CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

SJIF Impact Factor: 7.187

https://doi.org/10.5281/zenodo.8165042

Available online at: http://www.iajps.com *Research Article*

ANALYTICAL METHOD DEVELOPMENT AND METHOD VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF METFORMIN HYDROCHLORIDE AND PIOGLITAZONE HYDROCHLORIDE IN TABLET DOSAGE FORM BY RP-HPLC

[1]Madugula Sirisha, [2]Malla Pavithra Pavani, [3]Mamidi Sravani, [4]Mariserla Devayani, [5]Miriyala Renuka Swathi, [6]Mutyala Lohith, [7]Chandaka Madhu

[1]Avanthi Institute Of Pharmaceutical Sciences, Cherukupally, Near Tagarapuvalasa Bridge, Vizianagaram – 531162 AP, India.

Article Received: April 2023 **Accepted:** May 2023 **Published:** June 2023

Abstract:

A simple, sensitive and rapid reverse phase high performance liquid chromatographic method was developed for the estimation of Metformin Hcl (MET) and Pioglitazone (PIO) in pure and in pharmaceutical dosage forms. A BDS Hypersil C18column (250x4.6mm, 5µ) was used with a mobile phase containing a mixture of Acetonitrile and Potassium di hydrogen ortho phosphate buffer (pH-3) in the ratio of 50: 50. The flow rate was 1ml/min and effluents were monitored at 238nm and eluted at 2.81min (MET) and 4.57min (PIO). Calibration curve was plotted with a range from 40-240 µg / ml for MET and 12-72 µg / ml for PIO. The assay was validated for the parameters like accuracy, precision, specificity, robustness, ruggedness and system suitability parameters. The proposed method can be useful in the routine analysis for the determination on metformin and pioglitazone in pharmaceutical dosage forms.

Keywords: *MetforminHydrochloride, PioglitazoneHydrochloride, Reverse phase HPLC, Pharmaceutical dosage forms, simultaneous estimation.*

Corresponding author:

Chandaka madhu,

Asst.prof, Dept of pharmacology

Email id: pharmamadhuphd@gmail.com

QR code

Please cite this article in press Chandaka madhu 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.,*** *Indo Am. J. P. Sci, 2023; 10 (06).*

INTRODUCTION:

Metformin (I, *N*, *N*-dimethyldiguanide) and Pioglitazone, (±)-5-[p-[2-(5-ethyl-2-pyridyl)-ethoxy] benzyl]-2,4-thiazolidinedione[1] are used in the treatment of type 2 diabetes. Metformin improves hepatic and peripheral tissue sensitivity to insulin without the problem of serious lactic acidosis where as Pioglitazone hydrochloride has been shown to affect abnormal glucose and lipid metabolism associated with insulin resistance by enhancing insulin action on peripheral tissues. Many patients suffering from type 2 diabetes require treatment with more than one antihyperglycemic drug to achieve optimal glycemic control. The literature reveals that there are some of the methods have been reported for metformin UV[1, 2], HPLC[3] stability studies4 and potentiometry, spectrofluorimetry [5]. For pioglitazone HPLC method in pharmaceutical dosage forms6 determination of its metabolites in human plasma[7,8] and simultaneous determination of metformin and pioglitazone[9-12] in pharmaceutical dosage forms. The present paper describes a simple, accurate, validated and economic method for the simultaneous determination of metformin and pioglitazone.

MATERIALS AND METHODS:

Reagents:

Metformin Hydrochloride and Pioglitazone Hydrochloride were procured from CHANDRA LABS (Kukatpally, Hyderabad, A.P, India) which were claimed to contain 50mg and 3mg were used in analysis. Acetonitrile (HPLC grade, MERCK).
Other reagents were of AR grade.

Instrumentation:

HPLC system (Shimadzu prominence) equipped with UV- Detector. The data acquisition was performed by Spinchrom software.

Chromatographic conditions:

Column	→	BDS HYPERSIL C18, 250×4.6mm, 5µ
Flow rate	→	1ml/min
Wavelength	→	238nm
Run time	→	10mins
Column temperature	→	25^0c
Injection Volume	→	20µL
Pump mode	→	Isocratic
Retention time	→	2.81 and 4.57 respectively

Preparation of standard:

Accurately weighed about 50mg and 3mg of Metformin hydrochloride and Pioglitazone hydrochloride working standards and transferred into a 25ml volumetric flask, added 15ml of diluent, and sonicated to dissolve. Cooled to room temperature and diluted to volume with diluent.

Preparation of sample:

20 tablets of Metformin hydrochloride and Pioglitazone hydrochloride were weighed and powdered in glass mortar. The powder equivalent to 114mg was transferred into a 25 ml volumetric flask, 15 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 and allowed to stand until the residue settles before taking an aliquot for further dilution. 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 the HPLC system.

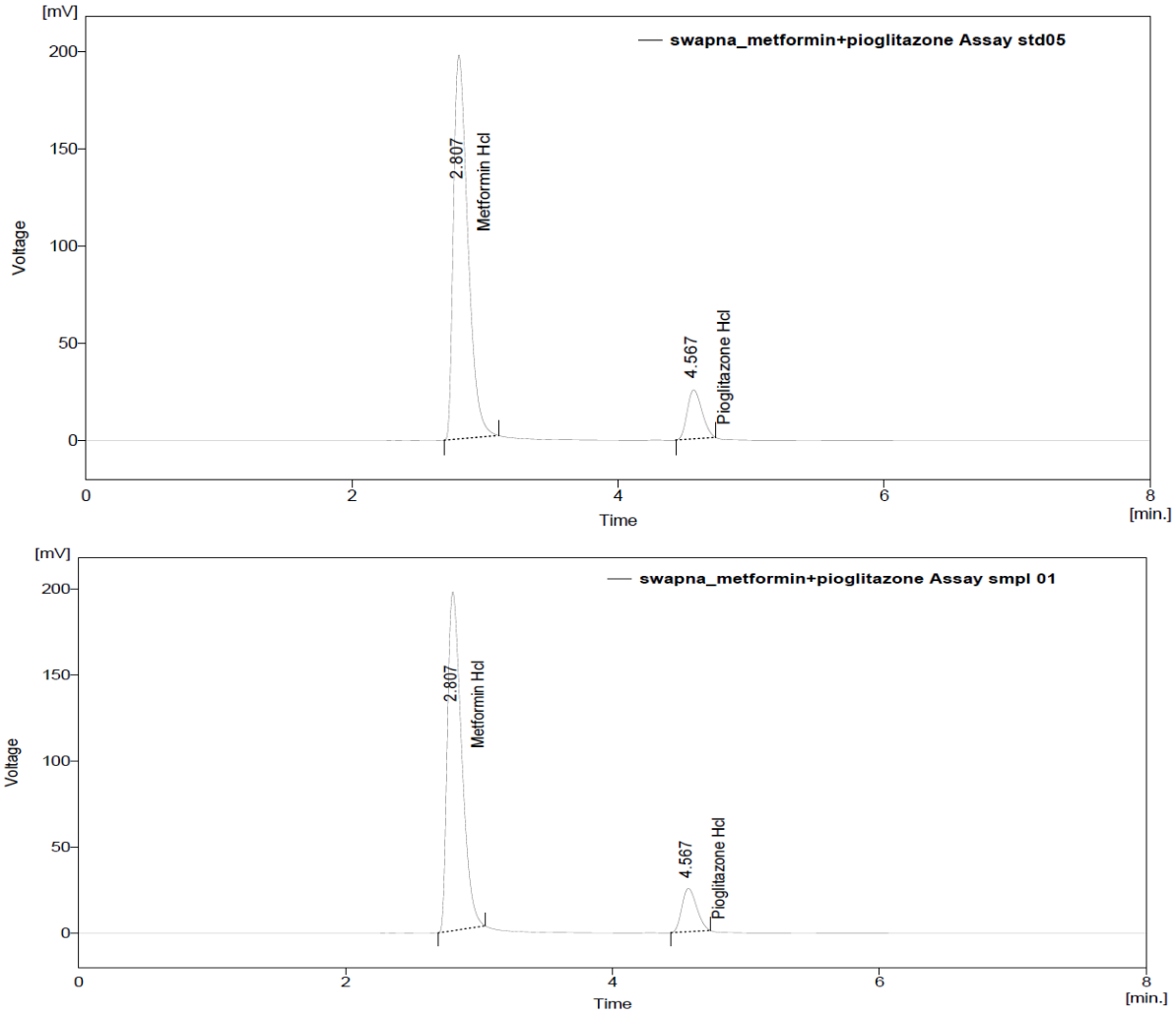
Procedure for assay:

20 µl of the Standard, Sample and Blank preparations in duplicate were injected separately into the HPLC system and the peak responses for Metformin hydrochloride and Pioglitazone hydrochloride were measured. The quantities from the peak area in mg of Metformin hydrochloride and Pioglitazone hydrochloride were calculated per tablet taken

RESULTS AND DISCUSSION:

A reversed-phase column procedure was proposed as a suitable method for the simultaneous determination of metformin and pioglitazone in combined dosage form. The chromatographic conditions were optimized by changing the mobile phase composition, pH, and buffers used in the mobile phase. Different ratios were experimented to optimize the mobile phase. Finally a mixture of Acetonitrile and potassium dihydrozen ortho phosphate anhydrous buffer (pH-3) in the ratio of 50:50 was used this mobile phase showed good resolution of Metformin and Pioglitazone peak. The wavelength of detection selected was 238 nm, as both the drugs showed optimum absorbance at this wavelength.

By our proposed method the retention time of metformin and Pioglitazone was about 2.803 and4.573 minute, respectively and none of the impurities were interfering in its assay (Fig. 1 & 2).



Validation of the method:

The developed method has been validated for the assay of MetforminHCl and PioglitazoneHCl as per ICH guidelines by using following parameters.

Specificity and Selectivity:

Specificity and selectivity were studied for the examination of the presence of interfering components. It was checked by subjecting the drug solution in different stress conditions like Acid, Base, Peroxide and the degradation was noted.

Acid Stress (0.1 M HCl)

Table 1: Specificity testing (Acid stress)

Concentration (µg/ml)		Time (hrs)	Retention time(min)		RT of degraded product
Metformin	Pioglitazone		Metformin	Pioglitazone	
15	3	0	2.807	4.573	-
		24	2.827	4.573	-

Base Stress (0.1M NaOH)

Table 2: Specificity testing (Base stress)

Concentration (µg/ml)		Time (hrs)	Retention time(min)		RT of degraded product
Metformin	Pioglitazone		Metformin	Pioglitazone	
15	3	0	2.807	4.573	-
		24	2.827	4.573	-

Peroxide stress (5% H2O2)

Table 3: Specificity testing (Peroxide stress)

Concentration (µg/ml)		Time (hrs)	Retention time(min)		RT of degraded product
Metformin	Pioglitazone		Metformin	Pioglitazone	
15	3	0	2.807	4.573	-
		24	2.827	4.573	-

Linearity:

Linearity was studied by preparing standard solutions of Metformin and Pioglitazone at different concentration levels (Fig. 3 & 4). The responses were found linear in the range of 40-240 µg / ml and 12-72 µg/ml for Metformin and Pioglitazone, respectively.

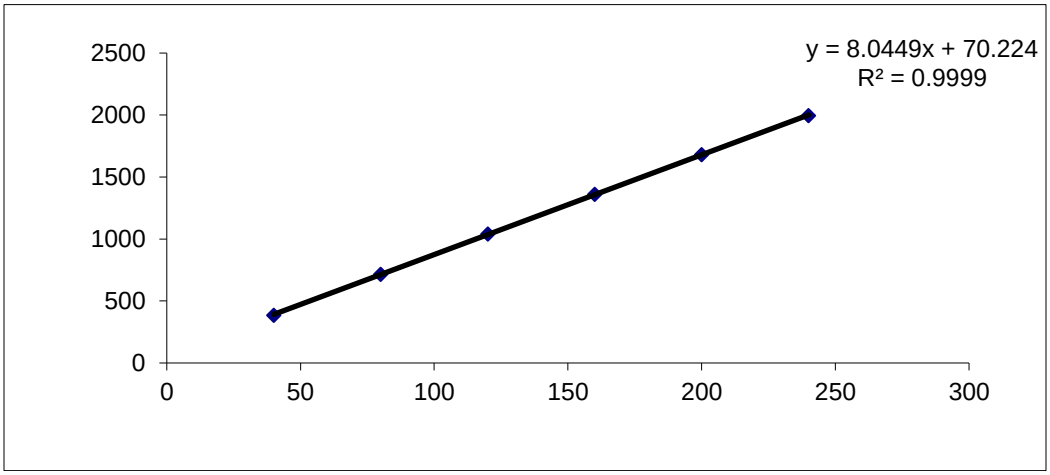


Figure 3: Linearity curve of standard Metformin HCl

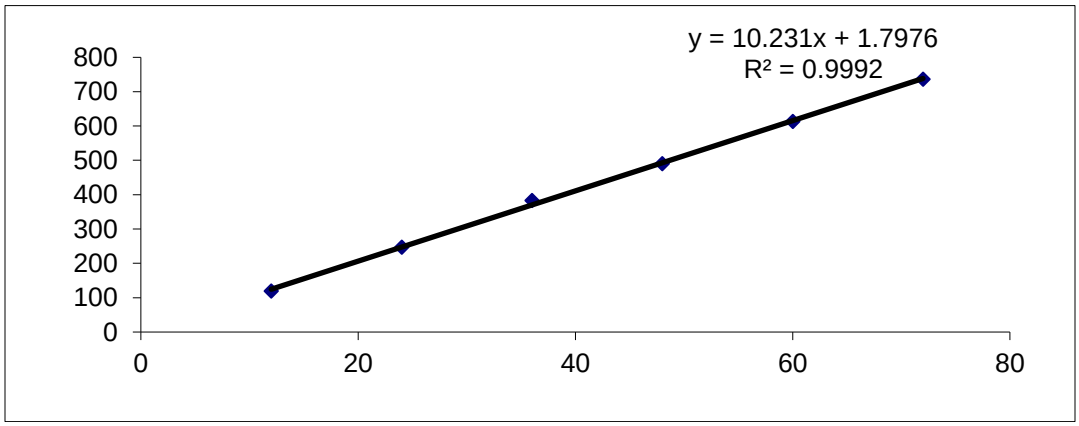


Figure 4: Linearity curve of standard Pioglitazone HCl

Accuracy:

Accuracy was performed in triplicate for various concentrations of Metformin and Pioglitazone equivalent to 80, 100 and 120 % of the standard amount was injected into the HPLC system per the test procedure. The average % recovery of Metformin and Pioglitazone was calculated. Table 4: Results of Analysis of Formulation and Recovery Studies.

Table 4: Results of Analysis of Formulation and Recovery Studies

Accuracy 80%

Pioglitazone

Sl.no		Area	Amt recov	%Amt recov
1	Std	504.866		
2	Accuracy 01	505.254	80.061	100.077
3	Accuracy 02	502.922	79.691	99.615
4	Accuracy 03	503.312	79.753	99.692

Metformin

	Area	Amt Recov	%Amt recov
Std	1426.908		
Accuracy 01	1425.054	79.896	99.87
Accuracy 02	1425.295	79.909	99.88
Accuracy 03	1423.756	79.823	99.78

Accuracy 100%

Pioglitazone

Sl.no		Area	Amt recov	%Amt recov
1	Std	606.133		
2	Accuracy 01	605.544	99.902	99.902
3	Accuracy 02	600.308	99.038	99.038
4	Accuracy 03	604.215	99.683	99.683

Metformin

	Area	Amt recov	%Amt recov
Std	1641.483		
Accuracy 01	1636.265	99.682	99.68
Accuracy 02	1625.414	99.021	99.02
Accuracy 03	1632.97	99.481	99.48

Accuracy 120%
Pioglitazone

Sl.no		Area	Amt recov	%Amt recov
1	Std	772.96		
2	Accuracy 01	774.645	120.261	100.21
3	Accuracy 02	776.519	120.525	100.46
4	Accuracy 03	777.65	120.728	100.60

Metformin

	Area	Amt recov	%Amt recov
Std	1992.869		
Accuracy 01	1991.368	119.909	99.92
Accuracy 02	1994.162	120.077	100.06
Accuracy 03	1990.781	119.874	99.89

Precision:
A) Method Repeatability
Six sample solutions of the same concentration (50%) were prepared and injected into the HPLC system as per test procedure.

Table 5: Results from determination of precision of analysis of Metformin and Pioglitazone
Metformin

Sl.no	Rt	Area
1	2.82	1704.821
2	2.8	1677.027
3	2.82	1700.461
4	2.827	1715.753
5	2.82	1714.368
6	2.82	1714.128
Avg	2.817	1704.41
Std dev	0.009	14.735
%RSD	0.325	0.864

Pioglitazone

Sl.no	Rt	Area
1	4.553	636.314
2	4.52	627.06
3	4.553	638.952
4	4.553	640.56
5	4.553	634.76
6	4.553	639.042
Avg	4.549	636.115
Std dev	0.015	4.904
%RSD	0.343	0.771

B) Intermediate Precision (Analyst to Analyst variability)
Two analysts as per test method conducted the study. For Analyst-1 Method Repeatability and for Analyst-2 six sample solutions of the same concentration (50%) were prepared and injected into the HPLC system as per test procedure.

Table 6: Results from determination of precision of analysis of Metformin and Pioglitazone
Metformin

Sl.no	Rt	Area
1	2.82	1675.571
2	2.82	1661.488
3	2.82	1654.72
4	2.82	1664.471
5	2.82	1672.099
6	2.827	1684.62
Avg	2.821	1668.735
Std dev	0.002	10.785
%RSD	0.101	0.634

Pioglitazone

Sl.no	Rt	Area
1	4.56	608.141
2	4.567	601.976
3	4.56	604.928
4	4.56	603.516
5	4.56	593.603
6	4.567	598.377
Avg	4.562	601.756
Std dev	0.003	5.135
%RSD	0.079	0.853

Robustness and Ruggedness:
Robustness was done by small deliberate changes in the chromatographic conditions and retention time of Metformin and Pioglitazone was noted. The factors selected were flow rate and variation in the mobile phase composition. The results remained unaffected by small variations in these parameters. Ruggedness of the method was checked by using different analysts and instruments. The relative standard deviation of the results obtained from different analysts and instruments was < 1.0%.

Validation parameter:
The method was validated by using the following parameters as shown in Table 7.

Table 7: Validation parameter of HPLC method for Metformin and Pioglitazone

Validation Parameter	**Metformin HCl**	**Pioglitazone HCl**
Linearity Range (µg/ml)	40-240	12-72
Regression equation	Y= 8.0449x+70.224	Y=10.231x+1.7976
Correlation Coefficient (r^2)	0.9999	0.9992
Accuracy	99.02 - 100.06	99.03 - 100.6
Precision		
Method Repeatability (RSD %)	0.8645	0.7709
IntermediatePrecision (RSD %)	0.634	0.853

CONCLUSION:

The proposed method is rapid,accurate and sensitive. It makes use of less amount of solvents and change of set of conditions requires a short time. Many samples can be simultaneously and suitably analysed for the routine quality control analysis of Metformin and Pioglitazone in bulk and its tablet dosage forms. It does not suffer from any interference due to common excipients present in pharmaceutical preparation and can be conveniently adopted for quality control analysis.

Acknowledgement:

"I certify that I have participated sufficiently in the conception and design of this work and the analysis of the data (wherever applicable), as well as the writing of the manuscript, to take public responsibility for it. I believe the manuscript represents valid work. I have reviewed the final version of the manuscript and approve it for publication. Neither has the manuscript nor one with substantially similar content under my authorship been published nor is being considered for publication elsewhere, except as described in an attachment. Furthermore I attest that I shall produce the data upon which the manuscript is based for examination by the editors or their assignees, if requested."

REFERENCES:

1. Lalhriatpuii TC, Kawathekar N,Derivative Spectrophotometric Estimationof Pioglitazone and Metformin hydrochloride, Ind Drugs 2005; 42: 740-743.
2. Ajithdas A, Nancy K, Simultaneous Estimation of Metformin hydrochloride and Glipizide in Solid Dosage Forms by Ultraviolet Spectrophotometry, Ind Drugs 2000; 37: 533-536.
3. Vasudevan M, Ravi J, Ravisankar S, Suresh B, Ion-Pair Liquid Chromatography
4. Technique for the Estimation of Metformin in its Multi Component Dosage Forms,
5. J. Pharm Biomed Anal, 2001; 25: 77-84.
6. Sakalgaonkar AA, Mirgane SR, Arbad BR, Validated High Performance Liquid Chromatographic Stability Indicating Method for Analysis of Metformin HCl,
7. Int. J. App Environ Sci 2008; 3: 65–73.
8. Saad SM Hassan, Wagiha H Mahmoud, Mohamed AF Elmosallamy, Abdel Hammeed M Othman, Determination of Metformin in Pharmaceutical Preparations using Potentiometry, Spectrofluorimetry and UV–Visible Spectrophotometry,
9. AnalChim Acta 1999; 378: 299-311.
10. Wanjari DB, Gaikwad NJ, Stability Indicating RP-HPLC Method for Determination of Pioglitazone from Tablets, Ind J. Pharm Sci 2005; 67: 256-258.
11. Kenji Yamashita, Hiromi Murakami, Teruaki Okuda, Michio Motohashi, High-Performance Liquid Chromatographic Determination of Pioglitazone and its Metabolites in Human Serum and Urine, J. Chromatogr B 1996; 677: 141-146.
12. Zhong WZ, Williams MG, Simultaneous Quantitation of Pioglitazone and its Metabolites in Human Serum by Liquid Chromatography and Solid Phase 166 Extraction, J. Pharm. Biomed. Anal 1996; 14: 465-473.
13. Kolte BL, Raut BB, Deo AA, Bagool MA, Shinde DB, Simultaneous High- Performance Liquid Chromatographic Determination of Pioglitazone and Metformin in Pharmaceutical-Dosage Form, J. Chrom Science 2004; 42: 27-31.
14. Shankar Madhira B, Modi Vaibhav D, Shah Dimal A, Bhatt Kashyap K, Mehta Rajendra S, Geetha Madhira et al.,Estimation of Pioglitazone hydrochloride and Metformin hydrochloride in tablets by Derivative Spectrophotometry and Liquid Chromatographic Methods, J. J. AOAC International 2005; 88: 1167-1172.
15. Sahoo PK, Sharma R, Chaturvedi SC, Simultaneous Estimation of Metformin hydrochloride and Pioglitazone hydrochloride by RP-HPLC method from combined tablet dosage form, Ind J.Pharm Sci 2008; 70: 383-386.
16. K.S.Lakshmi,T.Rajesh,Simultaneous,Determinat ionof Metformin and Pioglitazone by Reversed phase HPLC in Pharmaceutical dosageforms, International Journal of Pharmacy and Pharmaceutical Sciences, Vol. 1, Issue 2, Oct-Dec. 2009.