VOLUME-8, ISSUE-1, JANUARY-2019 • PRINT ISSN No 2277 - 8160

Original Research Paper

Pharmaceutical

METHOD DEVELOPMENT AND VALIDATION BY RP-HPLC FOR SIMULTANEOUS ESTIMATION OF GLIMEPIRIDE AND ROSIGLITAZONE IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

The proposed study, a new method development and validation by RP-HPLC has been developed for estimation of Glimepiride and Rosiglitazone in bulk and tablet dosage form. The present method was a sensitive, precise and accurate RP-HPLC method. To optimize the mobile phase, various combinations of buffer and organic solvents were used on Inertsil ODS-150x4.6mm, 5µ column. Altima-150x4.6mm, 5µ Then the mobile phase containing a mixture of phosphate buffer (pH 4.0) and Acetonitrile 60:40 %v/v were selected at a flow rate of 1ml/min for developing the peaks with good shape and resolution was found resulting in short retention time, baseline stability and minimum noise. Retention times of Glimepiride and Rosiglitazone were found to be 2.109min and 4.657min respectively. Quantitative linearity obeyed in the concentration range of 10-60 µg/ml and 20-120 µg/ml of Glimepiride and Rosiglitazone respectively. The limit of detection and limit of quantification were found to be 0.002 µg/ml and 0.06 µg/ml (Glimepiride) and 0.23 µg/ml and 0.70 µg/ml (Rosiglitazone) respectively, which indicates the sensitivity of the method. The high percentage recovery indicates that the proposed method is highly accurate. No interfering peaks were found indicating the excipients used in formulations didn't interfere with the estimation of the drugs.

KEYWORDS : Glimepiride and Rosiglitazone, RP-HPLC, Validation.

INTRODUCION

Glimepiride (1-10) is the first III generation sulphonyl urea it is a very potent sulphonyl urea with long duration of action. Chemically, Glimepiride is 3-ethyl-4-methyl-N-{2-[4-({[(4-methylcyclohexyl) carbamoyl] amino} sulfonyl) phenyl] ethyl}-2-oxo-2, 5-dihydro-1H-pyrrole-1-carboxamide. which increases the release of insulin from pancreatic beta cells, in addition, Glimepiride increases the activity of intracellular insulin receptors. Studies conducted on adiposities and skeletal muscle suggest that Glimepiride induces the PI3 kinase (PI3K) and Akt pathway, along with insulin receptor substrate-1/2 and endothelial nitric oxide synthase. Glimepiride also increases osteoblast proliferation and differentiation, which is thought to be related to its ability to activate the PI3K and Akt pathway. The chemical structure of Glimepiride was given in (Fig. 1).

Rosiglitazone (11-24) is an anti-diabetic drug in the thiazolidinedione class of drugs. Like other thiazolidinediones, the mechanism of action of Rosiglitazone is by activation of the intracellular receptor class of the peroxisome proliferator-activated receptors (PPARs), specifically PPARγ. Rosiglitazone is a selective ligand of PPARγ, and has no PPARα-binding action. Apart from its effect on insulin resistance, it appears to have an anti-inflammatory effect: nuclear factor kappa-B (NFκB) levels fall and inhibitor (IκB) levels increase in patients on Rosiglitazone. Recent research has suggested that Rosiglitazone may also be of benefit to a subset of patients with Alzheimer's disease not expressing the ApoE4 allele. This is the subject of a clinical trial currently underway. Chemically it is 5-[(4-{2-[methyl (pyridin-2-yl) amino] ethoxy} phenyl) methyl]-1, 3-thiazolidine-2, 4- dione. The chemical structure of Glimepiride was given in (Fig. 2).

The review of literature (25-30) revealed that several analytical methods have been reported for Glimepiride and Rosiglitazone in spectrophotometry, HPLC, HPTLC, and LC/MS individually and in combination. To date, there have been no published reports about the simultaneous estimation of Glimepiride and Rosiglitazone by RP-HPLC in bulk and tablet dosage forms. This present study reports for the first time method development and validation by RP-HPLC for simultaneous estimation of Glimepiride and Rosiglitazone in bulk and tablet dosage forms.

MATERIALS AND METHODS

Chemicals and reagents: Glimepiride and Rosiglitazone were obtained as gift sample from Spectrum Pharma Research laboratory in Hyderabad. Tablets (Avandaryl, GlaxoSmithKline.) containing Glimepiride -4mg and Rosiglitazone-8 mg Marketed formulation was purchased from local market. Acetonitrile, Water HPLC grade (Merck. Mumbai, India) Potassium dihydrogen ortho phosphate, Triethylamine (RANKEM, Mumbai, India.). Ortho Phosphoric Acid HPLC (Merck., Mumbai, India) All solvents used in this work are HPLC grade.

Instrument and chromatographic conditions: A Waters 2695 RP-HPLC separation module (Waters Corporation, Milford, USA) equipped with PDA detector having back pressure 5000psi, automatic injector and the chromatographic separation was achieved on Inertsil ODS-150x4.6mm, 5µ column using phosphate buffer (pH 4.0) and Acetonitrile 60:40 %v/v as mobile phase at a flow rate of 1ml/min. The injection volume was 10 µl and the total runtime was set as 8min. The determination of analytes was carried out at 235nm.

Preparation of samples and solutions:

Preparation of mobile phase: Preparation of 0.1M Phosphate buffer (pH 4.0): Accurately 13.6gms of KH2PO4 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 adjust pH 4.0 with ortho phosphoric acid. Sonicate to degas.

Preparation of glimepiride stock solution: Accurately Weighed and transferred 4mg of Glimepiride in to 10ml of clean dry volumetric flask, add 7ml of diluent, then sonicated for 10min and make up the volume with diluent.

Preparation of rosiglitazone stock solution: Accurately weighed 8mg of Rosiglitazone and transferred into 10ml of clean dry volumetric flask, add 7ml of diluent, then sonicated for 10 min and make up the final volume with diluent.

Preparation of glimepiride standard solution: From the above Glimepiride stock solution 1ml was pipette out into 10ml of clean dry volumetric flask and make up the final volume with diluent.

Preparation of rosiglitazone standard solution: From the above

Rosiglitazone stock solution 1ml was pipette out into a 10ml clean dry volumetric flask and make up the final volume with diluent.

Method validation: Method validation is the process of determining the performance of the developed method to meet the requirements for its analytical application. The proposed assay method was successfully validated according to ICH guidelines. The parameters studied for validation were specificity, accuracy, linearity, precision, robustness, limit of detection, limit of quantification and system suitability.

Preparation of sample solutions for method validation:

Accuracy: The accuracy of the proposed method was determined by standard addition method. It is the closeness of the analytical results obtained by the analysis to the true value. A known amount of standard drug was added to the fixed amount of tablet solution. Accuracy was expressed as percentage recovery. Recovery test was performed with three different concentrations i.e. 20 µg/ml, 40µg/ml and 60 µg/ml for Glimepiride and 40 µg/ml, 80 µg/ml and 120 µg/ml for Rosiglitazone. The %recovery results were calculated and given in table. 2.

Linearity: A series of six concentrations in the range of 10-60 µg/ml of Glimepiride and 20-120 µg/ml of Rosiglitazone has been prepared and peak areas were recorded at 235nm. A calibration curve was plotted between peak area versus concentration of respective Glimepiride and Rosiglitazone and the response of the drugs were found to be linear. The linear regression equation (y=mx+c) was found to be y = 62588x + 782.3 (Fig 4) for Glimepiride and y = 62832x + 1979 (fig 5) for Rosiglitazone respectively. The linearity results were given in table. 3 & 4.

Precision: Repeatability or Precision of the method was determined by injecting six replicates of standard solution at 40µg/ml of Glimepiride and 80µg/ml of Rosiglitazone into HPLC system. From the results obtained it was found that the proposed method was precise.

Robustness: Robustness study of the method was determined by changing the parameters such as flow rate, mobile phase ratio and temperature. Drug samples were analysed under small changed conditions and chromatogram was recorded. It was found that these deliberate changes did not affect the chromatograms of both drug samples.

Limit of Detection (LOD): Limit of detection is the known concentration of Glimepiride and Rosiglitazone and establishing minimum concentration at which the Glimepiride and Rosiglitazone can be reliably detected. It was calculated based on the standard deviation of the response and the slope of the standard calibration curve. The LOD was found to be 0.002 µg/ml of Glimepiride and 0.23 µg/ml of Rosiglitazone respectively.

Limit of Quantification (LOQ): Limit of quantification is the known concentration of Glimepiride and Rosiglitazone and establishing minimum level at which the Glimepiride and Rosiglitazone can be quantified with acceptable accuracy and precision. The LOQ was found to be 0.06 µg/ml of Glimepiride and 0.70µg/ml of Rosiglitazone respectively. The LOD and LOQ results were given table. 14.

System suitability: System suitability was performed by freshly preparing standard solutions containing Glimepiride 40µg/ml and Rosiglitazone 80µg/ml. From the prepared solutions 10µl solution of each was injected 6 times into the HPLC system and the suitability of the system was evaluated.

Application of developed method to formulation: Analysis of marketed formulation Tablets (Avandaryl, GlaxoSmithKline.) containing Glimepiride-4mg and Rosiglitazone-8 mg Marketed formulation was purchased from local market. 15 tablets were weighed and average weight was calculated. Then they were grind into fine powder, weighed a quantity equivalent to 10 tablets and transferred to 100ml volumetric flask, 70ml of diluent was added and sonicated for 25 min, further the volume was made up with diluent. From the filtered solution, 1ml was pipette out into 10ml volumetric flask and made up to 10ml with diluent. From the solution, 10µl was injected into HPLC system and peak area was recorded (fig 6) with detector at 235nm. The % assay was calculated with obtained peak area of detector response. The % assay was found to be 99.85% for Glimepiride and 99.72% for Rosiglitazone. This indicates that developed method can be used for routine analysis. The % assay results were given below table. 15.

RESULTS AND DISCUSSION

Optimized chromatographic conditions: Literature survey revealed that few analytical methods 25-30 have been reported for Glimepiride, Rosiglitazone in individual and combination with other drugs. It was found that, few attempts have been made to developed for simultaneous estimation of Glimepiride and Rosiglitazone by RP-HPLC at the starting of my work. Several trials were made with different columns and mobile phases to develop a suitable method for stability studies and simultaneous estimation of Glimepiride and Rosiglitazone. Several solvents and solvent combinations were used for developing suitable mobile phase. A Reverse Phase C8 and C18 columns were tried initially to separate the analytes. After several systemic trials, a suitable C18 column was selected and good separation of the compounds was achieved with mobile phase consisting Phosphate buffer pH 4.0 and Acetonitrile in the ration of 60:40 %v/v. Finally, a simple precise, sensitive, accurate and economic RP-HPLC method has been developed for performing simultaneous estimation of Glimepiride and Rosiglitazone. The optimized chromatographic conditions were given in the below table 1.

CONCLUSION

A novel RP-HPLC method was developed and validated for simultaneous estimation of Glimepiride and Rosiglitazone in pharmaceutical dosage form. The proposed method was capable of giving faster elution of both analytes and showed good separation within the less retention time of Glimepiride and Rosiglitazone. The percentage recovery and precision studies showed that the method is accurate and precise. Thus, the present RP-HPLC method was shown to be simple, specific, accurate, precise and robust and this method is suitable for determination of Glimepiride and Rosiglitazone both in formulation and biological matrix.

Table 1: Optimized chromatographic conditions

Parameter	Condition
RP-HPLC	Water 2695 separation module with PDA detector
Mobile phase	Phosphate buffer pH 4.0: Acetonitrile 60:40%v/v
Column	Altima-150x4.6mm, 5µ column
Column Temperature	25 0C
Wavelength	235nm
Diluent	Water: ACN (50:50)
Injector volume	10µl
Flowrate	1ml/min
Runtime	8min
Retention time	Glimepiride -2.109min and Rosiglitazone-4.657min
Theoretical Plates	Glimepiride -2849 and Rosiglitazone -3412

Table 2: % Recovery results of Glimepiride and Rosiglitazone

Conc.	Glimepiride			Rosiglitazone		
	Amount added (µg/ml)	Amount recovered (µg/ml)	% Recovery	Amount added(µg/ml)	Amount recovered (µg/ml)	% Recovery

50%	20	19.83	99.16	40	40.05	100.12
	20	20.01	100.05	40	40.15	100.38
	20	19.84	99.18	40	39.56	98.90
100%	40	40.07	100.18	80	79.48	99.35
	40	40.21	100.53	80	80.89	101.12
	40	39.94	99.85	80	79.64	99.55
150%	60	59.62	99.37	120	119.50	99.58
	60	59.78	99.63	120	119.49	99.58
	60	59.58	99.30	120	118.89	99.08

Table 3: Linearity results of Glimepiride

Concentration (µg/ml)	Area	Average area	% RSD
10	623558	624935	0.4
	623358		
	627889		
20	1245857	1245914	0.1
	1247785		
	1244101		
30	1855675	1855688	0.1
	1856647		
	1854742		
40	2562525	2574181	0.8
	2563332		
	2596686		
50	3122145	3141574	1.0
	3124475		
	3178101		
60	3735892	3736512	0
	3736658		
	3736985		

Table 4: Linearity results of Rosiglitazone

Concentration (µg/ml)	Area	Average area	% RSD
20	1254786	1252680	0.2
	1253369		
	1249886		
40	2536625	2539461	1.0
	2514775		
	2566982		
60	3715692	3738638	0.6
	3744525		
	3755698		
80	5066256	5072601	0.2
	5082214		
	5069332		
100	6233586	6241743	0.2
	6255748		
	6235895		
120	7566658	7558255	0.2
	7541148		
	7566958		

Table 5: Slope and intercept value of Glimepiride and Rosiglitazone

Linearity curve	Glimepiride		Rosiglitazone	
	Slope	Intercept	Slope	Intercept
Value	62588	782.3	62832	1979
Correlation coefficient (r2)	0.999	0.999		

Table 6: Precision data

Injection	Glimepiride concentration	Area	Rosiglitazone concentration	Area
1	40µg/ml	2501124	80µg/ml	5080274
2		2503325		5080796
3		2503336		5022719
4		2506665		5044743
5		2501425		5074843
6		2502258		5026719
Mean		2503022		5055016
STDV		2009.9		27000.1
%RSD		0.1		0.5

Table 7: Actual conditions and proposed variations of the method

Parameters	Actual conditions	Proposed variations
Flow rate	1ml/min	0.8, and 1.2ml/min
Mobile phase ratio	40:60 % v/v	±10%
Temperature	25 0C	20 0C, 30 0C

Table 8: Robustness data at flow rate 0.8ml/min of Glimepiride and Rosiglitazone

Parameters	RT	Area	Average area	% RSD
Glimepiride	4.82	2514447	2545263	0.8
	4.83	2566525		
	4.82	2541558		
	4.82	2566398		
	4.83	2541258		
	4.82	2541392		
Rosiglitazone	2.35	5069851	5049581	0.6
	2.36	5014789		
	2.34	5032568		
	2.35	5066987		
	2.34	5087745		
	2.37	5025544		

Table 9: Robustness data at 1.2ml/min of Glimepiride and Rosiglitazone

Parameters	RT	Area	Average area	% RSD
Glimepiride	4.42	2511458	2530332	0.9
	4.41	2566635		
	4.4	2541475		
	4.42	2514475		
	4.42	2533698		
	4.41	2514253		
Rosiglitazone	2.01	5011424	5051457	1.0
	2.02	5087458		
	2.03	5142545		
	2	5033625		
	2.01	5022214		
	2.03	5011475		

By changing the flow rate (1ml/min ±0.2ml) no drastic changes were seen in chromatographic parameters

Table 10: Robustness data at mobile phase ration 65:35% v/v

Parameters	RT	Area	Average area	% RSD
Glimepiride	4.75	2533696	2548154	0.7
	4.76	2547899		
	4.75	2547458		
	4.75	2533699		
	4.76	2584755		
	4.75	2541415		
Rosiglitazone	2.25	5022258	5043842	0.5
	2.26	5022145		
	2.25	5036987		
	2.25	5044789		
	2.26	5088978		
	2.24	5047895		

Table 11: Robustness data at mobile phase ration 55:45 v/v

Parameters	RT	Area	Average area	% RSD
Glimepiride	4.56	2596855	2544532	1.3
	4.56	2514155		
	4.57	2547858		
	4.56	2511477		
	4.57	2530147		
	4.56	2566698		
Rosiglitazone	2.05	5096571	5061272	0.4
	2.06	5066985		
	2.05	5063221		
	2.06	5047128		
	2.06	5047828		
	2.05	5045896		

In mobile phase, organic phase was changed to ±10%. It was found that change in mobile phase did not affect the chromatogram parameters.

Table 12: Robustness data at temperature 250C

Parameters	RT	Area	Average area	% RSD
Glimepiride	4.71	2511478	2547720	1.3
	4.72	2569865		
	4.72	2568123		
	4.71	2511996		
	4.72	2531485		
	4.71	2593374		
Rosiglitazone	2.21	5022565	5047266	0.3
	2.21	5033698		
	2.2	5047869		
	2.21	5047125		
	2.2	5069856		
	2.2	5062485		

Table 13: Robustness data at temperature 30 0C

Parameters	RT	Area	Average area	% RSD
Glimepiride	4.60	2525525	2538277	0.9
	4.61	2533355		
	4.62	2514456		
	4.61	2536698		
	4.61	2579914		
	4.62	2539711		
Rosiglitazone	2.08	5066692	5074206	0.2
	2.09	5087455		
	2.07	5071554		
	2.08	5084751		
	2.07	5071458		
	2.07	5063325		

Temperature of the column was changed to ±50C and chromatogram was recorded. From the results, it was found that change in temperature did not affect the chromatogram parameters.

Table 14: LOD and LOQ results of Glimepiride and Rosiglitazone

Sample	LOD	LOQ
Glimepiride	0.002	0.06
Rosiglitazone	0.23	0.70

Table 15: % Assay results of Glimepiride and Rosiglitazone in formulation

Tablet	Drug	Dosage (mg)	Sample concentration (µg/ml)	Amount found (µg/ml)	% Assay
PREALDONIL	Glimepiride	4	40	39.94	99.85
	Rosiglitazone	8	80	79.78	99.72

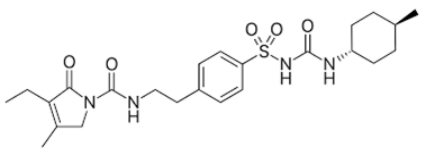

Figure 1: Chemical structure of Glimepiride

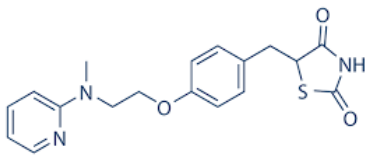

Figure 2: Chemical structure of Rosiglitazone

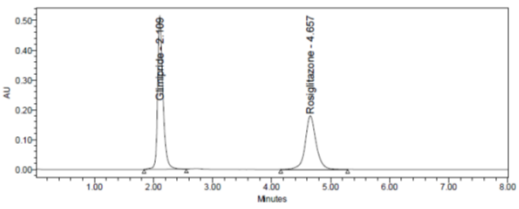

Figure 3: Chromatogram of Glimepiride and Rosiglitazone in API

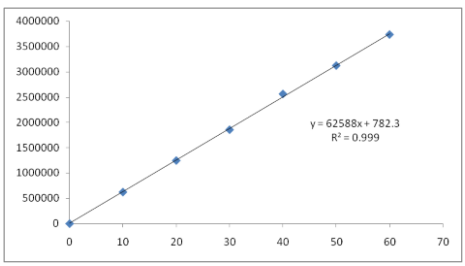

Figure 4: Calibration curve of Glimepiride

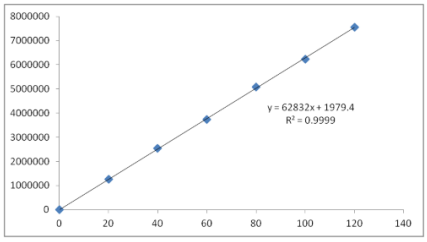

Figure 5: Calibration curve of Rosiglitazone

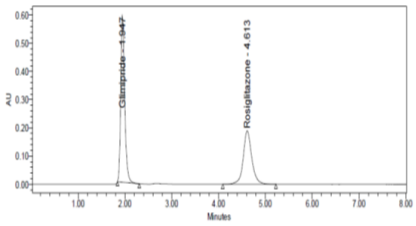

Figure 6: Chromatogram of Glimepiride and Rosiglitazone in formulation

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