Research Article

RP-HPLC Analytical Method Development and Validation for Simultaneous Estimation of Three Drugs: Glimepiride, Pioglitazone, and Metformin and Its Pharmaceutical Dosage Forms

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Developing a single analytical method for estimation of individual drug from a multidrug composition is a very challenging task. A simple, rapid, precise, and reliable reverse phase HPLC method was developed for the separation and estimation of three drugs glimepiride, pioglitazone and metformin in bulk drug mix and pharmaceutical dosage forms. The estimation was carried out using Inertsil ODS-3V (250 mm × 4.6 mm, 5 μm) column; mobile phase consisting of acetonitrile, tetrahydrofuran, and buffer at pH 5; the flow rate of 1.7 mL/min and ultraviolet detection at 228 nm. All the three drugs were properly resolved having run time of 5 minutes, 3.9 minutes and 1.3 minutes for glimepiride, pioglitazone, and metformin, respectively. The method was validated as a final verification of method development with respect to precision, linearity, accuracy, ruggedness, and robustness. The validated method was successfully applied to the commercially available pharmaceutical dosage form, yielding very good and reproducible result.

1. Introduction

In the current Indian scenario, most commonly attacking disease to a common man has been found to be diabetes. Recent studies indicate that prevalence of type-2 diabetes is rapidly increasing in the society. Type-2 diabetes is a progressive disorder with a consistent and steady increase in glycosylated hemoglobin (HbA_{1c}) overtime associated with enhanced risk of micro- and macrovascular complications and a substantial reduction in life expectancy. There are three major pathophysiologic abnormalities associated with type-2 diabetes: (i) impaired insulin secretion, (ii) excessive hepatic glucose output, and (iii) insulin resistance in skeletal muscles, liver and adipose tissue. These defects have been treated by use of oral insulin secretagogues (sulphonyl ureas/glinides) or insulin, biguanides, and thiazolidinediones, respectively [1].

Glimepiride is a medium-to-long acting sulphonyl urea antidiabetic drug. It is chemically 1-[[p-[2-(3-Ethyl-4-methyl-2-oxo-3-pyrroline-1 carboxamido)ethyl]phenyl]sulfonyl]-3-(trans-4-methyl cyclohexyl) urea. The primary mechanism of action of glimepiride in lowering blood glucose appears to be dependent on stimulating the release of insulin from functioning pancreatic beta cells. Metformin hydrochloride is also antidiabetic drug in the biguanide class and it is chemically 1,1-dimethyl biguanide monohydrochloride. It decreases hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity by increasing peripheral glucose uptake and utilization. Pioglitazone is a prescription drug of thiazolidinedione class with hypoglycemic (antihyperglycemic, antidiabetic) action; it is chemically (±) 5-[[4-[2-(5-Ethyl-2-pyridinyl)ethoxy]phenyl]methyl]-2,4 thiazolidinedione monohydrochloride. It selectively stimulates the nuclear

FIGURE 1: Chemical structures of the drugs.

FIGURE 2: Individual drugs.

FIGURE 3: Drug mixture.

FIGURE 4: Dosage forms.

receptor peroxisome proliferator activated receptor-gamma (PPAR- γ) and to a lesser extent PPAR- α . It modulates the transcription of the insulin-sensitive genes involved in the control of glucose and lipid metabolism in the muscle, adipose tissue and the liver. As a result, pioglitazone reduces insulin resistance in the liver and peripheral tissues, increases the expense of insulin dependent glucose, decreases withdrawal of glucose from the liver, and reduces quantity of glucose, insulin, and glycosylated hemoglobin in the bloodstream. The combination of glimepiride, pioglitazone, and metformin sustained release complements each other and provides better glycemic control in the management of Type-2 Diabetes and probably in the prevention of its associated macrovascular and microvascular complications [2].

The chemical structures of the drugs are as shown in Figure 1. Keeping the medical importance in mind, a group of drugs used for treating/maintaining diabetes, namely, glimepiride, pioglitazone, and metformin has been selected for method development and validation. All the three drugs are antidiabetic drugs. These drugs are very potent and are normally prescribed either individually or in combinations as per the demand of the situation. These three drugs are also available in the market as a combination, dosage forms.

TABLE 1: System suitability results.

Standard	Average	% RSD
Glimepiride		
Retention time	5.043	0.26
Area	14711933	0.13
Resolution	4.924	
Theoretical plates	7063	
Asymmetry	0.898	
Pioglitazone		
Retention time	3.977	0.27
Area	11302936	0.1
Resolution	15.52	
Theoretical plates	6782	
Asymmetry	0.94	
Metformin		
Retention time	1.342	0
Area	11142195	0.31
Resolution	0	
Theoretical plates	1347	
Asymmetry	1.17	
Dosage form		
Glimepiride		
Retention time	5.071	0.11
Area	14719434	0.1
Resolution	4.99	
Theoretical plates	7142.925	
Asymmetry	0.88	
Pioglitazone		
Retention time	3.992	0
Area	11285309.5	0.11
Resolution	15.775	
Theoretical plates	6893.845	
Asymmetry	0.93	
Metformin		
Retention time	1.342	0
Area	11109070.5	0.31
Resolution	0	
Theoretical plates	1396.245	
Asymmetry	1.205	

For individual estimation of each drug, several methods are available in the literature [3–7] even there are couple of methods available for estimation of two drugs at a time [8–14]. There are some methods where even more than 2 drugs are estimated at a time [15–18]. Very limited work has been done [19] for the simultaneous estimation of all the three drugs, namely, glimepiride, pioglitazone, and metformin.

For contributing such a novel cause, through this article, we have tried our best to develop a fast and user-friendly methodology for the simultaneous estimation of glimepiride, pioglitazone, and metformin, using reverse phase-HPLC method in bulk drug mix and pharmaceutical dosage forms.

FIGURE 5: Graphs for linearity of the drugs.

TABLE 2: Linearity results.

	Linearity range	Correlation coefficient
Glimepiride	50–150%	0.995
Pioglitazone	50–150%	0.9951
Metformin	50–150%	0.992

2. Experimental Section

In the present work, efforts have been made for the simultaneous estimation of glimepiride, pioglitazone, and metformin and its pharmaceutical dosage forms. Several trials have been made with respect to the mobile phase composition, columns, as well as UV detector's wavelength to develop a suitable and fast method for the analysis of all the three drugs, simultaneously. The ultimate method of analysis has been provided in Section 2.2.3.

2.1. Materials, Reagents, and Chemicals. Samples of glimepiride, pioglitazone, and metformin Hydrochloride Standards were obtained from Startech Labs. Combination drug tablets, Triblend 1, used for the experiment was manufactured by Akesis Pharma Pvt. Ltd. and Zoryl MP2 was manufactured by Intas Pharmaceuticals. HPLC-grade acetonitrile, tetrahydrofuran, dipotassium orthophosphate, trimethylamine, and orthophosphoric acid were obtained from Merck, Darmstadt, Germany.

2.2. Equipments. UV-Visible spectrophotometer used was Shimadzu, Model-2450. The HPLC instrument used was

Schimidzu make, model-LC-2010 CHT. Class VP Software was used for data acquisition.

2.3. Chromatographic Conditions. The Chromatographic column, Inertsil ODS-3 V (250 mm × 4.6 mm, 5 μm), column was used as a stationary phase. Mobile phase was prepared with buffer, acetonitrile, tetrahydrofuran (40 : 50 : 10). Buffer was prepared by dissolving 7.1 g of dipotassium hydrogen orthophosphate in 1000 mL of water. The pH was adjusted to 5.0 with orthophosphoric acid. Injection volume was 20 μL. The pump flow rate was 1.7 mL/min. The eluent was detected at 228 nm at 25°C.

2.4. Preparation of Standard Solution. Standard solution of 0.4 mg/mL (treat this as 100% for various experimental purpose) was prepared by taking 10 mg each of glimepiride, pioglitazone, and metformin Hydrochloride in 25 mL volumetric flask and 0.5 mL tetrahydrofuran was added and diluted upto the mark with mobile phase.

2.5. Preparation of Linearity Solutions. For linearity 150%, 125%, 100%, 75%, and 50% solutions were prepared. 150% solution was prepared by using 60 mg each of glimepiride, pioglitazone, and metformin hydrochloride was dissolved in 100 mL for 150% solution. 20.83 mL of 150% solution was taken in a 25 mL volumetric flask and make up with mobile phase for 125% solution. 16.67 mL of 150% solution was taken in a 25 mL volumetric flask and make up with mobile phase for 100% solution. 12.5 mL of 150% solution was taken in a 25 mL volumetric flask and make up with mobile phase for 75% solution. 8.33 mL of 150% solution is taken in a 25 mL

volumetric flask and make up with mobile phase for 50% solution.

2.6. Sample Preparation for Accuracy. Five different solutions were prepared for performing the accuracy studies. The first solution was prepared by dissolving 10 mg each of glimepiride, pioglitazone, and metformin in 25 mL volumetric flask and make up the solution with 50% linearity solution. The second solution was prepared by dissolving 10 mg each of glimepiride, pioglitazone, and metformin in 25 mL volumetric flask and make up the solution with 75% linearity solution. The third solution was prepared by dissolving 10 mg each of glimepiride, pioglitazone, and metformin in 25 mL volumetric flask and make up the solution with 100% linearity solution. The fourth solution was prepared by dissolving 10 mg each of Glimepiride, pioglitazone, and metformin in 25 mL volumetric flask and make up the solution with 125% linearity solution. The fifth solution was prepared by dissolving 10 mg each of glimepiride, pioglitazone, and metformin in 25 mL volumetric flask and make up the solution with 150% linearity solution.

2.7. Preparation of Sample Solution for Batch Analysis. Two commercial samples were used for batch analysis. Ten tablets were weighed and their average weight was calculated. The tablet was crushed to a homogeneous mixture and 20.19 mg of Triblend 1 tablet and 23.23 mg of Zoryl MP2 tablet have been dissolved in 25 mL each of the mobile phase. To extract the drug in solution, sonicate for 5 minutes followed by cyclomixing for 5 minutes. The resulting solution was filtered using Millipore syringe filter (0.42 μ). The resulting clear solution was injected in HPLC in duplicate as per the developed method.

2.8. Analytical Method Validation

2.8.1. Specificity of the Method. The terms selectivity and specificity are often used interchangeably. Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities. This parameter was performed to know the retention time of each drug in a mixture and in the sample to understand if any drug-drug interaction or drug-excipient interaction is present.

2.8.2. System Suitability. System suitability test is used to verify that the resolution and reproducibility of the chromatographic systems are adequate for the analysis to be done. The tests are based on the fact that the equipment, electronics, samples to be analyzed constitute an integral system that can be evaluated as such. The limits for system suitability were set for theoretical plates, resolution, and asymmetry.

2.8.3. Linearity. Five concentrations of the standard mixture, 50%, 75%, 100%, 125%, and 150%, were injected and chromatogram was recorded. A graph was plotted for the concentration of the corresponding drug versus area. The correlation coefficient (r) for each drug was calculated.

2.8.4. Accuracy. To determine the accuracy in sample preparation method of standard additions was made for measuring the recovery of the drugs. To the standard solution known concentrations of the drug (50%, 75%, 100%, 125%, and 150%) was added. Five different solutions were prepared as mentioned in Section 2.2.6. The accuracy was expressed as the percentage of the analytes recovery.

2.8.5. Method Precision. It is very important that the method developed should be precise. Six replicates of the sample prepared from the commercial tablets were injected and Assay was calculated to measure the repeatability of retention times and peak area of standard and sample.

2.8.6. Robustness. To verify the robustness of the method, the analysis was done under variable flow rates. The flow rate as per the developed method is 1.7 mL/min. This has been purposely changed to 1.5 mL/min and 1.9 mL/min and the chromatogram was obtained.

2.8.7. Ruggedness. To test the ruggedness of the method, the analysis was done on different days and different chemists to check for any changes in the chromatograph. The percentage RSD for the retention time and area was calculated.

2.8.8. Performance Test of the Method/Batch Analysis. The method is said to be reliable if it can be applied for the analysis of glimepiride, pioglitazone, and metformin simultaneously to the pharmaceutical dosage forms or commercial tablets. For this purpose, performance test of the method has been conducted on two market samples manufactured by Akesis Pharma Pvt. Ltd., brand name Triblend 1, and batch number TBD IP0310, and Zoryl MP2 manufactured by Intas Pharmaceuticals, Batch number TF 10D160.

3. Results and Discussion

After several permutation and combinations, above method has been optimized. It is evident from this method that this is a very fast method of analysis compared to the literature available [19]. We have been able to elute all the three drugs within 5 min. In the current days, industries are looking for the methodology which can save sophisticated instruments and chemist's valuable time, and as a result they can release their product analysis report within lesser time. This is the reason why people are more attracted toward ultra-fast liquid chromatography (UFLC) [20, 21]. In this regard, the current method developed by us is very fast and encouraging. The developed method was validated with a holistic approach according to ICH guidelines and details of findings are as below.

3.1. Specificity of the Method. The retention times of the standard drugs individually were measured and it was found to be 5.000 min, 3.942 min, and 1.342 min for glimepiride, pioglitazone, and metformin respectively. The drugs were taken as mixture and injected for taking the chromatogram. All the three drugs (glimepiride, pioglitazone, and metformin

TABLE 3: Results for accuracy of the method.

		Initial conc area	Sol 1 area	50% area	Sol 1—50% area	% Recovery
Sol 1	Glimepiride	14657264	22563825	8023992	14539833	99.2
	Pioglitazone	11302419	17393625	6189621	11204004	99.13
	Metformin	11072363	20056814	9030106	11026708	99.59
		Initial conc area	Sol 2 area	75% area	Sol 2—75% area	% Recovery
Sol 2	Glimepiride	14657264	26129857	11630341	14499516	98.92
	Pioglitazone	11302419	20320163	8954005	11366158	100.56
	Metformin	11072363	21365397	10146889	11218508	101.32
		Initial conc area	Sol 3 area	100% area	Sol 3—100% area	% Recovery
Sol 3	Glimepiride	14657264	29367481	14657264	14710217	100.36
	Pioglitazone	11302419	22539487	11302419	11237068	99.42
	Metformin	11072363	22249678	11072363	11177315	100.95
		Initial conc area	Sol 4 area	125% area	Sol 4—125% area	% Recovery
Sol 4	Glimepiride	14657264	32182741	17567370	14615371	99.71
	Pioglitazone	11302419	28483584	13618562	11225022	99.32
	Metformin	11072363	22950236	11878593	11071643	99.99
		Initial conc area	Sol 5 area	150% area	Sol 5—150%	% Recovery
Sol 5	Glimepiride	14657264	34362870	19549044	14813826	101.07
	Pioglitazone	11302419	26320185	15120715	11199470	99.09
	Metformin	11072363	23472537	12416531	11056006	99.85

TABLE 4: Method precision results.

	Assay 1	Assay 2	Assay 3	Assay 4	Assay 5	Assay 6	Average	% RSD
Glimepiride	99.55%	99.05%	99.21%	99.15%	99.21%	99.72%	99.31%	0.21
Pioglitazone	99.37%	99.44%	99.31%	99.54%	99.41%	99.26%	99.38%	0.099
Metformin	99.20%	98.95%	98.65%	99.63%	98.85%	99.05%	99.06%	0.34

hydrochloride) were resolved very nicely in a mixture. Retention time of all the three drugs in standard mix was found to be 5.017 min, 3.958 min, and 1.342 min for glimepiride, pioglitazone, and metformin hydrochloride, respectively. This indicates there is no chromatographic interference between the analytes. The sample solution (pharmaceutical dosage form) was then injected and the chromatogram was obtained. The retention time of the drugs in the dosage form (tablet) was found to be 5.358 min, 4.083 min, and 1.308 min for glimepiride, pioglitazone, and metformin hydrochloride, respectively. Respective HPLC chromatograms are represented in Figures 2, 3, and 4. There is no specific change in the chromatogram. This indicates that there is no drug-excipient interference and the drugs are properly resolved by this method. Therefore, this is a suitable method for the simultaneous estimation of glimepiride, pioglitazone, and metformin in drug mixture and dosage forms.

3.2. System Suitability. The suitability of the system was studied by performing the experiment and looking for changes in separation, retention times, and asymmetry of the peaks. Five injections of the standard and two injections of the sample were injected for this purpose. The resolution, areas, retention time, theoretical plates values and peak asymmetry

were calculated for standard and sample solutions. Results obtained are given in Table 1.

3.3. Linearity. The correlation coefficient (r) was calculated, and it was between 0.98 to 1.00 which is well within the acceptance criteria. The results are shown in Table 2. The concentration was found to be proportional to the area, and the response of the detector was determined to be linear over the range of 0.2 to 0.6 mg/mL as shown in the Figure 5.

3.4. Accuracy. The percentage recovery of the results obtained is listed in Table 3. The results indicate that the recoveries are well within the acceptance range, therefore, method is accurate and it can be used for the simultaneous estimation of glimepiride, pioglitazone, and metformin.

3.5. Method Precision. The percentage RSD values for the assays in precision study were calculated. The results as shown in Table 4 indicate that the method developed is precise.

3.6. Robustness. Due to deliberate change in the method, no changes were found in the chromatogram, the method developed is robust. The results are shown in Table 5.

TABLE 5: % RSD at different flow rates.

	Retention times % RSD	Areas % RSD	Sample area % RSD
1.5 mL/min			
Glimepiride	0.05	0.14	0.4
Pioglitazone	0.04	0.09	0.22
Metformin	0.06	0.52	0.22
1.9 mL/min			
Glimepiride	0.07	0.69	0.14
Pioglitazone	0.03	0.05	0.01
Metformin	0	0.21	0.18

TABLE 6: RSD of the drugs on different days and different analysts.

	Retention time % RSD	Areas % RSD	Sample area % RSD
Day 1—Analyst 1			
Glimepiride	0.03	0.1	0.04
Pioglitazone	0.08	0.1	0.04
Metformin	0.03	0.01	0.02
Day 2—Analyst 2			
Glimepiride	0.04	0.04	0.05
Pioglitazone	0.06	0.01	0.03
Metformin	0.08	0.1	0.06

TABLE 7: Estimation of the drugs in commercial samples.

	Label claim	Acquired data	Assay%
Triblend 1			
Glimepiride	1 mg/tab	1.01 mg/tab	101%
Pioglitazone	15 mg/tab	15.03 mg/tab	100.2%
Metformin	500 mg/tab	497.33 mg/tab	99.46%
Zoryl MP2			
Glimepiride	2 mg/tab	2.01 mg/tab	100.5%
Pioglitazone	15 mg/tab	15 mg/tab	100%
Metformin	500 mg/tab	501.02 mg/tab	100.2%

3.7. *Ruggedness.* Data acquired and compared, % RSD of area and RT has been calculated and tabulated in Table 6. Based on the data, it is evident that the method is Rugged.

3.8. *Performance of the Drug/Batch Analysis.* Two market samples have been analysed to see the performance of the method. First tablet taken was Triblend 1 which contains 1 mg of glimepiride, 15 mg of pioglitazone, and 500 mg of metformin hydrochloride; the second tablet ZorylMP2 contains contains 2 mg of glimepiride, 15 mg of pioglitazone, and 500 mg of metformin hydrochloride. Results obtained have been summarized in the Table 7.

4. Conclusion

It is concluded from the above study that the current method is fast, reproducible, and simple. By adopting this method one can elute all the three drugs in 5 minutes. Hence this

method is definitely time saving to enable the simultaneous estimation of glimepiride, pioglitazone, and metformin. The proposed method is found to be accurate, precise, linear, robust, and rugged.

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