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Research Article

Method development and validation of simultaneous estimation of metformin and pioglitazone in bulk and pharmaceutical formulation by UPLC

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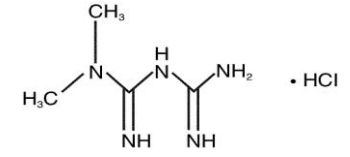
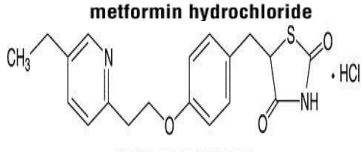
ABSTRACT
In this work, a rapid, precise and specific ultra performance liquid chromatography (UPLC) method was developed and validated for the simultaneous determination of Metformin and pioglitazone in bulk and pharmaceutical dosage forms. . The chromatographic separation of the drug components were achieved on Waters Acquity BEH C18, 50×2.1 mm, 1.7 µm UPLC column using 0.2% triethylamine in water : acetonitrile(70:30);pH adjusted with 3.8 with o-phosphoric acid as mobile phase 0.5ml/min and detection wavelength was 243nm. Within a short runtime of 5.0 min. The newly developed UPLC method was statistically validated for accuracy, precision, linearity, ruggedness, robustness, system suitability and selectivity, limit of detection and limit of quantitation.
Keywords- UPLC; Simultaneous determination; Metformin and pioglitazone; Validation.

INTRODUCTION

Metformin's insulin sensitizing effect occurs mainly at liver combination with thiazolidinediones (TZDS), which mainly sensitive muscle to insulin-mediated glucose uptake is a rational therapeutic strategy. For the simultaneous estimation of Metformin and Pioglitazone a few analytical methods UV, RP-HPLC, HPTLC methods are reported. [1-5] Till to date no accurate and precise UPLC method is developed for the combined estimation of Metformin and pioglitazone. The aim of the present analytical research is to develop a simple, precise, accurate, rapid and economic UPLC method for the assay of Metformin and Pioglitazone in combined tablet formulation. The objective of this is to validate the developed method.[6-7]



metformin hydrochloride



pioglitazone hydrochloride

MATERIALS AND METHODS

Chemicals and Reagents

Metformin and pioglitazone were obtained from gift samples of aurobindo pharma limited, bollarum, Hyd. water and acetonitrile (uplc grade), triethyl amine and o-phosphoric acid were of A.R.grade. The pharmaceutical preparations obtained from commercial drug store of combination MET-PIO (500mg, 30mg).

Instrumentation

Ultra performance liquid chromatography

A Waters Acquity UPLC system (Waters, USA) equipped with binary, auto sampler, column oven and photodiode array detector (PDA) was employed for analysis. Chromatographic data was acquired using Empower 2 software.

Chromatographic Conditions

The mobile phase containing triethyl amine in water: acetonitrile (70:30), pH was adjusted to 4.0 with o-phosphoric acid was found to resolve these two drugs-phosphoric acid was used for pH adjustment of buffer. The mobile phase was filtered on a 0.20 micron membrane filter and then

ultrasonicated for 5 min. The floe rate was set to 0.5ml/min. The wave length was selected 243nm for further analysis this was common wavelength for these two drugs. All determinations performed at constant column temperature (ambient).

Standard Solution Preparation

Accurately weigh and transfer 10 mg of Metformin and 10mg of Pioglitazone working standard into a 10ml clean dry volumetric flask add about 7ml of Diluent and sonic ate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).Further pipette 5ml of Metformin & 0.3ml of Pioglitazone the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

Sample Solution Preparation

A total of ten tablets were weighed and triturated with glass mortar and piston. An amount equivalent to one tablet (transfer 958.4 mg of Metformin and Pioglitazone Tablet Powder) into a 100ml clean dry volumetric flask add about 70ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).Further pipette 1ml of Metformin & Pioglitazone the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

Calibration Curve

Calibration curve was prepared by taking appropriate aliquots of standard and sample stock solutions (MET AND PIO) in different 10ml volumetric flasks and diluted up to the mark with mobile phase to obtain final concentrations of 300,400,500,600,700 ppm for Metformin and 10, 20,30,40,50 ppm for pioglitazone. Standard solutions were injected through 20 µl loop system and chromatograms were obtained using 0.5ml/min. The effluent was monitored at 243nm. Calibration curve was constructed by plotting average peak area against concentration and regression equation was computed.

VALIDATION OF THE METHOD

`The developed method was validated in terms of, specificity, precision, accuracy, linearity, Limit of detection, limit of quantification, robustness and ruggedness.

Standard Solution Preparation

Accurately weigh and transfer 10 mg of Metformin and 10mg of Pioglitazone working standard into a 10ml clean dry volumetric flask add about 7ml of Diluent and sonic ate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).Further pipette 5ml of Metformin & 0.3ml of Pioglitazone the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Sample Solution Preparation

A total of ten tablets were accurately weighed and triturated with glass mortar and pestle.an amount equivalent to one tablet (transfer 958.4 mg of Metformin and Pioglitazone Tablet Powder) into a 100ml clean dry volumetric flask add about 70ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).Further pipette 1ml of Metformin & Pioglitazone the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

RESULT AND DISCUSSION

To develop a precise, accurate, and suitable UPLC method for the simultaneous estimation of Metformin and pioglitazone, different mobile phases were tried and the proposed chromatographic conditions were found to be appropriate for the quantitative determination. The chromatogram obtained from pure Metformin and pioglitazone in fig 1 while for Metformin and pioglitazone in tablet dosage form. System suitability results were carried out as per USP XXIV and parameters are summarized in the table 1. The results obtained by the assay of marketed formulation are summarized in table 2.

Method Validation

The proposed method was validated as per ICH guidelines.

Specificity

The peak purity of Metformin and pioglitazone were assessed by comparing the retention time of standard Metformin and pioglitazone. Good correlation was obtained between the retention time of standard and sample of Metformin and pioglitazone.

Linearity

The linearity was studied by preparing standard solutions at different concentration levels. The linearity range for Metformin 300-700 ppm and for pioglitazone 10-50 ppm. The correlation coefficient was found to be 0.999.

Precision

Precision was evaluated by injecting five times of standard and sample solutions of Metformin and pioglitazone. Percentage relative standard deviation (% RSD) was found to be less than 2%, which proves the method is precise.

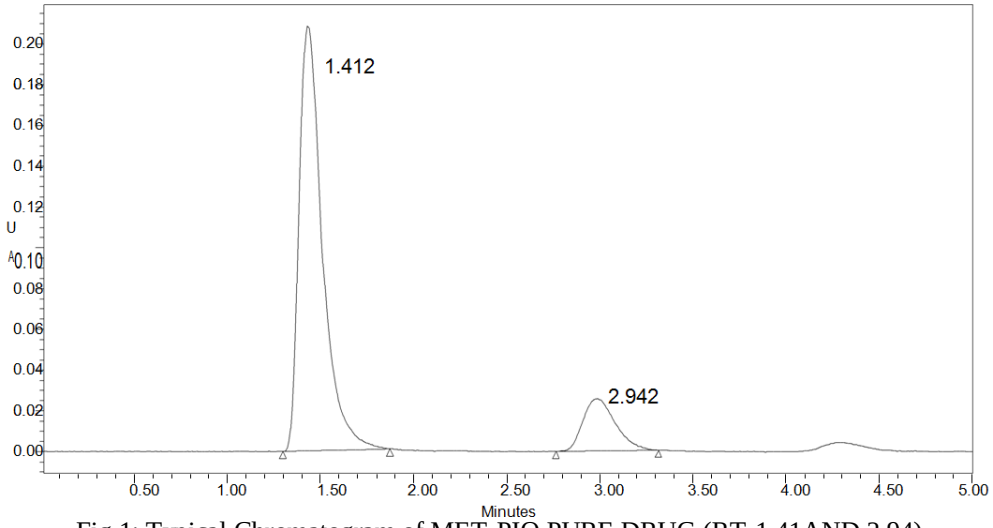


Fig 1: Typical Chromatogram of MET-PIO PURE DRUG (RT-1.41AND 2.94).

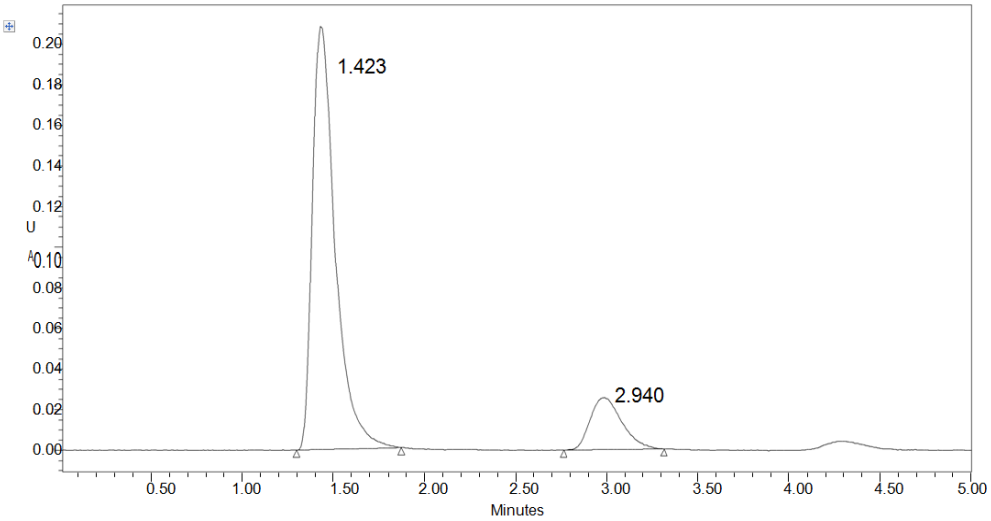
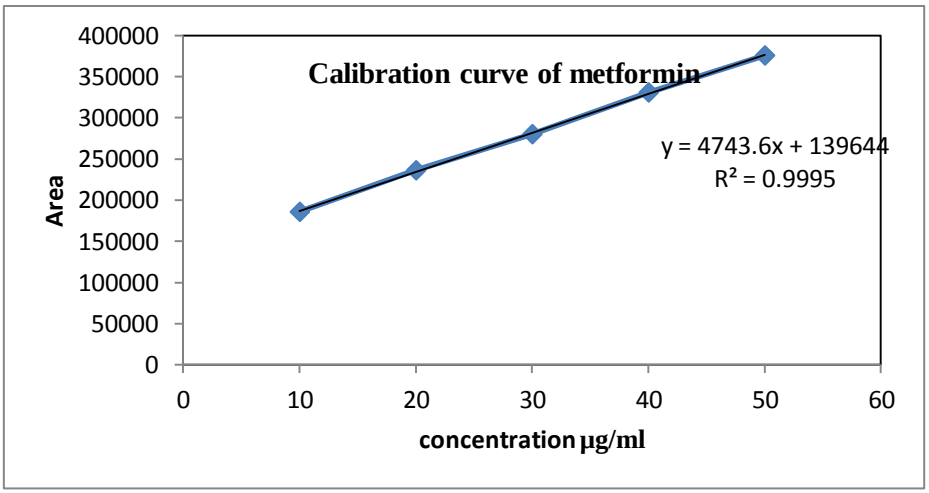


Fig 2: Typical Chromatogram of MET-PIO sample (RT-1.42AND 2.94).

Table1: System Suitability Parameters

Parameters	Metformin	Pioglitazone
Linearity range	300-700 ppm	10-50 ppm
Correlation coefficient	0.999	0.999
Slope	3076.435	4712.39
USP plate count	2725.2	2546.5
Tailing factor	1.7	1.4
Limit of Detection (LOD)	2.97	3.0
Limit of quantification(LOQ)	9.93	10.0



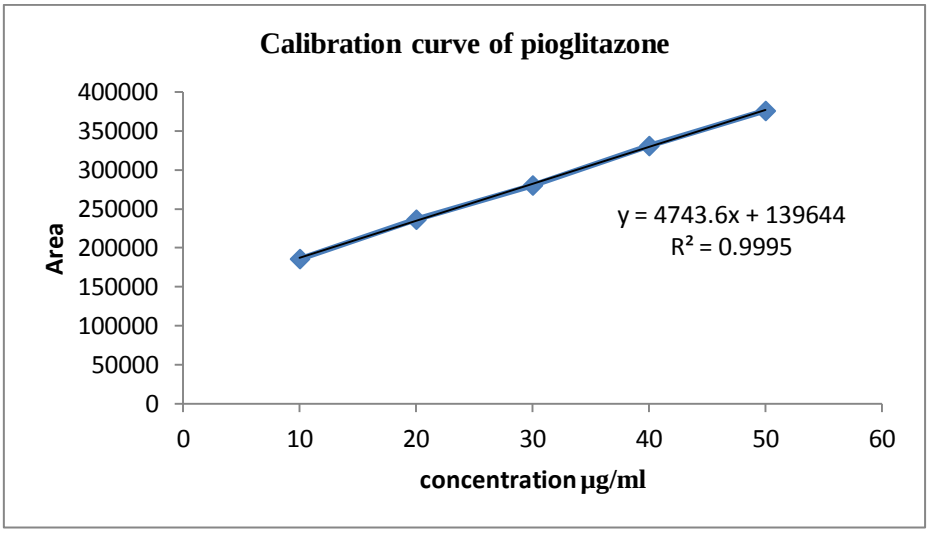


Table 2: Intraday and inter day precision result of Metformin and Pioglitazone

Drug	%RSD(Intra-day)	%RSD(Inter-day)
Metformin	0.14	0.55
Pioglitazone	1.04	0.26

Table 3: Accuracy (% recovery) results of Metformin and pioglitazone

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	758423	5.0	4.99	99.8%	99.6%
100%	1517711	10.0	9.99	99.9%	
150%	2255740	15.0	14.8	99.0%	

The accuracy results for Pioglitazone

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	126129	5.0	4.95	99.0%	99.9%
100%	254341	10.0	9.98	99.8%	
150%	385735	15.0	15.1	100.9%	

Results for robustness test of Metformin and pioglitazone

Results for Metformin

S. l.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.4	2854.7	1.6
2	0.5	2725.2	1.7
3	0.6	2725.8	1.7

Results for Pioglitazone:

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.4	2475.8	1.3
2	0.5	2546.5	1.4
3	0.6	2318.6	1.3

For Metformin:

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	3125.8	1.7
2	*Actual	2725.2	1.7
3	10% more	3012.8	1.7

*

For Pioglitazone:

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	2958.6	1.3
2	*Actual	2546.5	1.4
3	10% more	2802.0	1.4

Accuracy

To check the degree of accuracy of the method, recovery studies were performed in triplicate by standard addition method at 50%, 100%, 150%. A known amount of standard sample was added to pre analyzed samples and was subjected to the proposed UPLC method.

Robustness of Method

To evaluate the robustness of the developed UPLC method, small deliberate variations in the optimised parameters were done. The effect of change in flow rate and mobile phase composition on retention time and tailing factor were studied. The method was found to be unaffected by small changes like ± 0.1 change in flow rate and ± 0.1 change in mobile phase.

CONCLUSION

The proposed method is simple, sensitive, rapid and economic UPLC method was developed and validated for the assay of Metformin and pioglitazone in bulk and pharmaceutical formulation. This method yielded high recoveries with good linearity and precision. It can be concluded that the proposed method is a good approach for obtaining reliable results and found to be suitable for the routine analysis of Metformin and pioglitazone in pharmaceutical preparation.

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