Research Article **ISSN: 2349 – 7106**

Asian Journal of Research in Chemistry and Pharmaceutical Sciences

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

M. Alagar raja*[1], CH. Usha Rani[1], David banji[1], K. N. V. Rao[1], D. Selva Kumar[2]

[1*]Department of Pharmaceutical Analysis, Nalanda College of Pharmacy, Cherlapally, Nalgonda-508001, Telangana, India.

[2]School of Pharmacy, Taylors University, Subang Jaya, Malaysia.

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 ODS HG-5 RP C18 column (150x4.6mm,i.d 5mm) was used with a mobile phase containing a mixture of Acetonitrile and Potassium dihydrogen phosphate buffer (pH-3) in the ratio of (80:20.V/V). The flow rate was 1ml/min and effluents were monitored at 242nm and eluted at 1.93min (MET) and 5.29min (PIO). Calibration curve was plotted with a range from 0-750 µ g / ml for (MET) and 0-25 µ g / 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

Metformin Hydrochloride, Pioglitazone, Reverse phase HPLC, Bulk, Tablet dosage forms and Simultaneous estimation.

Author of Correspondence:

Alagar raja M,
Department of Pharmaceutical Analysis,
Nalanda College of Pharmacy, Cherlapally, Nalgonda-508001, Telangana, India.

Email: madurairaja@hotmail.com

INTRODUCTON

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 whereas 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 ant hyperglycemic 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 studies[4] and potentiometry, spectrofluorimetry[5]. For pioglitazone HPLC method in pharmaceutical dosage forms[6] determination of its metabolites in human plasma[7,8] and simultaneous determination of metformin and pioglitazone[9] 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 Hlppo labs pvt ltd. , India which were claimed to contain.

500mg and 15mg were used in analysis. Acetonitrile (HPLC grade, MERCK).

Other reagents were of LR grade.

Instrumentation

HPLC system [Hitachi lachrome] equipped with UV-Detector. The data acquisition was performed by Elico India.

Chromatographic conditions:

Column	:	ODS Develosiln HG-5 RP C18 {150x 4.6mm i.d 5mm]
Wavelength	:	242nm
Retention time	:	1.97 and 5.43
Flow rate	:	1ml|mn
Runtime	:	10min
Column temperature	:	room temperature
Injection volume	:	20ml
Pump mode	:	lsocartic

Preparation of Standard solution of Metformin and Pioglitazone

Accurately weighed 10 mg of Metformin and 10 mg of Pioglitazone were transferred to two different10 ml volumetric flask. About 4 ml of mobile phase was added and sonicated to dissolve. The volume was made up to mark with same solvent. Then 5 ml of metformin and 0.15 ml of Pioglitazone were diluted to 10 ml with the solvent system. The resultant solution was filtered through a 0.45 μm membrane filter and degassed under ultrasonic bath prior to use. From the above standard solution several working standard solutions are prepared by serial dilution technique.

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. Phosphate buffer (pH-3) in the ratio of 80:20 was used this mobile phase showed good resolution of Metformin and Pioglitazone peak. The wavelength of detection selected was 242 nm, as both the drugs showed optimum absorbance at this wavelength.

Validation of the method

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

Specificity and Selectivity

Specificity Preparation and running of synthetic mixture of Metformin and Pioglitazone For the specificity of the method the marketed formulations has been taken and the solution was injected into the HPLC system. No peaks were found at the retention of Metformin and Pioglitazone. Specificity studies indicating that the excipients did not interfere with the analysis.

Linearity and Range

Linearity was established by least squares linear regression analysis of the calibration curve. The calibration curves were linear over the concentration range of 0-750.0 μg/ml for Metformin HCL and 0-25 μg/ml for Pioglitazone, Peak areas were plotted versus respective concentrations and linear regression analysis was performed on the resultant curves.

Accuracy

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of pure drugs of

Metformin and Pioglitazone were taken and added to the pre-analyzed formulation of concentration. From that percentage recovery values were calculated.

Precision

Repeatability The precision of each method was ascertained separately from the peak areas obtained by actual determination of six replicates of a fixed amount of drug. Pioglitazone and Metformin. The percent relative standard deviations were calculated for Pioglitazone and Metformin.

Intermediate precision

For intra-day studies the drug having concentration value 80%, 100 % and 120% of the target concentration (n = 3), were injected in triplicate into the HPLC system and for inter-day studies the drug at above three concentrations were injected in triplicate into the HPLC system for three days. Data were subjected to statistical treatment for the calculation of SD and RSD.

Table No.1: Standard Curve for Metformin

S.No	Conc.(µg/ml)	Mean AUC (n=6)
1	0	0
2	250	3124838
3	300	3904737
4	500	6191960
5	600	7746831
6	750	9450813

Table No.2: Standard Curve for Pioglitazone

S.No	Conc.	AUC
1	0	0
2	5	1228747
3	10	2138031
4	15	3342166
5	20	4249436
6	25	5342166

Table No.3: Accuracy Result data

S.No	Sample	Amount Taken (µg/ml)	Amount Recovered (µg/m)	Recovery (%)	% RSD
1	MET	400	399.54	99.885	0.68
		500	501.01	100.202	0.84
		600	600.02	100.0034	0.92
2	PIO	12	11.83	98.58	0.65
		15	15.012	100.08	0.86
		18	18.21	101.16	0.98

Table No.4: Data Showing Repeatability Analysis for Metformin

S.No	HPLC Injection Replicates of Metformin	Area	Retention Time
1	Replicate – 1	1.97	6130775
2	Replicate – 2	1.97	6022268
3	Replicate – 3	1.97	6164471
4	Replicate – 4	1.96	6043413
5	Replicate – 5	1.96	6191960
6	Replicate -6	1.97	6094321
7	**Average**	1.966	6110577
8	**Standard Deviation**	0.005477	74574.12
9	**% RSD**	0.278597	1.22041

Table No.5: Data Showing Repeatability Analysis for Pioglitazone

S.No	HPLC Injection Replicates of Pioglitazone	Retention Time	Area
1	Replicate – 1	5.43	3389698
2	Replicate – 2	5.43	3481595
3	Replicate – 3	5.42	3394919
4	Replicate – 4	5.43	3442166
5	Replicate – 5	5.43	3342926
6	Replicate -6	5.43	3382585
7	**Average**	5.428	3410261
8	**Standard Deviation**	0.004472	53147.64
9	**% RSD**	0.08239	1.558463

Table No.6: Data for Pioglitazone Analysis

S.No	Conc. of Pioglitazone (API) (µg/ml)	Observed Conc. of Pioglitazone (µg/ml) by the proposed method			
		Intra-Day		Inter-Day	
		Mean (n=3)	% RSD	Mean (n=3)	% RSD
1	12	11.81	0.86	11.83	0.87
2	15	15.02	0.30	15.03	0.32
3	18	17.97	0.13	17.95	0.11

Table No.7: Data for Metformin Analysis

S.No	Conc. Of Metformin (API) (µg/ml)	Observed Conc. Of Metformin (µg/ml) by the proposed method			
		Intra-Day		Inter-Day	
		Mean (n=3)	% RSD	Mean (n=3)	% RSD
1	400	399.95	1.05	400.01	0.24
2	500	500.98	0.55	500.051	0.41
3	600	599.84	0.18	599.95	0.18

Table No.8: LOD and LOQ Results

S.No	Parameter	MET	PIO
1	LOD	0.1	0.03
2	LOQ	0.3	0.09

Table No.9: Data of System Suitability Parameter

S.No	Parameter	Limit	Result
1	Resolution	Rs > 2	7.29
2	Asymmetry	T ≤ 2	Metformin =0.14 Pioglitazone =0.19
3	Theoretical plate	N > 2000	Metformin =3971 Pioglitazone= 4861

Table No.10: Recovery Data for Estimation Metformin and Pioglitazone

S.No	Drugs	Labeled amount(mg)	Amount found(mg)	% Label claim	*% RSD
1	MET	500	500.04	100.008	1.2
2	PIO	15	14.98	99.86	0.8

Table No.11: Validation Parameters

S.No	Parameters	Metformin HCL	Pioglitazone
1	Calibration range(mcg/ml)	0-750	0– 25
2	Optimized wavelength	232	232
3	Mobile phase (Acetonitrile: Buffer)	80:20	80:20
4	Column	Develosil ODS HG-5 RP C18 column (150 × 4.6 mm i.d 5□□	Develosil ODS HG-5 RP C18 column (150 × 4.6 mm i.d 5□□
5	Retention time	1.97	5.43
6	Regression equation(Y*)	y = 12650x + 9975	y = 21129x + 75541
7	Correlation coefficient(r^2)	0.999	0.999
8	Precision (% RSD*)	1.22	1.55
9	% Recovery	100.202	101.2
10	LOD(mcg/ml)	0.1	0.03
11	LOQ(mcg/ml)	0.3	0.09

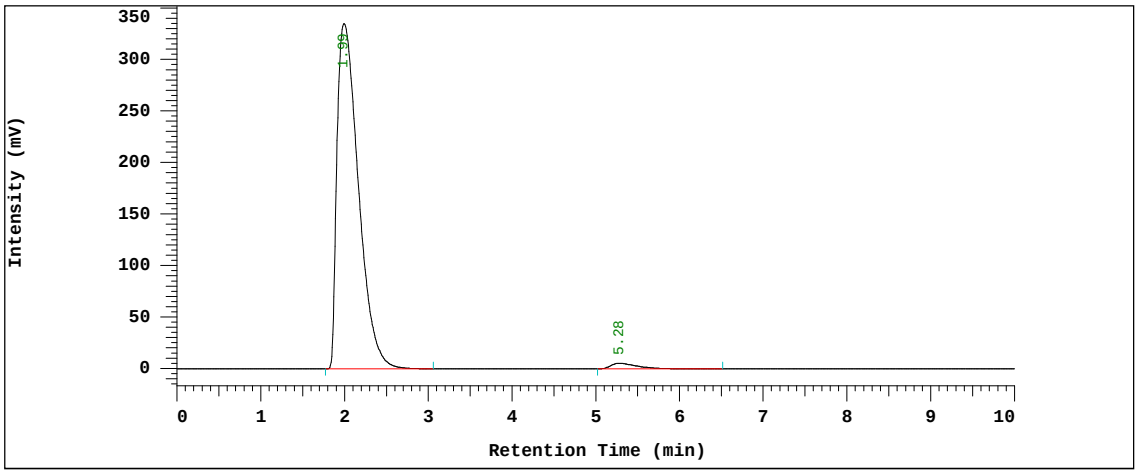

Figure No.1: Chromatogram of Metformin

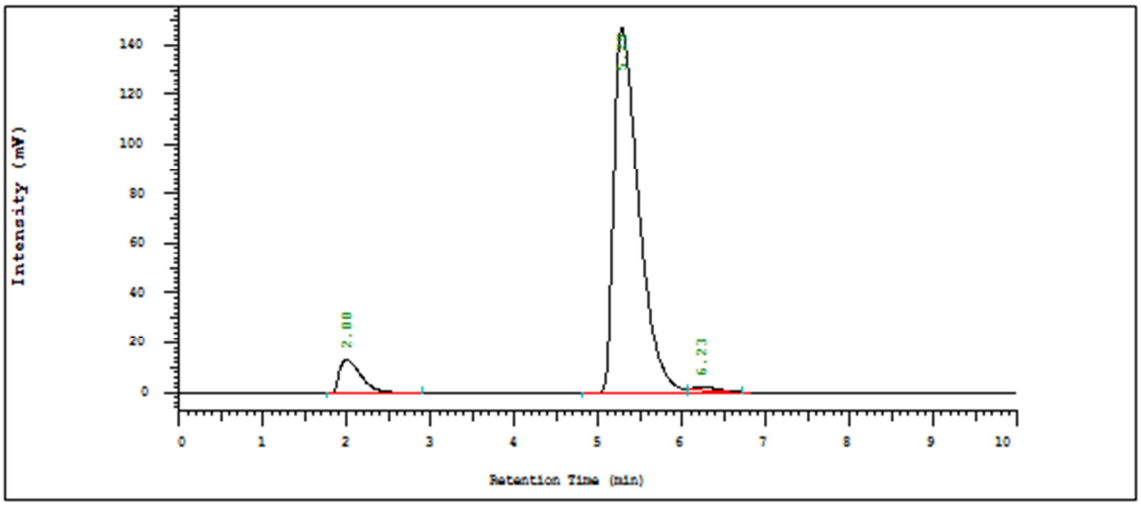

Figure No.2: Chromatogram of Pioglitazone

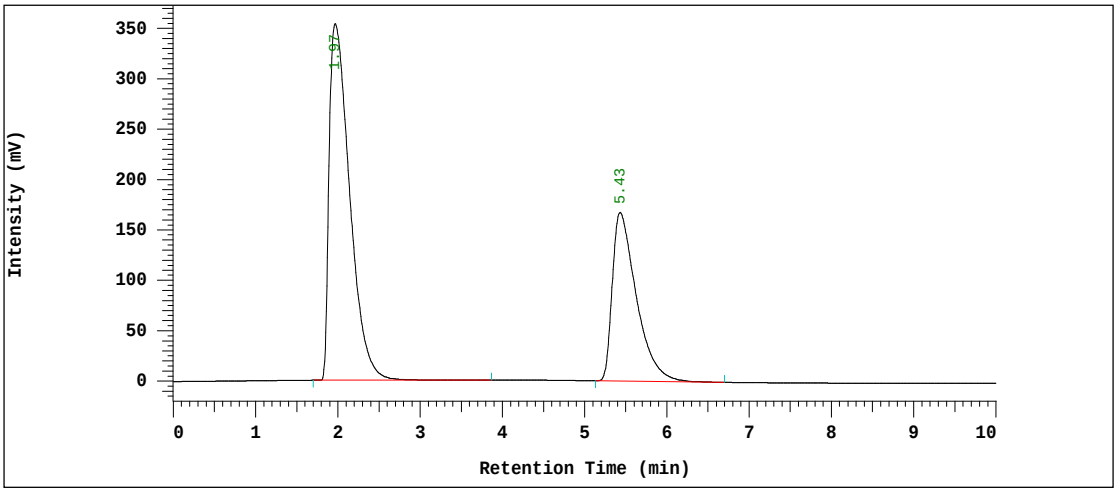

Figure No.3: Chromatogram of Metformin and Pioglitazone

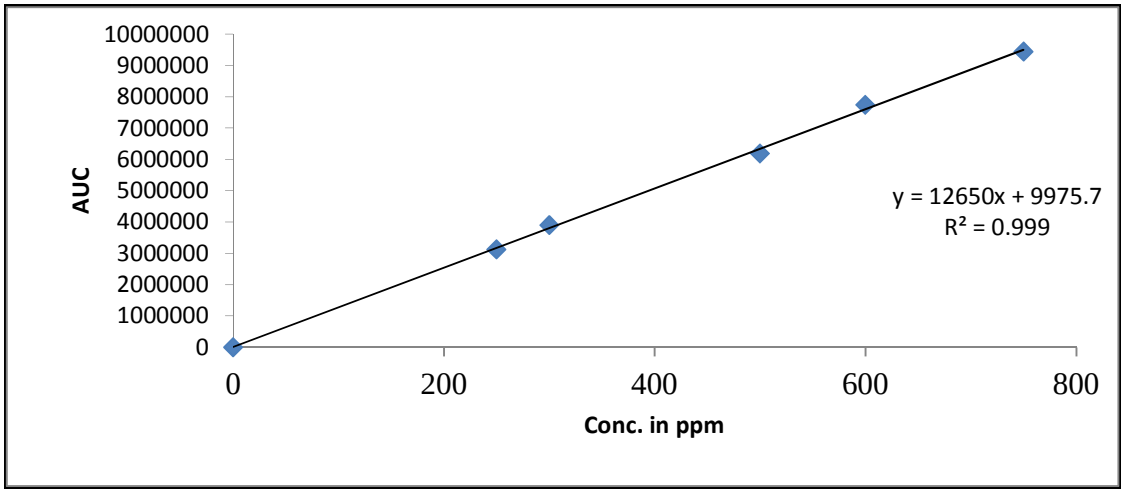

Figure No.4: Standard Curve for Metformin

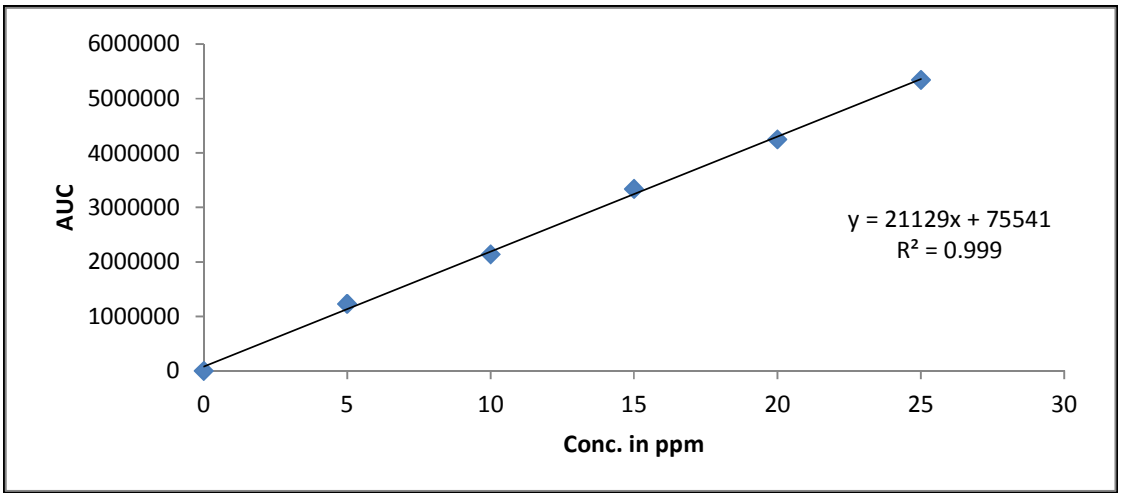

Figure No.5: Standard Curve for Pioglitazone

CONCLUSION

The developed isocratic Reverse Phase-HPLC method offers simplicity, selectivity, precision and accuracy. In this proposed method symmetrical peaks with good resolution were obtained. Out of all the methods developed, the RP-HPLC method was more sensitive and accurate. However, all these methods can be used for the simultaneous analysis of Metformin HCL and Pioglitazone in combined dosage form.

ACKNOWLEDGMENT

This work was supported by Nalanda College of Pharmacy, Charllapalli, and Nalgonda, for their continuous support and encouragement and for providing the necessary facilities.

CONFLICT OF INTEREST

We decleare that we have no conflict of interest.

BIBLIOGRAPHY

1. Michael E S, Ira S K. ''Analytical Method Development and Validation", *Marcel Dekker, Inc., New York*, 1997, 25-29.
2. Connors K A. "A text Book of Pharmaceutical Analysis", *Wiley-Inter Science, Singapore*, 1999, 175.
3. Skoog D A, West D M, Holler F J. Fundamentals of Anal tical Chemistry, *Saunders College Publishing, New York*, 6th Edition, 2001, 713.
4. Willard-H H, Lynne L M, Jr, John A, Dean F A." Instrumental Methods of Analysis", 7th Edn, *CBS*

Publishers and Distributors, New Delhi, 1-12, 1991, 580-610, 614-652.

5. Davidson A G. "Basis of Spectrophotometer", 4th Edn, Part-2, *CBS Publishers, New Delhi*, 2002, 264 74.
6. International Conference on Harmonization, "Q2A, Text on Validation of Analytical Procedures," Federal Register, 60(40), 1995, 11260-11262.
7. International Conference on Harmonization, "Q2B Validation of Analytical Procedures Methodology Availability," Federal Register, 62(96), 1997, 27463-27467.
8. FDA, "Analytical Procedures and Methods Validation Chemistry, Manufacturing and Controls Documentation Availability," Federal Register (Notices), 65(169), 2000, 52776-52777.
9. Shabir G A, "Validation of HPLC Chromatography Methods for Pharmaceutical Analysis, Understanding the Differences and Similarities between Validation Requirements of FDA, the US Pharmacopeia and the ICH," *J. Chromatogr. A.* 987(1-2), 2003, 57-66.
10. Dunn C J, Peters D H. "Metformin: A review of its pharmacological properties and therapeutic use in non-insulin-dependent diabetes mellitus, *Drugs*, 49(5), 1995, 721-49.
11. Hundal R S, Inzucchi S E. "Metformin: new understandings, new uses", *Drugs* 63(18), 2003, 1879-94.
12. Riddle M C. "Editorial: Sulfonyl urea Differ in Effects on Ischemic Preconditioning Is It Time to Retire Glyburide?" *J. Clin. Endocrinol, Metab*, 88(2), 2003, 528-30.
13. Gangji A S, Cukierman T, Gerstein H C, Goldsmith C H, Clase C M. "A Systematic Review and Meta-Analysis of Hypoglycaemia and Cardiovascular Events, A Comparison of Glyburide with Other Secretagogues and With Insulin", *Diabetes Care* 30(2), 2007, 389-394.

Please cite this article in press as: Alagar Raja. M *et al*. RP-HPLC Method Development and Method Validation for the Simultaneous Estimation of Metformin Hydrochloride and Pioglitazone in Bulk and Tablet Dosage Form, *Asian Journal of Research in Chemistry and Pharmaceutical Sciences*, 3(2), 2015, 76 - 83.