RESEARCH ARTICLE - OPEN ACCESS

Journal of Pharmacy and Experimental Medicine DOI: http://dx.doi.org/10.51521/JPEM.2021.22111

Contents lists available at bostonsciencepublishing.us

Journal of Pharmacy and Experimental Medicine

QBD BASED DEVELOPMENT AND VALIDATION OF A STABILITY INDICATING UHPLC METHOD FOR PIOGLITAZONE AND GLIMEPIRIDE

Nikhil Arun Shete[1*], Anil Pawar[2], Vaijayanti Lokhande[3], Vishwajeet Swami[4], V. K. Deshmukh[5]

[1]*Mula Education Societys College of Pharmacy, Sonai, Ahemadnagar, Newasa, Maharashtra-414105.*

[2]*Mula Education Societys College of Pharmacy, Sonai, Ahemadnagar, Newasa, Maharashtra-414105.*

[3]*Mula Rural Institute College of Pharmacy Sonai, Ahemadnagar, Newasa, Maharashtra-414105.*

[4]*Dr. D.Y. Patil institute of Pharmaceutical Sciences and Research Pimpri Pune - 18.*

[5]*Mula Education Societys College of Pharmacy, Sonai, Ahemadnagar, Newasa, Maharashtra-414105.*

ARTICLE INFO

Article history:
Received 28 July 2021
Revised 01 October 2021
Accepted 17 October 2021
Published 21 October 2021

KEYWORDS:
Quality By Design,
Reverse Phase High Performance Liquid Chromatographic,
Pioglitazone,
Glimepiride,
Optimization,
Mobile Phase.

ABSTRACT

Quality by Design (QbD) refers to the achievement of certain predictable quality with desired and predetermined specifications. In an attempt to reduce rising development costs and regulatory barriers to innovation and creativity. In the pharmaceutical world, is considered as any other organic material, besides the drug substance, or ingredients arise out of synthesis or an unwanted chemical that remains with API's. The present study describes a simple, accurate, precise and cost effective Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method for determination pioglitazone and Glimepiride from bulk and formulation. RP-HPLC method was developed to identify and quantify the pioglitazone and Glimepiride in bulk and formulation as per ICH Q2 (R1) guidelines. Optimization was done by response surface methodology, applying a three level full factorial design. Two factors selected were methanol concentration in mobile phase, and flow rate. The separation was carried out using gilent 4.5 X 100mm 2.5um column. Detection was done using UV detector at 225nm. The developed method employed mobile phase methanol: (0.1%) water (75:25) (pH 3.2) temperature 27°C and flow rate 0.7 ml/min, which was optimized with the help of design expert software. High linearity of the developed method was confirmed over concentration range of 15-75&2-10 µg/mL for pioglitazone and Glimepiride with correlation coefficient of 0.998 and 0.999. The percentage RSD for precision and accuracy of the method was found to be less than 2%. Peaks were obtained at retention times of 2.9333 & 6.9667 min respectively for pioglitazone and Glimepiride The proposed method can be successfully used to determine the drug contents of marketed formulation.

INTRODUCTION

Quality is the heart of pharmaceutical industry. Quality is one of the fundamental criteria in addition to safety and efficacy for any entity to be qualified and approved as a drug. For ensuring consistency of performance of pharmaceutical products and systems, the recent emphasis has been on building the quality rather than merely testing it. This philosophy forms the basis of Quality by Design (QbD).

The twenty-first century began with the pharmaceutical industry using manufacturing technologies that have been employed since the 1940s and did not make significant changes in manufacturing process unless significant compliance or costs saving advantages could justify the high costs and long cycle time needed to gain approval. This often resulted in inefficient, overly expensive processes that were ultimately not in the best long-term interests of patients. As a result, the FDA (Food and Drug Administration) and other agencies around the world have embraced a new paradigm for regulation. The desired state was to shift manufacturing from being empirical to being more science, engineering, and risk based. Juran is often credited with introducing the concepts behind Quality by Design (QbD) [1].

The Food and Drug Administration (FDA) Office of Generic Drugs (OGD) has developed a Question based Review (QbR) for its Chemistry, Manufacturing and Controls (CMC) evaluation of Abbreviated New Drug Applications (ANDAs). QbR is a new quality attributes. It is a practical implementation of some underlying concepts and principles outlined by the FDA„s Pharmaceutical CGMPs for the twenty first century and Quality by Design (QbD) initiatives.

* Corresponding author.
Nikhil Arun Shete, Mula Education Societys College of Pharmacy, Sonai, Ahemadnagar, Newasa, Maharashtra-414105
E-mail address: nikhilshete300@gmail.com

METHOD DEVELOPMENT IN HPLC

Method development and optimization in liquid chromatography is still an attractive field of research for theoreticians (researchers) and attracts also a lot of interest from practical analysts. Optimized column, mobile phase, best detection wavelength, efforts in separation can make a world of difference while developing HPLC method for routine analysis. Determining the ideal combination of these factors assures faster delivery of desired results and a validated method of separation. Before proceeding with development of method for a particular sample it is absolutely essential to have detailed information about the sample and separation goal should be clearly defined.

Information about sample:

1. Number of components present in the sample.
2. pKa values of different components.
3. UV spectra of each analyte.
4. Concentration range of each component.
5. Solubility behaviour.
6. Nature of sample (solid, liquid, semisolid)
7. Formula

VALIDATION OF ANALYTICAL METHOD

Validation is defined as "documented evidence which gives a high degree of confidence that a process, system, facility will consistently produce a product meeting its predetermined specifications and quality attributes".

Method Validation

Method validation is the process of proving that an analytical method is acceptable for its intended purpose. For pharmaceutical methods, guidelines from the United States Pharmacopoeia (USP), International Conference on Harmonization (ICH), World Health Organization (WHO) and the Food and Drug Administration (FDA) provide a framework for performing such validations.

Parameters for method validation:

The parameters for method validation as defined by ICH (International Conference on

Harmonization) guidelines are summarized below:

a) Accuracy:

The accuracy of an analytical procedure expresses the closeness of agreement between the values, which is accepted either as a conventional true value or an accepted reference value found. The results of the accuracy studies are expressed as percent recovery (The results must be followed within range of 98%-102%).

b) Precision:

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. It is normally expressed as % relative standard deviation. Precision may be considered at three levels:

i) Repeatability: Repeatability expresses the precision under the same operating conditions over a short interval of time. It is also termed as intra-assay precision. (Relative Standard Deviation of repeatability studies must be <2%).

ii) Intermediate precision: Intermediate precision expresses the precision within laboratories variations, different days, different analysts, different equipment,,s, reagents etc. (Relative Standard Deviation of Intermediate precision studies must be <2%).

iii) Reproducibility: Reproducibility expresses the precision between different laboratories, (collaborative studies, usually applied for standardization of methodology).

c) Specificity:

Specificity is the ability to assess unequivocally the analyte in the presence of components, which may be expected to be present. Typically these might include impurities, degradents, matrix etc.

d) Limit of Detection:

The detection limit of an individual analytical procedure is the lowest amount of analyte in sample, which can be detected but not necessarily quantitated as an exact value. Limit of Detection (LOD) is expressed as a concentration at a specified signal to noise ratio. In chromatography detection limit is the injected amount that results in a peak with a height at least twice or three times as high as baseline noise level.

S/N= 2/1 or 3/1

Where, S= Signal, N=Noise

It may be calculated based on standard deviation (SD) of the response and slope of the curve(S).

LOD= 3.3(SD)/S

Where, SD= Standard deviation, S= Slope

e) Limit of Quantitation:

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in sample, which can be quantitatively determined with suitable precision and accuracy. Limit of Quantitation (LOQ) is expressed as a concentration at a specified signal to noise ratio. In chromatography quantitation limit is the injected amount that results in a peak with a height ten times as high as baseline noise level.

EXPERIMENTAL WORK

Preliminary Characterization Identification of drug

1. Colour, odour and appearance:

2. Melting point determination:

3. Determination of Solubility:

4. Ultraviolet (UV) spectroscopy:

i) UV calibration curve in Methanol:

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) METHOD FOR ANALYSIS OF PIOGLITAZONE AND GLIMEPIRIDE.

1. Selection of analytical wavelength

Standard stock solution of Pioglitazone and Glimepiride was diluted with diluent to obtain final concentration of 1500 µg/ml and 200µg/ml. Each solution was scanned using UV- Visible Spectrophotometer in the spectrum mode between the wavelength range of 400 nm to 200 nm and their spectra were overlaid. The wavelength selected was 224nm & 238 nm respectively for Pioglitazone and Glimepiride.

2. Selection of mobile phase

The API Pioglitazone and Glimepiride were injected into the HPLC system and run in different solvent systems. Mixture of different solvents were tried in order to determine optimum chromatographic conditions for effective separation of both drugs and Pioglitazone and Glimepiride. After several permutation and combination, it was found that the Methanol: (0.1%opa)Water (70:30) of pH 3.2 gives satisfactory results as compared to other mobile phases. Finally, the optimal composition of the mobile phase takes as per design, as it gave good peak shape of Pioglitazone and Glimepiride with minimal tailing.

The summary of the method development is given in table:

a) Simultaneous estimation of Pioglitazone and Glimepiride

b) Determination of absorptive values of drugs at selected wavelengths

1. Validation of method for analysis of Pioglitazone and Glimepiride

1. Linearity:

Concentration (µg/mL)	
Pioglitazone	Glimepiride
15	2
30	4
45	6
60	8
75	10

Table 1: Table of linearity for RP -HPLC Method Concentration (µg/mL).

2. Accuracy (recovery):

Sample	Amount Added (mg)	
	Pioglitazone	Glimepiride
Accuracy 80%	30	4
Accuracy 100%	30	4
Accuracy 120%	30	4

Table 2: Table of Accuracy for Table 3: Table of Accuracy for UV HPLC Method.

Sample	Amount Added (mg)	
	Pioglitazone	Glimepiride
Accuracy 80%	4.8	0.64
Accuracy 100%	6	0.8
Accuracy 120%	7.2	0.96

3. Repeatability

4. Precision

4.1. Intra-day precision

4.2 Inter-day precision

5. Robustness

6. Detection Limit

7. Quantitation Limit

8. Analysis of marketed formulation

FORCED DEGRADATION STUDIES

Procedure for Pioglitazone And Glimepiride degradation

1. Acid hydrolysis

2. Alkaline hydrolysis

3. Oxidation

4. Neutral

RESULTS AND DISCUSSION

Statistical data analysis (DOE)

The layout of actual design of DOE with the subsequent response results are shown in table no.10.6 as given below,

		Factor 1	Factor 2	Response 1	Response 2
Std	Run	A:Flow rate	B:Methanol	RT	Peak area
		ml/min	%	Min	
3	1	0.8	70	2.5	2861.2
1	2	0.6	70	3.4	3793.69
4	3	0.6	75	3.4	3753.13
5	4	0.7	75	2.9	3229.5
2	5	0.7	70	2.9	3256.8
8	6	0.7	80	2.9	3164.5
9	7	0.8	80	2.5	2789.7
7	8	0.6	80	3.39	3697.8
6	9	0.8	75	2.57	2825.4

Table 4: Layout of Actual Design of DOE.

Layout of Actual Design of DOE of Pioglitazone

		Factor 1	**Factor 2**	**Response 1**	**Response 2**
Std	**Run**	**A:Flow rate**	**B:Methanol**	**RT**	**Peak area**
		ml/min	**%**	**Min**	
3	1	0.8	70	3.1	512.7266
1	2	0.6	70	4.1	654.2380
4	3	0.6	75	4.1	660.1245
5	4	0.7	75	3.5	578.67480
2	5	0.7	70	3.5	567.8891
8	6	0.7	80	3.4	359.7924
9	7	0.8	80	3.0	460.6467
7	8	0.6	80	4.02	611.8284
6	9	0.8	75	3.07	521.78

Table 5: Layout of Actual Design of DOE.

Layout of Actual Design of DOE of Glimepiride

ANOVA for response surface Quadratic model

The analysis of variance (ANOVA) was performed to identify the significant and insignificant factors. The results of ANOVA for the retention time of DOE are as following Table 6.

Source	**Sum of Squares**	**Df**	**Mean Square**	**F-value**	**p-value**	
Mean vs Total	77.79	1	77.79	-	-	-
Linear vs Mean	**1.14**	**2**	**0.5720**	**326.36**	**< 0.0001**	**Suggested**
2FI vs Linear	0.0000	1	0.0000	0.0119	0.9173	-
Quadratic vs 2FI	0.0084	2	0.0042	6.21	0.0858	-
Cubic vs Quadratic	0.0014	2	0.0007	1.13	0.5533	Aliased
Residual	0.0006	1	0.0006	-	-	-
Total	78.95	9	8.77	-	-	-

Table 6: ANOVA table for retention time of pioglitazone.

The Model F-value of 4.56 implies the model is significant. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B are significant model terms. The "Pred R-Squared" of 0.5720 is in reasonable agreement with the "Adj R-Squared" of 0.0006; i.e. the difference is less than 0.2. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Here the ratio of 78.95 indicates an adequate signal. This model can be used to navigate the design space.

Model assessment for the retention time response as dependent variable:

A) Graphical Presentation: For Retention Time of Pioglitazone:

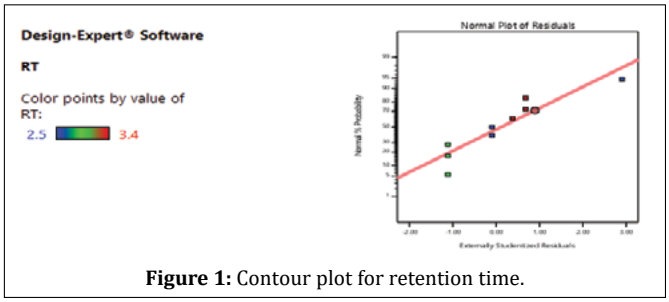

Figure 1: Contour plot for retention time.

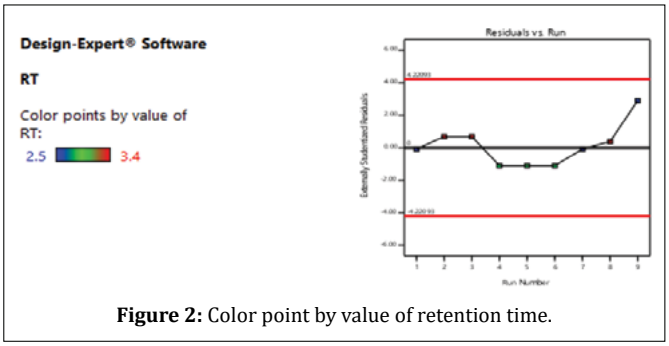

Figure 2: Color point by value of retention time.

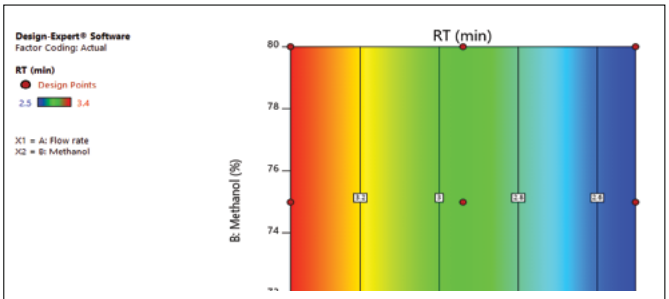

Figure 3: Contour plot for retention time (min) of Pioglitazone against Mobile phase and Flow rate.

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Mean vs Total	9.586E+07	1	9.586E+07	-	-	-
Linear vs Mean	1.289E+06	2	6.443E+05	378.42	< 0.0001	-
2FI vs Linear	148.72	1	148.72	0.0739	0.7966	-
Quadratic vs 2FI	**9920.66**	**2**	**4960.33**	**102.24**	**0.0017**	**Suggested**
Cubic vs Quadratic	43.11	2	21.55	0.2104	0.8390	Aliased
Residual	102.45	1	102.45	-	-	-
Total	9.715E+07	9	1.079E+07	-	-	-

Table 7 ANOVA table for retention time of Glimepiride.

The Model F-value of 0.210 implies the model is significant. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B are significant model terms. The "Pred R-Squared" of 9920.66 is in reasonable agreement with the "Adj R-Squared" of 0.0006; i.e. the difference is less than 0.2. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Here the ratio of 78.95 indicates an adequate signal. This model can be used to navigate the design space.

B) Graphical Presentation: For Retention Time of Glimepiride:

Mobile phase and Flow rate.

A) Optimization solution:

This plot elaborates that the optimized values of both independent variables in the required target range of retention times & Resolution lie within the yellow region which is the useful optimum region where the design space can be determined.

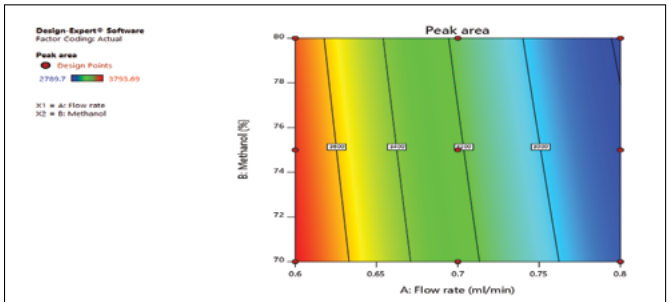

Figure 4: Contour plot for retention time (min) of Glimepiride against.

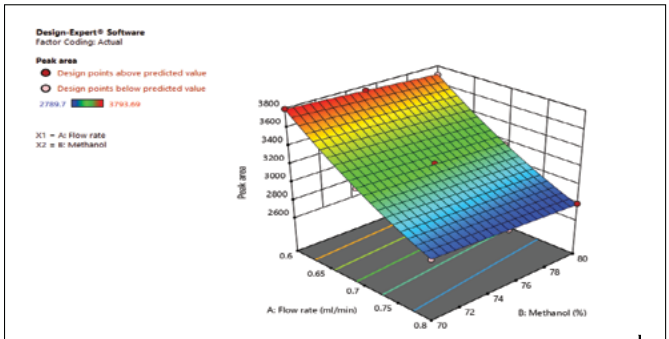

Figure 5: Predicted Vs Actual for DOE of Retention time (min) of Glimepiride against Mobile phase and Flow rate.

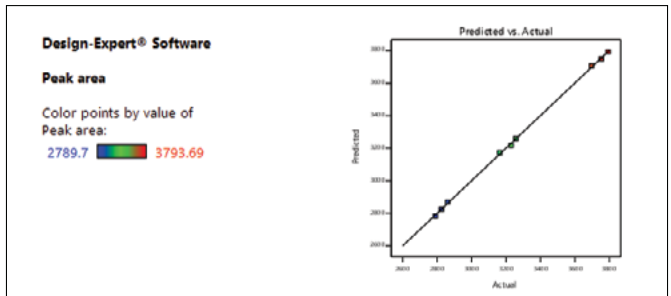

Figure 6: Predicted Vs Actual for DOE of Retention Time.

Optimized Method:

Flow rate (Ml/min)	Mobile phase composition (mL)
0.7mL	Methanol: Water (75:25)

Table 8: Optimized Method.

The final chromatographic conditions selected were as follow:

- Analytical column: Agilent C18 Column (100mm x 4.6mm, 2.5µm partical size).
- Injection volume: 20µl
- Flow rate: 0.7ml/min
- Mobile phase: Methanol+0.1OPA (75+25% v/v)
- Detection: 225nm
- Run Time: 15 min

In the standard mixture of Pioglitazone and Glimepiride theoretical plates were found above 2000 i.e. for Pioglitazone 3505.5 and Glimepiride 6728.3 at minimum RT 2.9333 and 6.9667 respectively.

Analytical of Method Validation:

Linearity:

From Pioglitazone standard stock solution, different working standard solution (15-75 µg/ml) were prepared in mobile phase Likewise from Glimepiride standard stock solution different working standard solution (2-10µg/ml) were prepared in mobile phase 20 µl of

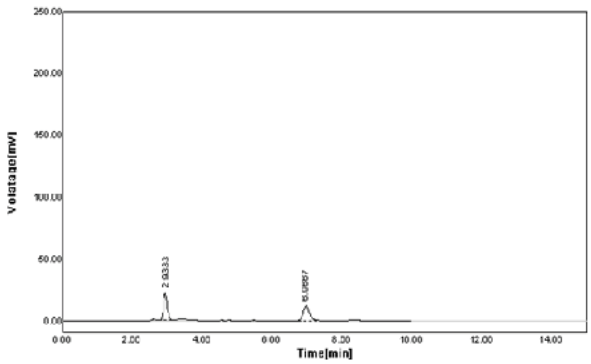

Figure 7: Chromatogram of standard Combination of Pioglitazone and Glimepiride .

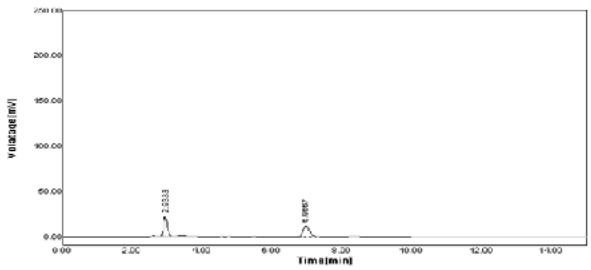

Figure 8: Chromatogram of linearity .

No.	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	2.9333	146.262	51.9	3505.5	1.25	0
2	6.9667	74.8328	48.1	6728.3	1.25	12.7368

Table 10: Chromatogram of linearity.

Concentration µg/ml	Area Pioglitazone
15	144.969
30	291.402
45	471.014
60	617.934
75	767.878

Table 11: Linearity of Pioglitazone.

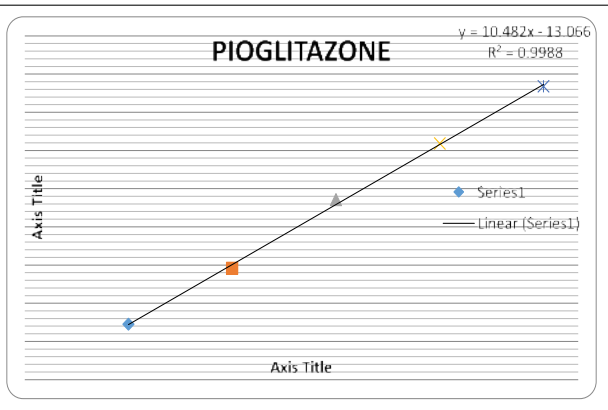

Figure 9: Calibration curve for HPLC method .

Regression Equation Data Y=mx+c	
Slope(m)	10.48x
Intercept(c)	13.06
Correlation Coefficient	0.998

Table 12: Regression equation data for Pioglitazone.

Concentration µg/ml	Area Glimepiride
2	73.87
4	134.312
6	201.4
8	252.654
10	323.5

Table 13: Linearity of Glimepiride.

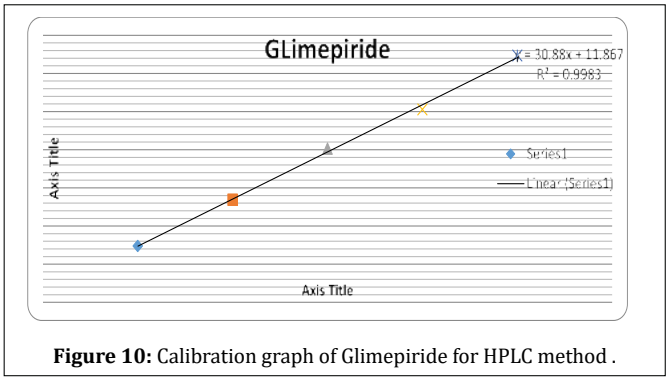

Figure 10: Calibration graph of Glimepiride for HPLC method .

Regression Equation Data Y=mx+c	
Slope(m)	30.88X
Intercept(c)	11.86
Correlation Coefficient	0.998

Table 14: Regression equation data for Glimepiride.

sample solution was injected into the chromatographic system using fixed volume loop injector. Chromatograms were recorded. The area for each concentration were recorded (Table 10). The Calibration curves are shown in Fig. 8.

Linearity of of Pioglitazone and Glimepiride was observed in the range of 15-75µg/ml and 2-10µg/ml. Detection wavelength used was 225 nm (Tables19 and 21).

The plot should be linear passing through the origin, Correlation Coefficient should not be less than 0.998 that concluded (Table 14).

Accuracy:

Recovery studies were performed to validate the accuracy of developed method. To pre analyzed tablet solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed (Table 15). Statistical validation of recovery studies shown in

*Denotes average of three determinations for RP-HPLC and UV method

Accuracy of RP-HPLC method and UV Spectrophotometric method is ascertained by recovery studies performed at different levels of concentrations (80%, 100% and 120%). The % recovery was found to be within 99-101% (Table 15).

Precision:

The method was established by analyzing various replicates standards of Pioglitazone and Glimepiride. All the solution was analyzed thrice in order to record any intra-day & inter-day variation in the result that concluded. The result obtained for intraday is shown in (Table 16) respectively.

Intraday and Inter day Precision studies on RP-HPLC and UV method for Pioglitazone and Glimepiride which shows the high precision %amount in between 97% to 102% indicates to analytical method that concluded.

Robustness:

The Robustness of a method is its ability to remain unaffected by small deliberate changes in parameters. To evaluate the robustness of

METHOD	Drug	Level (%)	Amt. taken (µg/ml	Amt. Added (µg/ml	Absorbance Mean* ± S.D.	Amt. recovered Mean *±S.D.	%Recovery Mean *± S.D.
RP-HPLC Method	PIO	80%	30	24	54.47±0.37	24.47±0.37	101.95±1.56
		100%	30	30	59.57±0.23	20.58±0.23	98.57±0.76
		120%	30	36	67.00±0.40	37.00±0.40	101.58±1.10
	GLIME	80%	4	3.2	7.20±0.06	3.20±0.06	99.93±1.89
		100%	4	4	8.05±0.02	20.58±0.02	101.15±0.49
		120%	4	4.8	8.71±0.01	4.71±0.01	101.58±0.26
UV Method	PIO	80%	6	4.8	10.79±0.021	4.79±0.021	99.79±0.44
		100%	6	6	19.81±0.07	49.53±0.23	99.07±0.46
		120%	6	7.2	12.07±0.018	6.07±0.018	101.13±0.29
	GLIM	80%	0.8	0.64	1.45±0.006	0.65±0.006	101.24±0.90
		100%	0.8	0.8	1.60±0.006	0.80±0.006	99.87±0.72
		120%	0.8	0.96	1.76±0.004	0.96±0.004	99.70±0.45

*mean of each 3 reading for RP-HPLC method and UV method

Table 15: Result of Recovery data for Pioglitazone and Glimepiride.

METHOD	Level of Recovery (%)	Drug	Mean % Recovery	Standard Deviation*	% RSD
Rp-HPLC Method	80%	PIO	101.95	1.56	1.53
		GLIME	99.93	1.89	1.89
	100%	PIO	98.57	0.76	0.77
		GLIME	101.15	0.49	0.49
	120%	PIO	101.58	1.10	1.09
		GLIME	98.58	0.26	0.26
UV Method	80%	PIO	99.79	0.44	0.44
		GLIME	101.24	0.90	0.89
	100%	PIO	101.13	0.29	0.29
		GLIME	99.87	0.72	0.72
	120%	PIO	98.65	0.74	0.75
		GLIME	99.70	0.45	0.45

*Denotes average of three determinations for RP-HPLC and UV method

Table 16: Statistical Validation of Recovery Studies Pioglitazone and Glimepiride.

the proposed method, small but deliberate variations in the optimized method parameters were done. The effect of changes in mobile phase composition and flow rate, wavelength on retention time and tailing factor of drug peak was studied.

The mobile phase composition was changed in (±1 ml/min-1) proportion and the flow rate was varied by (±1ml/min-1), and wavelength change (±1 ml/min-1) of optimized chromatographic condition. The results of robustness studies are shown in (Table 16). Robustness parameters were also found satisfactory; hence the analytical method would be concluded.

1) Flow Rate Change 0.6ml

2) Robustness Study of Pioglitazone:

The changes were doing flow rate (±1 ml/min-1), PH of mobile phase composition (±1 ml/min-1), and Wavelength (±1 ml/min-1). %RSD for peak area was calculated which should be less than 2%.the result shown in analytical method that concluded (Table 17).

Parameters	Conc.(µg/ml)	Amount of detected (mean ±SD)	%RSD
Chromatogram of flow change 0.6ml	6	253.66±0.36	0.36
Chromatogram of flow change 0.8 ml	6	235.82±2.84	1.20
Chromatogram of comp change 46 74Meoh+ 26 WATER	6	202.1±1.22	0.60
Chromatogram of comp change 76Meoh+ 24 WATER	6	266.86±4.89	1.83
Chromatogram of comp change wavelength change 224nm	6	201.2±1.32	0.65
Chromatogram of comp change wavelength change 226nm	6	210.96±0.62	0.29

Table 17: Result of Robustness Study of Glimepiride.

Stress conditions	PIO		GLIME	
	(%) Degradation 1Hr	Degradation (%) 2Hr	(%) Degradation 1Hr	Degradation (%) 2Hr
Acidic hydrolysis	20.3938	20.3618	78.2715	77.7900
Alkaline hydrolysis	19.8823	20.3471	77.3118	77.7114
Peroxide Degradation	19.8475	20.0423	77.2625	77.3654
Neutral Degradation	20.0961	20.2555	78.0440	78.1479

Table 18: Results of Forced degradation studies.

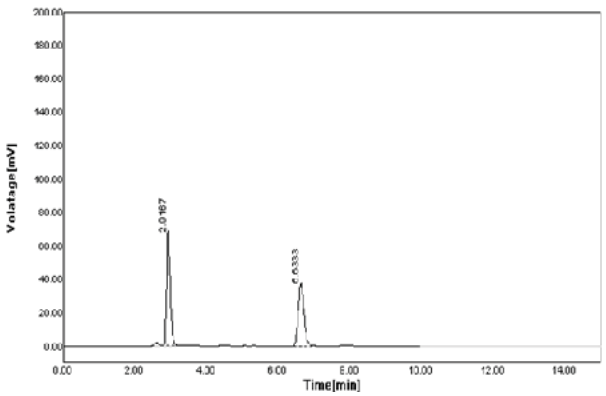

Figure 11: Chromatogram of Precision.

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 (0.1 N HCl), base (0.1 N NaOH), Hydrogen peroxide (H2O2) and Neutral. Investigation was done for the degradation products.

In this chromatogram of H2O2 degradation has lead to formation of degrading and calculate % Degradation of drug19.84-77.26%. Stability study of Glimepiride & Pioglitazone indicated good results. Stress degradation of Glimepiride &Pioglitazone was carried out with the help of various degradation agents or methods like Acid, Base, Hydrogen peroxide, Neutralete.

After degradation, the development of chromatogram indicated the formation of degradation product. These degradation products with different Rf value were well separated from each other.

The % drug recovery was calculated based on how much degradation of the standard drug occurred after degradation. It was determined using the peak area of standard drug and the drug after degradation.

The generally recommended degradation varies between 5-10% degradation. Very mild degradation was observed during Acid, Base, Hydrogen peroxide & Neutral degradation for Glimepiride & Pioglitazone.

CONCLUSION

- RP-HPLC method was developed by implementing QbD methodology on analytical column- Reversed Phase Agilent C18 (100mm×4.6mm×2.5µm), with mobile phase Methanol: (0.1%opa) Water (75: 25 v/v). The flow rate used was 0.7 mL / min and UV detection was carried out at 238 nm. The retention time for Pioglitazone & Glimepiride was found to be 2.9333 min & 6.9667 min respectively.
- Systematic approach was utilized to develop an efficient and robust method which includes beginning with determination of target profile characteristics, risk assessment, design of experiment and validation.
- The study was done by using33 full fraction response surface designs. In this study interaction of 2 factors; flow rate, mobile phase composition at 2 levels.
- Method Operable Design Region (MODR) was developed to achieve the region of operation for drug and Glimepiride.
- The RP-HPLC method developed for estimation of Pioglitazone & Glimepiride was validated as per ICH Q2 (R1) guidelines using various parameters.
- The Limit of Detection (LOD) and Limit of Quantitation (LOQ) were established at a signal-to-noise ratio. LOD and LOQ were calculated as 3.3×δ/S and 10×δ/S respectively as per ICH guidelines
- System suitability test ensures that the analytical system is working properly and can give accurate and precise results. System suitability tests includes tailing factor, number of theoretical plates, area etc. The results of all system suitability parameters were acceptable in their limits defined by official guidelines.
- The proposed HPLC method has also been evaluated for accuracy, precision and robustness and proved to be convenient and effective for the quality control of Pioglitazone & Glimepiride.
- Moreover, the lower solvent consumption along with the short analytical run time of 10 min leads to a cost effective and environmentally friendly chromatographic procedure.
- Thus, the proposed methodology is rapid, selective, requires a simple sample preparation procedure, and represents a good procedure for Pioglitazone & Glimepiride.

REFERENCES

1. ICH Q8 (R2), Pharmaceutical Development, Part I: Pharmaceutical Development., (2009) http://www.ich.org/LOB/media//MEDIA4986.pdf.
2. ICH Q9, Quality Risk Management (2005) http://www.ich.orghttp://www.ich.org/LOB/media//MEDIA1957.pdf.
3. ICH Q10, (2008). Pharmaceutical Quality Systems., http://www.ich.org

4. US Food and Drug Administration (FDA), Department of Health and Human Services, Pharmaceutical Quality for the 21st Century A Risk-Based Approach Progress Report, (2013).
5. US Food and Drug Administration (FDA), Guidance for industry PAT-A framework for innovative pharmaceutical manufacturing and quality assurance, FDA, Washington, DC, USA, (2004).
6. Trivedi B, Quality by design (QbD) in pharmaceuticals. Int J Pharm Pharm Sci 4(1), 17-9.
7. Serena O, Sergio P, Andra F. Application of quality by design to the development of analytical separation methods. Anal Bioanal Chem. 2012.
8. Bhutani H, Kurmi M, Singh S, Beg S, Singh B. Quality by Design (QbD) in Analytical Sciences: An Overview. Pharma Times. 2014;46(08),71-75.
9. Reid G.L, Morgado J, Barnett K., Harrington B., Wang J., Harwood J., Fortin D., (2013). Analytical Quality by Design (AQbD) in Pharmaceutical Development. Amer Pharm Rev, 1-7.
10. Rozet E, Lebrun P, Debrus B, Boulanger B, Hubert P. Design Spaces for analytical methods. Trends in Analytical Chemistry. 2013;42:157-167.
11. Molnar I, Rieger H.J, Monks K.E. Aspects of the "Design Space" in high pressure liquid chromatography method Development. J Chrom Anal. 2010;12173193–3200.
12. Monks, K.E, Rieger, H.J, Molnar, I. Expanding the term "Design Space" in high performance liquid chromatography (I). J. Pharm. Biomed. Anal. 2011;56(5):874879.
13. Sangshetti JN, Deshpande M, Zaheer Z, Shinde D.B, Arote R. Quality by design approach: Regulatory need. Arab J Chem. 2014;1-14.
14. Sumithra M, Ravichandiran V. Review on Quality by Design. Int J Front Sci Tech. 2015;2(4):47-56.
15. Bhatt D.A., Rane S.I. Qbd Approach to Analytical RP-HPLC Method Development and its Validation. Int J Pharm Pharm Sci. 2011;3(1):179-187.
16. Sanford, Boltan, Charl, S. Bon. Factorial Design. Pharmaceutical Statistics Practical and Clinical Application. fourth edition. 2004;265
17. Armstrong N, Kenneth C, James, Factorial Design of experiments. Pharmaceutical experimental design and interpretation, London. 1996;131-162.
18. Box GEP, Behnken DW. Some new three levels designs for the study of Quantitative variables. Technometrics. 1960;2(4):455 -75.
19. Ayre D, Varpe R, Nayak1 N. Impurity profiling of pharmaceuticals", Advance research in pharmaceuticals and Biologicals. 2011;1(2).
20. ICH Q3A(R), Oct (2006) Draft Revised Guidance on Impurities in New Drug Substances.
21. ICH Q3B (R), June (2006) Draft Revised Guidance on Impurities in New Drug Products.
22. ICH Q3B (R), Feb (2012) Draft Revised Guidance for Residual solvents.
23. ICH Q3D July (2013) Draft Consensus Guideline for Elemental Impurities.