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Research Article

SIMULTANEOUS ESTIMATION AND VALIDATION OF EZETIMIBE AND GLIMEPRIDE IN BULK AND PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

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ABSTRACT

A simple, specific and accurate reverse phase high performance liquid chromatographic method was developed for the simultaneous determination Ezetimibe and Glimepride in pharmaceutical dosage form. The column used was AltimaC18 (150mm x 4.6 mm, 5m) in isocratic mode, with mobile phase containing phosphate buffer and acetonitrile (20:80v/v). The buffer is prepared by adding 1.36gm of potassium dihyrogen ortho phosphate in a 1000ml of volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then added 1ml of triethylamine then pH adjusted to 4.8 with dil. orthophosphoric acid solution. The flow rate was 1.0ml/ min and effluents were monitored at 228 nm. The retention times of Ezetimibe and Glimepride were found to be 2.149 min and 2.717 min, respectively. The linearity for Ezetimibe and Glimepride were in the range of 50-300 µg/ml and 5-30 µg/ml respectively. The recoveries of Ezetimibe and Glimepride were found to be 98.13 to 100.90% w/v and 98.80 to 100.45% w/v, respectively. The proposed method was validated and successfully applied to the estimation of Ezetimibe and Glimepride in combined tablet dosage forms.

Keywords: Ezetimibe, Glimepride, Buffer, Acetonitrile, Validation and ICH Guidelines.

INTRODUCTION

Ezetimibe is an anti-hyperlipidemic medication which is used to lower cholesterol levels. Chemically, Ezetimibe is (3R, 4S) - 1- (4 - fluorophenyl) – 3 - [(3S) – 3 - (4-fluorophenyl) - 3 hydroxypropyl]-4-(4-hydroxyphenyl)azetidin-2-one. The chemical formula is $C_{24}H_{21}F_2NO_3$, The molecular weight is 409.425 g/mol. Ezetimibe localizes and appears to act at the brush border of the small intestine and inhibits the absorption of cholesterol. This leads to a decrease in the delivery of intestinal cholesterol to the liver[1].

Chemically, Glimepride is 3-ethyl-4-methyl-N-{2-[4-({[(4-methylcyclohexyl)carbamoyl]amino}sulfonyl)phenyl]ethyl}-2-oxo-2,5-dihydro-1H-pyrrole-1-carboxamide. The chemical formula is C24H34N4O5S. The molecular weight is 490.616 g/mol. The mechanism of action of glimepiride in lowering blood glucose appears to be dependent on stimulating the release of insulin from functioning pancreatic beta cells, and increasing sensitivity of peripheral tissues to insulin. Glimepiride likely binds to ATP-sensitive potassium channel receptors on the pancreatic cell surface, reducing potassium conductance and causing depolarization of the membrane[2, 3.] Different analytical methods have been reported in the literature for the assay of Atorvastatin and Ezetimibe in pharmaceuticals and include spectrophotometry and HPLC4-12. The present study was to establish a simple, sensitive and low cost RP-HPLC method for simultaneous estimation of Ezetimibe and Glimepride in bulk as well as in other dosage forms. The developed method was validated as per ICH guidelines[13, 14].

EXPERIMENTAL

Reagents Ezetimibe and Glimepride were kindly supplied by Dr. Reddy's Laboratory Ltd, Hyderabad. Acetonitrile,

water (HPLC grade, Merck) and all the other reagents of AR grade were purchased from M R Enterprisers. A tablet EZIWA (Kaytross Healthcare Limited) containing 10mg of Ezetimibe and 1mg of Glimepride were used.

Instrumentation

The LC system consisted of a Waters model 515, PDA detector 2998 with 20 µL sample loop. The output signals were monitored and integrated using Empower 2 software.

Chromatographic conditions

The elution was isocratic and the mobile phase consisted of a mixture of buffer (Accurately weighed 1.36gm of Potassium dihyrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then added 1ml of Triethylamine then PH adjusted to 4.8 with dil. Orthophosphoric acid solution) and acetonitrile (20:80 v/v). The mobile phase was filtered through a 0.45-µm (HVLP, Germany) membrane filter prior to use. AnAltima C18 (150mm x 4.6 mm, 5m) was used for determination. The flow rate was 1.0 ml/min and the column was operated at ambient temperature (~30oC). The volume of sample injected was 20 µL. Prior to injection of the solutions, column was equilibrated for at least 30min with mobile phase flowing through the system. The UV detector was set at wavelength of 228nm. A typical RP-HPLC chromatogram of Ezetimibe and Glimepride is shown in Figure-1.

Diluent: Water and acetonitrile (20:80).

Standard Preparation

Accurately weighed and transferred 100mg of Ezetimibe and 10mg of Glimepride working Standards into a 100 ml clean dry volumetric flask, add 70ml of diluent, sonicated for 30 minutes and make up to the final volume with diluent. From the above stock solution, 2ml was pipetted out in to a 10ml volumetric flask and then make up to the final volume with diluent.

Sample Preparation

About 20 tablets were taken and their average weight was calculated. The tablets were crushed to a fine powder and drug equivalent to 100mg of Ezetimibe and 10mg of Glimepride were transferred to a 100ml volumetric flask, dissolved in diluent. Transfer 2ml from the above solution into 10ml volumetric flask and filtered through 0.45µ membrane filter to get concentration of 200µg/ml and 20µg/ml for Ezetimibe and Glimepride.

Method Validation

The developed method was validated as per ICH guidelinesfor its accuracy, linearity, precision, specificity, robustness, ruggedness, limit of detection and limit of quantification by using the following procedures. The parameters are validated as shown in table-9.

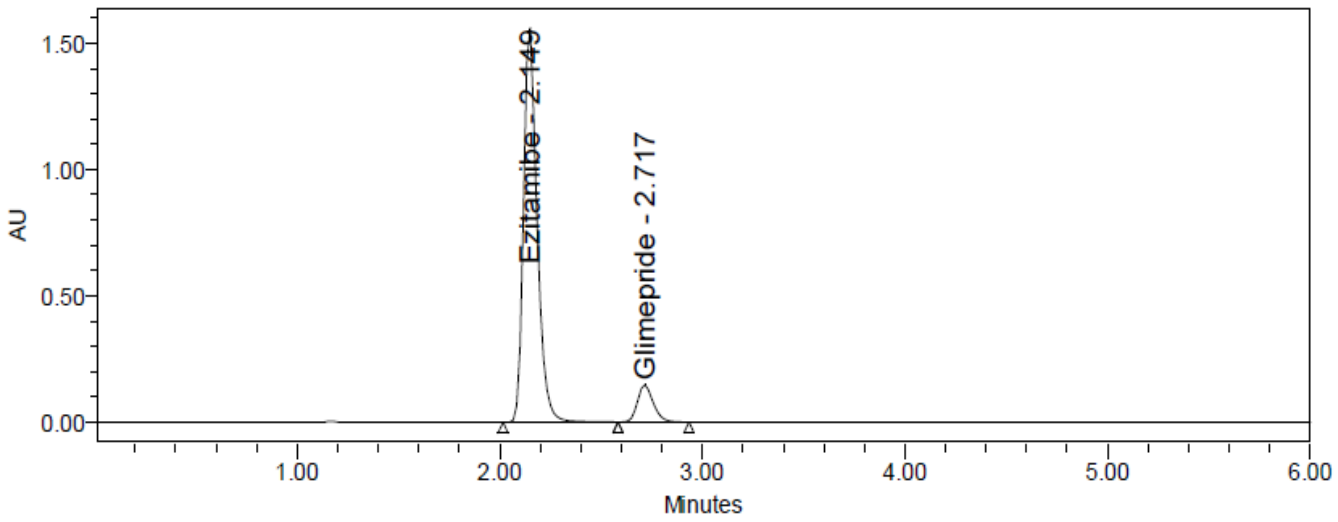

Figure - 1: HPLC chromatogram of Ezetimibe and Glimepride in optimized chromatographic conditions

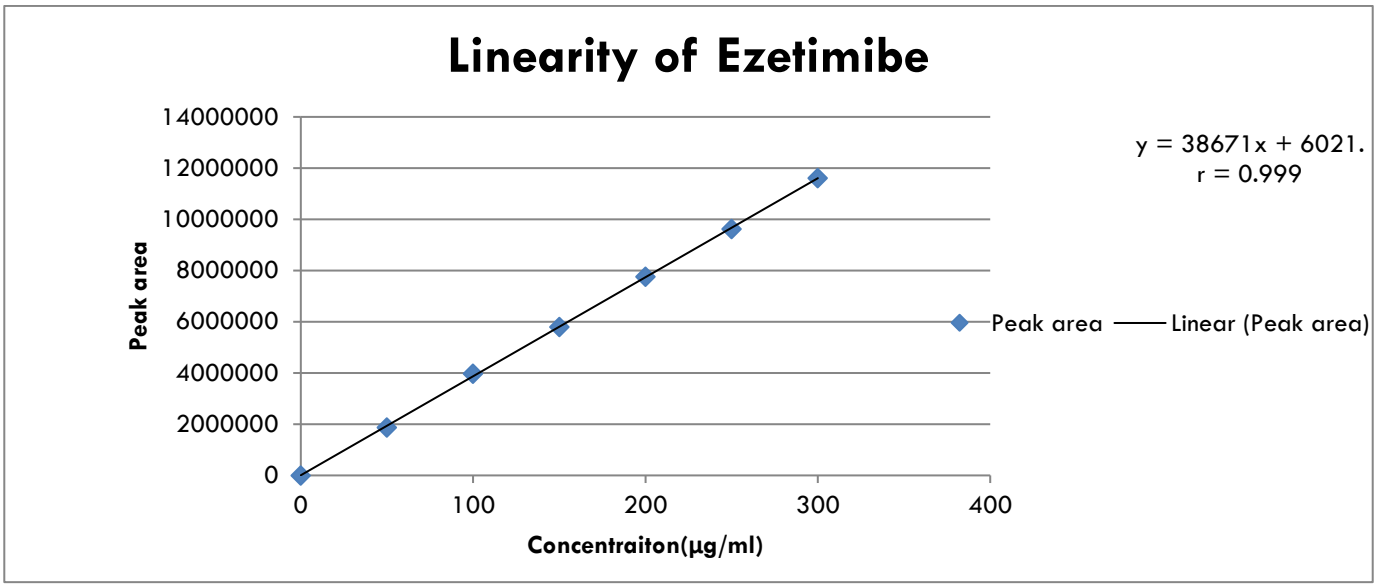

Fig – 2: Linearity of Ezetimibe in the range 50 to 300µg/ml.

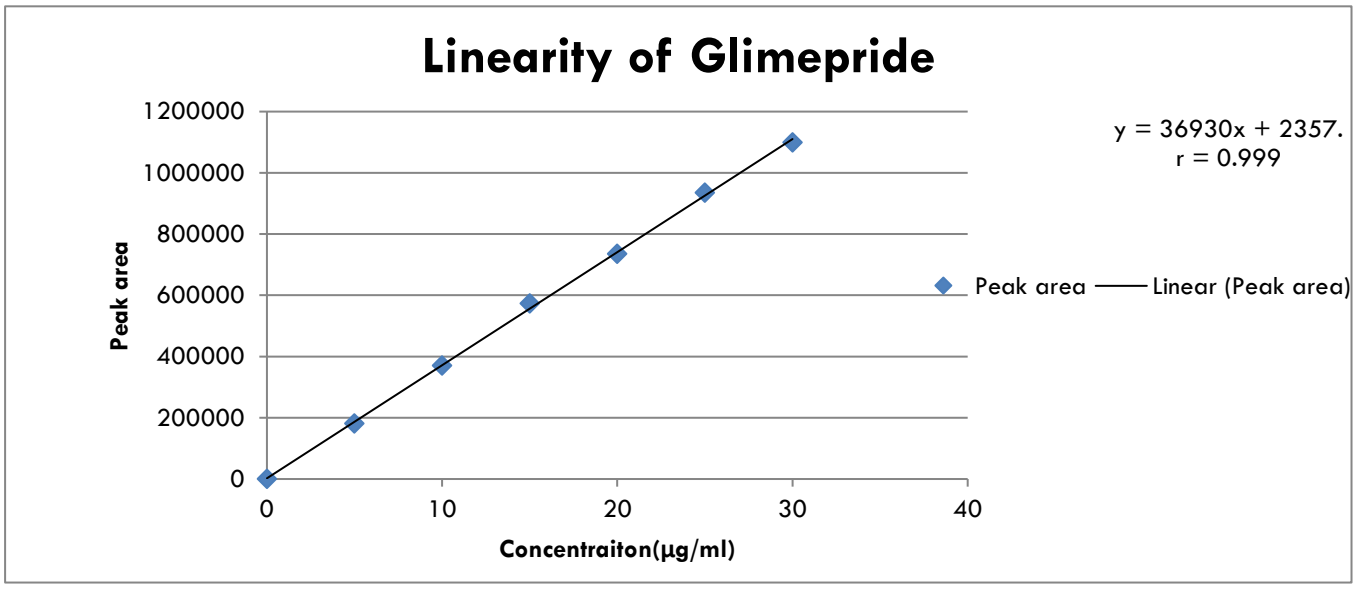

Fig – 3: Linearity of Glimepride in the range 5 to 30µg/ml.

Table-1: Linearity data of Ezetimibe and Glimepride

S.No	Ezetimibe			Glimepride		
	Conc(µg/ml)	Rt(mins)	Area	Conc(µg/ml)	Rt(mins)	Area
1	50	2.138	1871136	5	2.715	181032
2	100	2.134	3973903	10	2.700	370364
3	150	2.14	5798179	15	2.705	573403
4	200	2.139	7762924	20	2.700	735190
5	250	2.138	9629222	25	2.696	935021
6	300	2.124	11611456	30	2.683	1099123
	r = 0.9998 y = 38671x + 6021			r = 0.9995 y = 36930x + 2357		

Table-2: Precision data of Ezetimibe and Glimepride

S.No	Ezetimibe			Glimepride		
	Conc(µg/ml)	Rt(mins)	Area	Conc(µg/ml)	Rt(mins)	Area
1	100	2.149	7596921	10	2.717	806802
2	100	2.148	7653326	10	2.745	809123
3	100	2.130	7656124	10	2.723	804213
4	100	2.135	7592025	10	2.733	808626
5	100	2.139	7670228	10	2.711	803653
6	100	2.134	7641027	10	2.721	806705
Mean			7634942			806520
SD			32730			2230
%RSD			0.43			0.28

Table-3: Accuracy data of Ezetimibe and Glimepride

S.No	Ezetimibe				Glimepride		
	Spiked level	Amount added (µg/ml)	Amount present (µg/ml)	Average %Recovery* ± %RSD	Amount added (µg/ml)	Amount present (µg/ml)	Average %Recovery* ± %RSD
1	50%	50.00	49.91	98.83±1.20	5.00	5.01	100.13± 0.32
2	100%	100.00	98.51	98.51± 0.38	10.00	9.94	99.45± 0.33
3	150%	150.00	149.52	99.68±1.04	15.00	15.00	99.05± 0.24

*n=3 (Average of 3 determinations)

Table-4: Robustness data relating to change in flow rate (1.0ml/min)

S.No	Ezetimibe				Glimepride		
	Flow rate (ml/min)	Average Peak Area*	SD	%RSD	Average Peak Area*	SD	%RSD
1	0.9ml/min	7734185	22787	0.29	736506	1529	0.21
2	1.0ml/min	7780541	11670	0.15	738187	921	0.12
3	1.1ml/min	7756306	23515	0.30	734580	2733	0.37

*n=3 (Average of 3 determinations)

Table 5: Robustness data relating to change in mobile phase composition

S.No	Ezetimibe				Glimepride		
	Mobile phase variation (%)	Average peak area*	SD	%RSD	Average peak area*	SD	%RSD
1	M.P-1-(BUFFER:ACN::21:79)	7753431	38828	0.50	736216	3648	0.50
2	M.P-2-(BUFFER:ACN::20:80)	7767225	18128	0.23	737579	1454	0.20
3	M.P-3-(BUFFER:ACN::19:81)	7764902	30609	0.39	734957	3543	0.48

*n=3 (Average of 3 determinations)

Table 6: Ruggedness data relating to change of day

S.No	Inter-day precision					
	Day-1			Day-2		
	Peak area			Peak area		
	Conc (µg/ml)	Ezetimibe	Glimepride	Conc (µg/ml)	Ezetimibe	Glimepride
1	200	7759383	738472	20	7787913	736514
2	200	7772927	736182	20	7791785	739068
3	200	7782232	735121	20	7726451	737179
4	200	7732927	732191	20	7746159	739981
5	200	7723223	738171	20	7753418	732187
6	200	7622922	731199	20	7776291	731739
Mean		7732269	738977		7763670	736111
SD		58178	3019		25824	3451
%RSD		0.75	0.41		0.33	0.47

Table 7: Ruggedness data relating to change of instrument

S.No	Instrument to Instrument					
	Inst-1			Inst-2		
	Peak area			Peak area		
	Conc (µg/ml)	Ezetimibe	Glimepride	Conc (µg/ml)	Ezetimibe	Glimepride
1	200	7783654	732724	20	7793737	736228
2	200	7728464	738971	20	7726352	731313
3	200	7718437	739876	20	7746252	739877
4	200	7793645	736775	20	7719263	734821
5	200	7743532	731588	20	7773822	736592
6	200	7762521	737545	20	7759249	738462
Mean		7755042	736247		7753113	736216
Std.dev		30145	3367		28376	2986
%RSD		0.39	0.46		0.37	0.41

Table-8: Results of analysis of laboratory samples (Assay)

S.No	Sample	Label	Ezetimibe		Glimepride	
			Amount found	%Purity ± RSD*	Amount found	%Purity ± RSD*
1	Brand-1(EZIWA)	200mg/20mg	9.97	99.70± 0.14	1.00	99.57± 0.24

*n=3 (Average of 3 determinations)

Table 9: System suitability parameters

Validation parameter	Results	
	Ezetimibe	Glimepride
Linearity range (µg/ml)	50 – 300	5 – 30
Regression equation	y = 38671x + 6021	y = 36930x + 2357
Correlation Coefficient(r)	0.9998	0.9995
Precision (%RSD)	0.43	0.28
Accuracy	98.13% to 100.90%	98.80% to 100.45%
Robustness (%RSD)		
Flow rate: (0.9ml/min & 1.1ml/min)	NMT 0.30	NMT 0.37
Mobile phase: Buffer : ACN(20:80)	NMT 0.50	NMT 0.50
Ruggedness (%RSD)		
Interday – (Day 1 & Day 2)	NMT 0.75	NMT 0.47
Instrument to Instrument (Inst-1 & Inst-2)	NMT 0.39	NMT 0.46

System suitability

System suitability and chromatographic parameters were validated such as asymmetry factor, tailing factor and number of theoretical plates were calculated.

Linearity

Linearity of this method was evaluated by linear regression analysis and calculated by least square method and studied by preparing standard solutions of Ezetimibe and Glimepride at different concentration levels. Absorbance of resulting solutions was measured and the calibration curve was plotted between absorbance vs concentration of the drug (Figure: 2 & 3). The response was found to be linear in the range 50-300µg/ml & 5-30µg/ml for Ezetimibe and Glimepride. The data was given in table-1.

PRECISION

A) Method Repeatability

Six sample solutions of the same concentration (100%) were prepared and injected into the HPLC system as per test procedure. The results were given in table-2.

B) Intermediate Precision (Day to Day variability)

Two days as per test method conducted the study. For Day-1 and Day-2, six sample solutions of the same concentration (100%) were prepared and injected into the HPLC system as per test procedure. The results were given in table-6.

ACCURACY

Accuracy was performed in triplicate for various concentrations of Ezetimibe and Glimepride equivalent to 50%, 100% and 150% of the standard amount was spiked to 100% sample solution and they were injected into the HPLC system per the test procedure. The average % recovery was calculated. The data was given in table-3.

Limit of detection and Limit of Quantification

LOD and LOQ were calculated from the average slope and standard deviation from the calibration curve as per ICH guidelines. The LOD and LOQ of Ezetimibe were found to be 2.193 µg/ml and 8.464µg/ml respectively. The LOD and LOQ of Glimepride were found to be 0.199µg/ml and 0.604µg/ml respectively.

Robustness and Ruggedness

Robustness was done by small deliberate changes in the chromatographic conditions and retention time of Ezetimibe and Glimepride were noted. The factors selected were flow rate and variation in the mobile phase composition. The results remained unaffected by small variations in these parameters as shown in table-4 and 5.

Ruggedness of the method was checked by using different days and instruments. The relative standard deviation of the results obtained from different days and instruments was <2.0%. The results were given in table-6 and 7.

Assay

The assay and % purity were calculated for brand EZIWA with label claim 10mg and 1mg of Ezetimibe and Glimepride. The observed value was compared with that of standard value without interference from the excipients used in the tablet dosage form. The results were given in table-8.

RESULTS

A reverse-phase column procedure was proposed as a suitable method for the simultaneous estimation of Ezetimibe and Glimepride dosage form. The chromatographic conditions were optimized by changing the mobile phase composition. Different ratios were experimented to optimize the mobile phase. Finally, phosphate bufferand acetonitrile in the ratio 20:80v/v was used as mobile phase, which showed good resolution of Ezetimibe and Glimepride peak. The wavelength of detection selected was 228nm, as the drug showed optimized absorbance at this wavelength. By our proposed method the retention time of Ezetimibe and Glimepride were about 2.149mins and 2.717mins and none of the impurities were interfering in its assay.

DISCUSSION

The statistical analysis of data and the drug recovery data showed that the method was simple, rapid, economical, sensitive, precise and accurate. It can thereby easily adopt for routine quality control analysis. The results of this analysis confirmed that the proposed method was suitable for determination of drug in pharmaceutical formulation with virtually no interference of additives. Hence the proposed method can be successfully applied in simultaneous estimation of Ezetimibe and Glimepride in marketed formulation.

CONCLUSION

The proposed method is rapid, accurate and sensitive. It makes use of fewer amounts of solvents and change of set of conditions requires a short time. This method can be suitably analyzed for the routine analysis of Ezetimibe and Glimepride in bulk and its pharmaceutical dosage forms. It does not suffer from any interference due to common excipients present in pharmaceutical preparation and can be conveniently adopted for quality control analysis.

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