SIMULTANEOUS DETERMINATION OF METFORMIN AND PIOGLITAZONE BY REVERSED PHASE HPLC IN PHARMACEUTICAL DOSAGE FORMS

K.S. LAKSHMI[1], T. RAJESH*[1], SHRINIVAS SHARMA[2]

[1]Department of Pharmaceutical Analysis, SRM College of Pharmacy, SRM University, Kattankulathur- 603203, Tamil Nadu, India.

[2]Department of Pharmacology, SRM College of Pharmacy, SRM University, Kattankulathur- 603203, Tamil Nadu, India.

Corresponding author e-mail: rajeshtirumala@hotmail.com

Ph. No-+91-442-745-3160, +91-995-201-4288

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 Gemini C_{18} column (150x4.6mm, 5μ) was used with a mobile phase containing a mixture of Acetonitrile and Ammonium Acetate buffer (pH-3) in the ratio of 42: 58. The flow rate was 0.3ml/min and effluents were monitored at 255nm and eluted at 5.17min (MET) and 8.1min (PIO). Calibration curve was plotted with a range from 0.5-50 µg/ml for MET and 0.3-30 µg/ml for PIO. The assay was validated for the parameters like accuracy, precision, robustness 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.

Key words: Metformin Hcl, Pioglitazone, Reverse phase HPLC, Pharmaceutical dosage forms

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 anti-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[9-11] in pharmaceutical dosage forms. The present paper describes a simple, sensitive, validated and economic method for the determination of metformin and pioglitazone.

MATERIALS AND METHODS

Reagents

Metformin and Pioglitazone were obtained from Macleoids Pharmaceuticals Ltd., Mumbai, India. Acetonitrile and Methanol (HPLC grade, MERCK), water (Milli Q). Other reagents were of AR grade.

Instrumentation

The HPLC system consisted of a Shimadzu Class LC-10AT vp and LC-20AD pumps connected with SPD-10A vp UV-Visible detector. The data acquisition was performed by Spinco Winchrome software.

Chromatographic conditions

The HPLC system consisted of Shimadzu Class LC-10AT vp and LC-20AD pumps

connected with SPD-10A vp UV-Visible detector. The data acquisition was performed by Spincotech 1.7 software. Analysis was carried out at 255nm using a phenomenex C_{18} reverse phase column of 150x 4.6 mm i.d., 5 µm dimensions at ambient temperature. The mobile phase consisted of Acetonitrile: Ammonium acetate buffer (pH 3) in the ration of (42: 58, v/v) that was set at a flow rate of 0.3ml/min.

Preparation of stock and sample solutions

The standard stock solutions were prepared with methanol to give the final concentration of 1000 µg/ml. The working standard solutions of MET and PIO were prepared by taking suitable aliquots of drug solution from the standard solutions and the volume was made up to 10 ml with mobile phase to get concentrations of 0.5-50 µg/ml for MET and 0.3-30 µg/ml for PIO.

A mixed standard solution was prepared by transferring 0.2 ml of each from the stock (1000 µg/ml) into 10 ml volumetric flask and made up the volume with mobile phase to get 20 µg/ml each solution.

For the analysis of pharmaceutical dosage forms, ten tablets were weighed and powdered. A quantity equivalent to one tablet containing 500 mg of metformin HCl and 30 mg of pioglitazone was transferred into extraction flask, to this suitable amount of methanol was added and the mixture was subjected to vigorous shaking for 30 min for complete extraction of drugs, and then centrifuged at 5000 rpm for 20 min (Remi R8C laboratory centrifuge). Supernatant was collected from each set and diluted with mobile phase and injected to HPLC system for the analysis.

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 Ammonium Acetate buffer (pH-3) in the ratio of 42:58 was used.

A typical chromatogram obtained by using the aforementioned mobile phase from 20 µL of the assay preparation is illustrated in Fig. 1. The retention times of MET and PIO were 5.16 and 8.1 min, respectively.
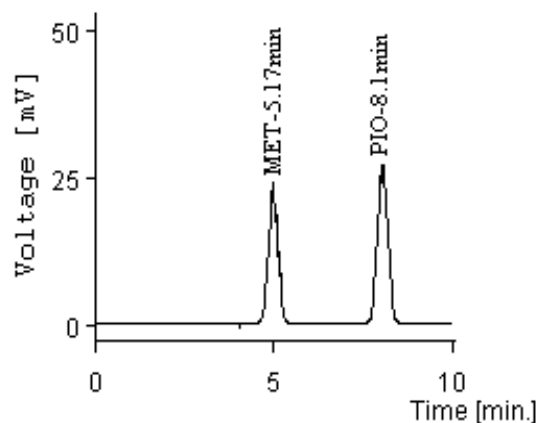

Fig. 1 : A typical chromatogram showing the peaks of metformin (5.17 min) and pioglitazone (8.1 min) in pharmaceutical dosage forms

The linearity of the method was tested from 0.5- 50 µg/ml for MET and 0.3- 30 µg/ml for PIO. Linearity solutions were injected in triplicate and the calibration graphs were plotted as peak area of the analyte against the concentration of the drug in µg/ml. In the simultaneous determination, the calibration graphs were found to be linear for both

Table 1 : Recovery of MET and PIO (n=3)

Sample ID	Concentration of drug (µg/ml)				% Recovery	
	Pure Drug		Formulation			
	MET	PIO	MET	PIO	MET	PIO
80%	40	2.4	50	3	99.22	97.87
100%	50	3.0	50	3	98.23	100.11
120%	60	3.6	50	3	99.49	98.73

the analytes in the mentioned concentrations and the correlation coefficients for the regression line were 0.9968 and 0.9986 for MET and PIO respectively. The accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts of the drug to the placebo. The recovery was determined at three levels, viz. 80%, 100%, and 120% of the selected concentrations.

Table 2 : Precision data for MET and PIO

Nominal concentrations (µg/ml)		Mean±S.D, %RSD	
MET	PIO	MET	PIO
25	1.5	24.67± 0.42, 1.75	1.46± 0.019, 1.30
50	3	48.76± 0.95, 1.95	2.89± 0.051, 1.76
100	6	98.35± 1.89, 1.92	5.86± 0.113, 1.93

Each mean value is the result of triplicate analysis for three times a day

%R.S.D= (S.D/mean) x100

Three samples were prepared for each recovery level. The recovery values for MET and PIO ranged from 98-102% and 97-103%, respectively (Table 1). The precision (repeatability and intermediate precision) of the method was determined from one lot of combined dosage form. Intra and Inter day studies were performed by taking six replicates of three concentrations. The results are shown in (Table 2). The limit of detection (LOD) and limit of quantitation (LOQ) for MET, PIO was 0.003 µg/ml, 0.0061 µg/ml and 0.01 µg/ml, 0.02 µg/ml, respectively. To determine the robustness of the developed method experimental conditions were purposely altered and RSD of the peak areas of MET and PIO were found not greater than 2.0 illustrate the robustness of the method.

Application of the method to pharmaceutical dosage forms: The method is sensitive and specific for the quantitative determination of MET and PIO and also subjected to validation for different parameters, hence has been applied for the estimation of drug in pharmaceutical dosage forms. Tablets from two different manufacturers (Piocon Forte MET 500 mg and PIO 30 mg, Orchid Chemicals and Pharmaceuticals, and Diavista M MET 500 mg and PIO 15 mg, Dr. Reddy's Laboratories) were evaluated for the amount of MET and PIO present in the formulations. Each sample was analyzed in triplicate after extracting the drug as mentioned above in experimental section. The amount of metformin and pioglitazone was found to be within the range of 95%-105%. None of the tablet excipients were found to interfere with the analyte peak and the results were shown in Table 3.

Table 3 : Results of the determination of metformin and pioglitazone in Tablets (n=6)

	Labeled amount (mg)	Amount (µg) Taken	Amount (µg) Found±S.D	Assay %RSD	Assay %w/w
PIOZ					
MET	500	500	491.76±6.75	1.37	98.4
PIO	30	30	29.79±0.45	1.51	99.3
PIOGLAR					
MET	500	500	487.32±5.46	1.12	97.4
PIO	15	15	28.67±0.31	1.08	95.6

CONCLUSION

The proposed method was found to be simple, precise, accurate and rapid for simultaneous determination of Metformin and Pioglitazone from pure and in pharmaceutical dosage forms. The mobile phase is simple to prepare and economical. The sample recoveries in all formulations were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. Hence, the method can be easily and conveniently adopted for routine analysis of Metformin and Pioglitazone in combined dosage forms and can also be used for dissolution or similar studies.

REFERENCES

1. Lalhriatpuii TC, Kawathekar N, Derivative Spectrophotometric Estimation of 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 Technique for the Estimation of Metformin in its Multi Component Dosage Forms, J. Pharm Biomed Anal, 2001; 25: 77-84.

4. Sakalgaonkar AA, Mirgane SR, Arbad BR, Validated High Performance Liquid Chromatographic Stability Indicating Method for Analysis of Metformin HCl, Int. J. App Environ Sci 2008; 3: 65–73.

5. 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, Anal Chim Acta 1999; 378: 299-311.

6. Wanjari DB, Gaikwad NJ, Stability Indicating RP-HPLC Method for Determination of Pioglitazone from Tablets, Ind J. Pharm Sci 2005; 67: 256-258.

7. 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.

8. Zhong WZ, Williams MG, Simultaneous Quantitation of Pioglitazone and its Metabolites in Human Serum by Liquid Chromatography and Solid Phase

Extraction, J. Pharm. Biomed. Anal 1996; 14: 465-473.

9. 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.

10. 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.

11. 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.