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# SIMULTANEOUS ESTIMATION OF METFORMIN AND PIOGLITAZONE BY RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF BULK AND PHARMACEUTICAL DOSAGEFORM

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## ABSTRACT

A specific, accurate, precise and reproducible HPLC method has been developed and subsequently validated for Metformin and Pioglitazone in commercial tablets. The proposed HPLC method utilizes Agilent eclipse XDB $C_{18}$ column (150 mm - 4.6 mm i.e., 5 µm) and mobile phase consisting of Acetonitrile: $NaH_2PO_4$ pH 5.00 (50:50 v/v) at a flow rate of 1.0 mL/min. Quantitation was achieved with UV detection at 232 nm and 246 nm based on peak area with linear calibration curves at concentration range 50-800 µg/mL and 7.5-120 µg/mL for Metformin and Pioglitazone ($R^2$> 0.999 and 1.0). The method was validated in terms of accuracy (% recovery 99.6% and 99.5), precision (%RSD 0.04 and 0.05), linearity, limits of detection (4.1 ng/ml), limits of quantitation (12.6ng/ml), assay (100.5%), and robustness. This method has been successively applied to pharmaceutical formulation.

**KEYWORDS:** HPLC method, Metformin, Pioglitazone, successively.

## INTRODUCTION

Compound-1-Metformin, marketed under the trade name Glucophage among others, is the first-line medication for the treatment of type 2 diabetes, particularly in people who are overweight. It is also used in the treatment of polycystic ovary syndrome. Chemically Metformin N,N-Dimethylimidodicarbonimidic diamide.

Compound-2- Compound-1- Pioglitazone, marketed under the brand name Actos among others, Pioglitazone is a medication belonging to the thiazolidinedione class of drugs that are used as adjuncts to diet, exercise, and other diabetes medications to manage type 2 diabetes mellitus. Chemically Metformin N,N-Dimethylimidodicarbonimidic diamide.[1-10]

In this research work we developed, optimized, and validated the method using HPLC. The main objective of this method should be time saving and cost effective.

## MATERIALS AND METHOD

### Chemicals and Reagents

Reference standard of Metformin and Pioglitazone were gifted by Hetero laboratory. The formulation used for assay is Metformin and Pioglitazone and the solvents used in this method were acetonitrile, methanol, $NaH_2PO_4$ and water of HPLC grade.

### Instrumentation

Simultaneous estimation method development and validation was carried out by HPLC (Shimadzu Tokyo, Japan) with PDA detector module with auto-sampler. Column used was Agilent Eclips XBD (150*4.6 * 5µm), and data recorded using LC Solutions software.

**Mobile Phase: ACN:** 0.1 M $NaH_2PO_4$ (50:50) (pH 5.00 with Ortho phosphoric acid).

**Diluent:** Mobile phase is used as a diluent.

### Preparation of Standard

### Metformin Standard stock solution

Weigh accurately about 100mg of working standard drug and transferred into 50 ml volumetric flask to it added 30 ml of diluent, sonicated 5 minutes and finally make up the volume with diluent.

### Pioglitazone Standard Stock Solution

Weigh accurately about 15mg of working standard drug and transferred into 50 ml volumetric flask to it added 30 ml of diluent, sonicated 5 minutes and finally make up the volume with diluent.

### Metformin and Pioglitazone mixed Standard solution

Pipette out 1 mL above solution (Met & Pio) into 10 mL volumetric flask to it made up to volume with diluent.

Obtained standard concentration is 200µg/ml and 30µg/ml solution.

**Preparation of Sample**
Twenty tablets of Metformin and pioglitazone combination taken and powdered, weigh accurately about 100 mg of equivalent weight of drug and transferred into 50 ml volumetric flask to it added 30 ml of diluent, sonicated 5 minutes and finally make up the volume with diluent .Pipette out 1 mL above solution into 10 mL volumetric flask to it made up to volume with diluent. Obtained standard concentration is 200µg/ml and 30µg/ml solution.

**Method Optimization**
Based on literature of Metformin and Pioglitazone and its combinations, one method was developed after conducting several trials and developed method was optimized. Chromatogram was given in fig.1

**Validation**
Developed method was validated for different parameters like specificity-Stability, Accuracy, Precision, linearity, LOD, LOQ, and robustness as per ICH guidelines $Q_2R_1$.[11]

**System Suitability**
By injecting it six times of standard into the system, the chromatograms of 200µg/ml and 30µg/ml solution were analyzed. Form chromatogram the system suitability parameters like plate count, tailing factor, capacity factor and reproducibility were determined.

**Specificity**
**Interference from Blank:** By injecting the mobile phase in to the system we can determine the interference from blank. The mobile phase used in this method was ACN: 0.1 M $NaH_2PO_4$ (50:50) (pH 5.00 with Ortho phosphoric acid). We performed the filter variation i.e. PVDF, Nylon, Glass, filter. There were no blank, filter peaks was observed the at the retention time of analyte.

**Interference from Excipients:** The excipients from the tablet should not show any response (peak) at the retention time of the drug.

**Interference from Impurities:** Metformin and Pioglitazone impurity (A, B, C, D, E, and F) interference of degradation product generated in stress testing. The drug product and drug substances peak should be homogenous and there should be no co-eluting peaks. Peak purity for drug peak should meet the peak purity.

**Linearity**
A series of solutions were prepared at concentration levels as Metformin 25 µg/ml,50 µg/ml, 100 µg/ml, 200 µg/ml,400 µg/ml, and 800 µg/ml and Pioglitazone 3.5 µg/ml,7.5 µg/ml, 15 µg/ml, 30 µg/ml,60 µg/ml, and120 µg/ml. A 10µl volume from each concentration of solutions was injected twice into the HPLC system. Chromatograms were recorded under optimized chromatographic conditions. A graph was plotted considering peak areas on Y-axis and concentration on X-axis. The linear equation, Y-intercept, slope of regression line and regression constant ($r^2$) were calculated and given in table no 1 and Fig no 2&3.

**Accuracy**
A series of solutions were prepared in triplicate by spiking the known standard concentrations of Metformin and Pioglitazone the range of 50-200% on the tablet solution and analyzed. The accuracy of method was provided at three different concentration levels at 250, 500, and 1000 µg/ml of Metformin standard and 15, 30, and 60 µg/ml of Pioglitazone. The percentage recoveries of three different concentrations were found to be within the range of 98.0 to 102.0 % as per ICH $Q_2R_1$ guidelines. The results were given in table no 2.

**Precision**
Repeatability or intra-day precision: The peak areas of test samples were analyzed on the same day by injecting it six times into the system. The chromatogram was recorded and RSD was calculated and given in table3.

**Limit of Detection and Limit of Quantitation**
LOD and LOQ can be calculated based on the signal to noise ratio approach, visual evaluation and standard deviation of the response and slope of the calibration curve. The slope (S) is calculated from the equation of straight line in calibration curve of theanalyte. The standard deviation (σ) is calculated based on its blank response or they-intercepts of regression line. Formulas were given below.
LOD= (3.3X SD)/Slope
LOQ= (10 X SD)/Slope

**Robustness and Ruggedness**
The robustness of a method is its ability to remain unaffected under changes in parameters. Robustness was carried out by altering the flow rate (±0.2ml/min) and mobile phase (60:50 & 50:60) .The standard solution comprising of Metformin and Pioglitazone (100µg/ml and 30µg/ml) was injected six times and the % RSD was calculated for the resultant area of the peak.

**Assay**
Twenty tablets of Metformin and Pioglytazone combination dosage form were taken and powdered, weigh accurately about 100 mg of equivalent weight of drug and transferred into 50 ml volumetric flask to it added 30 ml of diluent, sonicated 5 minutes and finally made up the volume with diluent .Pipette out 1 mL above solution into 10 mL volumetric flask to it made up to volume with diluent. Obtained standard concentration is 200 and 30 µg/ml solution.

$$\% \text{ Assay} = \frac{(\text{Area of unknown X Conc Of standard})_X 100}{(\text{Area of standard X Conc of unknown})}$$

## RESULTS AND DISCUSSION

**Optimization Of Chromatographic:** Developed method was optimized for different parameters. Optimized chromatogram was given in Fig: 1.

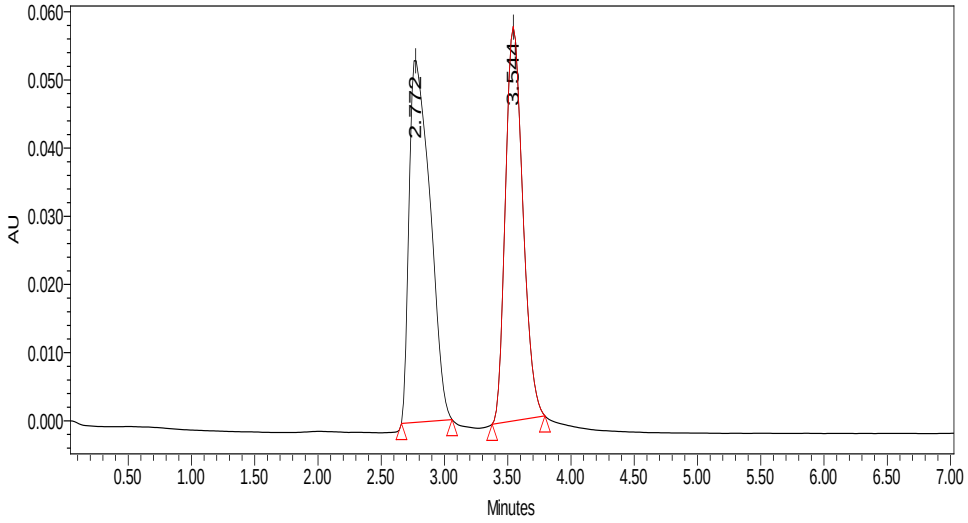


**Fig. 1: Optimized Chromatogram.**

### Linearity

The calibration curve was made by plottingthe concentration on X-axis against peakarea on Y-axis. A series of Metformin and Pioglitazone standard solution were prepared inthe range of 50 to 800µg/ml and 7.5 to 120 µg/ml. Thecorrelation coefficient of the curve was foundto be 0.999 and 1.0 with a regression equation of Y=317228x - 248444. This is shown in figure-4 and results were given in table:3.

**Table 1: Results of Linearity.**

| S. NO. | MET | PIO | MET | PIO |
|---|---|---|---|---|
| | Linearity concentration | Linearity concentration | Peak Area | Peak Area |
| 1 | 800 | 120 | 1167224 | 4673736 |
| 2 | 400 | 60 | 578612 | 2336868 |
| 3 | 200 | 30 | 290306 | 1168434 |
| 4 | 100 | 15 | 147153 | 584217 |
| 5 | 50 | 7.5 | 98202 | 292109 |
| 6 | 25 | 3.5 | 46015 | 146054 |

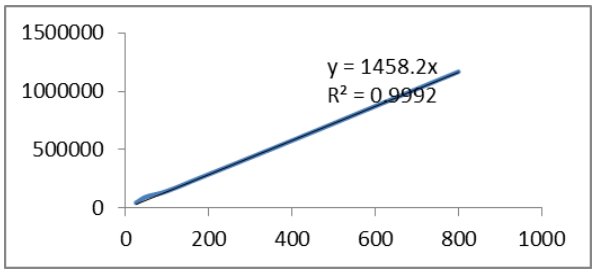


**Fig. 2: Calibration curve of Metaformin.**

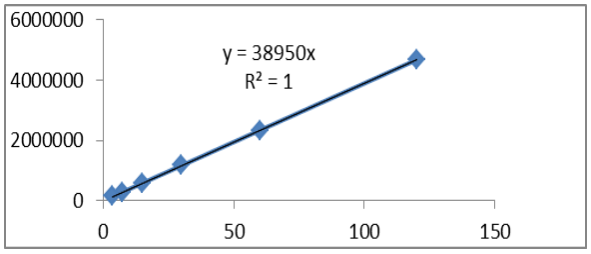


**Fig. 3: Calibration curve of Pioglytazone.**

### Accuracy

A series of solutions were prepared in triplicate by spiking the known standard concentrations of Metformin and Pioglitazone in the range of 50-150% on the tablet solution and analyzed. The accuracy of method was provided at three different concentration levels at 100, 500, and 250 µg/ml of Metformin and 15, 30, and 60 µg/ml of Piogliazone. Each concentration triplicate samples were injected and average % recovered was calculated. The average % recovery was found to be 99.6%. Results were given in the Table: 2

**Table 2: Results of Accuracy.**

| S. NO. | Metformin | | Pioglitazone | |
|---|---|---|---|---|
| | Level | Percent recovery | Level | Percent recovery |
| 1 | 50% | 98.90 | 50% | 98.9 |
| 2 | 50% | 99.35 | 50% | 99.35 |
| 3 | 50% | 99.35 | 50% | 99.35 |
| 4 | 100% | 99.87 | 100% | 99.87 |
| 5 | 100% | 99.67 | 100% | 99.67 |
| 6 | 100% | 99.20 | 100% | 99.2 |
| 7 | 200% | 99.96 | 200% | 99.96 |
| 8 | 200% | 99.76 | 200% | 99.76 |
| 9 | 200% | 99.96 | 200% | 99.96 |

**Precision**

Repeatability or intra-day precision: The peak areas of 200µg/ml and 30µg/ml were analyzed on the same day by injecting it six times into the system. % RSD was calculated. The %RSD was found to be 0.004 and 0.005. Results were given in table: 3.

**Table 3: Results of precision.**

| S. NO. | MET | PIO | MET | PIO |
|---|---|---|---|---|
| | PEAK AREA | PEAK AREA | % ASSAY | % ASSAY |
| 1 | 196539 | 1168796 | 99.86 | 100.03 |
| 2 | 196252 | 1168750 | 99.72 | 100.02 |
| 3 | 196757 | 1168868 | 99.97 | 100.03 |
| 4 | 196028 | 1168988 | 99.6 | 100.04 |
| 5 | 196803 | 1168434 | 99.9 | 100.1 |
| 6 | 196803 | 1168434 | 100 | 100 |

**LOD and LOD**

LOD and LOQ were calculated from linearity graph. The limit of Detection and limit of Quantification were found out to be 4.1ng/ml and 12.6 ng/mL respectively.

**Assay**

Tablet solution was injected into the HPLC system for three times and % assay of drug was found to be 99.9%.

**Robustness and Ruggedness**

**Change in The Mobile Phase:** On evaluation of the results, it can be concluded that the variation in mobile phase affected the method significantly. Hence it indicates that method was not affected even by change in the mobile phase. The system suitability parameters were within the limit.

**Change in The Flow Rate:** Results for actual flow (1.0 ml/min) have been considered from Assay standard. System suitability parameters were studied and the results were within the limit.

## CONCLUSION

An easy, rapid and efficient reverse-phase HPLC method was developed for quantitative estimation of Metformin and Pioglitazone in drug product and drug substance. The method was validated as per ICH Q2 (R1) guideline. A precise, accurate, linear, robust and rugged method was found during validation. In the assay 100.5% drug was found in the drug product. Limits of detection (4.1 ng/ml) and limits of quantitation (12.6ng/ml) also determined. A stability-indicating HPLC method has been developed for the estimation of Metformin and Pioglitazone in the presence of degradation products. The above method for Metformin and Pioglitazone was found to be selective and stability indicating under different stress conditions.

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