UPI Journal of Pharmaceutical Medical, and Health Sciences

Content Availabe at www.uniquepubinternational.com **ISSN: 2581-4532**

Research Article

A Validated Stability-Indicating High-Performance Liquid Chromatographic Method for the Simultaneous Quantification of Pioglitazone and Glimepride in Pharmaceutical Dosage Form

Upendra Rao Uttaravelli*[1], K. Satyavathi[2] And K.Eswar Kumar[3]

[1]Research Scholar, Jawaharlal Nehru Technological University, Kakinada- 533003, Andhra Pradesh, India.

[2]Principal, Veda College of Pharmacy, Battalur-518510- Andhra Pradesh, India.

[3]Professor, AU College of Pharmaceutical Sciences, Andhra University, Visakhapatnam-530003, Andhra Pradesh, India.

Article History

Received on: 11-09-2020
Revised on : 22-11-2020
Accepted on : 9-12-2020

Keywords

Stability-indicating, RP-HPLC, Pioglitazone, Glimepiride, Simultaneous estimation, Method validation.

***Corresponding Author**

Upendra Rao Uttaravelli

https://doi.org/10.37022/jpmhs.v3i4.157

Abstract

A simple, selective and precise Stability indicating RP-HPLC method was developed for the simultaneous estimation of Pioglitazone and Glimepiridein Pharmaceutical Dosage Forms. The chromatographic separation of the selected two drugs was achieved on a reverse phase ZORBAX Eclipse Plus C18,150 x 4.6 mm, 5µm usingacetonitrile: ethanol: buffer (20:30:50v/v/v) with a flow rate of 1.0 ml/min with injection volume 10 µL and the detection was carried out at 287 nm. The retention time of Pioglitazone and Glimepiride were found to be 3.8 and 5.4 min respectively. The drug products were subjected to stress conditions of acidic, alkaline, neutral, oxidation, UV and Thermal conditions. The degradation products were well resolved from Pioglitazone and Glimepiridepeaks, thus indicating the stability indicating nature of the method. The linear regression analysis data for the calibration plots showed good linear relationship in the concentration range of 15 – 90 µg/mlfor Pioglitazone, 1 -6µg/ml for Glimepiride. The developed method was successfully validated in accordance to ICH guidelines. Hence, this method can be conveniently adopted for the routine analysis in quality control laboratories.

Introduction

High-performance liquid chromatography (HPLC), being rapid, precise, accurate and economical, is one of the most widely utilized techniques for pharmaceutical analysis. HPLC is a versatile technique that is commonly used to quantify and identify active pharmaceutical ingredient and impurities. Chemical determination of related impurities in active pharmaceutical ingredients is crucial because prolonged exposure to low concentrations of pharmacologically active related substances can lead to unwanted side effects, toxicity, or interference with the drug's efficacy. A reliable assay method not only provides a valuable tool for ascertaining the finished pharmaceutical product's quality but it also provides insight into drug release kinetics. A significant advantage of HPLC techniques is that they can be developed to detect multiple analytes of interest simultaneously, saving considerable time and resources and providing deeper insight into formulation characteristics. For instance, Patel et al. Developed and validated a stability-indicating RP-HPLC method for simultaneous determination of glimepiride and pioglitazone in human plasma. Meanwhile Khan et al. Optimized a bioanalytical HPLC method for simultaneous quantification of glimepiride and pioglitazone in human plasma using liquid-liquid extraction (LLE) with ethyl acetate as extraction solvent. HPLC methods are generally validated to international regulatory standards to demonstrate that they are fit for the intended purpose. As per the International Conference of Harmonization (ICH) Q2(R2) guidelines, "A validated quantitative analytical procedure that can detect changes in relevant quality attributes of a product during storage is considered to be stability-indicating. To demonstrate specificity/selectivity of a stability-indicating test, samples

containing relevant degradation products should be included in the study". Moreover, an ideal stability-indicating method should effectively resolve the drug and its degradation products.

Pioglitazone is an antihyperglycemic used as an adjunct to diet, exercise, and other antidiabetic medications to manage type 2 diabetes mellitus. Pioglitazone enhances cellular responsiveness to insulin, increases insulin-dependent glucose disposal, and improves impaired glucose homeostasis. In patients with type 2 diabetes mellitus, these effects result in lower plasma glucose concentrations, lower plasma insulin concentrations, and lower HbA1c values [1].

Glimepiride is a member of the second-generation sulfonylurea (SU) drug class used for the management of type 2 diabetes mellitus (T2DM) to improve glycaemic control. Type 2 diabetes is a metabolic disorder with increasing prevalences worldwide; it is characterized by insulin resistance in accordance with progressive β cell failure and long-term microvascular and macrovascular complications that lead to co-morbidities and mortalities. Sulfonylureas are one of the insulins secretagogues widely used for the management of type 2 diabetes to lower blood glucose levels. Glimepiride works by stimulating the secretion of insulin granules from pancreatic islet beta cells by blocking ATP-sensitive potassium channels (KATP channels) and causing depolarization of the beta cells. Compared to other SUs, glimepiride was associated with a lower risk of developing hypoglycaemia and weight gain in clinical trials as well as fewer cardiovascular effects than other SUs due to minimal effects on ischemic preconditioning of cardiac myocytes. It is effective in reducing fasting plasma glucose, postprandial glucose, and glycosylated haemoglobin levels and is considered to be a useful, cost-effective treatment option for managing type 2 diabetes mellitus [2].

Literature survey revealed that few analytical methods were developed for the quantification of Pioglitazone and Glimepiride in pharmaceutical dosage forms [3-14].

Materials and methods

1Chemicals and Solutions

Pioglitazone and Glimepiridedrugs were obtained from Vyshno Bio Sciences Research & Development Lab.Snehamayi Nagar,Vanastalipuram, Hyderabad.HPLC-grade ethanol and acetonitrile were procured from Sigma-Aldrich. Analytical-grade reagents, including Potassium dihydrogen phosphate and Phosphoric acid were purchased from E. Merck Limited, Mumbai, India.

2 Chromatographic Conditions

Separations were performed with the Agilent 1260 Infinity III LC System is a high-performance liquid chromatography (HPLC) platform equipped with modular components including a quaternary pump, autosampler, column compartment, and diode array detector (DAD). The chromatographic column used was ZORBAX Eclipse Plus C18, 150x4.6 mm, 5µm. The mobile phase comprised of a mixture of acetonitrile, ethanol and buffer (20:30:50v/v/v).The pH of the solution was carefully adjusted to 3.5 using dilute phosphoric acid (10%). The mobile phase was filtered through 0.45-micron membrane filter, degassed in ultrasonic bath and pumped from the respective solvent reservoir to the column at the flowrate of 1.0 ml/min. Ambient column temperature was maintained and Autosampler temperature was maintained at 200 C. The detection wavelength was 287.0 nm. The injection volume was 10 µl. The column was equilibrated for 60 min prior to the injection of the drug solution.

3 Preparation of standard solution

Accurately weighed quantities of 30 mg of Pioglitazone and 2mg of Glimepiride working Standardswere transferred into separate 100 mL clean and dried volumetric flasks. Then mixed standard solution was prepared by dissolving in solvent and sonicated for 15 minutes to ensure complete dissolution. The final volume in was made up to 100 mL with the same solvent, resulting in stock solutions with concentrations of 3000µg/ml and 200 µg/ml for Pioglitazone and Glimepiride respectively.

4 Preparation of standard solution

A batch of twenty tablets was precisely weighed to determine the mean tablet weight. An amount of the finely powdered tablet blend, corresponding to 30 mg of Pioglitazone and 2 mg of Glimepiride, was accurately weighed and transferred into a 100 mL clean and dried volumetric flask. The sample was dispersed in the designated diluent and subjected to ultrasonication for 30 minutes to facilitate complete solubilization of the active pharmaceutical ingredients and filtered through 0.45µm membrane filter. The solution was then brought to volume with the same diluent to yield the sample stock solution.

5 method validation:

The proposed RP-HPLC method was validated as per ICH Guidelines (ICH Q2A, 1995, ICH Q2B 1996, ICH, Q2 R1 - 1995)

6 Procedure for Forced Degradation Studies

6.1 Diluent

The mobile phase comprised of a mixture of Acetonitrile, Ethanol and Buffer (20:30:50v/v/v) is used as Diluent.

6.2 Water Stress Degradation

20 ml of water was added to 10 ml of stock solution and was kept at 800Cfor about 3 H in water bath. The solution was allowed to attain ambient temperature. Then volume was made up to100 ml with diluent to achieve final concentration of 100 µg/ml.

6.3 Acidic Degradation

20 ml of 3 M HCl was added to 10 ml of stock solution and was kept at 800 Cfor about 3 H in water bath. The solution was allowed to attain ambient temperature. Then the solution was neutralised by 20 mL of 3M NaOH and volume was made up to 100 ml with diluent to achieve final concentration of 100 µg/ml.

6.4 Alkali Degradation

20 ml of 3 M NaOH was added to 10 ml of stock solution and was kept at 800 C for about 3 H in water bath. The

solution was allowed to attain ambient temperature. Then the solution was neutralised by sufficient amount of HCl and volume was made up to 100 ml with diluent to achieve final concentration of 100 µg/ml.

6.5 Oxidative Degradation

2 and 5 ml of 3% H2O2 1,2,5 and 20 ml of 30% H2O2 were added to six containers, each contained 10 ml of stock solution and was kept at 800 C for about 3 H in water bath. The solution was allowed to attain ambient temperature. Then the solution was neutralised by sufficient amount of HCl and volume was made up to 100 ml with diluent to achieve final concentration of 100 µg/ml.

6.6 Thermal Degradation

10 mL of stock solution was kept at 800 C for one week. The solution was allowed to attain ambient temperature. Then volume was made up to 100 ml with diluent to achieve final concentration of 100 µg/ml.

6.7 Photo Degradation

10 mL of stock solution was exposed to white light for one week then volume was made up to 100 ml with diluent to achieve final concentration of 100 µg/ml.

Results and Discussions

1 System Suitability

System suitability is checking of a system to ensure system performance before or during the analysis of unknowns. To evaluate the system suitability of the developed RP-HPLC method, six replicates of the working standard solution were prepared and injected, each with an injection volume of 10 µL. System suitability parameters assessed included retention time, peak area,plate count (theoretical plates), resolution, and Tailing factor. validating a method for Pioglitazone and Glimepiride, system suitability criteria should include Resolution between both peaks ($R_s \geq 2$), Tailing factor (≤ 2), Theoretical plates (e.g., > 2000 for each analyte), Repeatability of peak area (%RSD ≤ 2%). The results given in Table 1 were within acceptable limits (FDA 1994).

2 Method Validation

Specificity refers to the ability of the analytical method to unequivocally assess the analyte in the presence of components such as impurities, degradation products, excipients, or placebo. In this study, specificity was evaluated by analysing blank, placebo, and standard solutions under the optimized chromatographic conditions. No interfering peaks were observed at the retention times corresponding to Pioglitazone and Glimepiride in the chromatograms of the blank and placebo samples (Fig 1, 2, 3 and 4). This indicates that the method can clearly distinguish the analytes from other formulation components. Therefore, the method is specific for the estimation of Pioglitazone and Glimepiride.

Also, Degradation studies showed that the degradation products did not interfere with the detection of Pioglitazone and Glimepiride.

2.1 Accuracy

Accuracy of the method was evaluated using the standard addition method by spiking known quantities of the standard solution into the sample matrix at three concentration levels: 50%, 100%, and 150% of the target concentration. Each level was analysed in triplicate to ensure reliability.The percentage recovery was calculated for each level to determine how closely the measured values matched the true values. The results demonstrated that the % recovery for both PioglitazoneandGlimepiride was within the acceptable range of 95–105%, indicating that the method is accurate and free from matrix interference. (Table 2)

2.2 Precision

The precision of the analytical method was evaluated by performing repeatability (intra-day precision). Six replicate injections of a standard solution containing 50 µg/mL of Pioglitazone and 50 µg/mL of Glimepiride were analysed under the optimized HPLC conditions. The % Relative Standard Deviation (%RSD) of the peak areas was calculated for each drug to assess the method's consistency. The %RSD for both Pioglitazone and Glimepiride was found to be less than 2.0%, which is within the acceptable limit, demonstrating that the method is precise and capable of producing reproducible results. (Table 3)

2.3 Linearity and Range

The linearity of measurement was evaluated by analysing different concentrations of standard solutions of Pioglitazoneand Glimepiride.Calibration curves were constructed by plotting average peak area against concentration. The summary of Linearity parameters is shown in Table 4 and Fig 5 &6. As demonstrated in Table 4 the calibration curves cover concentrations of 15 to 90 µg/mlfor Pioglitazone and 1 to 6µg/ml for Glimepiride. (Table 4)

2.4 Robustness

The robustness of the developed RP-HPLC method was evaluated by making small, deliberate variations in critical chromatographic parameters, including flow rate, columntemperature, and mobile phase composition. Specifically, the flow rate was adjusted to 0.8mL/min (−0.2 mL/min) and 1.2 mL/min (+0.2 mL/min), column temperature was varied by ±2°C from the optimized condition, and the mobile phase composition was altered by ± 5 -10 % organic solvent proportion. (Table 5)

2.5 Detection Limit and Quantification Limit

Ratios of 3:1 and 10:1 signal to noise were considered acceptable for estimation of Detection limit and Quantification limit.The Limit of Detection (LOD) and Limit of Quantification (LOQ) were determined based on the standard deviation of the response (σ) and the slope (S) of the calibration curve, in accordance with ICH Q2(R1) guidelines. The LOD for Pioglitazone and Glimepirideis0.0231 µg/mL and 0.01196µg/mL.The LOQ is 0.0701 µg/mL and 0.03623 µg/mL respectively.

2.6 Analysis of Marketed Products

The validated method was applied for the analysis of Pioglitazone and Glimepiride from two different manufacturers. In both cases assay obtained weremore than 99%. (Table 5)

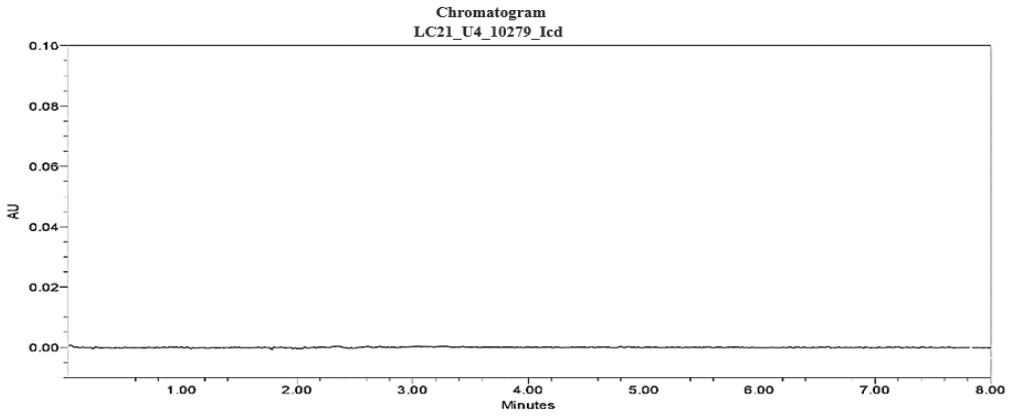

Figure 1: Blank Solution Chromatogram

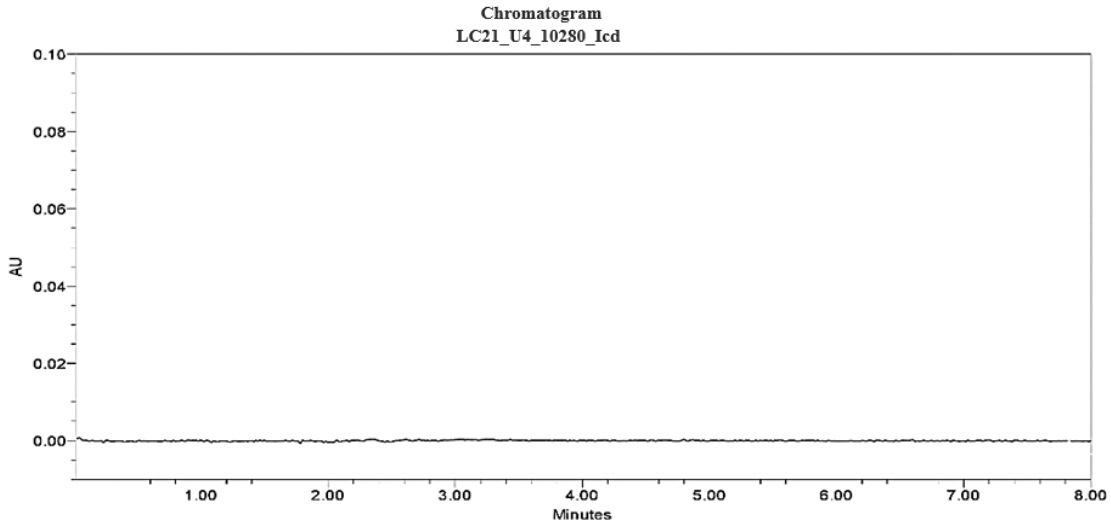

Figure 2: Placebo Solution Chromatogram

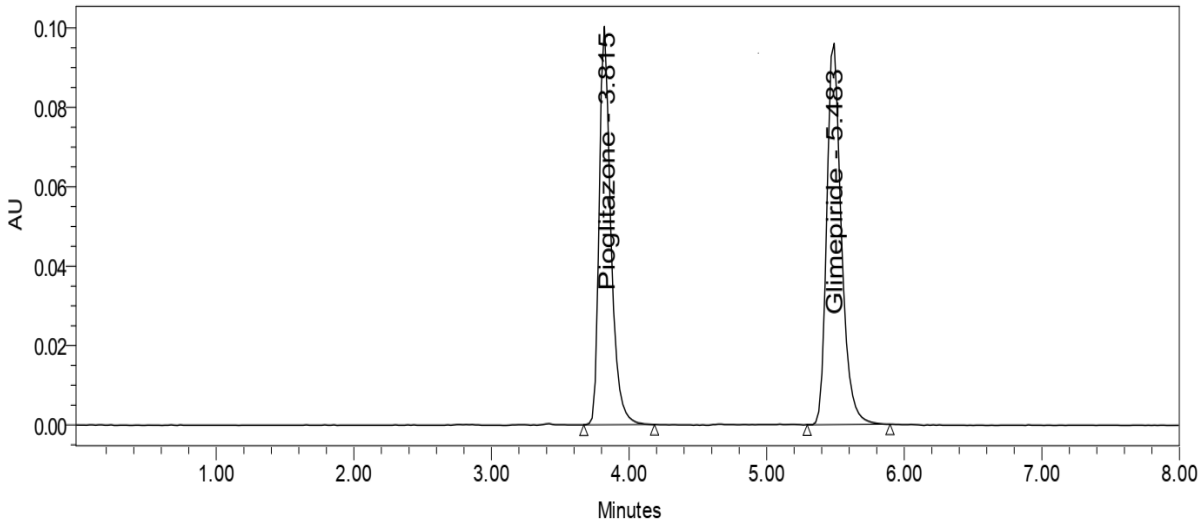

Figure 3: Standard Solution Chromatogram

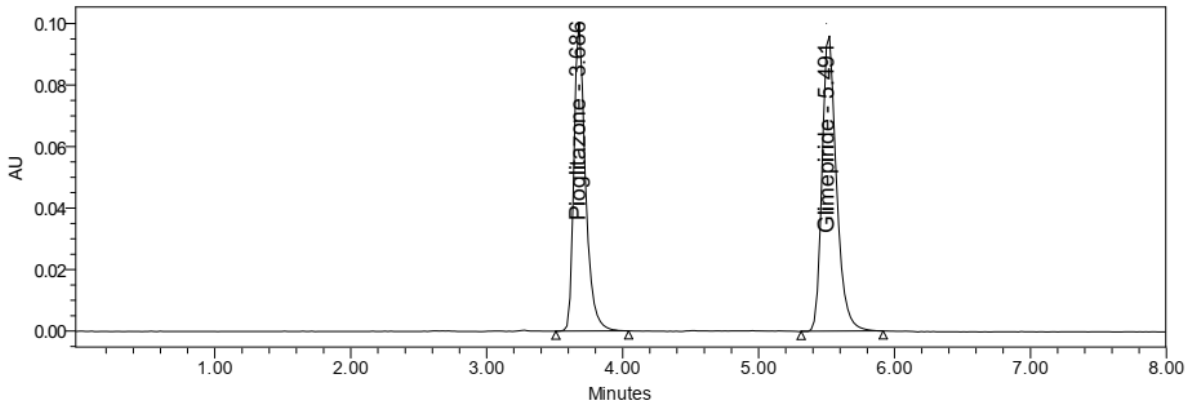

Figure 4: Sample Solution Chromatogram of Marketed Formulation

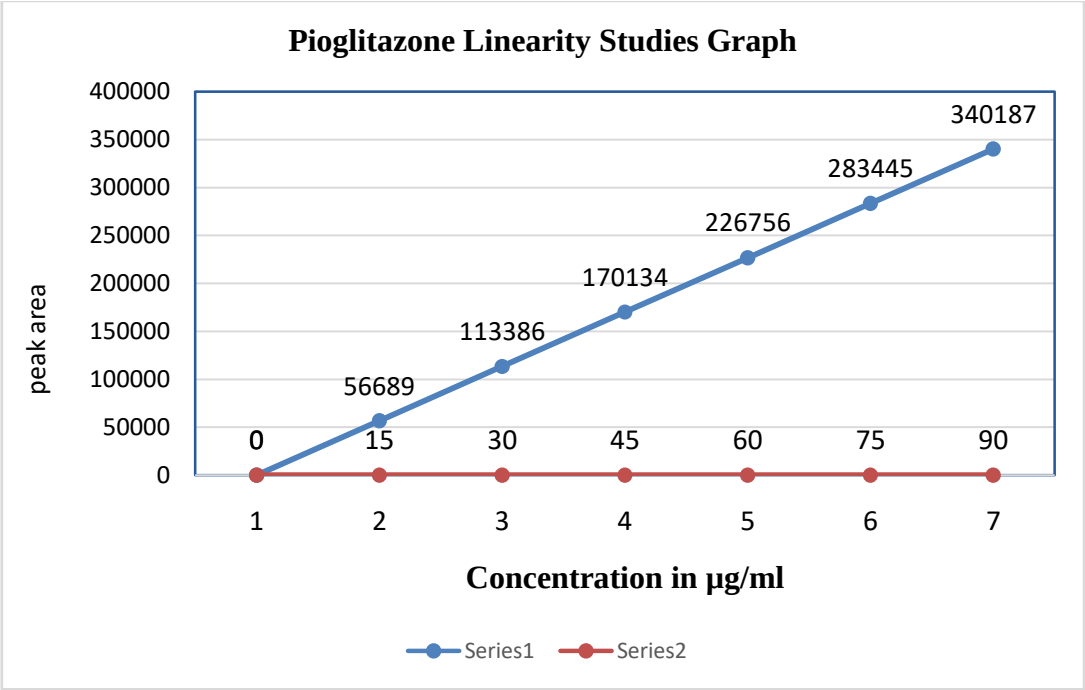

Figure 5: Linearity of Pioglitazone

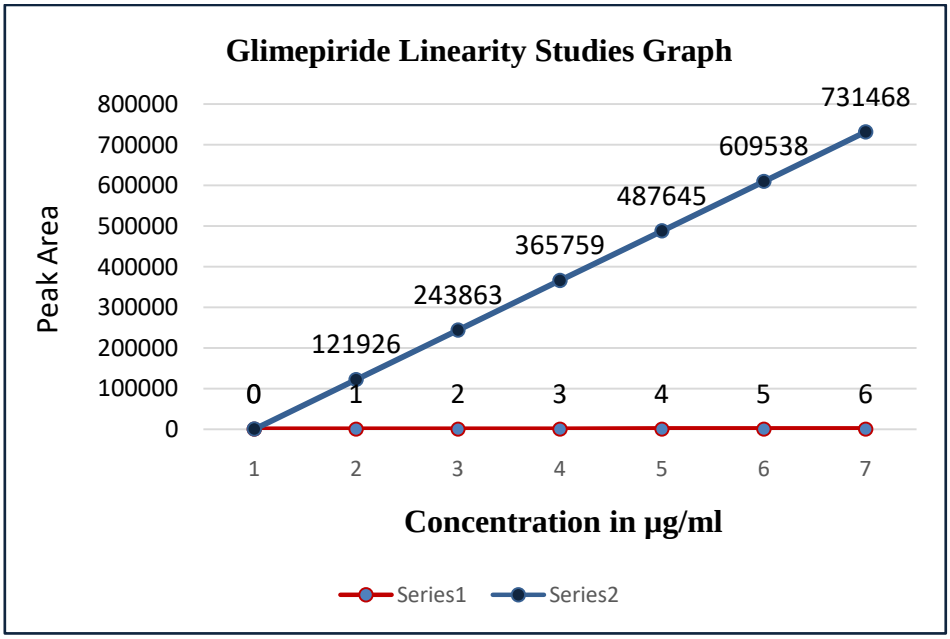

Figure 6: Linearity of Glimepride

Table 1: System Suitability Data

	Pioglitazone				Glimepiride			
Solutions	**Rt (Mint)**	**Peak Area**	**Plate Count**	**Asymmetry**	**RT(Mint)**	**Area**	**Plate Count**	**Asymmetry**
Std Sol injection-1	3.824	486327	6593.46	1.06	5.493	685862	8584.81	1.12
Std Sol injection-2	3.803	486758	6588.34	1.02	5.486	686022	8546.76	1.13
Std Sol injection-3	3.797	486566	6575.38	1.04	5.482	686153	8566.35	1.04
Std Sol injection-4	3.812	486826	6587.26	1.13	5.491	686256	8549.69	1.03
Std Sol injection-5	3.806	486834	6579.38	1.08	5.480	686781	8569.75	1.04
Std Sol injection-6	3.815	486908	6559.85	1.21	5.483	686709	8556.66	1.05
Mean	3.8095	487036.5	-	-	5.4858	686297.17	-	-
Standard deviation	0.00957	424.85	-	-	0.0052	371.83	-	-
% RSD	0.2512%	0.0873%	-	-	0.095%	0.0542%	-	-

Table 2: Accuracy data

% of Spiked Level Concentration	**Pioglitazone**			**Glimepiride**		
	Amount Added (µg/mL)	**% Recovery**	**% RSD & Mean Recovery**	**Amount Added (µg/mL)**	**% Recovery**	**% RSD & Mean Recovery**
50% - 1	15	100.8534	Mean - 100.3838 % RSD - 0.8498	1	99.6716	Mean- 100.0345 % RSD - 0.312%
50% - 2	15	99.9895		1	100.2528	
50% - 3	15	100.3084		1	100.179	
100% -1	30	99.6627	Mean - 99.9408 %RSD - 0.2441	2	100.0325	Mean - 100.0853 %RSD - 0.122
100% - 2	30	100.0418		2	99.9897	
100% - 3	30	100.1180		2	100.0362	
150% -1	60	99.97923	Mean - 100.2639 %RSD - 0.2360	3	100.0083	Mean - 99.9993 % RSD - 0.062
150% - 2	60	100.419		3	99.9326	
150% - 3	60	100.3935		3	100.0569	

Table 3: System Precision results

Pioglitazone					Glimepiride			
Solution	**RT**	**Area**	**Tailing Factor**	**Theoretical Plates**	**Solution**	**RT**	**Area**	**Tailing Factor**
Std Sol inj-1	3.686	685761	1.05	6859.26	5.494	9756841	1.25	7895.36
Std Sol inj - 2	3.681	685664	1.02	6985.23	5.491	9756856	1.23	7886.25
Std Sol inj - 3	3.684	685656	1.01	6985.37	5.487	9756884	1.25	7894.87
Std Sol inj - 4	3.687	685765	1.01	6875.44	5.490	9756877	1.26	7875.64
Std Sol inj - 5	3.674	685784	1.03	6878.49	5.493	9756819	1.24	7886.36
Std Sol inj - 6	3.665	685783	1.04	6885.36	5.495	9756877	1.23	7898.28
Mean	3.6795	686402.17	1.0267	6911.52	5.4917	9757025.67	1.2433	7889.46
Standard deviation	0.0088	52.78	0.0152	53.16	0.0027	183.87	0.0121	8.40
%RSD	0.239 %	0.0077 %	1.48%	0.77%	0.049%	0.00188 %	0.97%	0.106%
Bracketing STD	3.6843	686608	1.025	6857.81	5.484	9757157	1.20	7887.58

Table 4 - Linearity, LOD and LOQ data

Pioglitazone		Glimepiride	
Concentration (ppm)	***Peak Area**	**Concentration (ppm)**	***Peak Area**
0	0	0	0
15	56689	1	121926
30	113386	2	243863
45	170134	3	365759
60	226756	4	487645
75	283445	5	609538
90	340187	6	731468
y :	2.1071 + 3779.6262	20.2143 + 4876.3	
R^2	1.000	1.000	
Slope	3779.6262	4876.30	

Correlation (R)	Equals :1.0000	Equals :1.0000
SD:	26.48	17.67
%RSD:	0.0156%	0.00483%
LOQ	0.0701 (µg/mL)	0.03623 µg/mL
LOD	0.0231 µg/mL	0.01196 µg/mL

Table 5: Results of assay in tablet dosage form

Formulation	Label Claim(mg)	% Assay*
ZOLIGET	Pioglitazone 30 mg	100.021%w/w
	Glimepiride 2mg	100.061%w/w

Conclusion

A novel stability-indicating RP-HPLC method was developed and validated for the simultaneous quantification of Pioglitazone and Glimepiride in pharmaceutical dosage forms, in accordance with ICH guidelines. The method is simple, cost-effective, selective, precise, accurate, and suitable for routine quality control analysis

References

1. https://go.drugbank.com/drugs/DB01132
2. https://go.drugbank.com/drugs/DB00222
3. MadhaviLatha P., UmaSai Chaitanya A., BollaNagamani, Sivalalitha P., Uma Devi P.Method Development and Validation for the Simultaneous Estimation of Pioglitazone and Glimepiride in Bulk and Pharmaceutical Dosage Forms by RP-HPLC Zenodo (2024).
4. Dhani R., Guptha HKDR, Rajamanickam D. Earth-friendly Micellar UPLC Technique for Determination of Four Hypoglycemic Drugs in Different Pharmaceutical Dosage Forms and Spiked Human Plasma BMC Chemistry (2023)
5. Pandit V., Pai R.S., Devi K., Singh G., Narayana S., Suresh S. Development and Validation of the Liquid Chromatographic Method for Simultaneous Estimation of Metformin, Pioglitazone, and Glimepiride in Pharmaceutical Dosage FormsPharm Methods (2012).
6. Ramathilagam N, Solairaj P. Method Development and Validation of RP-HPLC Method for Glimepiride, Pioglitazone HCl, and Metformin HCl in Tablets. International Journal of Pharmaceutical and Analytical Research. 2014;3(1):46–54. DOI: 10.61096/ijpar.v3.iss1.2014.46-54.
7. Kande T, Barge V, Bhosale A, Khatal S, Dhekale P. Development and Validation of Analytical Methods for Simultaneous Estimation of Pioglitazone and Glimepiride in Bulk and Pharmaceutical Dosage Form by Using RP-HPLC Method. International Journal of Current Advanced Research. 2019;8(1):20129–20132.
8. Venkateswara Rao B, Vijetha P, Vidyadhara S, Kavitha K.A Novel RP-HPLC Method Development and Validation for the Determination of Pioglitazone and Glimepiride in Bulk and Pharmaceutical Formulations. Asian Journal of Pharmaceutical Analysis. 2017;7(3):145–150
9. Pawar J, Sonawane S, Chhajed S, Kshirsagar S, Wagh M.Development and Validation of RP-HPLC Method for Simultaneous Estimation of Pioglitazone HCl and Glimepiride in Tablets. International Journal for Pharmaceutical Research Scholars (IJPRS). 2016;5(2):167–172.
10. Sane RT, Menon SN, Inamdar S, Mote M, Gundi G. Simultaneous Determination of Pioglitazone and Glimepiride by High-Performance Liquid Chromatography. Chromatographia. 2004;59(7–8):451–453. DOI: 10.1365/s10337-004-0209-9
11. KS Nataraj, A Srinivasa Rao, KV Nagamani, P Divya and B Renuka Chandrasekhar. Analytical method development & validation of metformin, pioglitazone & glimepiride by RP-HPLC in tablet dosage forms. The Pharma Innovation Journal. 2019, 8(2), 266-273.
12. Simultaneous Estimation of Metformin Hydrochloride, Pioglitazone Hydrochloride, and Glimepiride by RP-HPLC in tablet formulation. Deepti Jain, Surendra Jain,Deepak Jain, Maulik Amin. Journal of Chromatographic Science. 2008, 46(6), 501-504.
13. MadhuriD.Game, KoshishB.Gabhane, Dinesh M. Sakarkar. Simultaneous Spectrophotometric Estimation of Glimepiride and Pioglitazone HCl in Combined Dosage Form using

Absorbance Correction Method. Asian J. Research Chem. 2011, 4(12), 1851- 1853.

14. R.Tsane,S.N.Menon, ShafiInamdar, Mandar mote and Gunesh gundi. Estimatedsimultaneous determination of pioglitazone and glimepiride by High Performance Liquid Chromatography. 2004, 59, 451-453.