of the method was confirmed by repeatability and interday precision and chromatograms are shown in Figures 5, respectively. The %RSD of repeatability was found to be 0.3730 and 0.3573 for Ezetimibe and Glimepiride, respectively. The %RSD of inter day precision was found to be 0.3763 and 0.3554 for Ezetimibe and Glimepiride, respectively. These results indicate that the method has good precision. The resulting data was shown in Tables 10, respectively. LOD value of Ezetimibe and Glimepiride was found to be 0.2, 0.7, respectively. LOQ value of Ezetimibe and Glimepiride was found to be 0.7, 2 respectively.

The robustness of the method was performed by changing the composition of mobile phase, flow rate and

temperature. The resulting chromatograms were shown in Figures 6, respectively. Robustness data was shown in Table 11, respectively. These results revealed that the developed method was found to be robust.

All the above parameters ensure that the developed method could be applied for the routine analysis of Ezetimibe and Glimepiride in pure from and in tablet dosage forms.

CONCLUSION

Simple, rapid, precise and accurate RP-HPLC method was developed and validated for the estimation of Ezetimibe and Glimepiride in pure and in combined tablet dosage forms. With the optimized chromatographic conditions, the drugs were linear in the concentration range of 25-150 µg/ml of Ezetimibe and 2.5-15 µg/ ml of Glimepiride. The correlation co–efficient was found to be 0.999 of two drugs. Regression equation OF Ezetimibe and Glimepiride was found to be y = 19127x + 1355 and y = 11306x + 2315, respectively. In the tablet dosage form (EZIWA) the percentage purity was found to be 99.34% and 99.91% of Ezetimibe and Glimepiride, respectively. The precision of method was confirmed by repeatability and interday precision and the %RSD of repeatability was found to be 0.3730 and 0.3577 for Ezetimibe and Glimepiride, respectively. The %RSD of inter day precision was found to be 0.3660 and 0.3501 for Ezetimibe and Glimepiride, respectively. The accuracy of the method was confirmed by recovery studies. The %recovery was found to be 99.78% for Ezetimibe, 99.98% for Glimepiride. LOD value of Ezetimibe and Glimepiride was found to be 0.2, 0.7, respectively. LOQ value of Ezetimibe and Glimepiride was found to be 0.7, 2 respectively. The developed RP-HPLC method of estimation Ezetimibe and Glimepiride in combined pharmaceutical dosage forms was simple, accurate, robust and economical and it can be applied in regular quality control studies.

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None.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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