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Research Article

SIMULTANEOUS DETERMINATION OF METFORMIN AND PIOGLITAZONE TABLETS IN PHARMACEUTICAL DOSAGE FORM BY RP-HPLC METHOD

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ABSTRACT
The present study reveals to determine the patterns of orthostatic blood pressure A simple, fast, and precise reverse phase, gradient HPLC method was developed for the separation and quantification of metformin and pioglitazone in pharmaceutical dosage form. The quantification was carried out using Zorbax XDB 4.6X150mm,5µm column and mobile phase comprised of Disodium hydrogen phosphate anhydrous, acetonitrile and Triethylamine in proportion of ratio 660:340:1 and adjusted pH to 7.10 with Orthophosphoric acid and degassed under sonication. The flow rate was 1.0 ml/min and the effluent was monitored at 225 nm. The retention time of metformin and pioglitazone were 3.2±0.1 and 7.3±0.1 min respectively. The method was validated in terms of linearity, precision, accuracy, and specificity, limit of detection and limit of quantitation. Linearity of metformin and pioglitazone were in the range of 8.5 to170.0µg/ml and 6.0-90µg/ml respectively. The percentage recoveries of both the drugs were 99.4% and 99.6% for metformin and pioglitazone respectively from the tablet formulation. The proposed method is suitable for simultaneous determination of metformin and pioglitazone in pharmaceutical dosage form.

Keywords: Metformin, Pioglitazone, HPLC, method validation.

INTRODUCTION

Metformin is a biguanidine chemically named as N,N-Dimethylimidodicarbonimidic diamine hydrochloride .Metformin decrease the gluconeogenisis while increasing the glucose uptake by muscles and fat cells.Pioglitazone is a thiazolidine Dione derivative. It is one of the PPAR-alpha agonist, insulin sensitizer used to reduce the insulin resistance. Pioglitazone is chemically[(±)-5-[[4-[2-[5-ethyl -2- pyridinyl) ethoxy] phenyl]-methyl]-2,4-]thiazolidine dionemonohydro-chloride.

The drugs are prescribed individually as well as multi component dosage forms available in the market. A number of methods have been published for the estimation of the above said analytes. Pioglitazone in human plasma (Venkatesh *et al*., 2007; Xue *et al*., 2006 and Sripalakit *et al*., 2006) and HPLC method for antidiabetic drugs (Yao *et al*., 2006; Jedlicka *et al*., 2004 and Kolte *et al*., 2004) were reported

Even though various methods were reported inthe literature for estimation of metformin and pioglitazone individually or in combination with other drugs no method had been reported for simultaneous estimation of these two drugs using HPLC in bulk drug and pharmaceutical dosage forms. The present study was aimed at the simultaneous estimation of metformin and pioglitazone by reverse phase HPLC method. The method was validated according to the ICH (Q2A 1995) guidelines.

EXPERIMENTAL

Materials, reagents and chemicals

Metformin and Pioglitazone were obtained as gift samples from Dr. Reddys Laboratories, Hyderabad. Triethylamine, Hydrogen peroxide -30%v/v , Orthophospharic acid , Hydrochloric acid , Sodium Dodecyl Sulphate and Methanol were A.R grade from MERCK chemicals Mumbai. Acetonitrile HPLC grade from Merck chemicals, Mumbai.

Chromatographic condition: Agilent 1200 series with VWD detector was used. Ezeochrome elite software version 4.0 is used for Data acquisition. Zorbax XDB 4.6X150mm,5µm column was used as a stationary phase. Mobile phase comprised of Disodium hydrogen phosphate buffer acetonitrile and Triethylamine the ratio 660:340:1 and adjusted pH to 7.10 with Orthophosphoric acid .Injection volume was 20µl and run time was 15min and flow rate 1.0ml/min. The column was maintained at ambient temperature and the eluent was detected at 225 nm.

Solutions

Standard solutions

Standard stock solution (1000 µg/ml) of metformin and pioglitazone, were prepared separately in methanol. Working standard solutions were prepared and further diluted in mobile phase to contain a mixture of Metformin and pioglitazone in over the linearity range from 5-200 µg/ml and 10-300 µg/ml respectively

% Recovery for Metformin at different levels:

S.No	Level	Area	mg added	Mg founded	%Recovery	Mean % Recovery
1	10%-01	5496953	0.0043	0.004345	99.1	
2	10%-02	5522291	0.0043	0.004365	99.6	
3	10%-03	5553106	0.0043	0.004390	100.2	
4	10%-04	5504432	0.0043	0.004351	99.3	99.7
5	10%-05	5545223	0.0043	0.004384	100.0	
6	10%-06	5548023	0.0043	0.004386	100.1	
7	50%-01	54137801	0.0428	0.04280	99.9	
8	50%-02	55096338	0.0428	0.04355	101.7	100.5
9	50%-03	54045135	0.0428	0.04272	99.8	
10	100%-01	105769692	0.0847	0.08361	98.8	
11	100%-02	106105603	0.0847	0.08388	99.1	99.0
12	100%-03	106275765	0.0847	0.08401	99.2	
13	150%-01	162104628	0.1270	0.1281	100.9	
14	150%-02	158857215	0.1270	0.1256	98.9	99.7
15	150%-03	159522795	0.1270	0.1261	99.3	
16	200%-01	210337229	0.1693	0.1663	98.2	
17	200%-02	210413321	0.1693	0.1663	98.2	
18	200%-03	212096229	0.1693	0.1677	99.0	98.5
19	200%-04	209470739	0.1694	0.1656	98.0	
20	200%-05	209468819	0.1694	0.1656	98.0	
21	200%-06	212992779	0.1694	0.1684	99.7	

RESULTS

Mean % recovery for Metformin from 5 levels is 99.4 %.

%RSD obtained for 21 determinations is 0.9%

% Recovery for Pioglitazone at different levels:

S.No	Level	Area	mg added	Mg founded	% Recovery	Mean % Recovery
1	10%-01	2910223	0.00300	0.003014	100.3	99.8
2	10%-02	2881042	0.00300	0.002983	99.3	
3	10%-03	2890612	0.00300	0.002993	99.6	
4	10%-04	2914121	0.00299	0.003018	100.4	
5	10%-05	2880017	0.00300	0.002982	99.4	
6	10%-06	2900775	0.00300	0.003004	100.0	
7	50%-01	13484505	0.014077	0.013963	99.2	99.1
8	50%-02	13340913	0.014044	0.013815	98.4	
9	50%-03	13753882	0.014295	0.014242	99.6	
10	100%-01	27965829	0.029470	0.028959	98.3	99.4
11	100%-02	29767792	0.303085	0.030825	101.4	
12	100%-03	28165344	0.029619	0.029166	98.5	
13	150%-01	5958635	0.047106	0.047591	101.0	101.1
14	150%-02	46035933	0.047124	0.047671	101.2	
15	150%-03	46061460	0.047167	0.047698	101.1	
16	300%-01	83909344	0.088632	0.086890	98.0	98.4
17	300%-02	84466275	0.088617	0.087466	98.7	
18	300%-03	83867972	0.088588	0.086847	98.0	
19	300%-04	84586934	0.088588	0.087591	98.9	
20	300%-05	84108400	0.088625	0.087096	98.3	
21	300%-06	84395079	0.088632	0.087393	98.6	

RESULTS

Mean % recovery forPioglitazone from 5 levels is 99.6 %.

%RSD from 21 determinations is 1.1 %.

Assay in formulations

Twenty tablets, Pioryl, (Panacea Biotech), each containing 15mg of pioglitazone and 850 mg of metformin

were weighed and finely powdered. A quantity of powder equivalent to 15mg of pioglitazone and 850 mg of metformin was weighed and transferred to a Standard flask. The drug was diluted using methanol to get a concentration of 10µg/ml of pioglitazone, 1µg/ml of metformin. The contents were mixed thoroughly and

filtered through a 0.45 µ filter. 10µ of the sample was injected in to HPLC system.

RESULTS AND DISCUSSION

The proposed HPLC method required fewer reagents and materials, and it is simple and less time consuming. This method could be used in quality control test in pharmaceutical industries. The chromatograms of pioglitazone and glimepiride were shown in (fig. 4). There was clear resolution between pioglitazone and glimepiride with retention time of 7.0 and 10.2 minutes respectively.

Validation of the method

Linearity

The response for the detector was determined to be linear over the range of 5 to 90µg/ml (5, 15, 30, 60, 75,90,) for pioglitazone and 10-200 µg/ml (10, 40, 80, 100, 120,200) for metformin . Each of the concentration was injected in duplicate to get reproducible response. The calibration curve was plotted as concentration of the respective drug versus the response at each level. The proposed method was evaluated by its correlation coefficient and intercept value calculated in the statistical study. They were represented by the linear regression Equation (figs. 2, 3).

Y Pioglitazone = 27494X+32335, 'r' value= 0.9995
Y Glimepiride = 37719X-3261, 'r' value= 0.9987

Slopes and intercepts were obtained by using regression equation (y=mx+c) and least square treatment of the results used to confirm linearity of the method developed.

Precision and accuracy

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out 6 times and the percentage recovery and % relative standard deviation was calculated. From the data obtained, recoveries of standard drugs were found to be accurate (table 1).

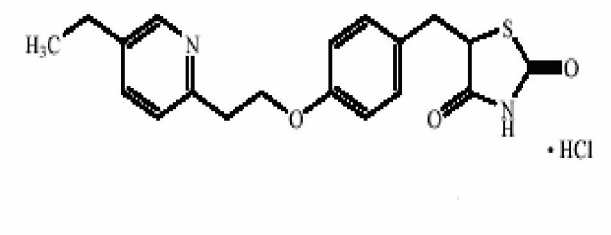
The %CV of interday and intraday precision obtained was less than 1% for both the drugs. The intraday and interday precision of pioglitazone was 0.47 and 0.86 and glimepiride was 0.76 and 0.94 respectively. From the data obtained, the developed HPLC method was found to be precise and accurate.

Specificity of the method

The PDA chromatograms of the pioglitazone and glimepiride in standard and sample were recorded. In the
chromatograms of the formulations, some additional peaks were observed which may be due to excipients
present in the formulations. These peaks however did not interfere with the standard peaks, which demonstrate thatthe assay method is specific. Furthermore, the purity of the peaks was studied by peak purity studies. The results revealed that the peak is free from interferences, which shows that the HPLC method is specific.

Quantification limit

The limit of detection (LOD) and limit of quantification (LOQ) of the developed method determined by injecting progressively low concentrations of the standard solutions using the developed methods. The LOD is the lowest concentration of the analyte that can be detected with signal to noise ratio (1:3) and LOQ is the lowest concentration that can be quantified with acceptable precision and accuracy with signal to noise ratio (1:10). The LOD of pioglitazone and glimepiride found to be 0.2µg/ml and 0.1µg/ml respectively. The LOQ of
pioglitazone and glimepiride found to be 2µg/ml and0.5µg/ml respectively



Pioglitazone

Robustness

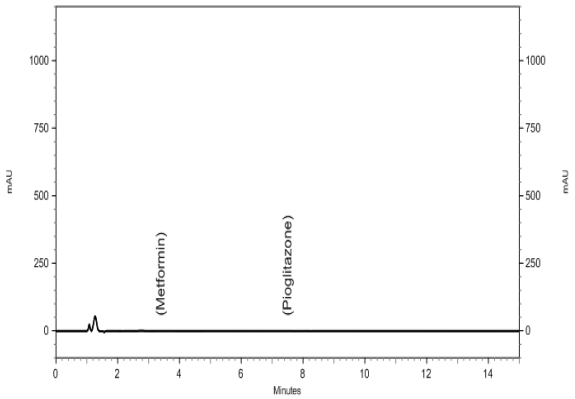
The robustness of the method was studied by deliberate changes in the method like alteration in pH of the mobile phase, percentage organic content, changes in the wavelength. It was observed that there was no marked changes in the chromatograms demonstrate that the HPLC methods have developed are robust.

Solution Stability

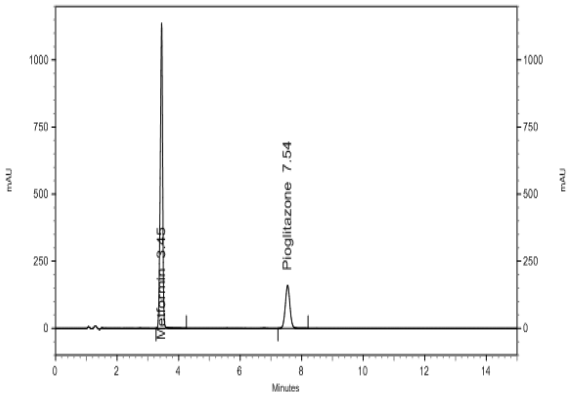
In this study, the mobile phase, the standard solutions, and the sample solution were subjected to long term (3 days) stability studies. The stability of these solutions was studied by performing the experiment and looking for changes in separation, retention, and asymmetry of the peaks which were then compared with the pattern of the chromatogram of freshly prepared solutions

System suitability

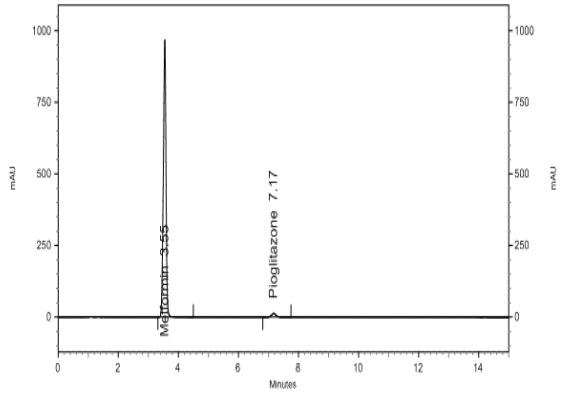
The resolution, capacity factor, theoretical plates/meter, Rt values and peak symmetry were calculated for the standard solutions. The values obtained demonstrated the suitability of the system for the analysis of the above drug combinations System suitability parameters might be fall within ± 3% standard deviation range during routine performance of the method. The summary of the method validation results were showed in the (table 2).



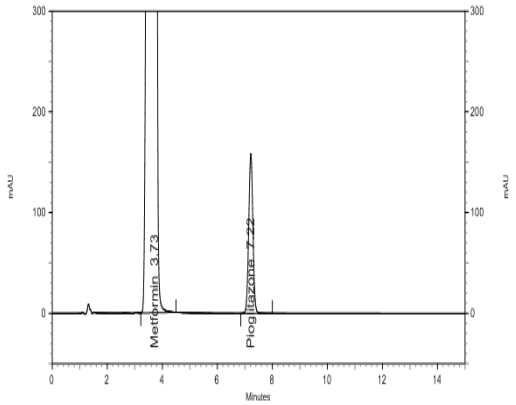
Chromatogram No 1: The Reference chromatogram of Blank



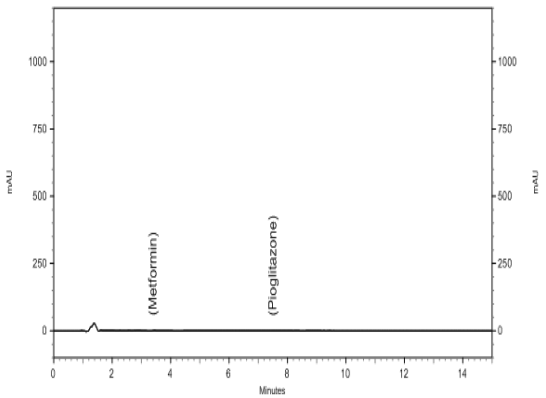
Chromatogram No 2: The Reference chromatogram of Standard



Chromatogram No 3: The Reference chromatogram of Test for Metformin



Chromatogram No 4: The Reference chromatogram of Test for Pioglitazone



Chromatogram No 5: The Reference chromatogram of Placebo

CONCLUSION

This method is simple, specific and easy to perform and requires short time to analyze the samples. Low limit of quantification and limit of detection makes this method suitable for use in quality control. This method enables simultaneous determination of Pioglitazone and Glimepiride because of good separation and resolution of the chromatographic peaks. The method was found to be accurate, precise, linear, robust and rugged.

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