International Journal of Medicine and Pharmaceutical Research

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Research Article **Open Access**

Analytical Method Development and Validation for Simultaneous Estimation of Metformine Hydrochloride and Pioglitazone by Using UV Spectrophotometry

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A B S T R A C T

A simple, Accurate, precise method was developed for the simultaneous estimation of the Metformine hydrochloride and Pioglitazone in Tablet dosage form. Chromatogram was run through C18, 4.6 mm, 5µm Make: x Terra Mobile phase containing Acetonitrile and Water in the ratio of 50:50 was pumped through column at a flow rate of 1ml/min. Optimized wavelength for Metformin and Pioglitazone was 242nm. %RSD of the Metformin and Pioglitazone were and found to be less than 2. Lnearity range of Metformin and Pioglitazone 25-125µg/ml. Correlation coefficient is 0.999.

Keywords: Metformin, Pioglitazone, UV-Spectrophotometry

A R T I C L E I N F O

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Article History: Received 24 October 2015, **Accepted** 27 November 2015, **Available Online** 10 February 2016

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Manuscript ID: IJMPR2787

PAPER-QR CODE

Citation: N. Geetha, *et al.* Analytical Method Development and Validation for Sumultaneous Estimation of Metformine Hydrochloride and Pioglitazone by Using UV Spectrophotometry. *Int. J. Med. Pharm. Res.*, 2016, 4(1): 16-22.

1. Introduction

Metformin is chemically named as 1-carbamimidamido-N, N-dimethyl methanimidamide [1]. Metformin decreases blood glucose levels by decreasing hepatic glucose production, decreasing intestinal absorption of glucose, and improving insulin sensitivity by increasing peripheral glucose uptake and utilization [2]. These effects are mediated by the initial activation by metformin of AMP-activated protein kinase (AMPK), a liver enzyme that plays an important role in insulin signaling, whole body energy balance, and the metabolism of glucose and fats. Activation

of AMPK is required for metformin's inhibitory effect on the production of glucose by liver cells[3]. Increased peripheral utilization of glucose may be due to improved insulin binding to insulin receptors. Metformin administration also increases AMPK activity in skeletal muscle [4].

Pioglitazone is chemically named as 5-({4-[2-(5-ethylpyridin-2-yl) ethoxy] phenyl}methyl)-1,3-thiazolidine-2,4-dione[5]. Pioglitazone acts as an agonist at peroxisome proliferator activated receptors (PPAR) in target tissues for insulin action such as adipose tissue, skeletal muscle, and liver[6]. Activation of PPAR-gamma receptors increases the transcription of insulin-responsive genes involved in the control of glucose production, transport, and utilization. In this way, pioglitazone both enhances tissue sensitivity to insulin and reduces hepatic gluconeogenesis. Thus, insulin resistance associated with type 2 diabetes mellitus is improved without an increase in insulin secretion by pancreatic β cells[7].

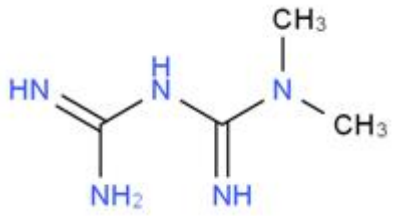

Figure 1: Structure of Metformin

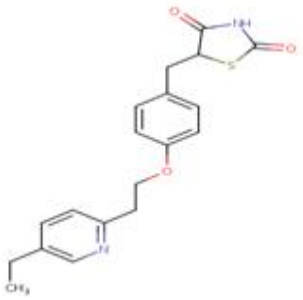

Figure 2: Structure of Pioglitazone

2. Materials and Methods

Preparation of standard solution:

10 mg of Metformin and 10mg of Pioglitazone were accurately weighed and transferred into a 10 ml clean dry volumetric flask, about 7 ml of diluent was added, sonicated to dissolve it completely and the volume was made up to the mark with the same solvent to give a concentration of 1000 µg/ml. (Stock solution) Further 0.75ml was pipetted out from the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent to give a concentration of 75 µg/ml and 75 µg/ml respectively[8].

Preparation of sample solution:

10 Tablets of contents were weighed and triturated in glass mortar. The quantity of powder equivalent to 10 mg of active ingredient present in Metformin and Pioglitazone was transferred into a 10 ml clean dry volumetric flask, 7 ml of diluent was added to it and was shaken by mechanical stirrer and sonicated for about 30 minutes by shaking at intervals of five minutes each and was diluted up to the mark with diluent to give a concentration of 1000 µg/ml and allowed to stand until the residue settles before taking an aliquot for further dilution (stock solution)[9]. 0.75 ml of upper clear solution was transferred to a 10 ml volumetric flask and diluted with diluent up to the mark to give the respective concentrations as par with standard solution. The solution was filtered through 0.45 µm filter before injecting into HPLC system [10].

Preparation of Placebo:

The amount of powdered inactive ingredient supposed to be present in 10 tablets were accurately weighed and transferred in to 10 ml volumetric flask, 7 ml of diluent was added and shaken by mechanical stirrer and sonicated for about 30 minutes by shaking at intervals of five minutes and was diluted up to the mark with diluent and allowed to stand until the residue settles before taking an aliquot for dilution [11]. 0.1 ml of upper clear solution was transferred to a 10 ml volumetric flask and diluted with diluent up to the mark and the solution was filtered through 0.45 µm filter before injecting into HPLC system [12].

Method Validation

The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. According toICH guidelines, typical analytical performance characteristics that should be considered in the validation of the type of methods are:

Linearity:

Preparation of sample stock solution:

About 50 mg of Metformin and 1.5 mg of Piogltazone samples was weighed in to 10 ml volumetric flask, it was dissolved with diluent and the volume was made up to the mark with same diluent (5000µg/ml of Metformin and 150µg/ml of Piogltazone)[13].

Preparation of Level – I (25µg/ml of Metformin & 1.5µg/ml of Piogltazone)

0.25 ml of stock solution had taken in 10ml of volumetric flask diluted up to the mark with diluent.

Preparation of Level–II (50µg/ml of Metformin &3µg/ml of Piogltazone)

0.5 ml of stock solution had taken in 10ml of volumetric flask diluted up to the mark with diluent.

Preparation of Level–III (75 µg/ml of Metformin &4.5 µg/ml of Piogltazone)

0.75 ml of stock solution had taken in 10ml of volumetric flask diluted up to the mark with diluent.

Preparation of Level–IV (100 µg/ml of Metformin &6 µg/ml of Piogltazone)

1.0 ml of stock solution had taken in 10ml of volumetric flask diluted up to the mark with diluent.

Preparation of Level–V (125 µg/ml of Metformin &7.5 µg/ml of Piogltazone).

1.25 ml of stock solution had taken in 10ml of volumetric flask diluted up to the mark with diluent. 10µl of each level were injected & placed into the HPLC and UV systems and recorded the peak response and absorbances.

Procedure: Each level solution was injected into the chromatographic system and the peak area was measured

[13]. A graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) was plotted and the correlation coefficient was calculated. The linearity of the method was demonstrated over the concentration range of 25-125µg / ml. Aliquots of five levels were prepared from sample solution and labeled as solution 1, 2, 3, 4 and 5 respectively. The solutions were injected in to HPLC system as per test procedure[14].

Each level solution was placed into the UV system and the Absorbance was noted. A graph of absorbance versus concentration (on X-axis concentration and on Y-axis absorbance) was plotted and the correlation coefficient was calculated [15].

Acceptance criteria

Correlation Coefficient should be not less than 0.9990.

% RSD of peak areas for Solution 1, 2, 3, 4 and 5 should be not more than 2.0 %.

Precision

Preparation of stock solution:

10 mg of Metformin and 10mg of Piogltazone were accurately weighed and transferred into a 10 ml clean dry volumetric flask, about 7 ml of diluent was added and sonicated to dissolve it completely. The volume was made up to the mark with the same solvent to give the concentration of 1000 µg/ml. (Stock solution) .Further 0.75 was pipette out from the above stock solutions into a 10ml volumetric flask and diluted up to the mark with diluent to give the concentration of 75 µg/ml and 75 µg/ml respectively[16].

Procedure:

The standard solutions were injected & placed into the HPLC and UV systems and recorded the peak response and absorbance,for five times and the areas for all five injections were measured in HPLC[17]. The %RSD for the area of five replicate injections was found to be within the specified limits[18].

Acceptance criteria:

% Relative standard deviation of peak areas and R_t should not be more than 2.0

Intermediate Precision/Ruggedness

To evaluate the intermediate precision (also known as Ruggedness) of the method Precision was performed on different day by using different make column of same Dimensions [19].

Preparation of stock solution:

10 mg of Metformin and 10mg of Piogltazone accurately weighed and transferred into a 10 ml clean dry volumetric flask, about 7 ml of diluent was added ,sonicated to dissolve it completely and the volume was made up to the mark with the same solvent to give the concentration of 1000 µg/ml. (Stock solution) Further pipette out 0.75 ml from the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent to give the concentration of 75µg/ml and 75 µg/ml respectively[20].

Procedure: The standard solution was injected for five times and the area was measured for all five injections in HPLC. The %RSD for the area and R_t of five replicate injections was found to be within the specified limits [21].

Acceptance criteria: % Relative standard deviation of peak areas and R_t should not be more than 2.0

% Accuracy: Assay was performed in triplicate for various concentrations of Metformin and Piogltazone equivalent to 50, 100, and 150 % of the standard amount was injected into the HPLC system as per the test procedure [22].

Preparation of Standard stock solution:

10 mg of Metformin and 10mg of Piogltazone accurately weighed and transferred into a 10 ml clean dry volumetric flask, about 7 ml of diluent was added, sonicated to dissolve it completely and volume was made up to the mark with the same solvent to give the concentration of 1000 µg/ml. (Stock solution)

Preparation Sample solutions:

Preparation of 50% solution (37.5 µg/ml of Metformin and 37.5 µg/ml of Piogltazone):

From the above stock solutions take 0.375ml into 10 ml dry volumetric flask, make up to the mark with diluent**.**

Preparation of 100% solution (75 µg/ml of Metformin and 75 µg/ml of Piogltazone):

From the above stock solutions take 0.3 ml and 1.8 ml into 10 ml dry volumetric flask, make up to the mark with diluent

Preparation of 150% solution (102.5 µg/ml of Metformin and 102.5 µg/ml of Piogltazone):

From the above stock solutions take 1.025 ml into 10 ml dry volumetric flask, make up to the mark with diluent. These solutions were filtered through 0.45µ membrane and then each concentration; three replicate injections were made under the optimized conditions

Procedure: The standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions were injected. The amount found and amount added for Metformin and Piogltazone individual recovery and mean recovery values were calculated and the results were found to be within the limit.

Acceptance criteria:

The mean % recovery of the Metformin and Piogltazone each spike level should be not less than 98.0 % and not more than 102.0 %[23].

Robustness

The robustness of the proposed method was determined by analysis of aliquots from homogenous lots by differing physical parameters like flow rate and mobile phase composition, temperature variations which may differ but the responses were still within the specified limits of the assay[24].

a) Effect of variation of flow rate*:*

A study was conducted to determine the effect of variation in flow rate. The flow rate was varied at 1.0 ml/min to 1.2 ml/min. Standard solution 75 ppm Metformin and 75 ppm Piogltazone were prepared and analysed using the varied flow rates along with method flow rate[25]. The results are summarized on evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly [26]. Hence it indicates that the method is robust even by change in the flow rate ±10%. The method is robust only in less flow condition. The effect of variation of flow rate was evaluated.

Acceptance criteria

1. The tailing factor for Metformin and Piogltazone should be not more than 2.0 for Variation in flow.

2. The % RSD of Asymmetry and retention time for Metformin and Piogltazone should be not more than 2.0 % for variation in flow.

b) Effect of variation of mobile phase composition:

A study was conducted to determine the effect of variation in mobile phase ratio by changing the ratio of mobile phase [27]. The Organic composition in the Mobile phase was varied from 40 % to 60 %.Standard solution 75 µg/ml of Metformin and 75 µg/ml Piogltazone were prepared and analysed using the varied mobile phase composition along with the actual mobile phase composition in the method[28,29].

Acceptance criteria

1. Tailing Factor of Metformin and Piogltazone drugs should not be more than 2.0 for Variation in composition of mobile phase.
2. The % RSD of tailing factor and retention times of Metformin and Piogltazone drugs should be not more than 2.0 for Variation in composition of mobile phase.

3. Results and Discussion

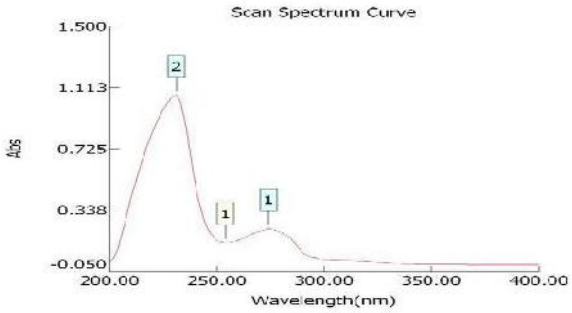

Figure 3: Spectrum showing the Metformin

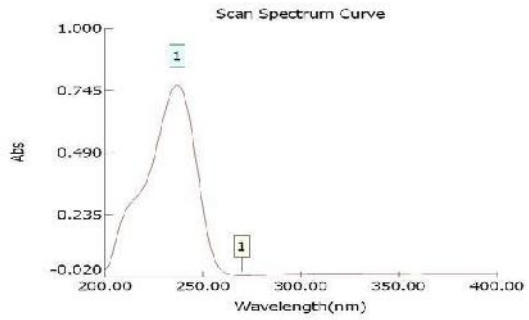

Figure 4: Spectrum showing the Pioglitazone

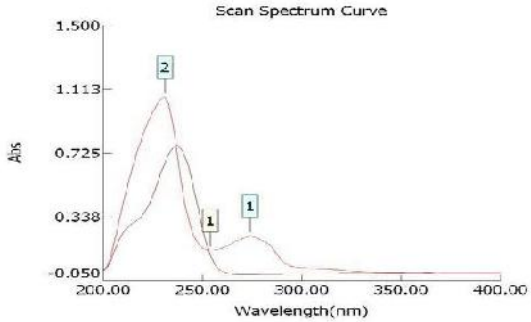

Figure 5: Spectrum showing the Metformin and Pioglitazone iso bestic point

Spectrum showing linearity:

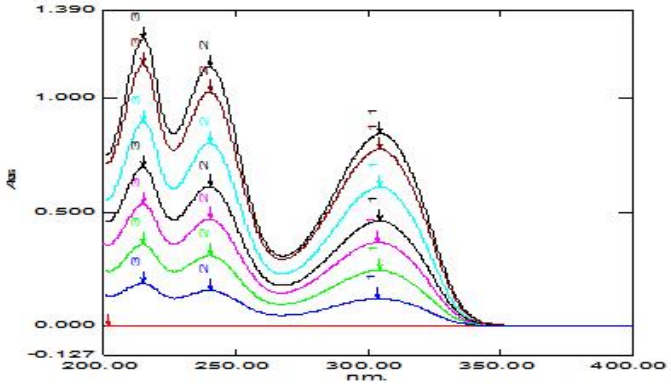

Figure 6: Spectrum showing the Metformin and Pioglitazone

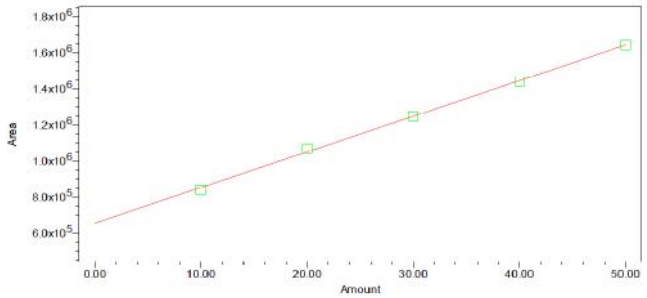

Figure 7: Calibration curve of Metformin

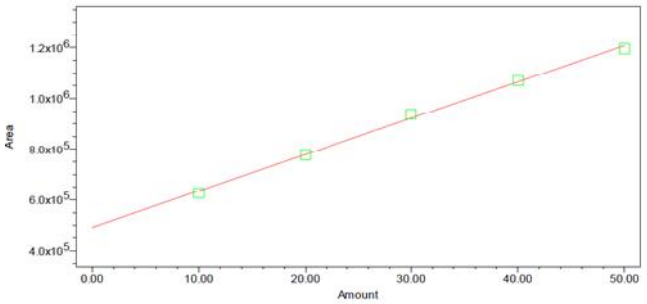

Figure 8: Calibration curve of Pioglitazone

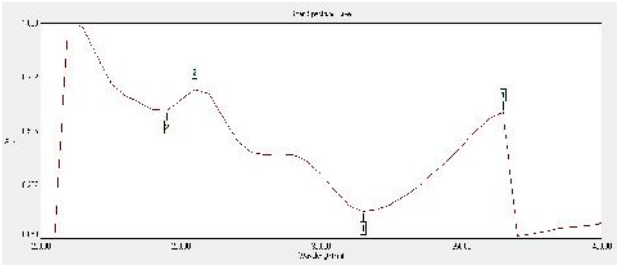

Figure 9: Spectrum showing accuracy for Metformin

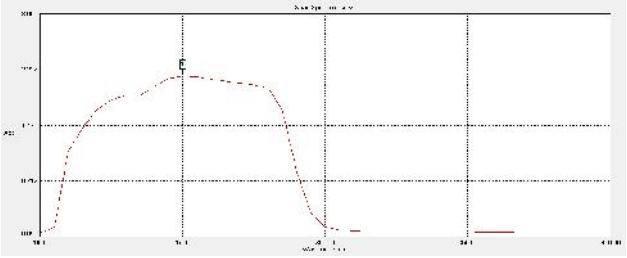

Figure 10: Spectrum showing accuracy for Pioglitazone

Table 1: List of Standard and Sample details

S.No	Name	Batch No	Manufaturer/ supplier
1.	Metformin working standard	-	KP labs pvt. Ltd
2.	Pioglitazone working standard	-	KP labs pvt. Ltd

Table 2: List of Equipment/Instrument details

S.No	Instrument name	Model
1.	HPLC system	WATERS, software: Empower, 2695 separation module. 2996 PDA detector.
2.	Semi micro balance	Sartorius ME235P
3.	P^H Meter	Lab India ph. Meter
4.	Sonicator	Ultrasonic cleaner power sonic 420
5.	UV/VIS spectrophotometer	LABINDIA UV
6.	Constant temperature water bath	Thermo lab GMP

Table 3: List of Chemicals and Reagents

S.No	Name	Manufacturer	Grade
1.	Potassium dihydrogen ortho phosphate	Merck	GR
2.	Sodium perchlorate	Merck	GR
3.	Perchloric acid	Merck	GR
4.	Ortho phosphoric acid	Merck	GR
5.	Methanol	Merck	HPLC
6.	Acetontrile	Merck	HPLC
7.	Water	Milli-pore	Milli-Q
8.	0.45 µm Nylon filter	Axivia	S0761009
9.	0.45µm PVDF filter	Rankem	D004A07

Table 4: Linearity results for Metformin

S.No	Linearity Level	Concentration	Abs
1	I	25 ppm	0.166
2	II	50 ppm	0.30
3	III	75 ppm	0.468
4	IV	100 ppm	0.610
5	V	125 ppm	0.767
Correlation Coefficient			0.999

Table 5: Linearity results for Pioglitazone

S.No	Linearity Level	Concentration	Abs
1	I	25 ppm	0.150
2	II	50 ppm	0.310
3	III	75 ppm	0.176
4	IV	100 ppm	0.627
5	V	125 ppm	0.810
Correlation Coefficient			0.999

Table 6: Spectrum values for Metformin

Sample No.	Spike Level	Amount (µg/ml) added	Amount (µg/ml) found	% Recovery	Mean % Recovery	Stdev	% Rsd
1	50 %	3	2.9	98.3%	99.2%	1.39	1.40
		3	2.9	98.5%			
		3	3.0	100.0%			
2	100 %	4	4.0	100.0%	100.01%	1.15	1.12
		4	3.9	99.0%			
		4	4.0	101.2%			

Acceptance Criteria: The % Recovery for each level should be between 98.0 to 102.0%

Table 7: Spectrum values for Pioglitazone

Sample No.	Spike Level	Amount (µg/ml) added	Amount (µg/ml) found	% Recovery	Mean % Recovery	Stdev	% Rsd
1	50 %	1.5	1.4	99.3%	100.2%	0.64	0.63
		1.5	1.5	100.5%			
		1.5	1.5	100.0%			
2	100 %	2	2.0	101.0%	101.01%	1.4	1.37
		2	2.0	100.0%			
		2	2.0	102.2%			
3	150 %	2.5	2.4	99.0%	99.29%	0.47	0.47
		2.5	2.4	98.80%			
		2.5	2.4	99.79%			

Acceptance Criteria: The % Recovery for each level should be between 98.0 to 102.0%

4. Conclusion

High performance liquid chromatography and spectroscopy are at present one of the most sophisticated tool of the analysis. The estimation of Metformin and Pioglitazone was done by RP-HPLC and UV Methods. The mobile phase was optimized with consists of Acetonitrile: Water mixed in the ratio of 50:50 % v/ v. A $C1_8$ column C18 (4.6 x 150mm, 5µm, Make: XTerra) or equivalent chemically bonded to porous silica particles was used as stationary phase.. The solutions were chromatographed at a constant flow rate of 1.0 ml/min. the linearity range of Metformin and Pioglitazone were found to be from 25-125 µg/ml. of Metformin and Pioglitazone. Linear regression coefficient was not more than 0.999.The maximum absorbance are found to be at 242 nm. The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 97-102% of Metformin and Pioglitazone. LOD and LOQ were found to be within limit. The results obtained on the validation parameters met ICH and USP requirements. It inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

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