DEVELOPMENT AND VALIDATION OF RP -HPLC METHOD FOR THE ESTIMATION OF PIOGLITAZONE AND GLIMEPIRIDE IN THE TABLET DOSAGE FORM.

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ABSTRACT

A simple, economical, accurate reverse-phase RP-HPLC method was established for the estimation of Pioglitazone and Glimepiride in tablet dosage form. The estimation was done by using an Inertsil ODS C 18 (4.6 x 150mm, 5.0µm) column with a flow rate of 1.5ml/min, with detection at 225nm. The separation was carried out by using a mobile phase containing 30% Buffer, 60% Acetonitrile, and 10% Methanol. The retention time obtained was 1.979 min and 3.666 min respectively. The current method was validated according to the ICH guidelines for accuracy, precision, linearity, specificity, and sensitivity. This method was shown to be linear in 15-75 µg/ml and 2-10 µg/ml concentration ranges (regression coefficient of 0.999 and 0.999) for Pioglitazone and Glimepiride respectively. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 2.97µg/ml and 9.97 µg/ml for Pioglitazone and 2.9 µg/ml and 9.98 µg/ml for Glimepiride respectively. The accuracy of the method was assessed by adding a fixed amount of pre-analyzed samples to different standard solutions (50%, 100%, and 150% of the tested concentration) in triplicate. The percentage mean recoveries were found to be 98% -102%. the method was found to be precise with the % RSD value found to be the limits for intraday and inter-day precision studied respectively. The method specificity and robustness were also established, new and sensitive RP-HPLC method for the estimation of Pioglitazone and Glimepiride has been developed concerning the reviewed analytical methods.

Key Words: Pioglitazone and Glimepiride, RP-HPLC, Accuracy, Precision.

INTRODUCTION

Pioglitazone is mostly used in the treatment of diabetes mellitus type 2. Pioglitazone selectively stimulates nuclear receptor peroxisome proliferator-activated receptor gamma (PPAR- gamma). The mechanism action of Pioglitazone is it acts as an agonist at peroxisome proliferator-activated receptors (PPAR) in target tissues for insulin action, such as skeletal muscle, adipose tissue, and liver. The activation of PPAR-gamma receptors increases the transcription of insulin-responsive genes which are involved in the control of glucose production, transportation, and utilization. It modulates the transcription of the insulin-sensitive genes involved in the control of glucose and lipid metabolism in the lipidic, muscular tissues, and the liver. Pioglitazone targets insulin resistance and, hence, is used alone or in combination with insulin, metformin, or sulfonylurea as an antidiabetic agent. IUPAC Name**:** 5-({4-[2-(5-ethylpyridin-2-yl)ethoxy]phenyl}methyl)-1,3-thiazolidine-2,4-dione, Chemical Formula: $C_{19}H_{20}N_2O_3S$ category is Hypoglycemic agents , melting point 183-184^{0}C and molecular weight is 356.44g/mol.

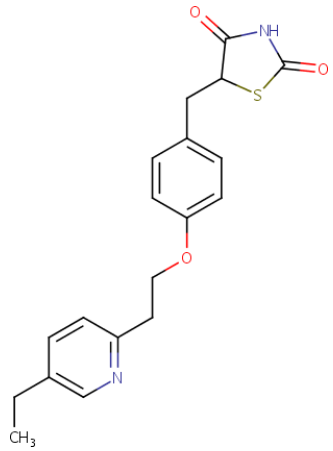

Fig:1 Chemical structure of Pioglitazone

Glimepiride is the first III-generation sulphonyl urea it is a very potent sulphonyl urea with a long duration of action. Mechanism of action of the mechanism of action of glimepiride in lowering blood glucose appears to be dependent on stimulating the release of insulin from functioning pancreatic beta cells and increasing the sensitivity of peripheral tissues to insulin. Glimepiride likely binds to ATP-sensitive potassium channel receptors on the pancreatic cell surface, reducing potassium conductance and causing depolarization of the membrane. It stimulates calcium ion influx through voltage-sensitive calcium channels and an increase in intracellular calcium ion concentration which induces the secretion of insulin. Glimepiride, like glyburide and glipizide, is a "second-generation" sulfonylurea agent. Glimepiride is used with diet to lower blood glucose by increasing the secretion of insulin from the pancreas and increasing the sensitivity of peripheral tissues to insulin. IUPAC Name is 3-ethyl-4-methyl-N-{2[4({[(4methylcyclohexyl)carbamoyl]amino}sulfonyl)phenyl]ethyl}-2-oxo-2,5-dihydro-1H-pyrrole-1-carboxamide, molecular formula $C_{24}H_{34}N_4O_5S$ molecular weight is 490.617 g/mol and the category is Hypoglycemic Agents, Immunosupressive Agents, Antidiabetic Agent.

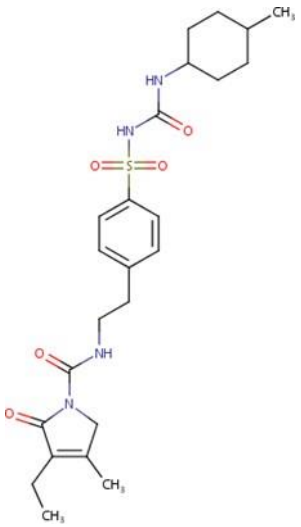

Fig:2 Chemical structure of Glimepiride

MATERIALS AND METHODS:

Table 1: Instruments used

S.no	Instrument	Model
1.	HPLC	WATERS, Software Empower, 29 Separation module 2487 UV detector
2.	UV/VIS Spectrophotometer	LABINDIA UV 3000+
3	pH meter	Adwa – AD 1020
4	Weighing machine	Afcoset ER-200A
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil

Table 2: Chemicals used:

S.no	Chemical	Brand
1	Pioglitazone	Supplied by Pharma train
2	Glimepiride	Supplied by Pharma train
3	Orthophosphoric acid	FINAR chemical LTD
4	Water and Methanol for HPLC	Standard solutions Ltd

5	Acetonitrile for HPLC	Standard solutions Ltd
6	HCl, H_2O_2, NaOH	MERCK

Method development

Preparation of Standard Solution:

Accurately weigh and transfer 15mg of Pioglitazone & 2mg of Glimepiride working standard into a 10ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 1ml of Pioglitazone & Glimepiride of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Further pipette 3ml of Pioglitazone & Glimepiride of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Sample Solution Preparation:

Accurately weigh and transfer equivalent to 15mg of Pioglitazone & 2mg Glimepiride equivalent weight of the sample into a 10ml clean dry volumetric flask add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 1ml of Pioglitazone & Glimepiride of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Further pipette 3ml of Pioglitazone & Glimepiride of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Procedure: Inject 20 µL of the standard, sample into the chromatographic conditions[7] measure the areas for the Pioglitazone & Glimepiride peaks, and calculate the % Assay by using the formulae, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines[8-10].

Method Validation Parameters[11]

Linearity:

Preparation of stock solution: Accurately weigh and transfer 15mg of Pioglitazone & 2mg of Glimepiride working standard into a 10ml clean dry volumetric flask add about 7 ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 1ml of Pioglitazone and glimepiride the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Preparation of Level – I (15ppm & 2ppm of Pioglitazone & Glimepiride):

1ml of stock solution was taken in 10ml of volumetric flask diluted up to the mark with Diluents.

Preparation of Level – II (30ppm & 4ppm of Pioglitazone & Glimepiride):

2ml of stock solution was taken in 10ml of volumetric flask diluted up to the mark with Diluents.

Preparation of Level – III (45ppm & 6ppm of Pioglitazone & Glimepiride):

3ml of stock solution was taken in 10ml of volumetric flask diluted up to the mark with Diluents.

Preparation of Level – IV (60ppm & 8ppm of Pioglitazone & Glimepiride):

4ml of stock solution was taken in 10ml of volumetric flask diluted up to the mark with Diluents.

Preparation of Level – V (75ppm & 10ppm of Pioglitazone & Glimepiride):

5ml of stock solution was taken in 10ml of volumetric flask diluted up to the mark with Diluents.

Procedure:

Inject each level into the chromatographic system and measure the peak area.

Plot a graph of peak area versus concentration (on X-axis concentration and Y- Y-axis peak area) and calculate the correlation coefficient.

Precision:

Preparation of stock Solution:

Accurately weigh and transfer 15mg of Pioglitazone & 2mg of Glimepiride working standard into a 10ml clean dry volumetric flask add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 1ml of Pioglitazone and glimepiride the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Further pipette 3ml of Pioglitazone and glimepiride the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents

Procedure:

The standard solution was injected five times and the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limit.

Intermediate precision/ruggedness:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days within the laboratory.

Preparation of stock solution:

Accurately weigh and transfer 15mg of Pioglitazone & 2mg of Glimepiride working standard into a 10ml clean dry volumetric flask add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (stock solution)

Further pipette 1ml of Pioglitazone and glimepiride the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Further pipette 3ml of Pioglitazone and glimepiride the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Procedure:

The standard solution was injected five times and measured the area for all Five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Accuracy:

For accuracy determination, three different concentrations were prepared separately i.e. 50%, 100%, and 150% for the analyte and chromatograms were recorded for the same.

Preparation of Standard stock solution:

Accurately weigh and transfer 15mg of Pioglitazone & 2mg of Glimepiride working standard into a 10ml clean dry volumetric flask add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 1ml of Pioglitazone and glimepiride the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Further pipette 3ml of Pioglitazone & Glimepiride the above stock solution into a10ml volumetric flask and dilute up to the mark with Diluents

Preparation Sample solutions:

For the preparation of 50% solution (Concerning target Assay concentration):

Accurately weigh and transfer 7.5mg of Pioglitazone and 1mg of Glimepiride working standard into a 10ml clean dry volumetric flask add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 1ml of Pioglitazone and glimepiride the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents

Further pipette 3ml of Pioglitazone & Glimepiride the above stock solution into a10ml volumetric flask and dilute up to the mark with Diluents

For the preparation of 100% solution (Concerning target Assay concentration):

Accurately weigh and transfer 15mg of Pioglitazone and 2mg of Glimepiride working standard into a 10ml clean dry volumetric flask add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 1ml of Pioglitazone and glimepiride the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Further pipette 3ml of Pioglitazone and glimepiride the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

For the preparation of 150% solution (Concerning target Assay concentration):

Accurately weigh and transfer 22.5mg of Pioglitazone and 3mg of Glimepiride equivalent weight of tablet powder into a 10ml clean dry volumetric flask add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 1ml of Pioglitazone and glimepiride the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents

Further pipette 3ml of Pioglitazone & Glimepiride the above stock solution into a10ml volumetric flask and dilute up to the mark with Diluents

Procedure:

Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions.

Calculate the Amount found, and Amount added for Pioglitazone and glimepiride and calculate the individual recovery and mean recovery values.

Limit of Detection:

Preparation of Pioglitazone solution:

Accurately weigh and transfer 15mg of Pioglitazone working standard into a 10ml clean dry volumetric flask add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 1ml of Pioglitazone the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent

Further pipette 3ml Pioglitazone of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

Preparation of 0.085µg/ml solution:

Further pipette 0.5ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

Further pipette 0.4ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

Preparation of Glimepiride solution:

Accurately weigh and transfer 2mg of Glimepiride working standard into a 10ml clean dry volumetric flask add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 1ml of Glimepiride the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

Further pipette 3ml of Glimepiride the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

Preparation 0.046µg/ml solution:

Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents

Further pipette 0.8ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents

Limit of Quantification

Preparation of Pioglitazone solution:

Preparation of 0.285µg/ml solution:

Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

Further pipette 0.6ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

Preparation of Glimepiride solution:

Preparation of 0.154µg/ml solution:

Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

Further pipette 2.5 of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

Robustness

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, and Temperature Variation was made to evaluate the impact on the method.

a) The flow rate was varied from 1.4 ml/min to 1.6 ml/min.

Standard solution 45 & 6 µg/ml of Pioglitazone & Glimepiride prepared and analyzed using the varied flow rates along with method flow rate.

b) The Organic composition in the Mobile phase was varied from 63% to 77%

Standard solution 45 & 6 µg/ml of Pioglitazone and glimepiride were prepared and analyzed using the varied Mobile phase composition along with the actual mobile phase composition in this method.

RESULTS AND DISCUSSION

Method development

Preparation of Standard Solution:

Accurately weigh and transfer 15mg of Pioglitazone & 2mg of Glimepiride working standard into a 10ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 1ml of Pioglitazone & Glimepiride of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Further pipette 3ml of Pioglitazone & Glimepiride of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Mobile Phase Optimization: Finally, the mobile phase was optimized to 30% buffer: 60% Acetonitrile: 10% Methanol here buffer used is Orthophosphoric acid**.**

Optimization of Column: The method was performed with various columns like C18 column, Symmetry, and X-Bridge. Inertsil ODS C 18 (4.6 x 150mm) 5µm Particle size Column was found to be ideal as it gave good peak shape and resolution at 1.5ml/min flow.

Preparation of 0.1% Ortho phosphoric acid buffer: Pipetted 1 ml of ortho phosphoric acid in 100 ml HPLC water.

Preparation of mobile phase: Mix a mixture of the above buffer 300 ml (30%), 600 ml Acetonitrile (60%), and 100 ml Methanol HPLC (10%) and degas in an ultrasonic water bath for 5 minutes. Filter through 045 µ filter under vacuum filtration.

Diluent Preparation: Use the Mobile phase as Diluents.

Optimized Chromatographic Conditions:

Instrument used: High-performance liquid chromatography equipped with

Auto Sampler and DAD or UV detector

Temperature: Ambient

Column: Inertsil ODS C 18 (4.6 x 150mm, 5.0ml)

Buffer : Ortho phosphoric acid buffer

Mobile phase : 30% Buffer: 60% Acetonitrile: 10% Methanol

Flow rate : 1.5 ml per min

Wavelength : 225 nm

Injection volume :20µl

Run time : 8min.

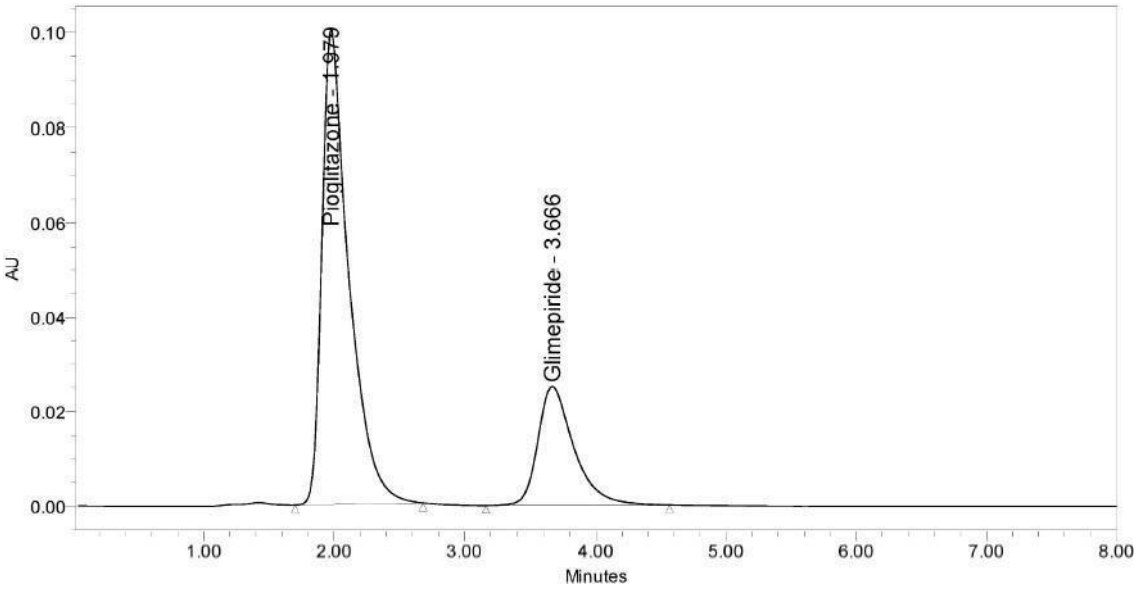

Fig:3 Optimized Chromatographic Condition

Method Validation:

Table: 3 -Area of different concentrations of Pioglitazone and Glimepiride

S. No	Pioglitazone		Glimepiride	
	Concentration (µg/ml)	Area	Concentration (µg/ml)	Area
1	15	497912	2	167152
2	30	909362	4	318510
3	45	1309957	6	445689
4	60	1751064	8	598598
5	75	2167600	10	744708

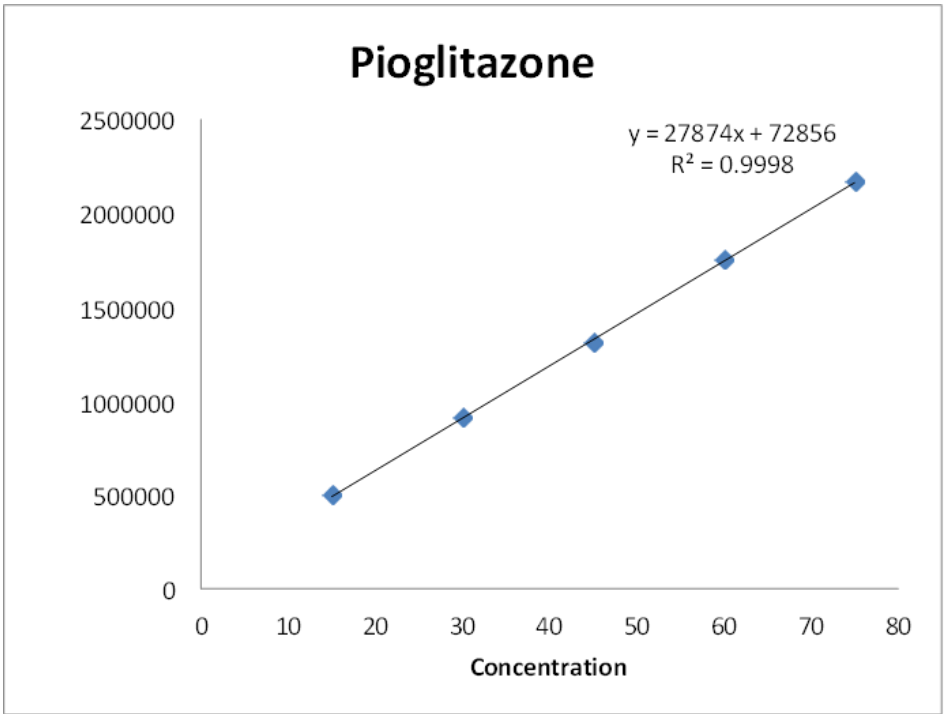

Fig:4 Calibration graph for Pioglitazone

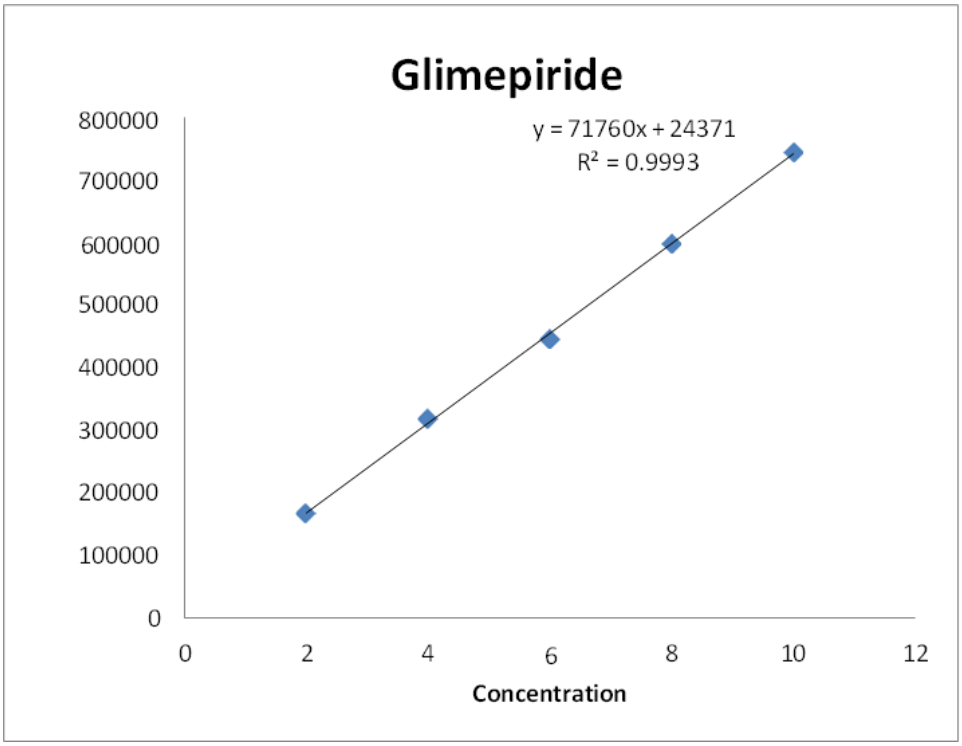

Fig:5 Calibration graph for Glimepiride

Table: 4 -Analytical performance parameters of Pioglitazone and Glimepiride

Parameters	Pioglitazone	Glimepiride
Slope(m)	27874	71760
Intercept(c)	72856	24371
Correlation coefficient(R^2)	0.999	0.999

Acceptance criteria: Correlation coefficient (R2) should not be less than 0.999

Conclusion: The correlation coefficient obtained was 0.999 which is in the acceptance limit.

PRECISION

The method was precise for both sample solutions as described under experimental work. The corresponding chromatograms and results are shown below.

Table: 5 -Results of Precision for Pioglitazone

INJECTION	**AREA**
Injection-1	1333648
Injection -2	1311342
Injection-3	1311831
Injection -4	1313475
Injection-5	1318756
Average	1317810.4
Standard Deviation	9328.9
%RSD	0.71

Table 6 -Results of Precision for Glimepiride

INJECTION	AREA
Injection-1	457296
Injection-2	452139
Injection-3	451559
Injection-4	453388
Injection-5	452856
Average	453447.6
Standard Deviation	2260.7
%RSD	0.50

Acceptance criteria: %RSD for the sample should be NMT 2

Conclusion: The %RSD for the standard solution is below 1, which is within the limits, hence the method to precise.

INTERMEDIATE PRECISION (ruggedness)

There was no significant change in assay content and system suitability parameters at different conditions of ruggedness like day-to-day and system-to-system variation

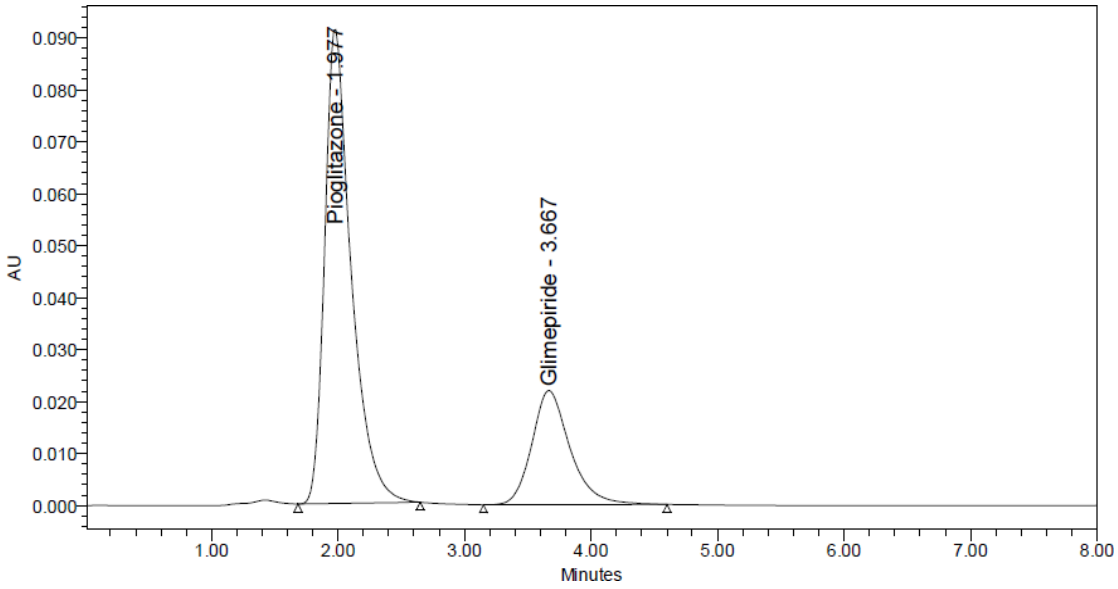

Fig:6-Chromatogram for ID Precision

Table:7-Results for intermediate precision for pioglitazone

INJECTION	**AREA**
Injection-1	1321427
Injection-2	1323183
Injection-3	1325174
Injection-4	1328935
Injection-5	1331407
Average	1326025
Standard Deviation	4104.2
%RSD	0.31

Table- Results for intermediate precision for Glimepiride

INJECTION	**AREA**
Injection-1	456412
Injection-1	456275
Injection-1	455601
Injection-1	458929
Injection-1	460580
Average	457559.4
Standard Deviation	2109.6
%RSD	0.46

Acceptance criteria: %RSD of five different sample solutions should not be more than 2

Conclusion: The %RSD obtained is within the limit, hence the method is rugged.

ACCURACY

Sample solutions at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated.

Table: 9- Accuracy (recovery) data for Pioglitazone

% Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	696587	7.5	7.67	101.43	100.50
100%	1351872	15	14.88	99.211	
150%	2061971	22.5	22.70	00.88	

Table:10-Accuracy (recovery) data for Glimepiride

% Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	226809	1	1.08	99.41	99.36
100%	453229	2	1.99	99.33	
150%	6799521	3	2.98	99.35	

Acceptance Criteria: The percentage recovery was found to be within the limit (97-103%).

Conclusion: The results obtained for recovery at 50%, 100%, and 150% are within the limits. Hence the method is accurate.

LIMIT OF DETECTION FOR PIOGLITAZONE AND GLIMEPIRIDE

The lowest concentration of the sample was prepared to the baseline noise and measured the signal to the ratio.

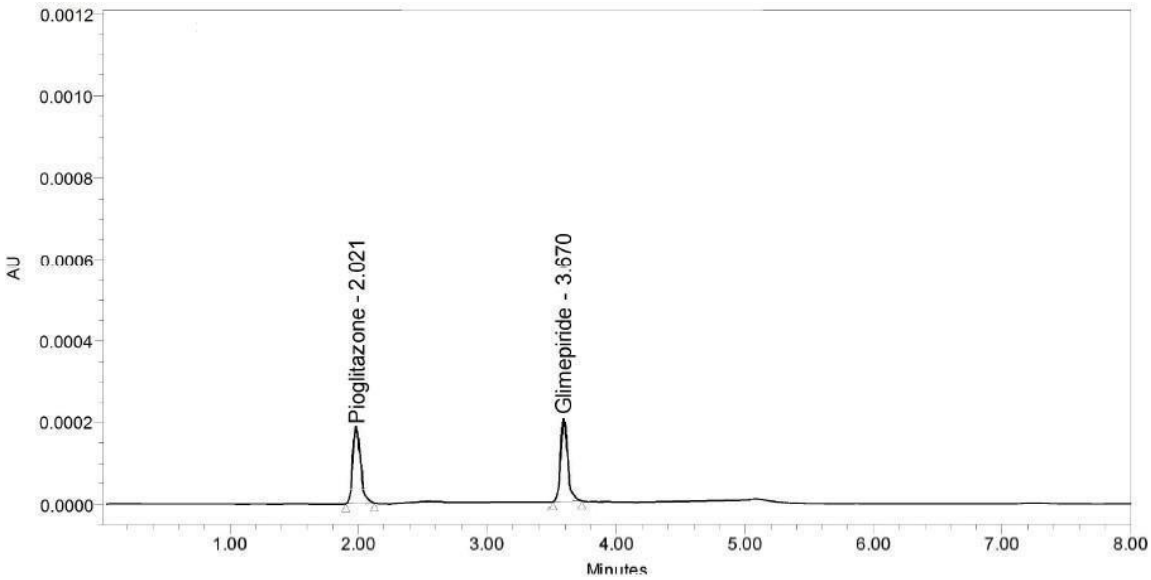

Fig:7-Chromatogram of Pioglitazone and glimepiride showing LOD

Table:11-Results of LOD

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Pioglitazone	61	181	2.97
Glimepiride	61	182	2.98

Acceptance criteria: Signal-to-noise ratio shall be 3 for the LOD solution

Conclusion: The result obtained is within the limit

LIMIT OF QUANTIFICATION FOR PIOGLITAZONE AND GLIMEPIRIDE

The lowest concentration of the sample was prepared to the baseline noise and measured the signal-to-noise ratio.

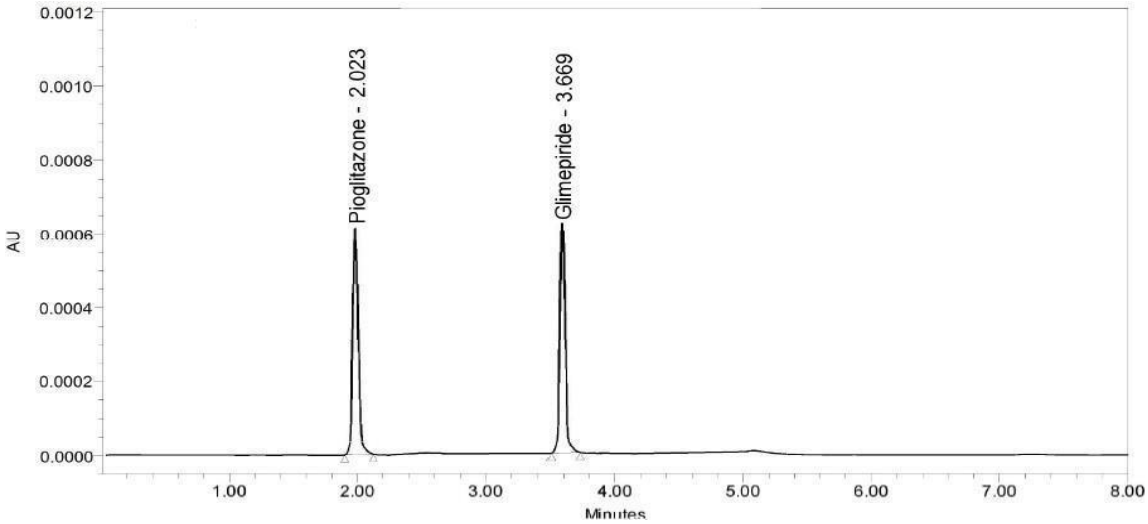

Fig:8-Chromatogram of Pioglitazone and Glimepiride showing LOQ

Table:12 Results of LOQ

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Pioglitazone	61	608	9.97
Glimepiride	61	609	9.98

Acceptance criteria: Signal to noise ratio shall be 10 for the LOQ solution

Conclusion: The result obtained is within the limit.

ROBUSTNESS

The standard and samples of Pioglitazone and Glimepiride were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

Table:13-Results for variation in flow for Pioglitazone

S. No	Flow Rate(ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	1.4	2491.77	1.55
2	1.5	2502.76	1.75
3	1.6	2374.78	1.32

Table:14-Results for variation in flow for Glimepiride

S. No	Flow Rate(ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	1.4	2919.05	1.21
2	1.5	2915.90	1.43
3	1.6	2519.01	1.13

Conclusion: Results for actual flow (1.5ml/min) have been considered from the Assay standard.

Table:15-Results for variation in mobile phase composition for Pioglitazone

S.no	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	2398.70	1.32
2	*Actual	2502.76	1.75
3	10% more	2478.80	1.49

Table:16-Results for variation in mobile phase composition for Glimepiride

S.no	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	2648.04	1.02
2	*Actual	2915.90	1.43
3	10% more	2684.45	1.26

Conclusion: Results for actual Mobile phase composition have been considered from the accuracy standard.

Acceptance criteria: The Retention time, USP plate count, USP tailing factor obtained for change of flow rate, and variation in mobile phase were found to be within the acceptance criteria. Hence the method is robust.

SUMMARY AND CONCLUSION

The estimation of Pioglitazone and Glimepiride was done by RP-HPLC.

The assay of Pioglitazone and Glimepiride was performed with tablets and the % assay was found to be 99.76 and 100.23 which shows that the method is useful for routine analysis.

The linearity of Pioglitazone and Glimepiride was found to be linear with a correlation coefficient of 0.999 and 0.999, which shows that the method can produce good sensitivity.

The acceptance criteria of precision are that RSD should be not more than 2.0% and the method shows precision of 0.71 and 0.50 for Pioglitazone and Glimepiride which shows that the method is precise.

The acceptance criteria of intermediate precision is that RSD should be not more than 2.0% and the method shows the precision of 0.31 and 0.46 for Pioglitazone and Glimepiride which shows that the method is repeatable when performed on different days.

The accuracy limit is the percentage recovery should be in the range of 97.0% - 103.0%. The total recovery was found to be 100.50% and 99.36% for Pioglitazone and Glimepiride. The validation of the developed method shows that the accuracy is well within the limit, which shows that the method can show good accuracy and reproducibility.

The acceptance criteria for LOD and LOQ are 3 and 10. The LOD and LOQ for Pioglitazone were found to be 2.97 and 9.97 and t h e LOD and LOQ for Glimepiride were found to be 2.98 and 9.98.

The robustness limit for mobile phase variation and flow rate variation is well within the limit, which shows that the method has good system suitability and precision under a given set of conditions.

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