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

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ABSTRACT: A rapid and accurate HPLC method was developed for determination of both metformin hydrochloride and pioglitazone hydrochloride in tablet dosage form. The chromatographic separation was conducted on Shimadzu (Prominence LC 20 UFLC XR) connected with PDA detector; using mixed column ODS/Cyano; ACE, (100 x 4.6 mm, 5 μm). The mobile phase was isocratic consisted of Acetonitrile : Phosphate buffer in ratio of (50 : 50 v/v) (buffer was composed of 3.55 gm disodium hydrogen phosphate per liter, adjusted by 85% phosphoric acid to pH 5) and was delivered to the system at a flow rate of 1.2 ml/min. An injection volume of 20 μl was used for pioglitazone hydrochloride and 5 μl for metformin hydrochloride. The detection wavelength (λ_{max}) was 235 nm for metformin HCl and 266 nm for pioglitazone hydrochloride. All assays were performed at ambient conditions. The calibration curve of metformin hydrochloride in mobile phase was linear with correlation coefficient (r^2) = 0.99995; over a concentration range of 30 – 750 mg/l for; with a retention time of 1.07 minutes. While the calibration curve of pioglitazone HCl in mobile phase was linear with correlation coefficient (r^2) = 0.99859; over a concentration range of 1 – 25 mg/l for; with a retention time of 1.85 minutes. The percentage recoveries of metformin hydrochloride and pioglitazone hydrochloride were 100.13% and 100.22%; respectively. The relative standard deviation (RSD) was found to be < 2. The proposed method was validated and successfully applied for simultaneous determination of metformin hydrochloride and pioglitazone hydrochloride HCl in tablets. The method described is quite suitable for routine analysis of tablets and for their dissolution quantitation.

INTRODUCTION: Metformin hydrochloride is a biguanidine chemically named as N,N-Dimethylimido-dicarbonimidic diamine hydrochloride.

Metformin hydrochloride decreases the gluconeogenesis while increasing the glucose uptake by muscles and fat cells.

Pioglitazone hydrochloride is a thiazolidine dione derivative. It is one of the PPAR-alpha agonist, insulin sensitizer, used to reduce the insulin resistance^{1 & 2}. Pioglitazone is chemically [(±)-5-[[4-[2-[5-ethyl -2- pyridinyl] ethoxy] phenyl]-methyl]-2,4] thiazolidine dionemonohydrochloride.

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These drugs are prescribed individually as well as multi component dosage forms available in the market. Different methods of analysis for pioglitazone hydrochloride in human plasma were reported³⁻⁵. Similarly, different HPLC methods for determination of pioglitazone hydrochloride and metformin hydrochloride in different dosage forms were reported⁶⁻⁸. The present study was aimed for the simultaneous estimation of metformin hydrochloride and pioglitazone hydrochloride by reversed phase HPLC method and its application in dissolution of these drugs in different dosage forms. The method was validated according to the ICH (Q2A 1995) guidelines⁹.

EXPERIMENTAL

Materials, reagents and chemicals: Metformin hydrochloride and Pioglitazone hydrochloride were obtained from Sinochem, China. Ortho-phosphoric was HPLC grade from Fluka chemicals. Acetonitrile was HPLC grade from Fisher chemicals. Di-Sodium hydrogen phosphate anhydrous & citric acid were purchased from local market.

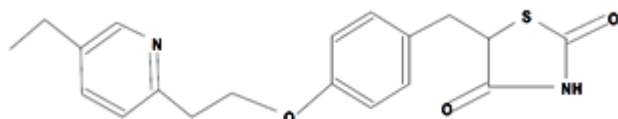


FIG. 1: CHEMICAL STRUCTURE OF PIOGLITAZONE

Analytical procedure for simultaneous determination of pioglitazone hydrochloride and metformin hydrochloride in tablet dissolution:-

- Dissolution condition:** The tablet dissolution was done according to FDA dissolution database guidelines using apparatus II (paddle) at 50 rpm with 900 ml dissolution medium constituted of McIlvaine buffer pH 2.5 (0.1 M citric acid adjusted to pH 2.5 with 0.2 M disodium hydrogen phosphate)¹⁰.
- Chromatographic condition:** Shimadzu LC prominence 20 (UFLC XR) connected with PDA detector was used. Shimadzu Lab solutions software was used for data acquisition. Mixed column ODS/Cyano; ACE, (100 x 4.6 mm, 5 μm) was used as a stationary phase. Mobile phase was isocratic consisted of

Acetonitrile: Phosphate buffer in ratio of (50: 50 v/v) (buffer is composed of: 3.55 gm disodium hydrogen phosphate anhydrous per liter, adjusted by 85% phosphoric acid to pH 5) delivered to the system at a flow rate of 1.2 ml/min, An injection volume of 20 μl was used for pioglitazone hydrochloride and 5 μl for metformin hydrochloride. The detection wavelength (λ_{max}) was 235 nm for metformin hydrochloride and 266 nm for pioglitazone hydrochloride, run time was 3.5 minute. The column was maintained at ambient temperature.

- Preparation of stock and working standard solution:** Standard stock solution (1000 μg/ml) of metformin hydrochloride and pioglitazone hydrochloride, were prepared separately in mobile phase. Working standard solutions were prepared and further diluted in mobile phase to obtain a mixture of metformin hydrochloride and pioglitazone hydrochloride over the linearity range from 30 - 750 μg/ml and 1 - 25 μg/ml respectively.
- Analytical method validation:**
 - Selectivity:** It provides an indication of the selectivity and specificity of the procedure. The method is to be selective, if the main peak is well resolved from any other peak by resolution of minimum 2. This could be done by injecting placebo and compare it with that of standard and dissolution samples, then peak purity was ascertained by use of PDA
 - Linearity:** is defined by the correlation coefficient, which should be found NLT 0.99, using peak area responses, Linearity for single point standardization should extend to at least 20% beyond the specification range and include the target Conc. This was performed by preparing 5 different concentrations, and then making 3 replicates of each concentration The linear working range were determined from the constructed standard calibration curve.
 - Intraday Precision:** This study was conducted by performing multiple analyses on a suitable number of portions of a homogeneous sample.

This was performed by assaying multiple aliquots with the same concentration starting from the first step to the final step of analysis.

The analytical precision of the method was determined by the relative standard deviation.

- d. **Inter-day Reproducibility (method ruggedness):** the degree of reproducibility determined by analysis of samples from homogeneous lot of materials, under different but typical test conditions. The method is to be rugged, at any item if the pooled %RSD of the total number of replicates that have been made in this item is within the acceptance criteria, 3 replicates of a single sample of powder material are used for each determination. First day: 3 replicates, on a second day: 3 replicates, then on third day: 3 replicates of freshly prepared test from the same sample are analyzed, under the same conditions.
- e. **Accuracy and Recovery:** Accuracy was evaluated by spiking standard solution. The measurements are made at a Conc. of

Standard Mix which is found to be the target concentration, and at suitable intervals around this point.

The dissolution samples was spiked with known quantities of St. Using three determinations over five Concentrations level covering the specified range (i.e. five concentrations and three replicates).

Relative recoveries of all concentrations of Pioglitazone hydrochloride and Metformin hydrochloride used in the standards were evaluated by comparing their peak area with those obtained from the calibration curve equation.

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 metformin hydrochloride and pioglitazone hydrochloride were shown in **figure 2 and 3**; respectively. There was clear resolution between Pioglitazone hydrochloride and Metformin hydrochloride with retention time of 1.07 and 1.85 minutes; respectively.

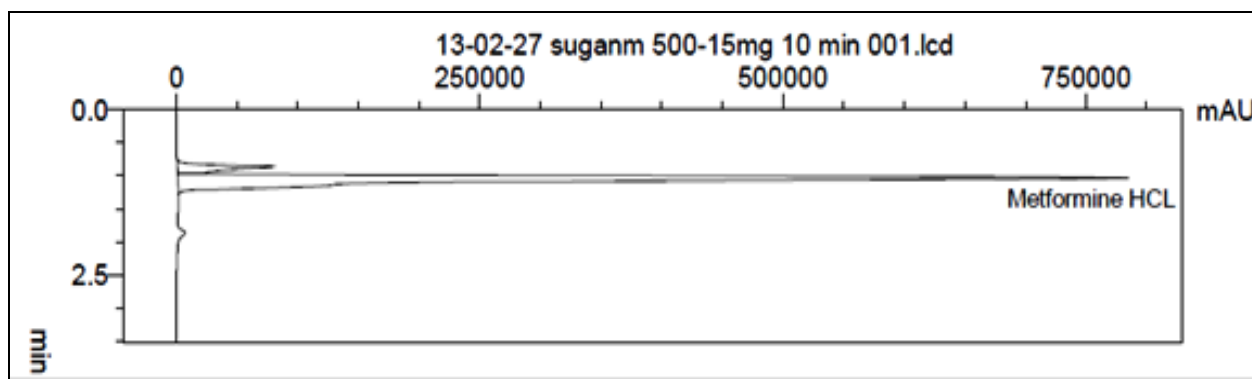


FIGURE 2: HPLC CHROMATOGRAM OF METFOMIN HYDROCHLORIDE

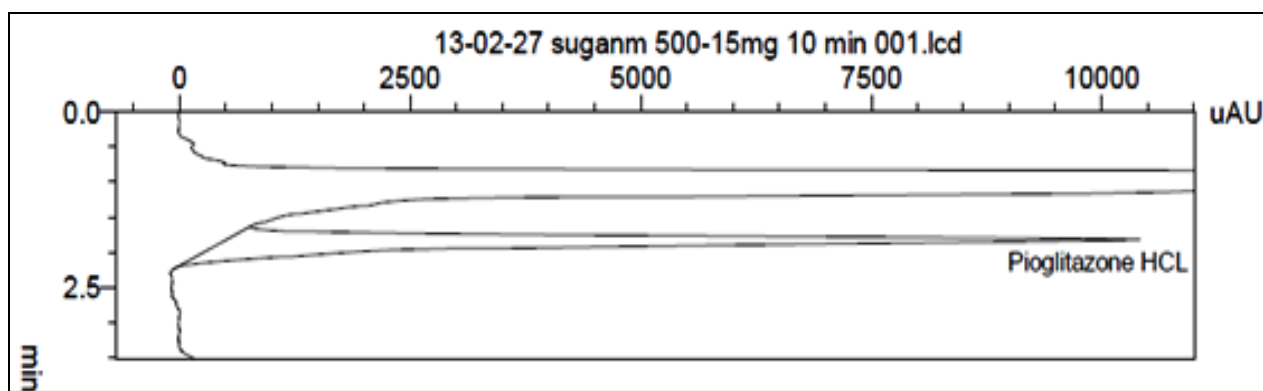


FIGURE 3: HPLC CHROMATOGRAM OF PIOGLITAZONE HYDROCHLORIDE

Specificity: The PDA chromatograms of the pioglitazone hydrochloride and metformin hydrochloride 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 that the 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.

Linearity: The response for the detector was determined to be linear over the range of 1-25 $\mu\text{g/ml}$ (1, 5, 10, 20 & 25) for pioglitazone hydrochloride as shown in **figure 4** and 30-750 $\mu\text{g/ml}$ (30, 150, 300, 600 & 750) for metformin hydrochloride as shown in **figure 5**. Each of the concentrations was injected in triplicate 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;

$$Y_{\text{Pioglitazone HCl}} = 75750 X - 360, r^2 = 0.99859$$

$$Y_{\text{Metformin HCl}} = 106020 X + 34961.11, r^2 = 0.99995$$

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.

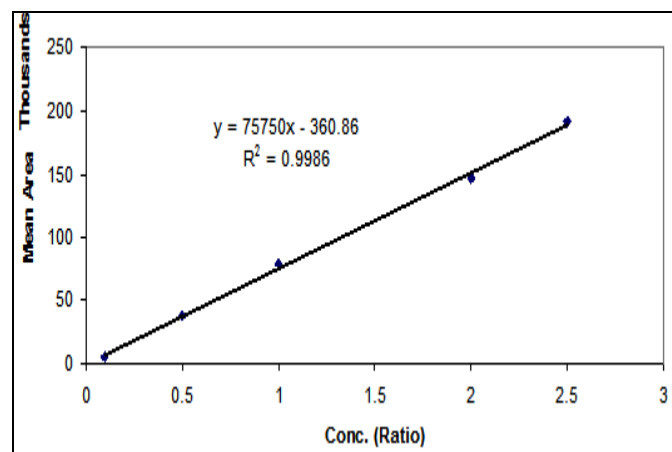


FIGURE 4: CALIBRATION CURVE OF STANDARD PIOGLITAZONE HYDROCHLORIDE

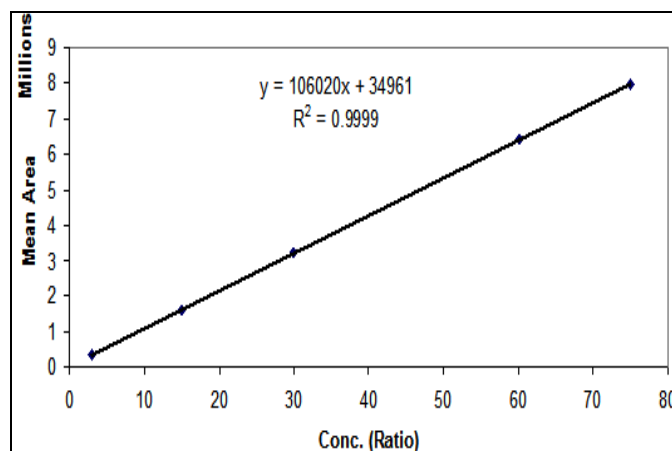


FIGURE (5): CALIBRATION CURVE OF STANDARD METFORMIN HYDROCHLORIDE

Quantification limit: The limit of detection (LOD) and limit of quantification (LOQ) of the developed method was 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 (3:1) and LOQ is the lowest concentration that can be quantified with acceptable precision and accuracy with signal to noise ratio (10:1). The LOD of pioglitazone hydrochloride and metformin hydrochloride found to be $0.33\mu\text{g/ml}$ and $3.3\mu\text{g/ml}$ respectively. The LOQ of pioglitazone hydrochloride and metformin hydrochloride found to be $1\mu\text{g/ml}$ and $10\mu\text{g/ml}$ respectively

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, R_t 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 $\pm 3\%$ standard deviation range during routine performance of the method.

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 hydrochloride and metformin hydrochloride because of good separation and resolution of the chromatographic peaks. The method was found to be accurate, precise, linear, and rugged.

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