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Research Article

METHOD DEVELOPMENT AND VALIDATION OF METFORMIN, GLIMEPIRIDE AND PIOGLITAZONE IN TABLET DOSAGE FORM BY RP-HPLC

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ABSTRACT

A rapid RP-HPLC method was developed and validated for simultaneous estimation of metformin, glimepiride and pioglitazone from pharmaceutical dosage forms. A sensitive chromatographic separation was accomplished on C_{18} column (100 × 4.6 mm, 5 µ) with mobile phase consisting of Methanol: phosphate buffer (p^H 3.6 adjusted with ortho phosphoric acid) in the ratio of 75:25 v/v, at a flow rate of 1.0 ml / min and monitored at 238 nm. The developed method was validated in terms of accuracy, precision, linearity and limit of detection, limit of quantification, robustness and solution stability. The proposed method can be used for the routine estimation of these drugs in combined pharmaceutical dosage forms.

Keywords: Simultaneous estimation, Metformin, Glimepiride and Pioglitazone.

INTRODUCTION

Metformin is chemically, 1, 1-dimethyl biguanide hydrochloride. It is the first line drug of choice for the treatment of type2 diabetes. Bio analytical, HPLC, HPTLC and UV-visible spectrophotometry methods have been reported for its individual determination of Metformin and in combination with other drugs[1-4]. Glimepiride (is chemically 2-(3-ethyl-4-methyl-2-oxo-3 pyrroline-1-carboxamido) ethyl-phenylsulfonyl-3-(trans-4-methylcyclohexyl) urea[8-11]. It is a medium to long acting sulphonyl urea anti-diabetic drug. Several spectrophotometric method, HPLC, HPTLC have been reported for estimation of Glimepiride[5-7]. Pioglitazone is one of the PPAR-alpha agonist, insulin sensitizer used to reduce the insulin resistance. It is a thiazolidine dione derivative and chemically (RS)-5-(4-[2-(5-ethylpyridin-2-yl)ethoxy]benzyl)thiazolidine-2,4-dione. HPLC and UV-visible spectrophotometry methods have been reported for its individual determination of Pioglitazone and in combination with other drugs. The present manuscript describes a novel LC method which is simple, rapid, precise, sensitive, selective and accurate isocratic reverse phase HPLC-UV method for simultaneous determination of metformin, pioglitazone and glimepiride in tablet dosage form[8-10].

Reagents and Materials

Pure metformin, glimepiride and pioglitazone used as working standards were gift samples from Ranbaxy Laboratories Ltd., Mumbai, India. Tablets containing 500 mg of metformin, 2 mg of glimepiride and 15 mg of pioglitazone (ziglimplus2-FDC) were obtained from local market and used within their shelf life period. Methanol and water (HPLC-grade) were purchased from Merck, India. All other chemicals employed of analytical grade are purchased from Merck, India.

Instrumentation

The chromatographic system comprised of Waters binary gradient pumps and Waters 2998 photo diode array detector (PDA). Data integration was carried out using Empower-2™ software. Samples were injected into X-Terra symmetry C_{18} column (100 × 4.6 mm, 5 µ). A Bandline sonerex sonicator was used for enhancing the dissolution of the compounds. A Digisum digital pH meter was used for pH adjustment.

Preparation of Standard Solutions of Metformin, Glimepiride and Pioglitazone

The standard stock solutions were prepared separately by transferring 500 mg of metformin, 2 mg of glimepiride and 15 mg of pioglitazone into 100 mL standard volumetric flask. To that about 50 mL of methanol was added, the solution was sonicated to dissolve and the volume is made up to the mark. Further secondary dilution was done with the mobile phase to get the final concentrations of metformin (400 – 600 µg / mL), pioglitazone (10 – 25 µg / mL) and glimepiride (1.5 - 3.5 µg / mL).

Preparation of Sample Solution

Twenty tablets each containing 500 mg of metformin, 2 mg of glimepiride and 15 mg of pioglitazone were weighed. Finely powder the tablets in a mortar, a quantity of the powder equivalent to one tablet content was accurately weighed and transferred into 100 mL of standard volumetric flask containing 50 mL of methanol, sonicated for 20 minutes and made up the volume with mobile phase. Further secondary dilutions were made with the mobile phase to get final concentration of metformin 500 µg / mL, glimepiride 2 µg / mL and pioglitazone 15 µg / mL. Quantification was achieved by peak area-ratio method with reference to the standards.

RESULTS AND DISCUSSION

In order to achieve simultaneous elution of the three components, initial trials were performed with the objective to select adequate and optimum chromatographic conditions. Parameters, such as ideal mobile phase and their proportions, detection wavelength, optimum pH, different columns and concentration of the standard solutions were carefully studied.

Table 1: Accuracy of the Method Data for Metformin, Glimepiride and Pioglitazone

Amount (%) of drug added	Theoreti-cal content (µg / mL)	Conc. Found (µg / mL) ± SD, N = 3	Recovery (%)	RSD (%)
Metformin				
0	500	500.96±0.39	100.19	0.38
75	875	874.82±0.49	99.97	0.49
100	1000	1000.21±0.23	100.02	0.22
125	1125	1124.89±0.14	99.99	0.14
Glimepiride				
0	2	2.14±0.216	107	0.20
75	3.5	3.54±0.314	101.14	0.31
100	4	3.99±0.169	99.75	0.16
125	4.5	4.52±0.145	94.44	0.15
Pioglitazone				
0	10	10.13±0.434	101.31	0.42
75	15	15.26±0.653	101.73	0.64
100	20	20.04±0.556	100.20	0.54
125	25	24.95±0.464	99.81	0.46

Table 2: Reproducibility Precision Report for Metformin, Glimepiride and Pioglitazone

Sample	Sample sets	Peak area N=3	SD	% RSD
Metformin	Set 01	3267748	23265.93	0.71
	Set 02	3267744	23274.67	0.71
	Set 03	3267386	23483.47	0.71
	Set 04	3267862	23269.64	0.71
	Set 05	3267678	23654.28	0.72
	Set 06	3267934	23745.62	0.72
Glimepiride	Set 01	36728	203.65	0.55
	Set 02	36549	206.38	0.56
	Set 03	36742	208.36	0.56
	Set 04	36682	196.52	0.53
	Set 05	36567	189.54	0.51
	Set 06	36754	198.42	0.53
Pioglitazone	Set 01	98976	451.54	0.45
	Set 02	95752	439.42	0.45
	Set 03	97753	458.94	0.46
	Set 04	94593	457.38	0.48
	Set 05	98974	460.04	0.46
	Set 06	96692	462.37	0.47

Table 3: Intermediate Precision Report for Metformin, Glimepiride and Pioglitazone

Sample	Sample sets	Peak area N=3	SD	% RSD
Metformin	Set 01	3264398	23693.42	0.72
	Set 02	3265987	23652.52	0.72
	Set 03	3261273	23639.54	0.72
	Set 04	3260986	23784.32	0.72
	Set 05	3262485	23693.85	0.72
	Set 06	3266783	23592.47	0.72
Glimepiride	Set 01	36684	194.38	0.52
	Set 02	36735	206.94	0.56
	Set 03	36764	202.59	0.55
	Set 04	36698	196.63	0.53
	Set 05	36654	186.74	0.50
	Set 06	36732	194.54	0.52
Pioglitazone	Set 01	98854	488.34	0.49
	Set 02	99237	449.64	0.45
	Set 03	96776	459.59	0.47
	Set 04	99684	450.42	0.45
	Set 05	98978	452.53	0.45
	Set 06	97684	452.42	0.46

Table 4: Change in Flow Rate Report for Metformin, Glimepiride and Pioglitazone

Drugs	Flow rate (mL / min)	Average area of six injections	SD	% RSD
Metformin	0.9	3262351	24154.22	0.74
	1.0	3262432	33456.46	1.02
	1.1	3266549	23258.34	0.71
Glimepiride	0.9	36453	183.48	0.50
	1.0	35342	175.52	0.49
	1.1	36543	197.63	0.54
Pioglitazone	0.9	99276	446.85	0.45
	1.0	98964	455.62	0.46
	1.1	97192	452.25	0.46

Table 5: Change in Mobile Phase Ratio Report for Metformin, Glimepiride and Pioglitazone

Drugs	Change in mobile phase ratio	Average area of six injections	SD	%RSD
Metformin	Methanol 76 % : buffer 24 %	3263564	32346.76	0.99
	Methanol 75 % : buffer 25 %	3264328	35276.53	1.08
	Methanol 74 % : buffer 26 %	3264819	34167.38	1.04
Glimepiride	Methanol 76 % : buffer 24 %	36765	178.54	0.48
	Methanol 75 % : buffer 25 %	36654	182.62	0.49
	Methanol 74 % : buffer 26 %	36357	195.68	0.53
Pioglitazone	Methanol 76 % : buffer 24 %	97652	437.46	0.44
	Methanol 75 % : buffer 25 %	89678	455.35	0.50
	Methanol 74% : buffer 26%	95764	451.76	0.47

Table 6: Assay Report of Metformin, Glimepiride and Pioglitazone

Tablet sample	Label claim (mg / tablet)	*Amount Present (mg / tablet) ± SD	% RSD	*Percentage Label claim (% w/w)
Metformin	500	500.98 ± 0.83	0.16	100.19
Glimepiride	2	2.01 ± 0.03	1.40	100.50
Pioglitazone	15	14.98 ± 0.12	0.80	99.37

* mean of six sampling

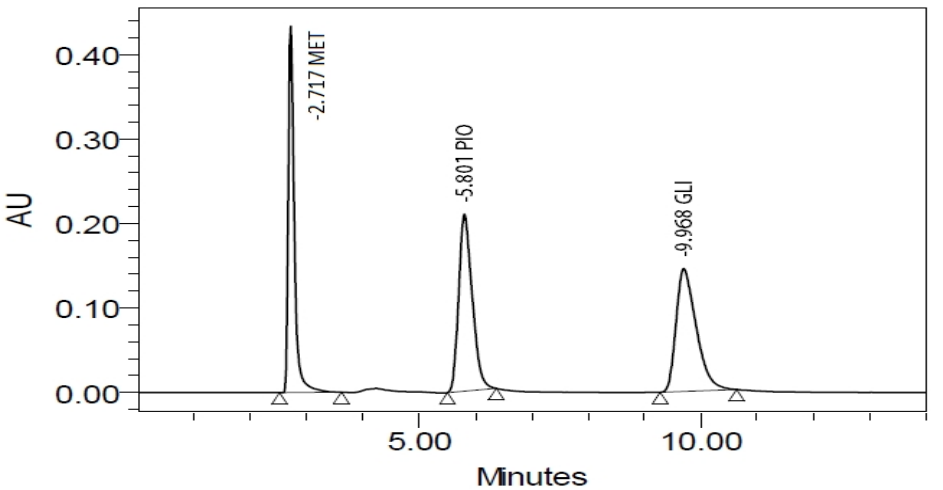

Figure 1: Chromatogram of Standard Metformin, Pioglitazone and Glimepiride

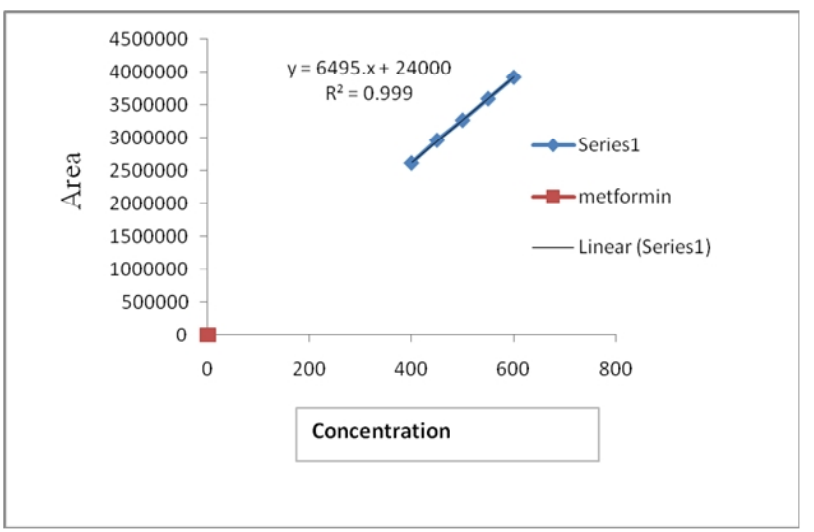

Figure 2: Linearity Curve of Metformin

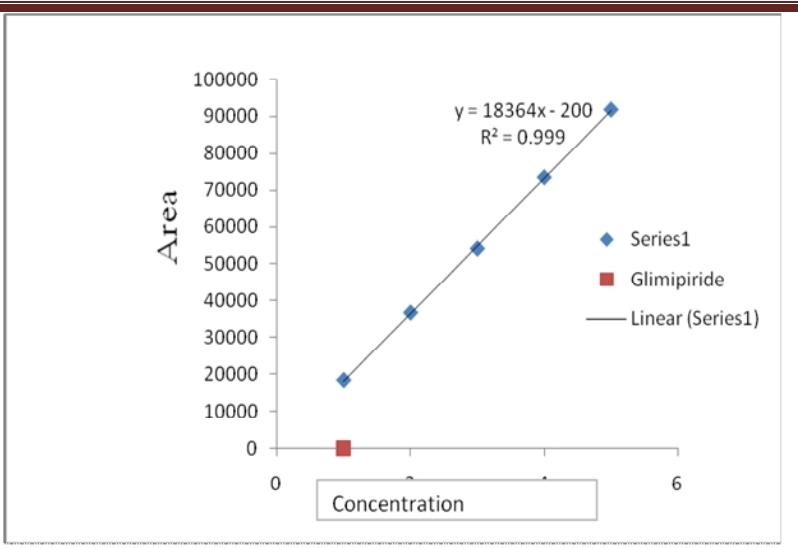

Figure 3: Linearity Curve of Glimepiride

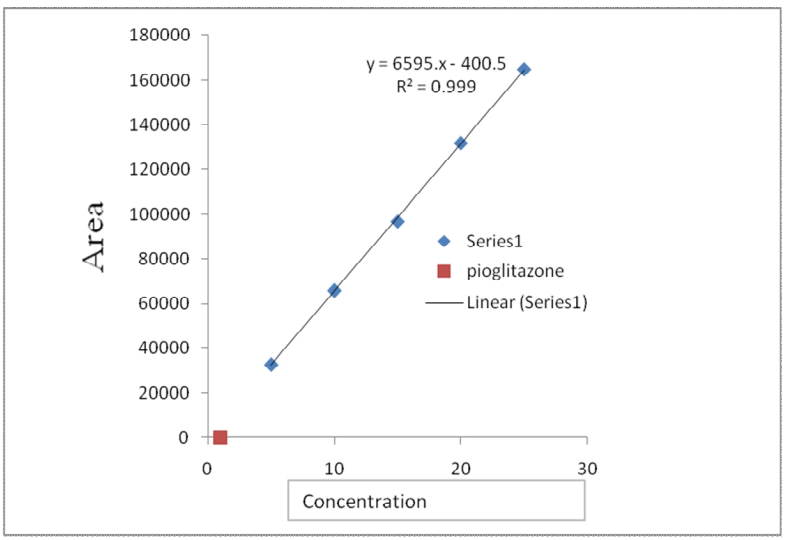

Figure 4: Linearity Curve of Pioglitazone

Several solvents were tested by using different proportions, such as methanol-water (80:20 v/v), acetonitrile-water (80:20 v/v), methanol-acetonitrile-0.05M phosphate buffer (80:10:10 v/v/v, pH 3.5-6.5 adjusted with ortho-phosphoric acid) and methanol-acetonitrile-0.05M phosphate buffer (60:20:20 v/v/v, pH 3.5-6.5 adjusted with ortho-phosphoric acid). Finally, methanol and phosphate buffer (adjusted to pH 3.6 with ortho-phosphoric acid) at a ratio of 75:25 v/v was selected as the optimum mobile phase and a flow rate of 1.0 mL / min. Under these conditions, the analyte peaks were well resolved and were free from tailing. The tailing factor was < 1.5 for all the analytes. The retention time of metformin, pioglitazone and glimepiride was found to be 2.717, 5.801 and 9.968 minutes respectively. The asymmetry factor of metformin, glimepiride and pioglitazone was found to be 0.76, 0.88 and 0.63 respectively which indicates symmetrical nature of the peak. The resolution (Rs) between metformin and pioglitazone was found to be 7.83, pioglitazone and glimepiride was found to be 5.42, indicating good separation of both analytes from each other. The theoretical plate number for metformin, glimepiride and pioglitazone were found to be 8235, 5632 and 3247 respectively, thus indicating good column efficiency. Typical chromatograms were recorded at 238 nm, shown in Figure 1. To prove that the above developed method can be useful for routine quality control of these drugs, the method is validated according to ICH guidelines as follows. The calibration plot was constructed by plotting peak area versus concentration (µg / mL) of metformin, glimepiride and pioglitazone which were found to be linear in the range of 400 – 600 µg / mL (r^2 = 0.999), 1.5 - 3.5 µg / mL (r^2 = 0.999) and 10 - 25 µg / mL (r^2 = 0.999), respectively (Figures 2, 3 and 4). Limit of detection (LOD) values of metformin, glimepiride and pioglitazone were experimentally verified to be 0.15 µg / mL, 0.02 µg / mL and 0.12 µg / mL respectively. Limit of quantitation (LOQ) values of metformin, glimepiride and pioglitazone were found to be 0.45 µg / mL, 0.06 µg / mL and 0.36 µg / mL respectively, which indicated that the method can be used for analysis of metformin, glimepiride and pioglitazone over a very wide range of concentrations. The percentage recoveries of metformin, glimepiride and pioglitazone were found to be in the range of 99 - 100.36 %, 98 - 101.95 % and 98 - 101.58 %, respectively. The results were shown in Table 1, which indicates that there is no interference with excipients, so the developed method is accurate. The precision of an analytical method is the degree

of agreement among the individual test results, when the method is applied repeatedly to multiple sampling homologous samples of metformin, glimepiride and pioglitazone. The precision of the method was checked by repeatability (intra-day) and intermediate precision (inter-day). Results for repeatability and intermediate precision, expressed as % RSD, results were given in Table. The low values of % RSD indicate that the method is precise. Reproducibility was checked by analyzing the samples by another analyst using same instrument and same laboratory. There was no significant difference between the % RSD values (Table 2 and 3), which indicates that the proposed method was reproducible. Robustness was done by small deliberate changes in the chromatographic conditions. There were no significant changes in the peak areas of metformin, glimepiride and pioglitazone when the mobile phase and flow rate of the mobile phase were changed (Table 4 and 5). The results indicated that the proposed method was robust.

CONCLUSION

The proposed method was applied to the simultaneous estimation of metformin, glimepiride and pioglitazone in tablets. The assay results show that the proposed method was selective for the simultaneous determination of metformin, glimepiride and pioglitazone without interference from the excipients used in the tablet dosage form. The values are shown in Table 6. The assay results and low % RSD values indicated that the developed method can be used for routine analysis of metformin, glimepiride and pioglitazone in pharmaceutical dosage forms.

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