RESEARCH REVIEW JOURNALS
Volume-04
Issue-05
May-2019

ISSN: 2455-3085 (Online)
RESEARCH REVIEW International Journal of Multidisciplinary
www.rrjournals.com[UGC Listed Journal]

Development of HPLC Method for Simultaneous Estimation for Pioglitazone, Glimepiride, Glimepiride Impurities from a Combination Drug Product

[1]Vivek Pravinchandra Nandani and [2]Dr. Pushpendra Sharma

[1]Research Scholar, Department of Chemistry, Sri Satya Sai University of Technology & Medical Sciences, Sehore, M.P.
[2]Research Guide, Department of Chemistry, Sri Satya Sai University of Technology & Medical Sciences, Sehore, M.P.

ARTICLE DETAILS

Article History
Published Online: 25 May 2019

Keywords
Stability Indicating; Peak Purity; Impurities; Validation.

ABSTRACT

The goal of this paper is to develop a single HPLC method for estimating pioglitazone, glimepiride, glimepiride impurity B, and impurity C from a combination drug product simultaneously. By dissolving sufficient amounts of glimepiride impurity B, impurity C, and glimepiride (1000 |ig/mL) in methanol, stock solutions of glimepiride impurity B, impurity C, and glimepiride (1000 |ig/mL) were prepared. By dissolving the required volume of pioglitazone and glimepiride standard in diluent, a standard solution containing 750 |ig/mL pioglitazone and 100 |ig/mL glimepiride was prepared. Many of the system validation criteria were found to be sufficient, and the method was thoroughly validated. The developed procedure, which can be used for routine examination to determine the compound, may support both quality control departments and commercial sample purity tests.

1. Introduction

Type 2 diabetes is a disease in which the blood glucose levels are abnormally elevated. Diabetes mellitus is the most common form of diabetes. People who are affected must live with the disease for the remainder of their lives. For the treatment of type 2 diabetes, a mixture of glimepiride, pioglitazone hydrochloride, and metformin hydrochloride extended release is used. Glimepiride's primary mechanism for reducing blood glucose is most likely based on inducing insulin release from working pancreatic beta cells (Sweetman 2009). Pioglitazone hydrochloride is a potent and highly selective agonist for the gamma subunit of the peroxisome proliferators-activated receptor (Sweetman 2009). Metformin hydrochloride works by increasing peripheral glucose uptake and consumption, lowering hepatic glucose production, lowering glucose absorption in the intestine, and improving insulin sensitivity. As a result, this combination aids in the treatment of type 2 diabetes by improving glycaemic function. It's also likely to play a part in reducing macrovascular and microvascular complications.

In the USP, glimepiride was estimated (2011). For the determination of purity of both raw materials and pharmaceutical formulations, the HPLC method is stated as the primary method. Liquid chromatography (Warnjari and Gaikwad 2005) and derivative spectroscopy are two methods for evaluating glimepiride in pharmaceutical dosage forms that have been published in the literature (Bonfilio et al 2011). Glimepiride-related substances and degradation pathway methods have been studied by Bansal et al (2008), Kovarlkova et al (2004), and Khan et al (2005). The degradation actions of pioglitazone and stability-indicating assay methods have also been published by Ramulu et al (2010), Shirkhedkar and Surana (2009), and Smita Sharma et al (2010). The drugs in the combination drug product have been estimated by Jain et al (2008), Karthik et al (2008), and Lakshmi et al (2009). Hossain et. al.(2013) a quick, easy, and cost-effective reversed phase high performance liquid chromatographic (RP-HPLC) method for simultaneous and quantitative studies of pioglitazone HCl and glimepiride in pharmaceutical dosage forms has been established and validated. Gadapa and Upendra (2013) For the separation and estimation of three drugs, glimepiride, pioglitazone, and metformin, in bulk drug mix and pharmaceutical dosage types, a quick, rapid, accurate, and reliable reverse phase HPLC method was created. Kinnari et. al.(2014),an accurate, responsive, specific, and stability demonstrating high performance thin layer chromatographic system for the estimation of Glimepiride and Metformin hydrochloride has been established and validated.

2. Objective of the study

The compounds glimepiride and pioglitazone are extremely unstable. Despite the fact that this combination drug product has been sold by many pharmaceutical firms, there is no empirical procedure for evaluating it by routine quality control and stability sample analysis processes. For the unstable molecules in glimepiride and pioglitazone, it is important to establish a stability-indicating assay process. Glimepiride significant degradations of impurity B and impurity C were also injected and estimated in the combination tablets to show the method's selectivity. The goal of this study is to develop a single HPLC method for estimating pioglitazone, glimepiride, glimepiride impurity B, and impurity C from a combination drug product simultaneously.

2.1 Experimental

➢ **Materials and Reagents**

Pharmaceutical grade standards of pioglitazone (chemically: 5-(4-[2- (5-ethylpyridin-2-yl)ethoxy]benzyl) thiazolidine-2,4-dione) and glimepiride (chemically: 3 -ethyl-4-methyl-N-(4- [N-((1 r,4r)-4-methyl cyclohexylcarbamoyl) sulfamoyl]phenethyl)-2-oxo-2,5-dihydro-1H-pyrrole-1 -carboxamide) were supplied by M/S Pharma Lab (Baddi, India). Glimepiride impurity B (chemically: 3-Ethyl-4-methyl-2-oxo-N-[2-(4- sulphamoylphenyl) ethyl]-2,3-dihydro-1H- pyrrole-1-carboxamide) and impurity C (chemically: Methyl [[4-[2-

[[(3-Ethyl-4- methyl-2-oxo-2,3-dihydro-1H-pyrrol-1 -yl) carbonyl]amino]ethyl]phenyl]- sulphonyl] carbamate) were purchased from LGC Standards (Mumbai, India). Chemical structures are shown in Figures 2.1 to 2.4. The researchers purchased commercially available combination tablets containing 15 mg pioglitazone, 2 mg glimepiride, and 500 mg metformin hydrochloride (PRICHEK GMP®-manufactured by Indoco Rem). Rankem provided HPLC grade acetonitrile, analytical reagent grade potassium dihydrogen phosphate, and orthophosphoric acid (India). Millipore water manufactured by the Milli-Q plus water purification system was used (Bedford, MA, USA).

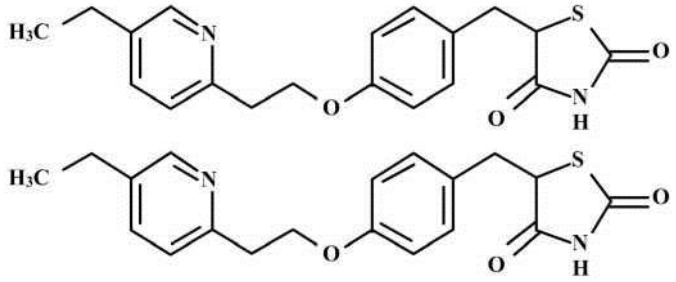

Figure 2.1 Chemical structure of pioglitazone (MF: $C_{19}H_{20}N_2O_3S$, MW: 356)

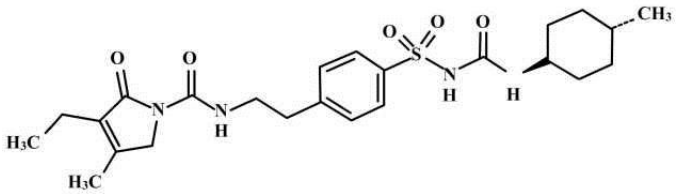

Figure 2.2 Chemical structure of glimepiride (MF: $C_{24}H_{34}N_4O_5S$, MW: 490)

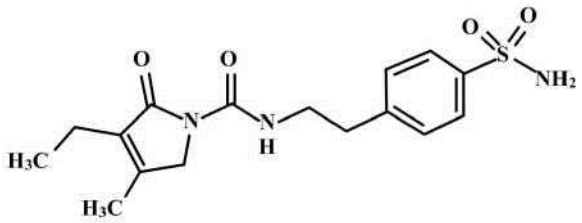

Figure 2.3 Chemical structure of glimepiride impurity B (MF: C8H9NO2, MW: 151)

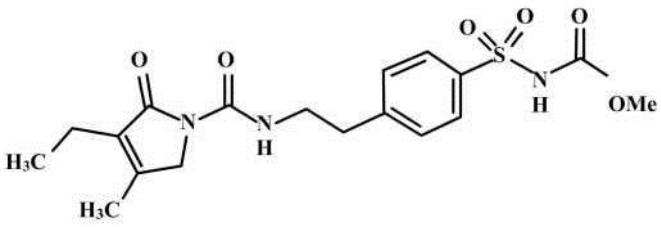

Figure 2.4 Chemical structure of glimepiride impurity C (MF: C18H23N3O6S, MW: 409)

➢ **Instrumentation**

For the construction and validation, a Waters HPLC device with a 2695 binary pump plus auto sampler, a 2996 photo diode array, and a 2487 UV detector (Waters Corporation, Milford, USA) was used.

➢ **System Suitability Solution**

By dissolving sufficient amounts of glimepiride impurity B, impurity C, and glimepiride (1000 |ig/mL) in methanol, stock solutions of glimepiride impurity B, impurity C, and glimepiride (1000 |ig/mL) were prepared. From the above-mentioned stock solutions, device suitability solutions of 0.2 |ig/mL impurity B and impurity C and 0.5 |ig/mL glimepiride were prepared using a diluent mixture of acetonitrile and water (8:2, v/v).

➢ **Standard Solution Preparation**

By dissolving the required volume of pioglitazone and glimepiride standard in diluent, a standard solution containing 750 |ig/mL pioglitazone and 100 |ig/mL glimepiride was prepared.

➢ **Sample Solution Preparation**

With the mortar and pestle method, twenty tablets were weighed and powdered. A 100 mL volumetric flask was filled with powder tablets containing 10 mg of glimepiride (equivalent to 75 mg of pioglitazone). Around 60 mL of diluent was added and held on a rotatory shaker for 10 minutes to fully disperse the material, then sonicated for 10 minutes (bath temperature was kept at 25°C during sonication) and diluted to 100 mL with diluent. Pioglitazone and glimepiride concentrations were 750 and 100 |ig/mL, respectively. The resulting solution was centrifuged for 5 minutes at 10000 rpm. The pioglitazone, glimepiride, and glimepiride

impurities were determined in the supernatant solution.

2.2 Results and discussion

2.2.1 Optimization of Chromatographic Method

The HPLC system was tweaked in order to create a method that could detect stability. The method for determining stability should accurately calculate the active ingredients without interference from degradation products or sample matrices. Since pioglitazone and glimepiride have degradation properties, the gradient method was chosen over the isocratic method in order to obtain a complete degradation product and good resolution between closely eluting compounds. The first experiments used pioglitazone and glimepiride in their pure drug forms, spiked with glimepiride impurity B and glimepiride impurity C. The effects of various buffer pH (27) and solvent systems containing methanol and acetonitrile were investigated. For the preliminary trial, C18 reverse phase column chemistry was used. With a flow rate of 0.8 mL/min, a successful separation was achieved in the gradient software containing solution. A (phosphate buffer at pH 3.2) and solution B (acetonitrile). Both forced degradation samples were injected in the optimised conditions to demonstrate the method's stability-indicating existence. Because of the interference of degradation compounds, peak purity of glimepiride and pioglitazone was not reached. A small adjustment in gradient, column temperature, and flow rate was made to solve this problem, but the trials did not yield the desired results. As a result, various column chemistry was attempted. The C8 column was chosen first, and a known compound was combined with it. One degradation peak emerged from the glimepiride peak by using the phenyl column. The peak purity of glimepiride was adequate, but the peak purity of pioglitazone remained unchanged. Finally, the cyano column was employed in the production phase. With more than 2.0 resolutions from the pioglitazone peak, the main base degradation peak appeared. To our knowledge, this is the first process in which, despite a large number of degradation peaks, the identified compound was resolved very well. At 230 nm, pioglitazone, glimepiride, glimepiride impurity B, and glimepiride impurity C were found to have adequate response. A chromatogram was collected with the entire range of 200-400 nm in the case of a stressed sample to search for a new impurity at different wavelengths, but no extra peak was found except at 230 nm wavelength observed peaks. Using 100 |ig/mL of glimepiride sample preparation and a 25 |iL injection volume, the required LOQ value for glimepiride impurity B and impurity C was calculated. During the growth, it was discovered that the impurity B forms very easily, so a new sample preparation was prepared and used to ensure a consistent result. During the preparation of the sample solution, the sonicator bath temperature was held below 25°C. In comparison to the current USP monograph glimepiride tablet process, the critical near eluting impurity of glimepiride impurity B and impurity C was found at a higher resolution. Table 2.1 shows the optimised chromatographic process.

Table 2.1 Optimized chromatographic method

Mobile phase-A	20 m mol/L potassium dihydrogen phosphate, pH adjusted to 3.2 using dilute ortho phosphoric acid		
Mobile phase-B	Acetonitrile		
Diluent	Mixture of acetonitrile and water (8:2, v/v)		
Column	Zorbax cyano, 250 mm x 4.6 mm, 5 micron		
Column oven temperature	25°C		
Detection wavelength	230 nm		
Injection volume	25 |iL		
Flow rate	0.8 mL/min		
Gradient programme	Time (min)	Mobile phase-A (%)	Mobile phase-B (%)
	0.01	80	20
	13	80	20
	50	50	50
	55	20	80
	60	20	80
	63	80	20
	70	80	20

2.2.2 Method Validation

The developed chromatographic method was validated for system suitability, selectivity, specificity, linearity, precision, accuracy, LOD, LOQ and robustness as per ICH and FDA guidelines.

- **System Suitability**

Table 2.2 shows the observed analyte retention time (RT) and relative retention time (RRT). The device suitability parameter (> 6.0) was set to the resolution between the near eluting pair of glimepiride impurity B and glimepiride impurity C. Also measured was the percent RSD of the peak area of pioglitazone and glimepiride. Figure 2.5 shows the chromatogram of device suitability.

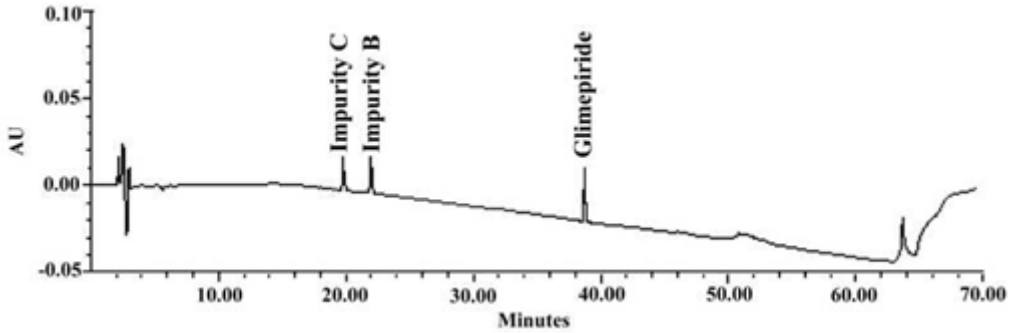

Figure 2.5 System suitability chromatogram (Containing glimepiride, glimepiride impurity B and glimepiride impurity C)

Table 2.2 results of System suitability

Parameter	Pioglitazone	Glimepiride	Impurity B	Impurity C
% RSD	1.1	1.3	4.1	3.2
Retention time	31.93	38.73	21.99	19.82
Relative retention time	-	1.00	0.57	0.51
USP resolution	-	-	6.50	-
USP tailing factor	1.01	0.99	1.22	1.13
USP theoretical Plates	15011	18123	8012	7532

➢ **Specificity and Selectivity**

Forced degradation experiments were used to determine the specificity of the established process. The established HPLC method's specificity was tested in the presence of degradation products and other sample matrices. The proposed method's stability-indicating property and specificity were determined through forced degradation studies on a tablet sample. Acid and base hydrolysis (using 0.1 N HCl and 0.1 N NaOH, respectively for 2 hours), oxidation (using 3 percent H2O2 for 2 hours), and UV radiation were all used on the sample solutions (254 nm for 48 hours). Minor degradation was observed when the drug was exposed to acid and a peroxide state, but significant degradation was observed when the drug was exposed to a base condition. Impurity B levels were higher in both acid, base, and peroxide-stressed samples, but impurity C was only present in peroxide-stressed samples. Photolysis had no effect on the drugs, and no deterioration was observed. Peak purity was found within reasonable limits in all stressed samples (purity angle less than purity threshold), suggesting the method's specificity. Table 2.3 displays the results.

Table 2.3 Results of Forced degradation

Condition	Time	% Assay of Glimepiride	% Assay of Pioglitazone
Unstressed sample	-	99.2	99.0
Acid hydrolysis (0.1 N HCl)	2 hours	96.0	97.2
Base hydrolysis (0.1 N NaOH)	2 hours	91.9	82.3
Oxidation (3 % H_2O_2)	2 hours	95.3	96.3
Light (254 nm)	48 hours	100.2	99.2

To prove the selectivity of the method, all individual compounds, i.e., pioglitazone, glimepiride, metformin, glimepiride impurity B and glimepiride impurity C were injected in the optimized method. Blank interference was checked by injecting the sample diluents. No interference was found with the discussed compounds. Specificity chromatograms are shown in Figures 2.6 to 2.15.

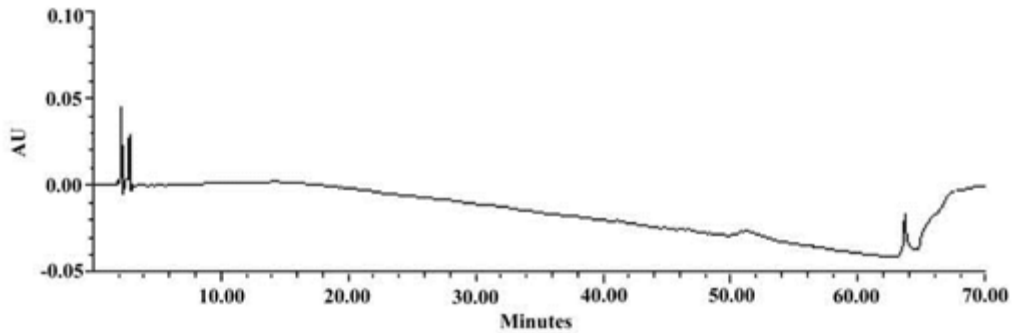

Fig 2.6 Blank Chromatogram

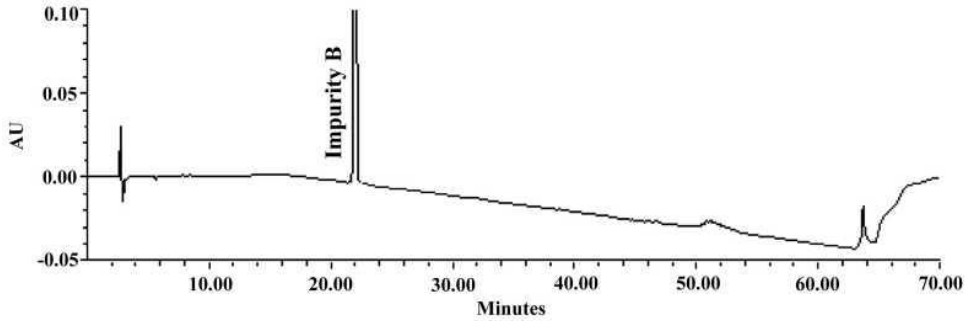

Figure 2.7 Chromatogram of glimepiride impurity B injection

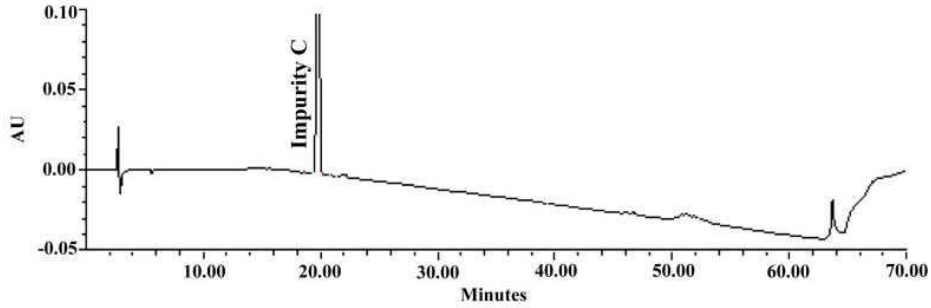

Figure 2.8 Chromatogram of glimepiride impurity C injection

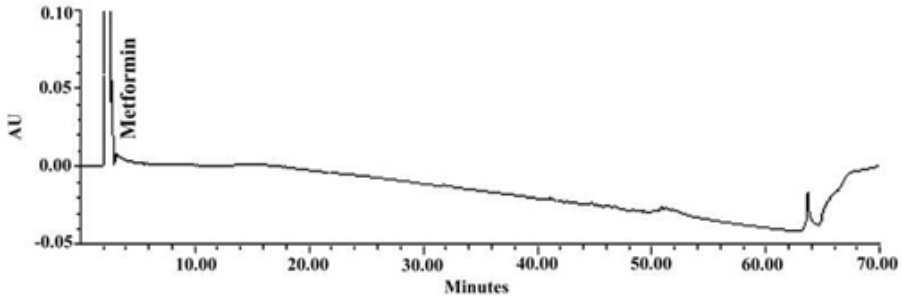

Figure 2.9 **Chromatogram of metformin injection**

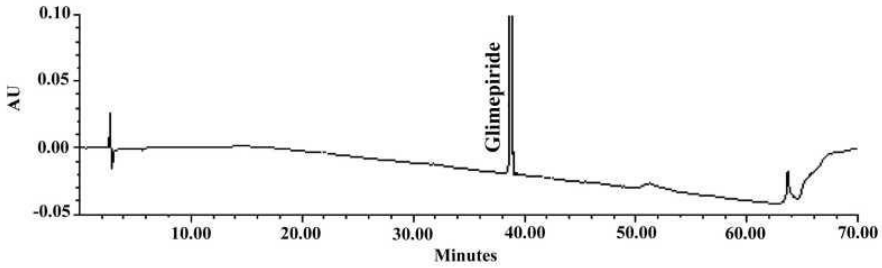

Figure 2.10 Chromatogram of glimepiride injection

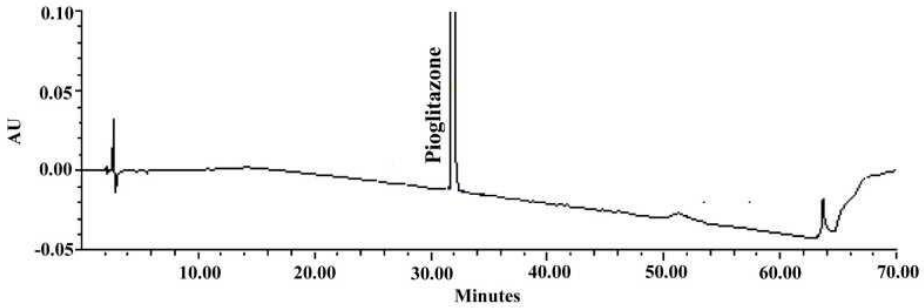

Figure 2.11 Chromatogram of pioglitazone injection

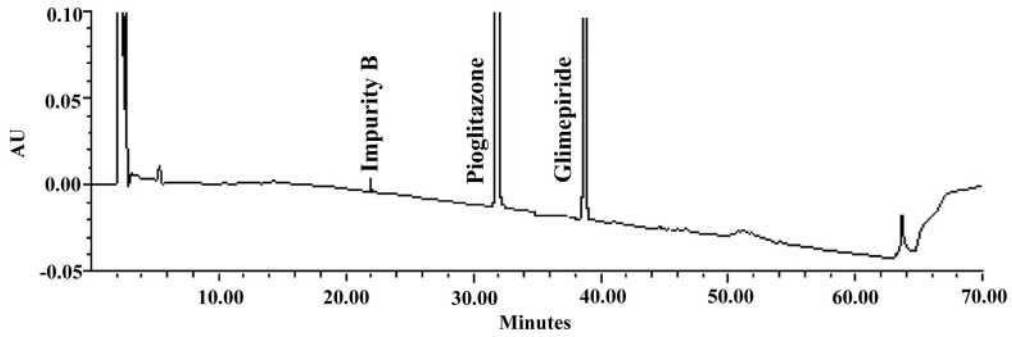

Figure 2.12 Chromatogram of unstressed sample injection

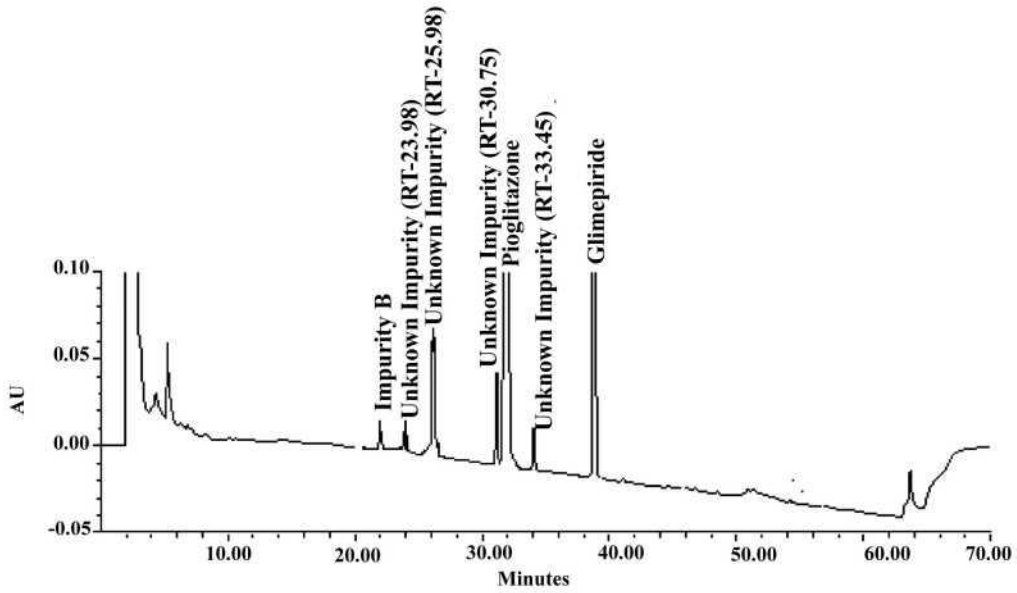

Figure 2.13 Chromatogram of base degradation sample injection

➢ **Limit of Detection (LOD) and Limit of Quantification (LOQ)**

The magnitude of analytical history was used to assess LOD and LOQ. Serial dilutions of glimepiride impurity B and impurity C solutions were used to measure the LOD and LOQ. After that, the signal-to-noise ratio was estimated. LOD and LOQ were described as signal-to-noise ratios of 3 and 10 respectively. 0.005 percent (i.e., 0.005 pg/mL) and 0.02 percent (i.e., 0.02 pg/mL) for a 25 pL injection volume were obtained by injecting six preparations of the LOD and LOQ solutions of glimepiride impurity B and glimepiride impurity C. For glimepiride impurity B and glimepiride impurity C, the precision at the LOQ concentration (six individual preparations) was less than 5.0 percent. Table 2.4 displays the results.

Table 2.4 LOQ level precision for impurities

Peak Area		
Injection	**Impurity B**	**Impurity C**
1	16950	15591
2	16985	15659
3	17001	16000
4	17500	15350
5	16680	15455
6	17100	15377
Mean	17036	15572
SD	267.34	241.71
% RSD	1.57	1.55

➢ **Linearity**

The assay method's linearity was tested by calculating five concentration levels at three preparations ranging from 50% to 150

percent of the analyte concentration, i.e. 750 |ig/mL for pioglitazone and 100 |ig/mL for glimepiride. On both compounds, the correlation was shown to be greater than 0.9999.

Table 2.5 Linearity data for drug substances and impurities

Compound	Range (^g/mL)	Regression Parameters (n = 3)	
		Equation of regression line	R^2 value
Pioglitazone	375-1125	Y = 332706x - 479991	0.9999
Glimepiride	50-150	Y =123014x +160007	0.9999
Impurity B	0.02-0.4	Y = 867436x - 115.18	0.9999
Impurity C	0.02-0.04	Y = 780417x - 524.47	0.9999

Six concentration levels of impurity B and impurity C were prepared by diluting the impurity stock solution to the appropriate concentrations, ranging from LOQ to 200 percent (LOQ, 25 percent, 50 percent, 100 percent, 150 percent, and 200 percent). The obtained correlation coefficient was higher than 0.9999. Table 2.5 displays the findings. Figures 2.14 to 2.17 display the linearity graphs.

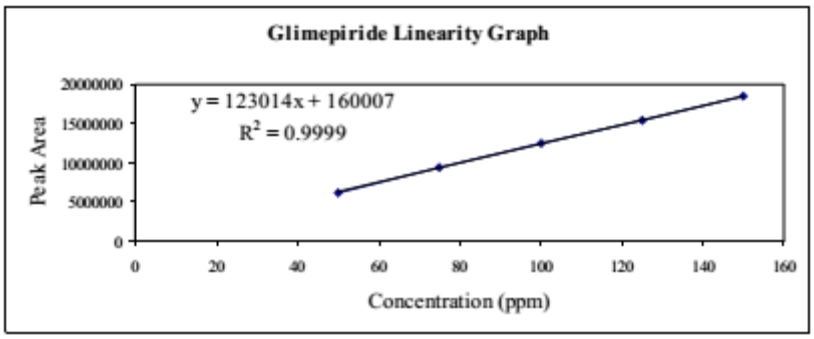

Figure 2.14 Linearity graph for glimepiride impurity C

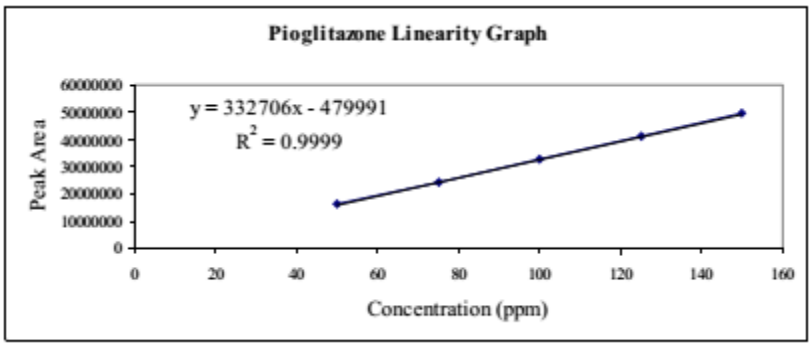

Figure 2.15 Linearity graph for glimepiride

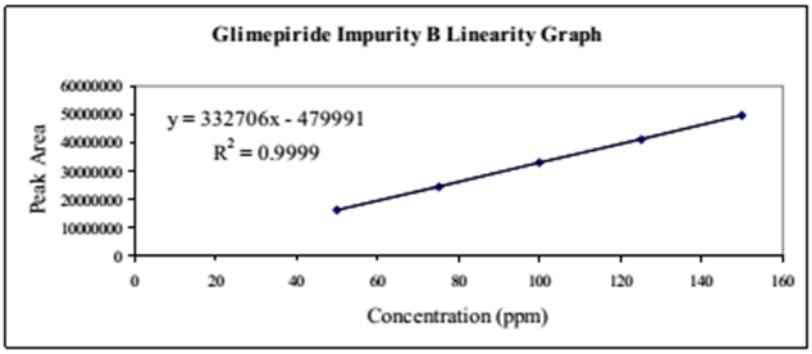

Figure 2.16 Linearity graph for pioglitazone

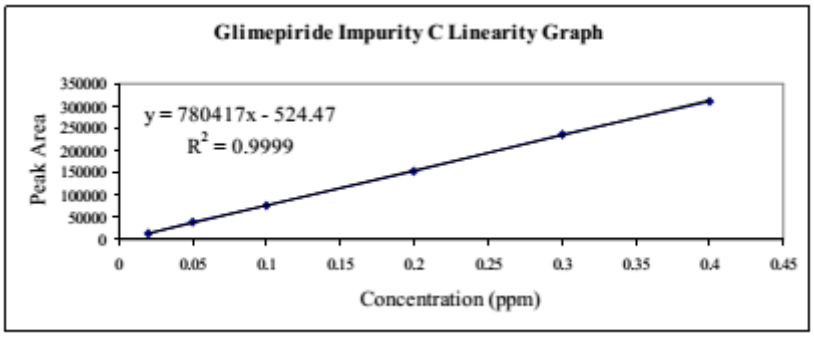

Figure 2.17 Linearity graph for glimepiride impurity B

➢ **Precision**

For pioglitazone, the percent RSD of six sample preparations assay value was 1.1, and for glimepiride, it was 0.9. The average assay for pioglitazone was 98.2 percent, and for glimepiride, it was 100.2 percent. Different tables, methods, and analysts were used to assess the assay method's intermediate precision. Both pioglitazone and glimepiride had percent RSDs below 2.0 on separate days.

Assay value was found between 98 % and 102 %, confirming the ruggedness of the method.

By injecting six individual formulations (in a single injection) of 100 g/mL glimepiride spiked with 0.2 percent of the above described impurities, the accuracy of impurity B and impurity C was confirmed. Impurity B and impurity C had percent RSDs of 3.2 and 2.9, respectively. The percent RSDs for impurities in the intermediate precision were well within the range of (5.0). Tables 2.6 and 2.7 indicate the results.

Table 2.6 Summary of method precision

Injection	Pioglitazone (%)	Glimepiride (%)	Impurity B (%)	Impurity C (%)
1	98.5	99.0	0.26	0.21
2	97.5	99.2	0.27	0.22
3	97.3	100.1	0.27	0.21
4	98.0	101.0	0.27	0.21
5	97.5	101.1	0.25	0.22
6	100.5	100.5	0.27	0.22
Mean	98.22	100.15	0.27	0.21
SD	1.20	0.89	0.01	0.01
% RSD	1.22	0.89	3.16	2.87

Table 2.7 Summary of intermediate precision

Injection	Pioglitazone (%)	Glimepiride (%)	Impurity B (%)	Impurity C (%)
1	98.9	97.1	0.27	0.20
2	99.3	98.3	0.26	0.21
3	99.1	99.2	0.26	0.20
4	98.4	99.5	0.26	0.19
5	98.0	99.8	0.27	0.20
6	100.1	99.1	0.26	0.20
Mean	98.97	98.83	0.26	0.20
SD	0.73	0.99	0.01	0.01
% RSD	0.74	1.00	1.96	3.16

➢ **Accuracy**

The recovery of pioglitazone, glimepiride, and glimepiride impurities from three sample preparations at five concentration levels, i.e. 50 percent, 75 percent, 100 percent, 125 percent, and 150 percent of working concentration levels, was calculated. The pioglitazone and glimepiride recovery rates were within the normal range, ranging from 98 to 102 percent. Impurity B and C recovery percentages ranged from 96.1 percent to 101.3 percent and 98.1 percent to 102.1 percent, respectively. Table 2.8 shows the recovery results.

Table 2.8 Accuracy results for developed HPLC method

Compound	Level (%)	Amount added (^g/mL)	Recovery (%)	% RSD (n = 3)
	50	375	98.3	1.1
	75	563	98.5	1.3
Pioglitazone	100	750	100.1	0.9
	125	938	100.3	1.2
	150	1125	99.2	0.8
	50	50	98.1	0.9
	75	75	99.3	1.1
Glimepride	100	100	99.1	1.2
	125	125	98.7	0.8
	150	150	100.2	0.5
	50	0.10	100.3	1.1
	75	0.15	101.2	1.4
Impurity B	100	0.20	96.1	3.1
	125	0.25	100.1	0.8
	150	0.30	101.3	0.9
	50	0.10	99.1	1.3
	75	0.15	98.1	1.2
Impurity C	100	0.20	98.7	2.0
	125	0.25	102.1	1.9
	150	0.30	101.5	0.9

➢ **Robustness**

To test the method's robustness, the chromatographic parameters were drastically changed. The recovery for the key ingredients in the sample solution, as well as the device suitability parameters, were investigated. In the mobile phase, the flow rate (0.1 mL/min), the pH of the buffer (0.2), and the organic composition (5%) were all modified. The deliberate adjustments yielded results that were well within the parameters. In all of the improvements, the sufficient resolution obtained between impurity B and impurity C was greater than 5.0. The assay value for pioglitatone and glimepiride was between 98 and 102 percent, suggesting that the method is accurate. Tables 2.9 to 2.11 demonstrate the robustness effects.

Table 2.9 Robustness result for flow rate variation

Compound	0.7 mL/min	0.8 mL/min	0.9 mL/min
Resolution between impurity B and impurity C	6.8	6.6	6.5
Pioglitazone (%)	98.3	99.1	98.9
Gimepiride (%)	99.1	99.5	98.6
Impurity B (%)	0.06	0.07	0.07

Compound	pH 3.0L.	pH 3.2	pH 3.4
Resolution between impurity B and impurity C	6.6	6.6	6.7
Pioglitazone (%)	98.8	99.1	99.2
Gimepiride (%)	99.5	99.5	98.2
Impurity B (%)	0.07	0.07	0.07

Table 2.11 Robustness result for organic concentration variation

Compound	Acetonitrile (95 %)	Acetonitrile (100 %)	Acetonitrile (105 %)
Resolution between impurity B and impurity C	7.1	6.6	6.1
Pioglitazone (%)	98.2	99.1	99.7
Gimepiride (%)	99.1	99.5	98.4
Impurity B (%)	0.06	0.07	0.07

➢ **Application of the Developed Method to Commercial Tablets**

Commercial preparations (PRICHEK GMP®-manufactured by Indoco Rem-Tablets containing 15 mg of pioglitazone, 2 mg of glimepiride, and 500 mg of metformin hydrochloride) were examined to assess the applicability of the established procedure. The contents of pioglitazone, glimepiride, glimepiride impurity B, and glimepiride impurity C were measured six times from commercial samples. Pioglitazone, glimepiride, and glimepiride impurity B had average assay values of 98.2 percent, 100.1 percent, and 0.07 percent, respectively. Glimepiride impurity C was not found in the commercial sample that was tested.

3. Conclusion

A single reversed step stability-indicating RP-HPLC method has been developed for the simultaneous estimation of pioglitazone, glimepiride, glimepiride impurity B, and impurity C from the combination drug product. Many of the system validation criteria were found to be sufficient, and the method was thoroughly validated. The developed procedure, which can be used for routine examination to determine the compound, may support both quality control departments and commercial sample purity tests.

References

1. Sweetman, S. C. “The Complete Drug Refernce (Domperidone)”, 36th Edition, Pharmaceutical Press, London, UK, pp. 1726, 2009.
2. Sweetman, S. C. “The Complete Drug Refernce (Glibenclamide)”, 36th Edition, Pharmaceutical Press, London, UK, pp. 440, 2009.
3. Sweetman, S. C. “The Complete Drug Refernce (Glimepiride)”, 36th Edition, Pharmaceutical Press, London, UK, pp. 441, 2009.
4. Wanjari, D. B. and Gaikwad, N. J. “Reversed phase HPLC method for determination of glimepiride in tablet dosage form”, Indian J. Pharm. Sci., Vol. 67, No. 2, pp. 253-255, 2005.
5. Bonfilio, R., Kovaříková, M. B. and Salgado, H. R. N. “Development and validation of UV-derivative spectrophotometric method for determination of glimepiride in tablets”, J. Braz. Chem. Soc., Vol. 22, No. 2, pp. 292-299, 2011.
6. Kovaříková, P., Klimes, J., Dohnal, J. and Tisovská, L. “HPLC study of glimepiride under hydrolytic stress conditions”, J. Pharm. Biomed. Anal., Vol. 36, No. 1, pp. 205-209, 2004.
7. Ramulu, K., Thilak Kumar, T., Radha Krishna, S., Vasudev, R., Kaviraj, M., Rao, B. M. and Someswara Rao, N. “Identification, isolation and characterization of potential degradation products in pioglitazone hydrochloride drug substance”, Pharmazie, Vol. 65, No. 3, pp. 162-168, 2010.
8. Shirkhedkar, A. A. and Surana, S. J. “Application of a stability- indicating densitometric RP-TLC method for analysis of pioglitazone hydrochloride in the bulk material and in pharmaceutical formulations”, J. Planar. Chromatogr., Vol. 22, No. 3, pp. 191-196, 2009.
9. Shirkhedkar, A. A. and Surana, S. J. “Application of stability- indicating RP-TLC densitometric determination of rabeprazole sodium in bulk drug and pharmaceutical formulation”, Eurasian J. Anal. Chem., Vol. 4, No. 1, pp. 87-97, 2009.
10. Jain, D., Jain, S. and Amin, M. “Simultaneous estimation of metformin hydrochloride, pioglitazone hydrochloride and glimepiride by RP-HPLC in tablet formulation”, J. Chromatogr. Sci., Vol. 46, No. 6, pp. 501-504, 2008.

