**IJPSR (2022), Volume 13, Issue 5** **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 31 July 2021; received in revised form, 04 September 2021 accepted, 09 September 2021; published 01 May 2022

# QUALITY BY DESIGN (QBD) APPROACH TO DEVELOP STABILITY-INDICATING RP-HPLC METHOD DEVELOPMENT FOR PIOGLITAZONE AND GLIMEPIRIDE

Saurabh Chaudhari [* 1], Sufiyan Ahmad [1], A. U. Tatiya [1] and Bakhshi Abdul Rahman [2]

Gangamai College of Pharmacy [1], Nagaon, Dist. Dhule - 424005, Maharashtra, India.
Royal College of Pharmaceutical Education and Research [2], Sayne Khurd, Malegaon Dist: Nashik - 423203, Maharashtra, India.

**Keywords:**

Pioglitazone, Glimepiride, QbD, RP-HPLC, Stability Study, Method development, Validation

**Correspondence to Author:**
**Mr. Chaudhari Saurabh**

Research Scholar,
Gangamai College of Pharmacy,
Nagaon, Dist. Dhule - 424005,
Maharashtra, India.

**E-mail:** sufimpharm@rediffmail.com

**ABSTRACT: Background and Objectives:** A sper requisition of current regulatory requirements, a simple, rapid, and sensitive method by 33 factorial QbD approach was established and validated for Pioglitazone (PGZ) Glimepiride (GPR) by RP-HPLC. **Method:** A simple RP-HPLC method has been developed and validated with different parameters such as linearity, precision, repeatability, LOD, LOQ, accuracy as per International Conference for Harmonisation guidelines (Q2R1). Statistical data analysis was done for data obtained from different aliquots Runs on Agilent Tech. Gradient System with Autoinjector, UV (DAD) & Gradient Detector. Results: Equipped with Reverse Phase (Agilent) C18 column (4.6 mm × 100 mm; 2.5µm), a 20µl injection loop and UV730D Absorbance detector at 231nm wavelength and running chemstation 10.1 software and drugs along with degradants were separated *via* **Methanol:** (0.1% OPA) Water (70:30) of pH 3.2 as mobile phase setting flow rate 0.7 ml/min at ambient temperature. The developed method was found linear over the concentration range of 15-75 µg/ml for PGZ and 2-10 µg/ml for GPR, while detection and quantitation limit was found to be 1.39 µg/ml and 0.28 µg/ml as LOD and 3.85 µg/ml and 0.77 µg/ml respectively for PGZ and GPR. **Conclusion:** There are no interfering peaks underperformed degradation conditions. Therefore, a sensitive, robust, accurate, and stability-indicating method was developed with a high degree of practical utility.

**INTRODUCTION:** The concept of "Quality by Design" (QbD) was defined as an approach that covers a better scientific understanding of the critical process and product qualities, designing controls and tests based on the scientific limits of understanding during the development phase and using the knowledge obtained during the life-cycle of the product to work on a constant improvement environment. QbD describes a pharmaceutical development approach referring to formulation design and development and manufacturing processes to maintain the prescribed product quality.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.13(5).2029-38

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.13(5).2029-38

Guidelines and mathematical models are used to establish and use the knowledge on the subject in an independent and integrated way [1, 2, 3]. Pioglitazone chemically, 5-[[4-[2-(5-ethyl pyridine-

2-yl) ethoxy] phenyl] methyl]-1, 3-thiazolidine-2, 4-dione; **Fig. 1.** hydrochloride is a selective agonist at peroxisome proliferator-activated receptor-gamma (PPARγ) in target tissues for insulin action such as adipose tissue, skeletal muscle, and liver. Activation of PPARγ increases the transcription of insulin-responsive genes involved in the control of glucose and lipid production, transport and utilization. Through this mechanism, pioglitazone enhances tissue sensitivity to insulin and reduces the hepatic production of glucose (*i.e.* gluconeogenesis)-insulin resistance associated with type 2 diabetes mellitus is improved without an increase in insulin secretion by pancreatic beta cells. Enhances cellular responsiveness to insulin, increases insulin-dependent glucose disposal, and improves impaired glucose homeostasis [4].

Glimepiride chemically is 4- ethyl-3- methyl- N-[2-[4-[(4- methyl cyclohexyl) carbamoyl sulfamoyl] phenyl] ethyl]-5-oxo-2 H-pyrrole-1-carboxamide **Fig. 1.** pancreatic beta cells, ATP-sensitive potassium channels play a role as essential metabolic sensors and regulators that couple membrane excitability with glucose-stimulated insulin secretion (GSIS), thus shows Anti-diabetic activity *via* polarization and depolarization [5].

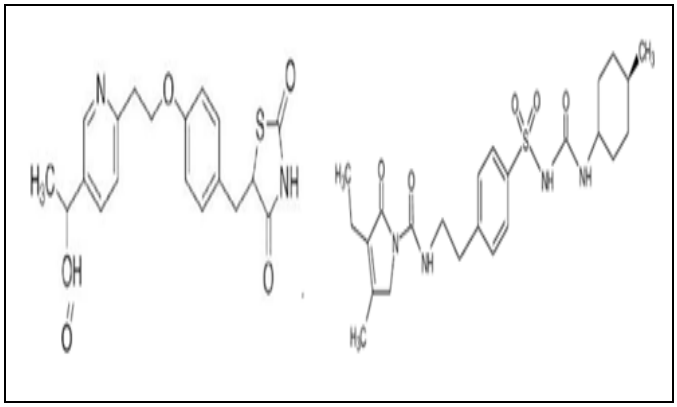

**FIG. 1: STRUCTURE OF PIOGLITAZONE AND GLIMEPIRIDE**

Literature surveys revealed that sensitive LC-MS methods are available for analysis of antidiabetic drugs and its metabolites in human plasma and urine [6, 11]. Several HPLC methods have been developed individually and combined dosage forms in human plasma [12, 13, 14]. Even though various methods were reported in the literature for estimation of metformin, glimepiride and pioglitazone alone and in combination with other drugs 15-20 no method has been reported for simultaneous estimation of these drugs in combination using QbD based 33 factorial designing.

**Chemicals and Reagents:** Reference standards of Pioglitazone hydrochloride were obtained as a gift sample from Dr. Reddy's Laboratories, Hyderabad, India, while Glimepiride was obtained as a generous gift from Micro Labs Ltd., Bangalore, India. The pharmaceutical formulation was purchased from the local market (Brand: Adride-P tablet labelled claim Pioglitazone 15 and Glimepiride 2 mg make Mankind Pharmaceuticals). The HPLC grade solvents used were of E-Merck (India) Ltd., Mumbai. HPLC grade Acetonitrile, Methanol, and Ortho Phosphoric Acid (Merck, Mumbai, India) were used in the analysis. HPLC grade water was prepared using a Millipore purification system.

**Instruments:** The analysis of the drug was carried out on Agilent Tech. Gradient System with an Auto injector, UV (DAD) & Gradient Detector. Equipped with Reverse Phase (Agilent) C18 column (4.6 mm × 100 mm; 2.5µm), a 20µl injection loop, and UV730D Absorbance detector and running chemstation 10.1 software.

**RP-HPLC Optimised Chromatographic Condition using QbD:** Column C18 (100 mm×4.6mm); particle size packing 5µm; detection wavelength 231 nm; flow rate 0.7 ml/min; temperature 260 ºC ambient; sample size 20 µl; mobile phase methanol: water (OPA 0.1% PH 3.2) (70:30); run time 15 min. The retention time for Pioglitazone and Glimepiride was found at 2.9333 min and 6.9667 min, respectively **Fig. 2.**

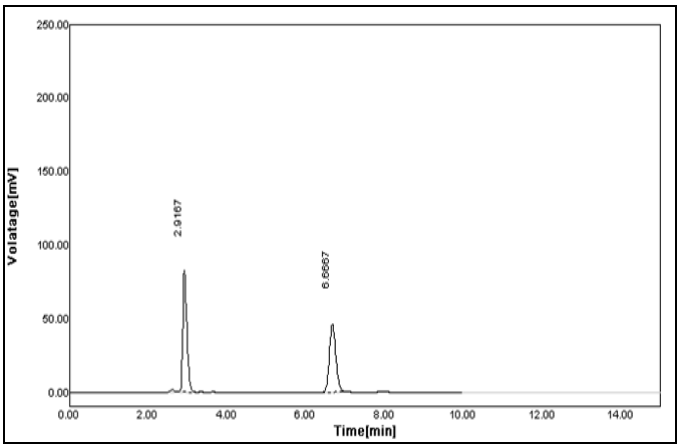


**FIG. 2: CHROMATOGRAM OF STANDARD PGZ AND GPR AT 231 NM**

**Preparation of Standard Solution:** All solutions were prepared on a weight basis. Solution concentrations were also measured on weight basis to avoid using an internal standard Pharmaceutical formulation available in the market in the proportion of 2:15.

**Stock Preparations:** Standard stock solution was prepared by dissolving 15 mg PGZ and 2 mg GPR in 10 ml clean dry volumetric flask, and dilution was up to the mark with Methanol to obtain the final concentration of PGZ (1500 µg/ml) and GPR (200 µg/ml). All the stock solutions were filtered through a 0.45 µm membrane filter.

**Detection of $\lambda_{max}$:** The sample solution has been prepared and scanned in the UV region of 200-400 nm and the spectrum showed the maximum absorbance at 231 nm **Fig. 3.**

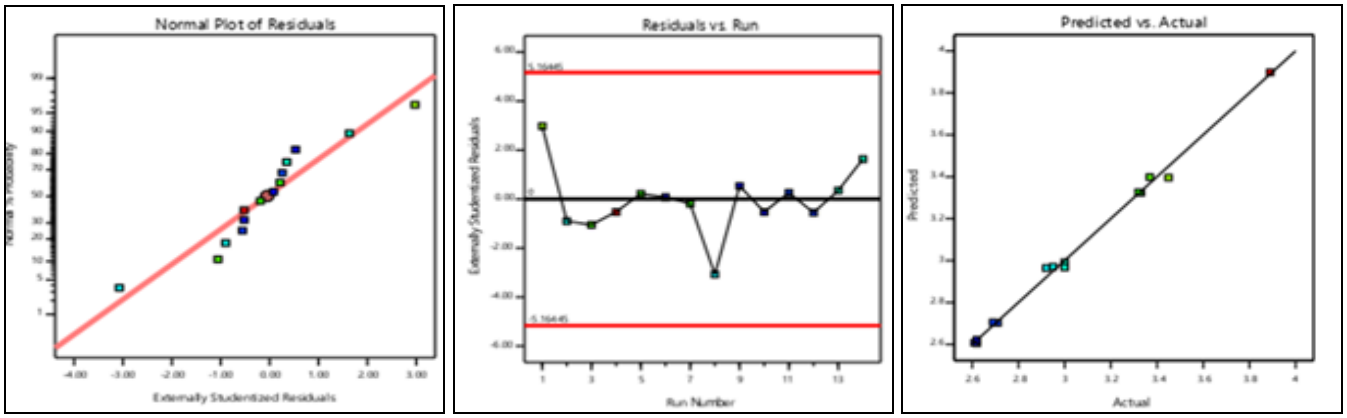


**FIG. 3A: NORMAL PLOT OF RESIDUALS FOR RETENTION TIME AND PLOT OF PREDICTED VS. ACTUAL DATA FOR RETENTION TIME OF CP**

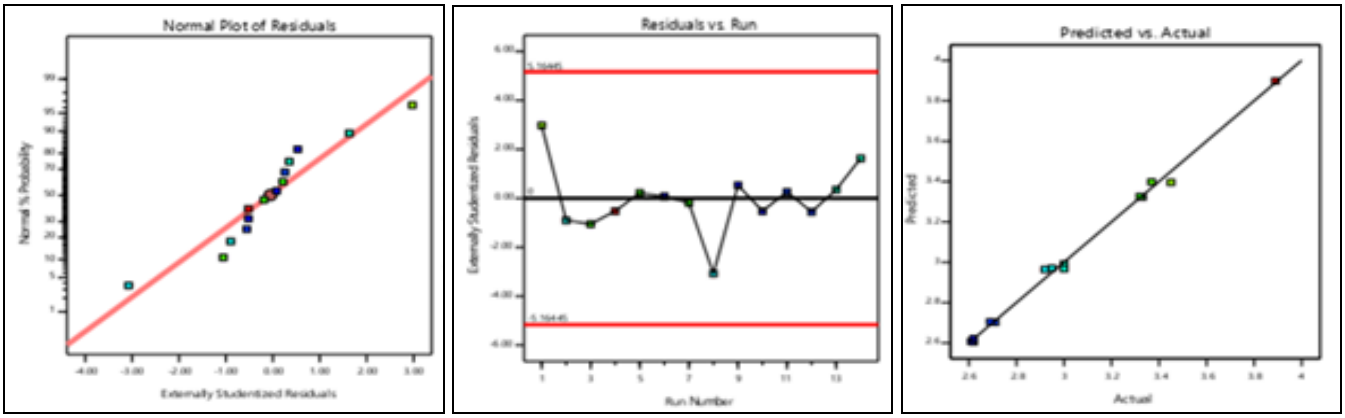


**FIG. 3B: NORMAL PLOT OF RESIDUALS FOR RETENTION TIME AND PLOT OF PREDICTED VS. ACTUAL DATA FOR RETENTION TIME BY THE VALUE OF 2.61 TO 3.89**

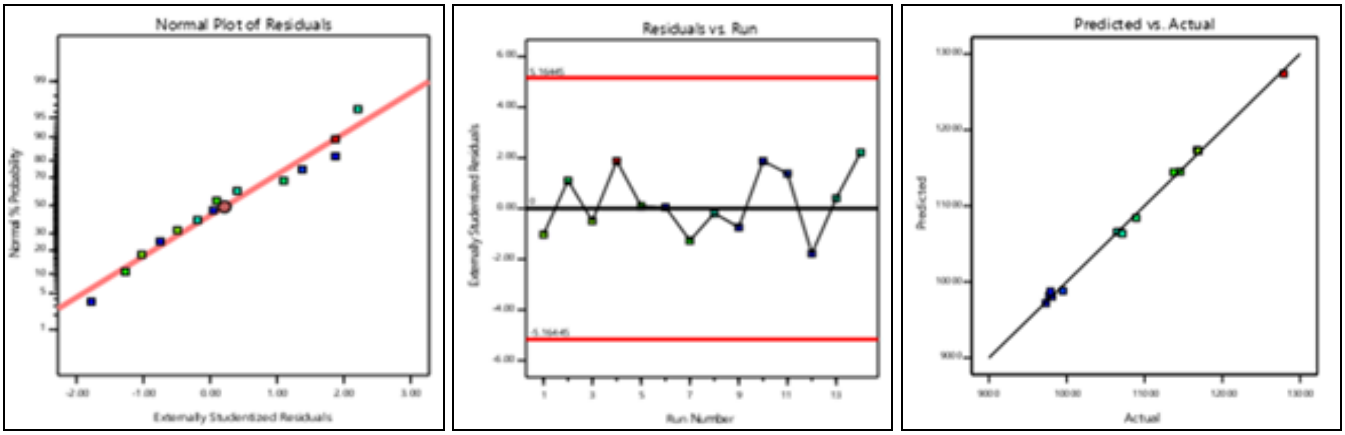


**FIG. 3C: NORMAL PLOT OF RESIDUALS FOR RETENTION TIME AND PLOT OF PREDICTED VS. ACTUAL DATA FOR RETENTION TIME BY THE VALUE OF 9733 TO 12789**

**Qbd Approach to Analysis:** The application of QbD in HPLC method development commences with establishing analytical objectives based on sound science to ensure consistent method performance characteristics are achieved [21]. The use of QbD for an analytical method commences with defining the target analytical profile in which the pre-defined objectives for method performance must be appropriately validated and documented [22, 23]. Thus the objective of this work was to perform experimental design by using Design Expert Software leading to develop a simple, rapid, and sensitive method by QbD approach and validated as per ICH Guidelines (Q2R1) for pioglitazone and Glimepiride and its stability-indicating method by RP-HPLC. Further statistical data analysis is to be done along with numerical and graphical optimization to develop Analytical Design Space.

**Method Validation:**

**Calibration Curve:** A calibration curve was constructed succeeding replicate (n=6) analysis of five standards of 15, 30, 45, 60, 75 µg/ml of PGZ and 2, 4, 6, 8, 10 µg/ml of GPR. The peak height ratio of drugs was calculated and plotted AUC versus concentration, after which least-squares linear regression analysis of data was undertaken to establish the equation for the best fit line and the correlation coefficient (R2) to authorize linearity. Samples were injected, and peaks were recorded at 231 nm, and the graph plotted as the concentration of drug versus peak area as shown in **Table 1.**

**TABLE 1: LINEARITY STUDY**

| PGZ | | | GPR | | |
|---|---|---|---|---|---|
| **Conc. [µg/ml]** | **Mean peak area ± SD [n=5]** | **%RSD** | **Conc. [µg/ml]** | **Mean peak area ± SD [n=5]** | **%RSD** |
| 15 | 208.8±3.70 | 1.77 | 2 | 173.6±3.05 | 1.76 |
| 30 | 412.2±6.87 | 1.67 | 4 | 311.4±5.98 | 1.92 |
| 45 | 619.4±5.81 | 0.94 | 6 | 453.0±7.28 | 1.61 |
| 60 | 823.2±7.12 | 0.86 | 8 | 571.6±4.83 | 0.84 |
| 75 | 1055.4±9.34 | 0.89 | 10 | 729.6±5.90 | 0.81 |

**Precision:** Intra-day (repeatability) precision was established following analysis of replicate samples (n=6) at three concentrations indicative of low, medium and high levels within the linear range *viz.*, 30, 45, 60, 75 µg/ml of PGZ and 4, 6, 8 µg/ml of GPR. Analysis was performed over a short period of time on the same day. Inter-day precision or reproducibility was assessed at low, medium and high concentrations on three consecutive days and the percent relative standard deviation (% RSD) was used to assess intra- and inter-day precision. An upper limit of 2% was used to confirm precision in our laboratory. The precision of an analytical method is usually expressed as standard deviation or relative standard deviation. **Table 2** and 3 describes the Intraday, Inter day and repeatability of the method.

**TABLE 2: RESULTS OF PRECISION STUDIES (INTRA-DAY AND INTER-DAY)**

| Drug | Conc. [µg/ml] | Intraday Amount Found [µg/ml] | | Inter day Amount Found [µg/ml] | |
|---|---|---|---|---|---|
| | | Mean ± S.D. | % RSD [n= 3] | Mean ±S.D. | % RSD [n= 3] |
| PGZ | 30 | 29.87 ±4.16 | 0.34 | 29.51 ± 8.50 | 0.69 |
| | 45 | 44.37±10.21 | 0.55 | 44.77±7.64 | 0.41 |
| | 60 | 59.50±6.66 | 0.27 | 59.50±9.45 | 0.38 |
| GPR | 4 | 3.77±2.00 | 0.29 | 4.13±2.00 | 0.28 |
| | 6 | 6.25±3.06 | 0.27 | 6.09±5.57 | 0.50 |
| | 8 | 8.39±5.51 | 0.35 | 7.65±5.03 | 0.34 |

**TABLE 3: RESULTS OF REPEATABILITY STUDY**

| Drug | Concentration [µg/ml] [n=6] | Peak Area | Mean [µg/ml] ± SD | % RSD |
|---|---|---|---|---|
| PGZ | 45 | 622.833 | 45.15 ± 0.94 | 1.26 |
| GPR | 6 | 377.677 | 6.25 ± 0.255 | 1.677 |

**Accuracy:** Recovery studies were performed to validate the accuracy of developed method. To pre-analyze tablet solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed. Statistical validation of recovery studies is shown in **Table 4.**

**TABLE 4: RESULTS OF RECOVERY STUDIES**

| Drug | Initial amount [µg/ml] | Amount added [µg/ml] | Amt. recovered ± S.D. [µg/ml, n =3] | % Recovery | % RSD |
|---|---|---|---|---|---|
| PGZ | 45 | 0 | 45.29 ±0.67 | 100.39 | 0.89 |
| | 45 | 36 | 80.89 ±0.89 | 99.81 | 1.49 |
| | 45 | 45 | 89.69 ±1.09 | 99.58 | 1.45 |
| | 45 | 99 | 144.49 ±1.28 | 100.55 | 1.42 |
| GPR | 6 | 0 | 6.15 ±0.27 | 100.99 | 1.83 |
| | 6 | 4.8 | 10.87 ±0.20 | 100.57 | 1.67 |
| | 6 | 6 | 12.08 ±0.24 | 100.51 | 1.58 |
| | 6 | 7.2 | 12.84 ±0.18 | 99.11 | 1.02 |

**Limits of Detection (LOD) and Quantitation (LOQ):** Several approaches for the calculation of the LOD and LOQ of a method have been suggested in different guidelines and include visual evaluation, use of signal-to-noise (S/N) ratio, calculations based on a standard deviation of response and the slope of the calibration curve [24]. By convention, the LOD is estimated as one-third of the LOQ.

A series of samples of 15, 20, 25, 30 µg/ml of PGZ and 2, 2.7, 3.4 and 4 µg/ml of GPR were prepared and analyzed using the optimized RP-HPLC method and the peak height ratio calculated.

The LOQ was determined by establishing the lowest concentration of drugs that resulted in a % RSD value for the precision of < 2%.

**Specificity:** The specificity of an analytical method is defined as the ability to ensure that the peak(s) of interest elute as distinct responses in the presence of excipients, impurities, or degradation compounds.

**Robustness:** To evaluate robustness, few parameters were deliberately varied. The parameters include a variation of flow rate, percentage of methanol as described in **Table 5.**

**TABLE 5: ROBUSTNESS EVALUATION OF THE HPLC METHOD**

| Chromatographic conditions | PGZ | | | GPR | | |
|---|---|---|---|---|---|---|
| | Tailing (T') | Capacity Factor (K') | Theoretical Plate (N) | Tailing (T') | Capacity Factor (K') | Theoretical Plate (N) |
| **A: Mobile phase pH** | | | | | | |
| 3.0 | 1.26 | 1.23 | 2683.9 | 1.28 | 0.99 | 7591.4 |
| 3.2 | 1.22 | 1.27 | 2683.5 | 1.23 | 1.09 | 7632.5 |
| 3. | 1.21 | 1.33 | 2625.5 | 1.25 | 1.15 | 7414.7 |
| Mean ±SD | 1.23±0.02 | 1.27±0.05 | 2687.63±36.80 | 1.25±0.02 | 1.07±0.02 | 7546.2±115.7 |
| **B: Flow rate (ml/min.)** | | | | | | |
| 0.5 ml | 1.23 | 0.98 | 2723.8 | 1.26 | 0.76 | 7587.3 |
| 0.7 ml | 1.16 | 1.08 | 2818.9 | 1.29 | 1.10 | 7668.8 |
| 1.0 ml | 1.15 | 1.09 | 2768.7 | 1.22 | 0.88 | 7423.5 |
| Mean ±SD | 1.18±0.04 | 1.05±0.06 | 2770.47±47.50 | 1.25±0.03 | 0.91±0.17 | 7593.2±75.82 |
| **C: Percentage methanol in mobile phase (v/v)** | | | | | | |
| 60 | 1.09 | 1.22 | 2646.2 | 1.18 | 0.87 | 7623.8 |
| 70 | 1.06 | 1.13 | 2687.4 | 0.94 | 0.95 | 7667.3 |
| 80 | 1.19 | 1.18 | 2638.3 | 1.23 | 0.87 | 7433.2 |
| Mean ±SD | 1.11±0.06 | 1.17±0.04 | 2657.3±26.36 | 1.11±0.15 | 0.89±0.04 | 7574±124.51 |

**TABLE 6: SYSTEM SUITABILITY TEST**

| PGZ | | GPR | |
|---|---|---|---|
| System suitability parameters | Proposed method | System suitability parameters | Proposed method |
| Retention time (Rt) | 2.9333 | Retention time (Rt) | 6.9167 |
| Capacity factor (K') | 1.18 | Capacity factor (K') | 0.98 |
| Theoretical plate (N) | 2838.7 | Theoretical plate (N) | 7465.8 |
| Tailing factor (T) | 1.16 | Tailing factor (T) | 0.95 |

**Study of System Suitability Parameters:** The system suitability is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. The test was performed by collecting data from five replicate injections of standard solution as shown in **Table 6.**

**Forced Degradation Studies:** Forced degradation study was performed to evaluate the stability of the developed method using the stress conditions like exposure of sample solution to acid, base, Hydrogen peroxides ($H_2O_2$) and Neutral. Investigation were done for the degradation products in different conditions and are shown in **Table 7.**

**Procedure for Pioglitazone and Glimepiride Degradation:**

**Acid Hydrolysis:** The acid hydrolysis performed using 0.1N HCl at 70 ºC for 1st hr and 2nd h for both Glimepiride and Pioglitazone indicated degradation. The major degradation products for Glimepiride and Pioglitazone were observed at relative retention time (RRT) for 1st and 2nd Hours.

**Alkaline Hydrolysis:** The alkaline hydrolysis condition was performed using 0.1N NaOH at 70 ºC for 1st h and 2nd h both Glimepiride and Pioglitazone. The major degradation products for Glimepiride and Pioglitazone were observed at relative retention time (RRT) for 1st and 2nd Hours.

**Oxidation:** In the oxidation condition with 3% $H_2O_2$ for 1st hr and 2nd hr both Glimepiride and Pioglitazone show oxidative stress degradation peak in the chromatogram.

**Neutral:** There was no major degradation observed for both Glimepiride and Pioglitazone and hence they were not sensitive to light at 70 ºC for 1st h and 2nd h.

**TABLE 7: FORCED DEGRADATION**

| Sample Exposure condition | Total Number of products with their Rt | PGZ | | GPR | |
|---|---|---|---|---|---|
| | | Degradation remained (150 µg/ml) | Recovery (%) | Degradation remained (30 µg/ml) | Recovery (%) |
| Acidic,1N, 1 Hr | 5 (2.95, 4.80, 6.05, 7.08, 7.65) | 136.224 | 90.81 | 28.25 | 94.18 |
| Basic, 1N, 1 Hr | 6 (2.61, 2.80, 2.95, 3.38, 4.51, 7.20) | 122.22 | 81.48 | 13.28 | 44.29 |
| Per oxide, 30%, 1 Hr | 4 (2.63, 2.83, 4.76, 7.03) | 128.50 | 85.67 | 20.92 | 69.73 |
| Heat, 50°C, 1 Hr | 3 (2.61,2.81,6.766) | 136.58 | 91.05 | 22.20 | 74.01 |

**Application of Analytical Method:** To determine the content of PGZ and GPR in marketed tablets (label claim 15 mg of Pioglitazone and 2 mg Glimepiride), 20 tablets powder weighed as 5.96 gm and average weight of powder was calculated in 0.298 gm. Tablets were triturated and powder equivalent to weighed in 298 mg. The drug was extracted from the tablet powder with 10 ml Methanol. To ensure complete extraction it was sonicated for 15 min. 0.1 ml of supernatant was then diluted up to 10 mL with mobile phase. The resulting solution was injected in HPLC and drug peak area was noted.

**RESULTS AND DISCUSSION:** Such analytical methods are, in fact, an indicator of a quality product and the robustness of that product for the duration on the lifecycle of that product. The main goal of any HPLC method is to separate and quantitate analyte(s) of interest from any impurity and/or excipients. Initially it is important to establish the critical quality attributes (CQA) of a system that may impact the quality of the analytical method. Development of Analytical RP-HPLC Method with Design Space and Control Strategy determination by optimization study all the computations for the current optimization study and statistical analysis were performed using Design Expert® software (Design Expert trial version). State-Ease Inc., Minneapolis, MN, USA).

**Application of Design of Experiments for Method Optimization Design of Experiments (DOE-1):** Thus, 3 randomized response surface designs with a full fraction design were used with 17 trial runs to study the impact of three factors on the three key response variables.

In this design 3 factors were evaluated, each at 3 levels, and experimental trials were performed at

all 3 possible combinations. The mobile phase composition (X1), Wavelength (X2) and flow rate (X3), were selected as independent variables and retention time (RT) and Resolution were selected as dependent variables.

The resulting data were fitted into Design Expert 10 Software and analyzed statistically using analysis of variance (ANOVA) and F-Test. **Fig. 3** indicates the normal plot of residuals for retention time with other chromatographic parameters.

The data were also subjected to 3-D response surface methodology to determine the influence of flow rate, wavelength and mobile phase composition on dependent variables as shown in **Fig. 4**. The probable trial runs using 33 full fraction designs are as shown in **Table 4.**

Further ANOVA and F-test with variables are shown in **Table 8-12.** More over degradation peaks of API were shown in **Fig. 5-8** from acidic, alkaline, peroxide and Heat.

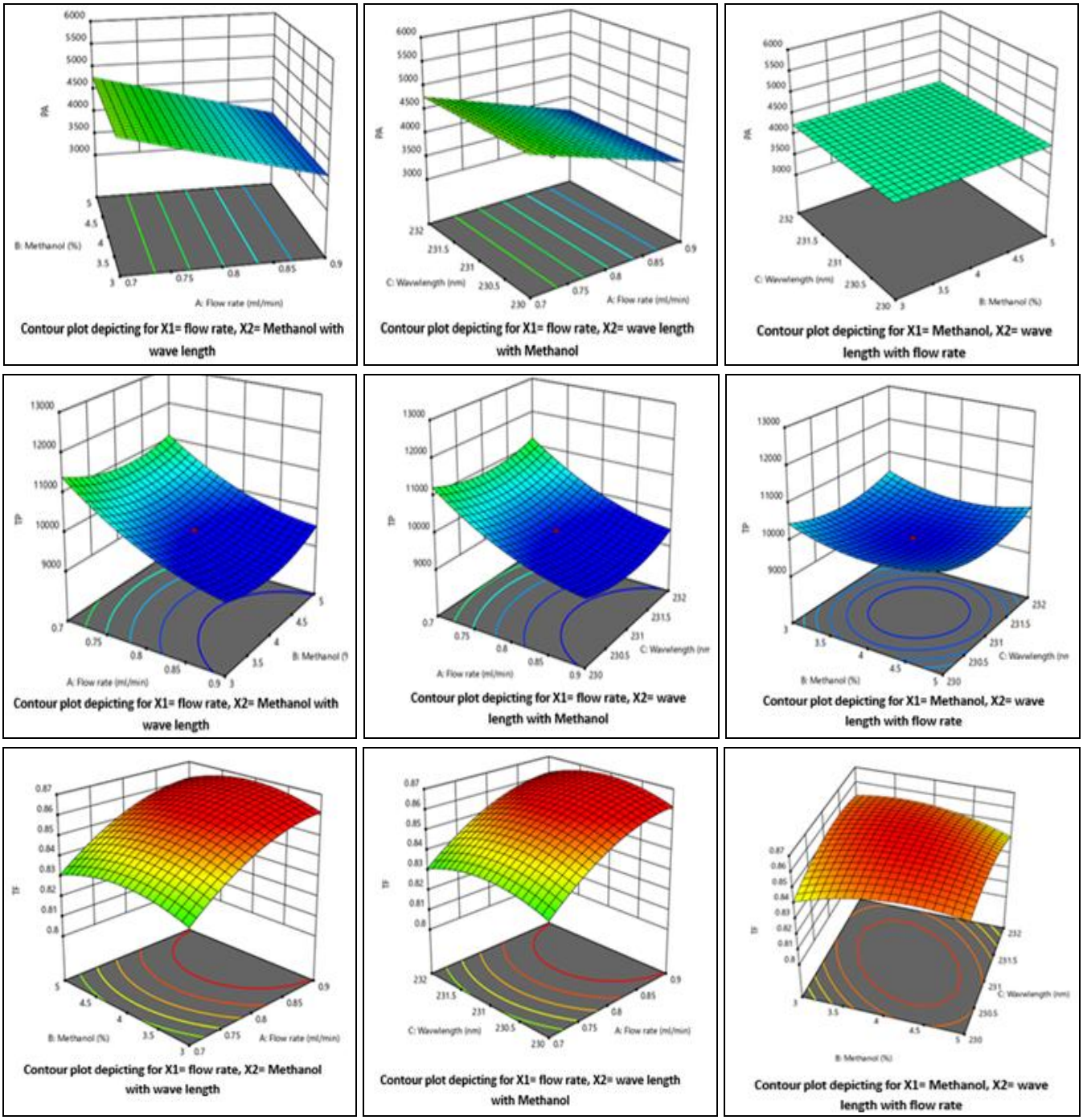


**FIG. 4: CONTOUR PLOT FOR FLOW RATE, MOBILE PHASE COMPOSITION AND WAVELENGTH**

**TABLE 8: PROBABLE TRIAL RUNS USING 33 FULL FRACTION DESIGNS**

| Std | Run | Factor 1 | Factor 2 | Factor 3 | Response 1 | Response 2 | Response 3 | Response 4 |
|---|---|---|---|---|---|---|---|---|
| | | A: Flow rate ml/min | B:Methanol % | C: Wavelength nm | RT | PA | TP | TF |
| 1 | 1 | 0.7 | 3 | 230 | 3.45 | 4850.37 | 11675 | 0.82 |
| 11 | 2 | 0.8 | 2.3 | 231 | 2.95 | 4019.71 | 10892 | 0.84 |
| 5 | 3 | 0.7 | 3 | 232 | 3.369 | 4521.28 | 11693 | 0.83 |
| 9 | 4 | 0.6 | 4 | 231 | 3.89 | 5516.24 | 12789 | 0.8 |
| 3 | 5 | 0.7 | 5 | 230 | 3.33 | 4896.5 | 11458 | 0.83 |
| 6 | 6 | 0.9 | 3 | 232 | 2.61 | 3555.04 | 9810 | 0.86 |
| 7 | 7 | 0.7 | 5 | 232 | 3.32 | 4665.06 | 11373 | 0.82 |
| 13 | 8 | 0.8 | 4 | 229.3 | 2.92 | 4373.36 | 10645 | 0.84 |
| 2 | 9 | 0.9 | 3 | 230 | 2.62 | 3755.37 | 9777 | 0.85 |
| 10 | 10 | 0.8 | 4 | 231 | 2.62 | 3707.75 | 9733 | 0.86 |
| 4 | 11 | 0.9 | 5 | 230 | 2.71 | 4018.88 | 9950 | 0.86 |
| 8 | 12 | 0.9 | 5 | 232 | 2.69 | 3785.56 | 9793 | 0.85 |
| 12 | 13 | 0.8 | 5.7 | 231 | 3 | 4326.7 | 10679 | 0.84 |
| 14 | 14 | 0.8 | 4 | 232.7 | 3 | 4484.22 | 10716 | 0.84 |

**TABLE 9: ANOVA FOR REDUCED QUADRATIC MODEL (RESPONSE 1: RT)**

| Source | Sum of Squares | df | Mean Square | F-value | p-value | |
|---|---|---|---|---|---|---|
| Model | 1.95 | 7 | 0.2783 | 211.69 | < 0.0001 | significant |
| A-Flow rate | 1.10 | 1 | 1.10 | 833.33 | < 0.0001 | |
| B-Methanol | 0.0005 | 1 | 0.0005 | 0.4083 | 0.5464 | |
| C-Wavelength | 0.0000 | 1 | 0.0000 | 0.0124 | 0.9149 | |
| AB | 0.0144 | 1 | 0.0144 | 10.93 | 0.0163 | |
| $A^2$ | 0.1479 | 1 | 0.1479 | 112.53 | < 0.0001 | |
| $B^2$ | 0.0882 | 1 | 0.0882 | 67.09 | 0.0002 | |
| $C^2$ | 0.0810 | 1 | 0.0810 | 61.60 | 0.0002 | |
| Residual | 0.0079 | 6 | 0.0013 | | | |
| Cor Total | 1.96 | 13 | | | | |

**TABLE 10: ANOVA FOR REDUCED LINEAR MODEL (RESPONSE 2: PA)**

| Source | Sum of Squares | df | Mean Square | F-value | p-value | |
|---|---|---|---|---|---|---|
| Model | 3.294E+06 | 1 | 3.294E+06 | 57.63 | < 0.0001 | significant |
| A-Flow rate | 3.294E+06 | 1 | 3.294E+06 | 57.63 | < 0.0001 | |
| Residual | 6.858E+05 | 12 | 57151.71 | | | |
| Cor Total | 3.979E+06 | 13 | | | | |

**TABLE 11: ANOVA FOR REDUCED QUADRATIC MODEL (RESPONSE 3: TP)**

| Source | Sum of Squares | df | Mean Square | F-value | p-value | |
|---|---|---|---|---|---|---|
| Model | 1.120E+07 | 7 | 1.600E+06 | 288.01 | < 0.0001 | significant |
| A-Flow rate | 6.682E+06 | 1 | 6.682E+06 | 1202.92 | < 0.0001 | |
| B-Methanol | 40072.40 | 1 | 40072.40 | 7.21 | 0.0363 | |
| C-Wavelength | 358.64 | 1 | 358.64 | 0.0646 | 0.8079 | |
| AB | 60031.13 | 1 | 60031.13 | 10.81 | 0.0167 | |
| $A^2$ | 7.371E+05 | 1 | 7.371E+05 | 132.68 | < 0.0001 | |
| $B^2$ | 7.252E+05 | 1 | 7.252E+05 | 130.54 | < 0.0001 | |
| $C^2$ | 5.848E+05 | 1 | 5.848E+05 | 105.27 | < 0.0001 | |
| Residual | 33330.78 | 6 | 5555.13 | | | |
| Cor Total | 1.123E+07 | 13 | | | | |

**TABLE 12: ANOVA FOR REDUCED QUADRATIC MODEL (RESPONSE 4: TF)**

| Source | Sum of Squares | df | Mean Square | F-value | p-value | |
|---|---|---|---|---|---|---|
| Model | 0.0040 | 7 | 0.0006 | 646.40 | < 0.0001 | Significant |
| A-Flow rate | 0.0020 | 1 | 0.0020 | 2334.61 | < 0.0001 | |
| B-Methanol | 0.0000 | 1 | 0.0000 | 0.0000 | 1.0000 | |
| C-Wavelength | 0.0000 | 1 | 0.0000 | 0.0000 | 1.0000 | |
| BC | 0.0002 | 1 | 0.0002 | 228.17 | < 0.0001 | |
| $A^2$ | 0.0004 | 1 | 0.0004 | 427.66 | < 0.0001 | |

| | | | | | |
|---|---|---|---|---|---|
| $B^2$ | 0.0003 | 1 | 0.0003 | 298.35 | < 0.0001 |
| $C^2$ | 0.0003 | 1 | 0.0003 | 298.35 | < 0.0001 |
| Residual | 5.259E-06 | 6 | 8.765E-07 | | |
| Cor Total | 0.0040 | 13 | | | |

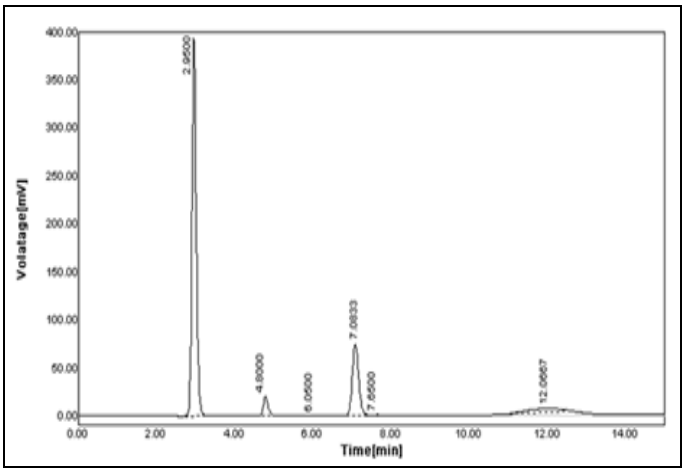


**FIG. 5: ACIDIC DEGRADATION**

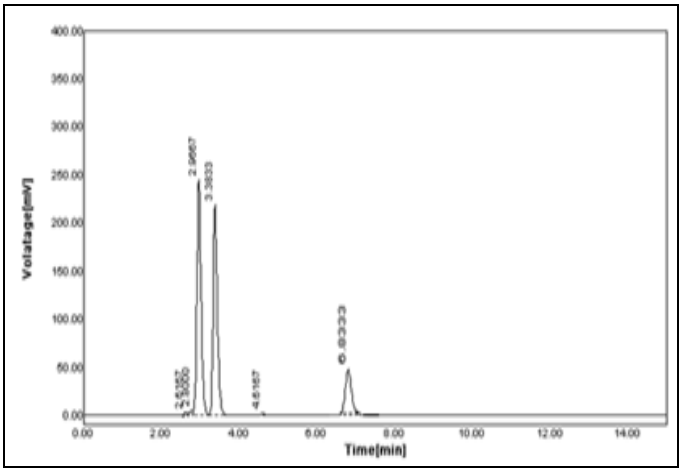


**FIG. 6: ALKALINE DEGRADATION**

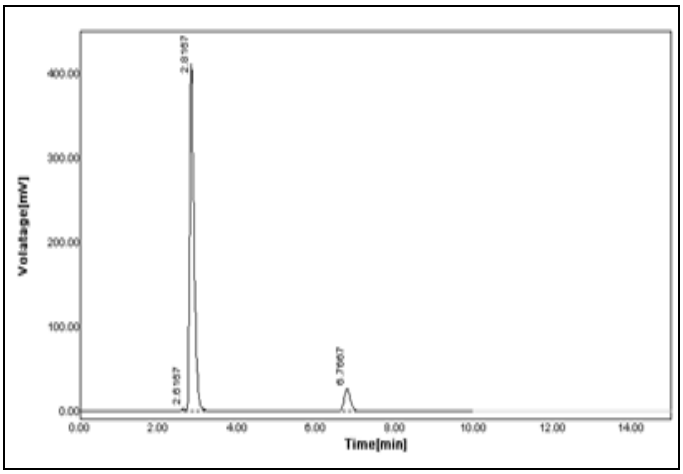


**FIG. 7: PEROXIDE DEGRADATION**

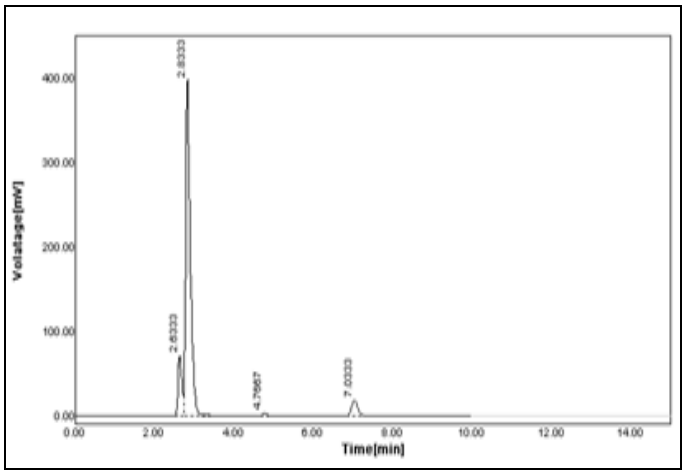


**FIG. 8: HEAT DEGRADATION**

**CONCLUSION:** A simple, rapid, reliable, robust, and optimized reversed-phase high-performance liquid chromatographic method for estimation of Pioglitazone and Glimepiride was successfully developed and validated as per International Conference on Harmonization guidelines.

Percentage of mobile phase, flow rate and wavelength were optimized by using QbD approach *i.e.*, 33 factorial design.

There are no interfering peaks underperformed degradation conditions. Therefore, a sensitive, accurate, and stability-indicating method was developed with high degree of practical utility.

**ACKNOWLEDGMENT:** The authors are grateful thanks to the Management and Principal of Gangamai College of Pharmacy, Nagaon, Dhule, Maharashtra, for providing timely support for the research work.

**CONFLICTS OF INTEREST:** The authors declare no conflicts of interest.

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**How to cite this article:**
Chaudhari S, Ahmad S, Tatiya AU and Rahman BA: Quality by design (QBD) approach to develop stability indicating RP-HPLC method development for pioglitazone and glimepiride. Int J Pharm Sci & Res 2022; 13(5): 2029-38. doi: 10.13040/IJPSR.0975-8232.13(5).2029-38.