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Method development and statistical comparison for Rosiglitazone and Gliclazide in combined dosage form

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ABSTRACT

A rapid reverse phase high performance liquid chromatography method has been developed and validated for the determination of Rosiglitazone maleate and Gliclazide simultaneously in tablets. Two methods were developed and individually validated and a comparative aspect has been shown between the methods. Isocratic chromatography was performed on a Xterra C_{18} (250X4.6mm) with acetonotrile: water (1.5%HCl) in the ratio of 40:60 as a mobile phase for Method I and Acetonitrile: Ammonium acetate buffer of pH=4.5 (55:45) as the mobile phase for Method II at a flow rate of 1.0 ml min-1 and the detection was monitored out by photodiode array detector at 235 nm. Retention time was found out to be 1.98min and 3.02min for Method I and 6.4min and 7.4min for Method II.Good linearity was demonstrated in the range of 5-250 µg/ml and 10-240 µg/ml for Rosiglitazone in Method I and Method II respectively and 2-300 µg/ml and 2-160 µg/ml for Gliclazide in Method I and Method II respectively (r~0.9999 in each case). Various chromatographic parameters including precision, accuracy, system suitability, specificity, LOD, LOQ and robustness have been evaluated. The proposed method was statistically evaluated and a statistical correlation has been shown and can be applied for routine quality control analysis of Rosiglitazone maleate and Gliclazide.

Keywords: Chromatographic method developments, Gliclazide, Rosiglitazone Maleate, Statistical Correlation, Validation.

INTRODUCTION

Rosiglitazone,have a unique mechanism of action, binding to a novel receptor known as the peroxisome proliferators activated receptor γ(PPAR γ).When activated, the receptor binds with response elements on DNA,altering transcription of a variety of genes that regulate carbohydrate and lipid metabolism. The primary effect is decreasing tissue insulin resistance thereby stimulating glucose uptake by peripheral tissues.Secondarily, thiazolidinediones may decrease hepatic glucose production and this may aid in overall blood glucose reduction. These agents do not stimulate the β cells of the pancreas to secrete insulin; however they may enhance the responsiveness and efficacy of the β cells.Prelimnary data suggest that these agents may actually prolong β cell survival and should be considered earlier in therapy. Typical reduction in fasting plasma glucose is 50-60 mg/dl and decrease in HgA1c by 1% to 2% [1-4].

Gliclazide selectively binds to sulfonylurea receptors (SUR-1) on the surface of the pancreatic beta-cells. It was shown to provide cardiovascular protection as it does not bind to sulfonylurea receptors (SUR-2A) in the heart. It

causes depolarization by conductance of ATP sensitive K^+channels.It enhances Ca^{+2} influx →degranulation.The rate of insulin secretion at any glucose concentration is increased [5-6].

Till date literature reports many methods on rosiglitazone and gliclazide mostly in biological fluids [7, 8]. Simple method development has also been carried out but all the methods have been reported with buffer. Use of buffer no doubt increases the sensitivity of the method but it simultaneously reduces the column longittivity.The present study has been given with a comparative study for method development of rosiglitazone and gliclazide simultaneously ,one without using buffer and one using buffer basing on certain existing methods [9-11].

MATERIALS AND METHODS

Chromatographic conditions

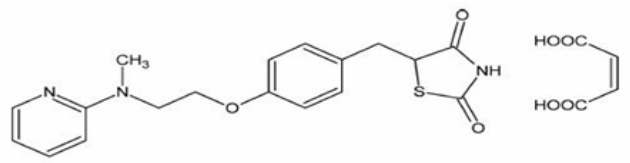
Chromatographic separation was performed on a Jasco HPLC system consisting of Jasco PU – 2089 pump, Jasco UV 2010 plus photo diode array detector. Rheodyne injection syringe with 20 µl loop volume and windows based chrompass software. An ODS C-18 RP- column (Intersite 4.6 mm X 2.5 cm, 10 µm) was used for separation. The elution was carried out isocratically at flow rate of 1 ml / min using acetonitrile: water (40: 60 v/v) as mobile phase.The run time was 10 min. Before analysis both mobile phase and sample solutions were degassed by sonication and filtered through 0.2-µm filter. The analytes were monitored at 248 nm. The analytes were identified by comparison of retention times obtained from sample and standard solutions. The work was performed in an air-conditioned room maintained at 25 ± 2°C.

Chemicals and Reagents

Pure rosiglitazone and gliclazide were received from Actavis pharmaceuticals LTD, Indrad, Dist. Mehsana (Gujarat) and Panacea Biotech LTD, Baddi, Dist.Solan (H.P.) Laboratory mixture of rosiglitazone maleate and gliclazide prepared in the laboratory. Formulations were collected from local market.HPLC Grade acetonitrile, water, acetic acid were collected from Merck, India LTD.

Experimental

(a)



(b)

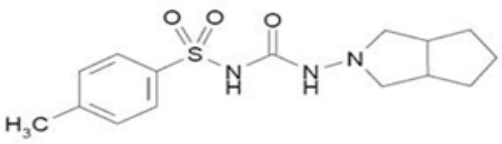


Figure.1: Structure of (a) rosiglitazone maleate (b) gliclazide

Selection of analytical wavelength

By appropriate dilution of each standard stock solution with acetonitrile, various concentrations of rosiglitazone maleate and gliclazide were prepared separately. The isobestic point of rosiglitazone maleate and gliclazide was selected at 236nm, where both components show good absorbance as shown in Fig.2

Preparation of standard solutions for rosiglitazone maleate and gliclazide
Accurately weighed quantity 2.5 mg of rosiglitazone maleate and gliclazide were dissolved in Acetonitrile and volume was made up to 5 mL mark. The stock solution was diluted further with water to get final concentration of about 100 µg/mL of rosiglitazone maleate and gliclazide respectively.

Construction of the Calibration Curve
For each drug, appropriate aliquots were pipette out from each standard stock solution into a series of 10 ml volumetric flask. The volume was made up to mark with water to get a solution of rosiglitazone maleate having range 5,10, 20, 50, 100, 150, 200, 250 µg/ml for Method I and 10, 20, 40, 80, 120, 160, 200, 240 µg/ml for Method II and for Gliclazide having concentration ranges 2, 5, 10, 20, 50, 100, 150, 200 ,250 ,300 µg/ml for Method I and 2, 4, 8, 12, 16, 20, 40, 80, 120, 160 µg/ml for Method II. Triplicate dilutions of each concentration of each drug were prepared separately.

Analysis of drugs in physical laboratory mixture-
Five solutions containing physical laboratory mixture of known concentration were prepared in the mobile phase. The proportion of the drugs in laboratory mixtures was same as that in tablets. The solutions were injected int--o the column and chromatograms were recorded. Concentrations of the drugs in the solutions were determined by comparison with their calibration curves.

Estimation of rosiglitazone maleate and gliclazide in tablet formulation
Sample solution preparation:
Twenty tablets were weighed and their average weight was determined. It was finely powdered and mixed thoroughly. Accurately weighed tablet powder equivalent to 10 mg of rosiglitazone maleate and 200 mg of gliclazide was transferred in a 100 mL volumetric flask and acetonitrile was added. It was shaken vigorously for 5 to 10 minutes. Later, the volume was made up to mark with water. The solution was filtered through membrane filter. Further dilution was done with water to get concentration of 4µg/mL of rosiglitazone maleate and 80µg/mL of gliclazide.

The test solution (10µl) was injected and the chromatogram was recorded. From the peak area obtained, concentrations (label claim) of both the drugs were calculated using their respective slope and intercept values from calibration data.

Method validation parameters
The developed methods were validated according to ICH guidelines [11]. The validation parameters were linearity, specificity, accuracy, precision, Limit of detection (LOD), Limit of Quantification (LOQ) and Robustness at both the wavelengths (Method I and II). Intra-day and Inter-day precision values were estimated by assaying the pharmaceutical dosage form containing three different concentrations of rosiglitazone and gliclazide in combined dosage form, six times on the same day and on three different days. Accuracy was determined by recovery study by standard addition method. The standard was added to a predetermined concentration at 80%, 100% and 120% level.

Statistical Analysis
To correlate the difference between the two developed methods of HPLC, six different samples were taken and quantification was done simultaneously. To test difference between the proposed HPLC methods statistical tests were performed for the level of confidence 95% ($P = 0.05$). Student's t – test were applied to test the significant difference between both the methods.

RESULTS

Two simple and efficient methods were developed and a comparative analysis was done to find the significant difference in developing the method with buffer and without buffer. Method I was developed by taking acetonitrile: water (1.5%HCl) in the ratio of 40:60 and Method II was developed by taking Acetonitrile: Ammonium acetate buffer of pH=4.5 (55:45) as the mobile phase.

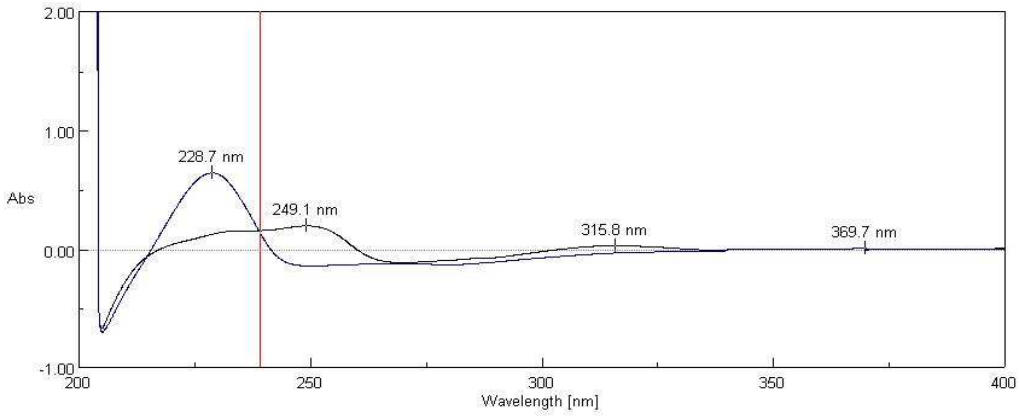
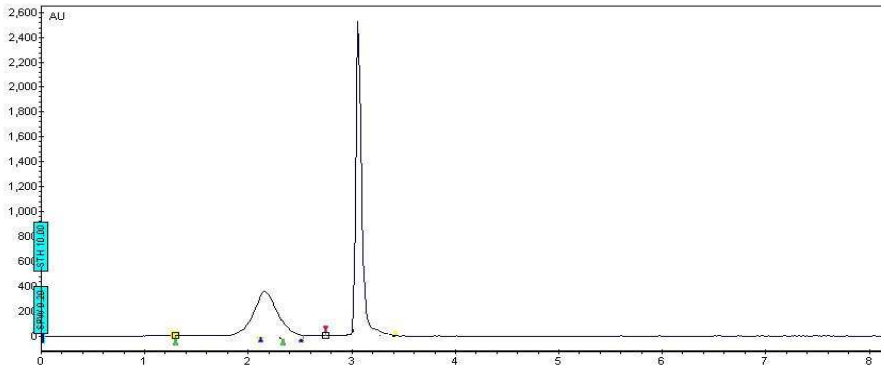


Figure.2: Overlain Spectra for rosiglitazone maleate (249.1nm) and gliclazide (228.7nm)

Calibration and Linearity

(a)



(b)

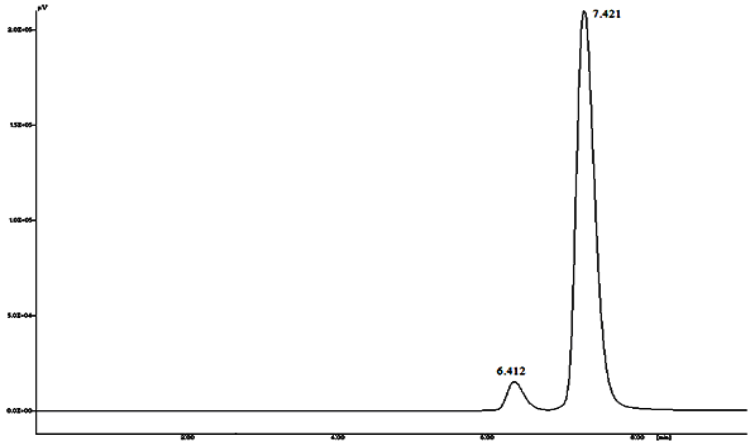


Figure.3 (a) A typical model chromatogram for rosiglitazone and gliclazide without using buffer. (b) A typical model chromatogram for rosiglitazone and gliclazide using buffer

The summary of linear regression data for Method I and Method II is shown in table 1.

The percentage assay results for Method I and Method II are depicted in table.

Method Validation

Accuracy: The mean recoveries are listed in table.

Precision: The interday –intraday precision and LOD & LOQ were computed after assaying the samples in triplicate and the results are depicted in Table.V and Table.VI respectively.

DISCUSSION

It was found that Method I gave a faster response than Method II, though both the methods were found to be very specific and accurate .The developed method with buffer and without buffer has given a very satisfactory result but with the point that use of buffer is more time consuming and less economical than Method 1. Retention time was found out to be 1.98min and 3.02min for Method I and 6.4min and 7.4min for Method II.The detecting wavelength was taken at 235nm.

Statistical Correlation

To correlate the difference between the two developed methods of HPLC, six different samples were taken from two different brands and quantification was done simultaneously. To test difference between the proposed HPLC methods statistical tests were performed for the level of confidence 95% (P = 0.05).One way ANOVA was applied to test both method – sample interaction and differences in method precision. In case of rosiglitazone **F stat is less than F crit**, signifying the method – sample interaction and the differences between the methods are not significant but in case of gliclazide **F stat is more than F crit,** signifying the method – sample interaction and the differences between the methods are significant.

Table.I: Linear regression data for calibration curve of rosiglitazone maleate and gliclazide (n=6)

Name of drug	Linearity range* (µg/ml)		Slope*		Intercept*		Regression coefficient*(r^2)	
	MethodI	Method II	MethodI	Method II	MethodI	Method II	MethodI	Method II
Rosiglitazone	5-250	10-240	1542.3	43601.03	351	72928.9	0.999	0.999238
Gliclazide	02-300	02-160	425.23	75614.83	-253	-5008.74	0.9999	0.99899

Table.II: Statistical parameters of linear regression of rosiglitazone maleate

Parameters	Method I			
	S.D.		% R.S.D.	
	RSGN	GLZ	RSGN	GLZ
Slope	1532	276	0.762	0.179
Intercept	89	16	0.19	0.104
Regression Coefficient	0.0022	0.0014	0.529	0.720
Parameters	**Method II**			
	S.D.		**% R.S.D.**	
	RSGN	**GLZ**	**RSGN**	**GLZ**
Slope	205.32	185.76	0.469	0.248
Intercept	265.0	92.57	0.85	0.182
Regression Coefficient	0.0035	0.0042	0.350	0.420

Table.III: Results and statistical data for estimation of rosiglitazone maleate and gliclazide in marketed formulation

Tablet Formulation	Drug	Label claim (mg/tab)	Mean	S.D.	% RSD	Mean	S.D.	% RSD
			Method I			Method II		
GLYROZ-4	RSGN	4	3.96	0.08	2.02	99.04	0.40	0.354
	GLZ	80	79.80	0.53	0.67	98.85	0.405	0.359

Table. IV: Accuracy of the method

Formulation	Drug	Level of % Recovery	Conc. Spiked (µg/ml)	Amount recovered*(µg/ml)		% Recovery*±S.D	
				Method I	Method II	Method I	Method II
GLYROZ-4	RSGN	80	3.2	3.19	3.198	99.74±0.05	99.94±0.02
	GLZ	80	64	64.08	63.95	100.14±0.12	99.93±1.03
	RSGN	100	4	4.02	4.035	100.54±0.06	100.88±0.05
	GLZ	100	80	79.96	80.89	100.14±0.12	101.13±0.4
	RSGN	120	4.8	4.79	4.787	99.86±0.04	99.73±0.6
	GLZ	120	96	96.05	96.875	100.05±0.08	100.92±0.12

Table.V: Precision data of the two methods

Parameters	Method I				Method II			
	Intraday Studies		Interday Studies		Intraday Studies		Interday Studies	
	RSGN	GLZ	RSGN	GLZ	RSGN	GLZ	RSGN	GLZ
Mean	99.62	100.59	100.02	99.98	98.95	98.88	98.24	98.86
S.D	0.12	0.043	0.03	0.208	0.70	0.78	0.98	0.95
%R.S.D	2.05	0.44	0.79	0.26	0.71	0.79	0.99	0.96

Table.VI: Limit of Detection and Limit of Quantification values

Parameter	Drug	Method I	Method II
LOD	**RSGN**	0.261	0.354
	GLZ	0.201	0.298
LOQ	**RSGN**	0.802	1.065
	GLZ	0.791	0.894

Table.VII: Assay results for rosiglitazone in two HPLC methods

Label claim(mg)	Amount in method without buffer (%)	Amount in method with buffer (%)
4	96.75	99.0
4	100.21	99.5
4	96.5	98.5
4	98.25	99.0
4	100.21	98.75
4	101.25	99.5

Table.VIII: Single Factor ANOVA study of rosiglitazone

SUMMARY						
Groups	***Count***	***Sum***	***Average***	***Variance***		
Amount in method without buffer	6	23.73	3.955	0.00639		
Amount in method with buffer	6	23.77	3.961666667	0.000256667		
ANOVA						
Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	0.000133	1	0.000133333	**0.040120361**	0.84526	**4.964603**
Within Groups	0.033233	10	0.003323333			
Total	0.033367	11				

Table.IX: Assay results for Gliclazide in two HPLC methods

Label claim(mg)	Amount in method without buffer (%)	Amount in method with buffer (%)
80	**100.11**	**99.19**
80	**99.09**	**98.56**
80	**99.6**	**98.94**
80	**98.93**	**98.71**
80	**100.55**	**98.41**
80	**100.28**	**99.33**

Table.X: Single Factor ANOVA study of Gliclazide

Anova: Single Factor						
SUMMARY						
Groups	***Count***	***Sum***	***Average***	***Variance***		
Amount in method without buffer	6	478.81	79.80166667	0.281816667		
Amount in method with buffer	6	474.5	79.08333333	0.080426667		
ANOVA						
Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	1.548008	1	1.548008333	**8.54678715**	0.015209352	**4.964602701**
Within Groups	1.811217	10	0.181121667			
Total	3.359225	11				

CONCLUSION

Two simple, selective and rapid chromatographic methods were developed for rosiglitazone and gliclazide simultaneously. Both the methods can be used for routine QC analysis and can be extended for further analytical works.

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