RESEARCH ARTICLE JPAR 2020

Journal of Pharmaceutical Advanced Research

(An International Multidisciplinary Peer Review Open Access monthly Journal)

Available online at: www.jparonline.com

Simultaneous estimation of Metformin and Pioglitazone in Pharmaceutical dosage form by HPLC

Rama Prasad Padhy*, Amrit Kumar Rath

Department of Pharmaceutical Analysis and Quality Assurance, Danteswari College of Pharmacy, Borapadar, Raipur Road, Jagadalpur- 494221, Chhattisgarh, India.

Received: 03.07.2019 Revised: 16.07.2020 Accepted: 20.07.2020 Published: 31.07.2020

ABSTRACT: Background: According to the literature survey it was found that few analytical methods have been reported for stability analysis of Metformin by Ultraviolet (UV), High Performance Liquid Chromatography (HPLC), Potentiometer and Spectrofluorimetry studies. Pioglitazone in Pharmaceutical dosage forms, it's metabolites in human plasma and simultaneous determination of Metformin and Pioglitazone in Pharmaceutical dosage forms can be analysed by HPLC analytical techniques. **Aim:** The present study was aimed to carry out a simple, sensitive and specific HPLC method development and validation for the quantification of Metformin and Pioglitazone in bulk and Pharmaceutical dosage form. **Method:** The HPLC study was carried out on X Tetra RP C18 (4.6×250 mm) 5 µ column using a mixture of water: Methanol (50:50 v/v) as the mobile phase at a flow rate of 1.0 ml/min. The detection was carried out at λ_{max} of 240 nm in ambient temperature. The validation of the proposed method was carried out as per ICH Guidelines. **Results:** The retention time for Metformin and Pioglitazone was found to be 1.948 and 3.594 min. The responses for Metformin and Pioglitazone were found to be linear and their co-relation coefficients (r^2) were found to be 0.9998 and 0.9999 (r^2 >0.999) respectively. The precision that is % RSD for Metformin and Pioglitazone were 0.10 and 0.30 respectively. The Accuracy that is mean percentage recovery fields Metformin and Pioglitazone were 100.63 and 99.8 % respectively. **Conclusion:** Based on the performance characteristic, the proposed HPLC method was found to be suitable for the estimation of Metformin and Pioglitazone in bulk and pharmaceutical dosage form.

Corresponding author*
Dr. Rama Prasad Padhy
Principal
Danteswari College of Pharmacy,
Borapadar, Jagadalpur- 494221,
Chhattisgarh, India.
Tel: +91-8895908528
Mail ID: rampadhy@gmail.com

Keywords: Metformin, Pioglitazone, HPLC, Validation, Precision, Accuracy.

INTRODUCTION:

The Metformin (Fig 1) is an anti-diabetic drug of the biguanide group, available as tablets at doses of 200, 500, 850 and 1000 mg. It is soluble in water but slightly soluble in methanol and acetone. Metformin activates AMP-activated protein kinase (AMPK), a liver enzyme that plays an important role in insulin signalling, 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 [1-3].

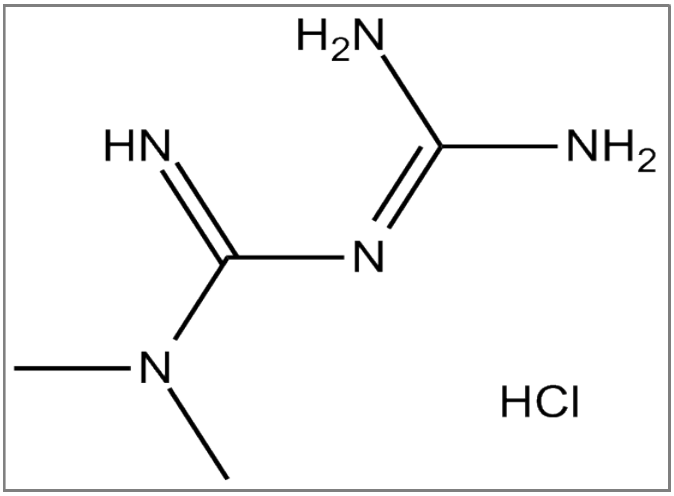

Fig 1. The chemical structure of Metformin Hydrochloride.

The Pioglitazone (Fig 2) is Thiazolidinediones group hypoglycemic drug available as tablet at doses of 7.5, 10, 30 and 45 mg. It is soluble in methanol, ethanol, acetonitrile and di-methyl formamide but slightly soluble in water. Pioglitazone acts as an agonist at peroxisome proliferator activated receptors (PPAR) in target tissues for insulin action [4-6].

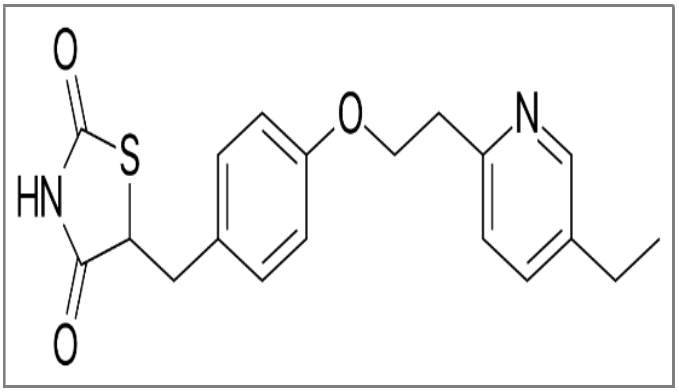

Fig 2. The chemical structure of Pioglitazone.

Validation is used for framing and documenting the capabilities of the developed method. The utility of the developed method to determine the content of drug in commercial formulation [7,8]. Validation of the method was done in accordance with USP and ICH guidelines for the assay of active ingredient [9,10]. The method was validated for parameters like system suitability, linearity, precision, accuracy, specificity, ruggedness, and robustness, limit of detection and limit of quantification [11,12]. The objective of the study was to develop a simple, sensitive and specific HPLC method for the analysis of Metformin and Pioglitazone in bulk and Pharmaceutical dosage forms.

MATERIALS AND METHOD:

The Metformin and Pioglitazone pure drug (API) were procured as gift samples from NATCO Pharma Pvt. Ltd., Kothur, Hyderabad, A.P, India. The tablets containing a combination of Metformin and Pioglitazone were purchased from Local Jagdalpur market. Methanol and ortho-phosphoric acid were purchased from Merck, India. All other chemicals used in this study were of analytical grade and procured from authorized dealers.

Preparation of Buffer:

Accurately 13.609 g of potassium dihydrogen phosphate (KH2PO4) was weighed and it was dissolved in 1000 ml of Milli-Q water. The pH was adjusted to 4.5 with ortho-phosphoric acid by using digital pH meter (Inolab WTW series, india). The solution was filtered through 0.45µm nylon membrane filter and finally the solution was degassed by sonication (Ultrasonic cleaner power sonic 420, Lab India, Mumbai).

Preparation of Mobile Phase (Phosphate buffer and Methanol:

The phosphate buffer pH 4.5 and methanol were mixed together in the ratio of 70:30 and the solution was degassed by sonication to remove any entrapped air.

Preparation of Blank solution (Diluent):

The Milli Q water and methanol were mixed within the ratio of 50:50 v/v and degassed by sonication.

Preparation of normal Stock Solution:

Accurately 200 mg of Metformin working standard was weighed and it was transferred to 100 volumetric flask containing 7.5 mg of Pioglitazone working standard into a 100 ml volumetric flask. To the flask, about 50 ml of diluent was added and the mixture was sonicated to dissolve the drugs. Finally the solution was dilute to required volume with diluents and blended well.

Preparation of normal solution with diluent:

About 5.0 ml of the above prepared solution were pipetted and put into a 50 ml volumetric flask. The solution was diluted to volume with diluents. The solution was filtered through 0.45 µ nylon membrane filter. First few ml of filtrate was discarded.

Preparation of Test solution of Metformin and pioglitazone (200/ 7.5 mg) Tablets:

Randomly about 20 tablets were selected and weighed. The average weight of 20 tablets was determined. All the tablets were crushed into the fine powder. The tablet powder equivalent to 396.50 mg was weighed and

transferred into a 100ml of volumetric flask. To the flask about 70 ml of diluent was added and mixed. The solution was sonicated for 10 min with intermittent shaking. From this solution, about 5 ml was pipetted out and transferred into a 50 ml volumetric flask. It was diluted to volume with diluent and mixed well. The solution was filtered 0.45 µ nylon membrane. First few ml of filtrate was discarded.

Drugs analysis (Quantification)n by HPLC study: The column was equilibrated with the mobile phase for sufficient time until a stable baseline is obtained. The blank (diluents) in single, standard solution in five replicates and every test solution in duplicate was injected into the chromatographic system and the chromatograms were recorded. The samples were injected into the HPLC as per the parameters given in Table 1.

Table 1. The HPLC study specification detail.

Sl. No.	Parameters	Specifications
1	Apparatus	WATERS-HPLC equipped with variable wave length PDA- Detector and an empower-2-software.
2	Column	X Terra RP 18 250 × 4.6 mm, 5µL
3	Wave length	240 nm
4	Mobile phase	Water: Methanol (50: 50)
5	Flow rate	1.0 ml/min
6	Temperature	Ambient
7	Injection volume	10 µl
8	Run time	20 min

Assay of Tablet dosage form:

Applicability of the proposed method for the simultaneous estimation of Metformin and Pioglitazone was studied by assay of Economic tablets Pioglit MF label to contain Metformin 200 mg and Pioglitazone 7.5 mg.

RESULTS AND DISCUSSION:

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Metformin and Pioglitazone was done by HPLC. A straight forward Reverse phase HPLC method was developed for the simultaneous estimation of Metformin and Pioglitazone in Pharmaceutical dosage form. The developed method was found to be simple, sensitive, rapid, precise, accurate, and price effective. Peaks are good and all system suitability parameters are within the limits (Fig 3 and Table 7 and 8). The retention time for Metformin and Pioglitazone was found to be 1.948 and 3.594 min (Fig 4 and Table 2). The responses for Metformin and Pioglitazone were found to be linear and their correlation coefficients (r^2) was found to be 0.9998 (Fig 5) and 0.9999 (Fig 6) (r2 >0.999) for Metformin and Pioglitazone respectively (Table 3 and 4). The precision that is % RSD for Metformin and Pioglitazone were 0.10 and 0.30 respectively (Table 9 and 10). The Accuracy that is mean percentage recovery fields Metformin and Pioglitazone were 100.63 and 99.8 % respectively (Table 5 and 6). The tactic is free from interference of the excipients utilized in the formulation. The linearity range of Metformin and Pioglitazone were found to be from 50 to 300 mg/ml and 4 to 12 mg/ml respectively. The results indicated that the quantity of every drug within the tablets was within the ranges of 98 to 102 % of the label claim (Table 11). LOD and LOQ were found to be within limit. The results obtained on the validation parameters met ICH and USP requirements.

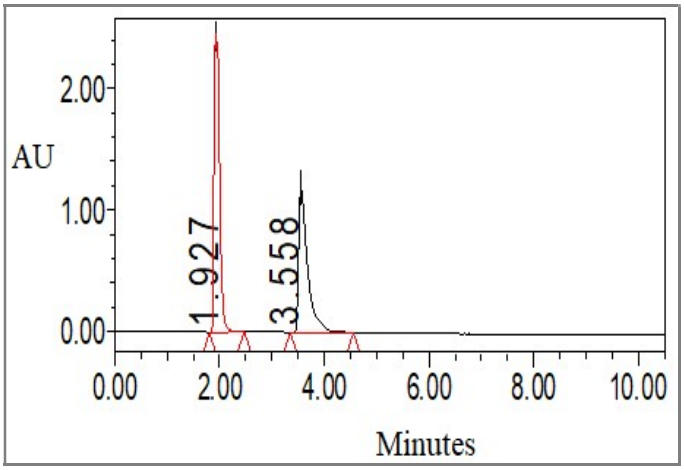

Fig 3. The chromatogram of Metformin and Pioglitazone for system suitability.

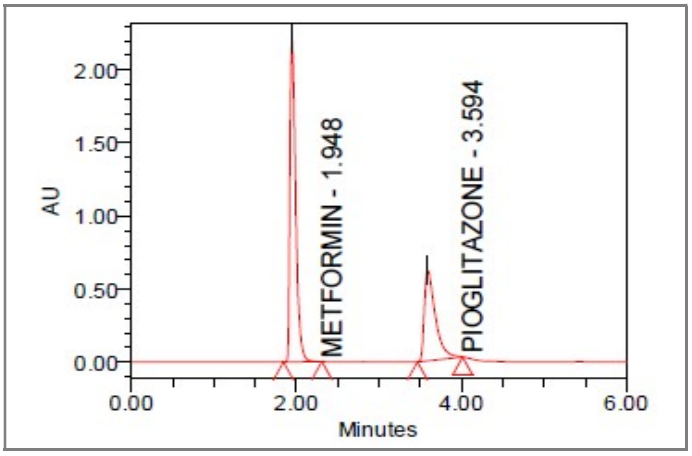

Fig 4. The retention time for Metformin and Pioglitazone.

Table 2. The HPLC chromatogram data of Metformin and Pioglitazone.

SL. No.	Drug	Retention Time	Area	Height	USP Resolution	s/n	USP Tailing	USP Plate Count
1	Metformin	1.948	18733243	2457066	--	1.998683	1.60	4132
2	Pioglitazone	3.594	14497206	1186369	8.9	0.965044	1.81	3689

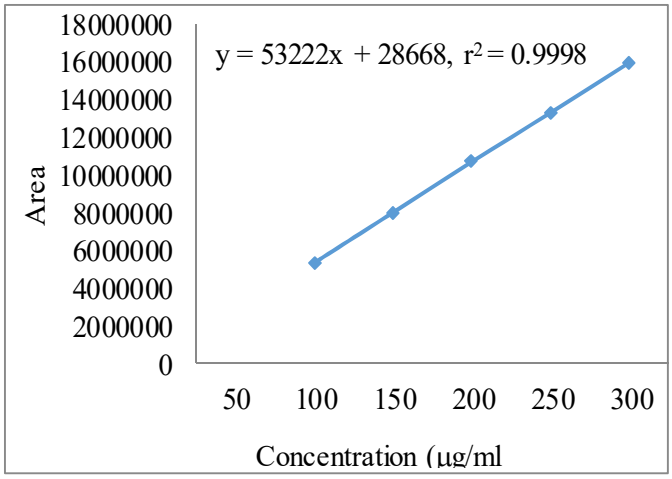

Fig 5. The standard curve of Metformin in Methanol using HPLC.

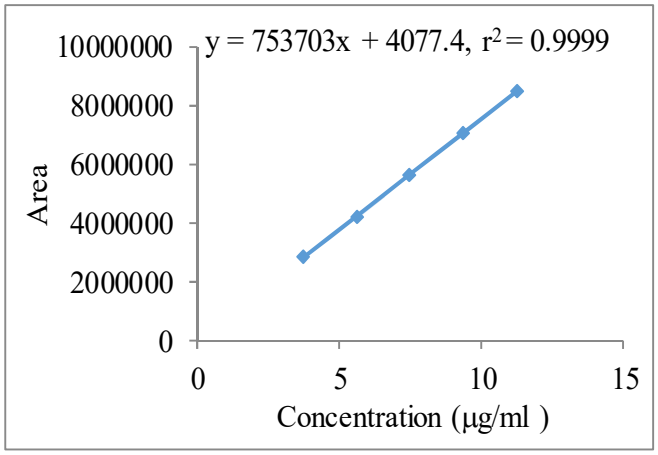

Fig 6. The standard curve of Pioglitazone in Methanol using HPLC.

Table 3. The linearity data of Metformin Hydrochloride.

Sl. No.	Concentration (µg/ml)	Inj	RT (min)	Area
1	100	1	1.969	5333244
2	150	1	1.962	8016544
3	200	1	1.950	10707877
4	250	1	1.970	13321307
5	300	1	1.950	15986360

RT – Retention time and Inj – Injection.

Table 4. The linearity data of Pioglitazone.

Sl. No.	Concentration (µg/ml)	Inj	RT (min)	Area
1	100	1	3.656	2822196
2	150	1	3.631	4231405
3	200	1	3.598	5656565
4	250	1	3.523	7058935
5	300	1	3.578	8474404

RT – Retention time and Inj – Injection.

Table 5. The components accuracy data of Metformin Hydrochloride (100 %).

Sl. No.	Sample name	Inj	RT (min)	Area
1	Acc – 100 % - 1	1	1.953	10696174
2	Acc – 100 % - 2	1	1.955	10624004
3	Acc – 100 % - 3	1	1.955	10669280
Mean	--	--	--	10663152
SD	--	--	--	36473
% RSD	--	--	--	0.3

RT – Retention time, Acc – Accuracy, SD – Standard deviation, RSD – Relative standard and Inj – Injection.

Table 6. The components accuracy data of Pioglitazone (100 %).

Sl. No.	Sample name	Inj	RT (min)	Area
1	Acc – 100 % - 1	1	3.595	5643263
2	Acc – 100 % - 2	1	3.598	56454463
3	Acc – 100 % - 3	1	3.594	5646091
Mean	--	--	--	5644939
SD	--	--	--	1485
% RSD	--	--	--	0.0

RT – Retention time, Acc – Accuracy, SD – Standard deviation, RSD – Relative standard and Inj – Injection.

Table 7. The System Suitability Data of Metformin.

System suitability parameters	Results	Acceptance criteria
% RSD for area count of six replicate injections of standard	0.1	Not more than 2.0
Tailing factor for peak	1.6	Not more than 2.0
Theoretical plates for peak	4239	Not less than 2000

Table 8. The System Suitability Data of Pioglitazone.

System suitability parameters	Results	Acceptance criteria
% RSD for area count of six replicate injections of standard	0.3	Not more than 2.0
Tailing factor for peak	1.8	Not more than 2.0
Theoretical plates for peak	3681	Not less than 2000

Table 11. The assay of Metformin and Pioglitazone.

Drug	Amount		% Assay
	Labelled	Measured	
Metformin	200 mg	199.88 mg	99.67
Pioglitazone	7.5 mg	7.46 mg	99.16

CONCLUSION:

The results obtained on the validation parameters satisfied 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 with high degree of accuracy and precision. Hence, the developed method may be used as an alternative method to the sooner reported ones for the routine analysis of Metformin and Pioglitazone in a much combined dosage form.

ACKNOWLEDGEMENT:

Authors wish to thank authority of Danteswari College of Pharmacy, Borapadar, Raipur Road, Jagadalpur, for providing laboratory facility to complete this research work Authors also wish to thank NATCO Pharma Pvt. Ltd., Kothur, Hyderabad, for providing Metformin and Pioglitazone as gift sample.

REFERENCES:

1. Lakshmi KS, Rajesh T, Sharma S. Simultaneous Determination of Metformin and Pioglitazone by Reversed phase HPLC in Pharmaceutical dosage forms. Int J Pharmacy Pharm Sci, 2009; 1(2): 162-166.
2. Rao SV, Gollu SR, Krishna AG, Ameer SK, Sekar KC. Estimation of Metformin Hydrochloride and Pioglitazone Hydrochloride in their Bulk and Formulation Dosage forms by using RPHPLC Method. Orient J Chem, 2011; 27(3): 1221-1227.
3. Shende MA, Budde BR. Novel RP-HPLC method development and validation for simultaneous estimation of metformin, voglibose and pioglitazone in bulk and triple fixed drug combinations pharmaceutical dosage form. J Drug Deliv Ther, 2019; 9(1): 30-37.
4. Kumara NS, Shantikumara S, Srinivas R. Pioglitazone: A review of analytical methods. J Pharm Anal, 2014; 4(5): 295-302.
5. Srinivas P, *et al*. Simultaneous determination of Metformin and Pioglitazone tablets in pharmaceutical dosage form by RP-HPLC method. Int J Pharm Chem Biol Sci, 2012; 2(1): 104-109.
6. Singh D, *et al*. Development and validation of a RP-HPLC method for simultaneous estimation of pioglitazone and metformin in bulk and tablet dosage form. Int J Biomed Adv Res, 2013; 3(3).
7. Babu BS, *et al*. Method development and validation of simultaneous estimation of Metformin and Pioglitazone in bulk and pharmaceutical formulation by UPLC. Int Res J Pharm Appl Sci, 2012; 2(4): 91-96.
8. Pola, *et al*. Simultaneous Determination of Metformin and Its Related Substances in Metformin and Pioglitazone Tablets in Pharmaceutical Dosage Form by RP-HPLC Method. Am J Pharm Tech Res, 2012; 2(2): 2249-3387.
9. Swapna, *et al*. Analytical Method Development and Method Validation for the Simultaneous Estimation of Metformin hydrochloride and Pioglitazone hydrochloride in Tablet Dosage Form by RP-HPLC. Asian J Pharm Ana, 2012; 2(3): 85-89.
10. Elrefay H, Ismaiel OA, Hassan WS, Shalaby A. development and validation of a stability-indicating HPLC-UV method for the determination of pioglitazone hydrochloride and Metformin hydrochloride in bulk drug and combined dosage form. Asian J Pharm Clin Res, 2013; 6(4): 116-120.

Table 9. Results of System Suitability (STD) of Metformin.

Sl. No.	Sample Name	Inj	Name	RT	Area	USP Tailing	USP Plate count
1	STD	1	Metformin	1.948	10601826	1.655	4129
2	STD	2	Metformin	1.945	10669388	1.582	4178
3	STD	3	Metformin	1.948	10697626	1.635	4239
4	STD	4	Metformin	1.948	10656454	1.615	4162
5	STD	5	Metformin	1.947	10681677	1.652	4115
6	STD	6	Metformin	1.953	10674782	1.578	4162
Mean	--	--	--	--	10675985	--	--
SD	--	--	--	--	15225	--	--
% RSD	--	--	--	--	0.1	--	--

STD – Standard, Inj – Injection, SD – Standard deviation, RSD – Relative standard.

Table 10. Results of System Suitability (STD) of Pioglitazone.

Sl. No.	Sample Name	Inj	Name	RT	Area	USP Tailing	USP Plate count
1	STD	1	Pioglitazone	3.594	5645416	1.782	3714
2	STD	2	Pioglitazone	3.595	5612758	1.872	3663
3	STD	3	Pioglitazone	3.592	5651831	1.785	3607
4	STD	4	Pioglitazone	3.593	5628076	1.845	3681
5	STD	5	Pioglitazone	3.593	5644606	1.852	3671
6	STD	6	Pioglitazone	3.595	5642682	1.776	3728
Mean	--	--	--	--	5635991	--	--
SD	--	--	--	--	1559.1	--	--
% RSD	--	--	--	--	0.3	--	--

STD – Standard, Inj – Injection, SD – Standard deviation, RSD – Relative standard.

11. Sujana K. *et al*. Simultaneous estimation of pioglitazone hydrochloride and metformin hydrochloride using UV spectroscopic method. J Biomed Sci Res, 2010; 2(2):110-5.
12. ICH. Topic Q2 (R1) Validation of analytical procedures: Text and methodology, note for guidance on validation of analytical procedures: text and methodology. 1995. (CPMP/ICH/381/95).

Conflict of Interest: None
Source of Funding: Nil
Paper Citation: Padhy RP*, Rath AK. Simultaneous estimation of Metformin and Pioglitazone in Pharmaceutical dosage form by HPLC. J Pharm Adv Res, 2020; 3(6): 926-931.