DEVELOPMENT AND VALIDATION OF STABILITY – INDICATING RP-HPLC METHOD FOR THE SIMULTANEOUS DETERMINATION OF PIOGLITAZONE HYDROCHLORIDE AND METFORMIN HYDROCHLORIDE IN BULK AND TABLET DOSAGE FORM

Surla Durga¹, Sappa Pavani Naidu¹, Revu Baby Nalanda¹, Atla Srinivasa Rao*¹, Bolla Nagamani²

¹Department of Pharmaceutical Analysis and Quality Assurance, Shri Vishnu College of Pharmacy, Vishnupur, Bhimavaram-534202, Andhra Pradesh, India.
²Corpuscle Research Solutions, Vishakhapatnam- 530017, Andhra Pradesh, India.

ABSTRACT
The aim of the present study is to develop stability-indicating reverse phase-high performance liquid chromatography method for the simultaneous estimation of pioglitazone hydrochloride and metformin hydrochloride. The method uses a Agilent Variant C₁₈ reverse phase column (150mm×4.6mm, 5µm) with mobile phase consisting of Methanol: 0.1 %v/v Ortho-phosphoric acid (45:55) under isocratic mode with an injection volume 20µL and both the analytes were monitored at 230 nm. Stress conditions were performed by subjecting the individual analytes to the hydrolysis (acidic, basic), oxidation and photolytic stress conditions. Considerable degradation of the both drugs was observed in alkaline hydrolysis and in oxidation stress conditions. The retention times of metformin hydrochloride and pioglitazone hydrochloride were 1.63 and 3.8 min, respectively and showed a good linearity in the concentration range of 5-50µg/ml for metformin hydrochloride and 5.02-50.19µg/ml for pioglitazone hydrochloride with a correlation coefficient of 0.999 for both drugs. None of the degradation products or excipients interfered with the retention time of the drugs. The validation parameters like specificity, system suitability, accuracy, precision, linearity, limit of detection, quantification, robustness and ruggedness were all within limits as per International Conference on Harmonization (ICH) guidelines. The proposed RP-HPLC-UV method is specific, accurate,
and precise and was successfully applied for the simultaneous estimation of pioglitazone hydrochloride and metformin hydrochloride in bulk and tablet dosage forms.

**KEYWORDS**: Pioglitazone hydrochloride, Metformin hydrochloride, Simultaneous estimation, Stability indicating assay, Reverse phase liquid chromatography, Degradation products.

**INTRODUCTION**

Diabetes is not a single disorder, but, is group of metabolic disorders characterized by hyperglycemia caused by inadequate insulin secretion with or without a simultaneous disease in hormone action at its receptor.[1] Type-1 Diabetes represents the diagnosis for 5-10% of the diabetic population, is caused by an absolute deficiency in insulin secretion. Type-2 diabetes, for which 800,000 new cases are diagnosed per year, is a more complex disease & characterized by end-organ insulin resistance and/or a relative deficiency in insulin secretion.[2] In many patients, insulin resistance causes an initial increase in plasma insulin levels as results of a compensatory increase in insulin secretion. At this point, blood glucose levels likely appear normal and the patient is asymptomatic. Diabetes is the leading cause of new-onset blindness, kidney failure & non-traumatic amputations and has a major role in the development of heart disease, hypertension, sexual dysfunction & dental disease. Many oral diabetes medications are available with different mechanism of actions using sulfonylurea, biguanides, thiazolidinediones and α-glycosidase inhibitors. One important aspect of treating a patient with diabetes is not to focus on blood sugar alone. The leading cause of death for patients with diabetes is heart disease; therefore, health care provides need to assess cholesterol, blood pressure & other risk factors for heart disease. The diagnosis of Type-1 and Type-2 diabetes requires the measurement of fasting plasma glucose levels and/or plasma glucose levels after an oral glucose challenges.[1]

Pioglitazone hydrochloride (PGZ), (±)-5-(((4-(2-(5-ethyl-2-pyridinyl)ethoxy)phenyl)methyl)-2-4-thiazoli-dinedione monohydrochloride, belongs to the thiazolidinedione class acts by binding to peroxisome proliferators activated receptors gamma (PPARγ) as an oral antihyperglycemic agent while increasing the receptor sensitivity to insulin in muscle and adipose tissues on inhibits hepatic gluconeogenesis. It is used either as a monotherapy or in combination with other hypoglycemic agents in the treatment of type-2 diabetes. PGZ decreases insulin resistant in the periphery, liver after administration resulting in increased insulin dependent glucose disposal and decreased output of hepatic glucose.[3-5] Metformin
hydrochloride (MET), 1,1-Dimethyl biguanides monohydrochloride, used for the treatment of type-2 diabetes mellitus as a biguanides hypoglycemic agent. In the skeletal muscle, MET acts by increasing the glucose transport across the cell membrane and it is recommended to the overweight patients.\textsuperscript{[6-8]} However, MET was used decades ago prescribed to use widely for the treatment of diabetes either as a monotherapy or combination with other components.\textsuperscript{[7]} It is not adequate for many type-2 diabetes patients by monotherapy with oral anti-diabetic agents; necessary to achieve an acceptable blood sugar control by multidrug therapy. Presently a combination of MET and second generation sulfonylureas (glipizide, gliclazide, glibenclamide or glimepiride) is commonly prescribed for type-2 diabetes.\textsuperscript{[9]} Determination of pioglitazone and its metabolites in biological fluids by liquid chromatography (LC) methods have been reported\textsuperscript{[10-12]} and for analysis of PGZ in bulk drug and in pharmaceutical formulations.\textsuperscript{[3]}

Simultaneous determination of PGZ with another six anti-diabetic drugs in a single run using UPLC and the same method has been applied for determination of these compounds in pharmaceutical formulations using UV detection.\textsuperscript{[4]} A validated method has been developed for the simultaneous estimation of PGZ and its two metabolites M-III (keto-derivative) and M-IV (hydroxyl derivative) in human plasma using liquid chromatography tandem mass spectrometry (LC-MS/MS).\textsuperscript{[13]} Another LC-MS/MS method has been reported for the simultaneous determination of PGZ and candesartan in human plasma for human pharmacokinetic and bioequivalence studies.\textsuperscript{[5]} Solid phase extraction\textsuperscript{[11]} and hallow fiber liquid phase micro-extraction (HF-LPME)\textsuperscript{[12]} procedures have applied for extraction and pre-concentration of PGZ from biological fluids before quantitative determination by high-performance liquid chromatography (HPLC). Applications of membrane-selective electrodes have been reported for the determination of PGZ hydrochloride in the presence of its acid degradants or MET hydrochloride in tablets and plasma using polyvinyl chloride membrane sensors.\textsuperscript{[14]} A simple HPLC-UV detection method has been developed and validated for the simultaneous determination of MET and rosiglitazone in human plasma.\textsuperscript{[15]} A stability indicating method has been reported for the analysis of MET in tablet formulation using capillary electrophoresis technique.\textsuperscript{[7]} HPLC method has been reported for the determination of MET in human plasma and urine using small sample volume and Octadecyl silane column.\textsuperscript{[16]} High performance thin layer chromatography (HPTLC) method has been used for simultaneous determination of MET and glyburide in tablets.\textsuperscript{[17]} Determination of MET and gluburide in an anti-hyperglycemic binary mixture has been studied using HPLC-UV and
spectrometric methods.\textsuperscript{[18]} A HPLC-UV method has been developed and validated for the determination of MET in tablets containing croscarmellose sodium as an additive.\textsuperscript{[19]}

Determination of MET such as interaction with ninhydrin in alkaline medium\textsuperscript{[20]} and oxidation with hydrogen peroxide\textsuperscript{[21]} have been reported using various spectrophotometric procedures. Simultaneous spectrophotometric determination of MET and repaglinide in a synthetic mixture has been also reported.\textsuperscript{[22]} Several methods like gas chromatography,\textsuperscript{[23]} NMR spectrometry\textsuperscript{[24]}, capillary electrophoresis\textsuperscript{[25]} potentiometry and spectrofluorimetry\textsuperscript{[26,27]} methods have been reported for determination of MET. Spectrophotometric and chemometric methods have been applied for determination of MET and PGZ in binary mixture and in their ternary mixture with pioglitazone acid degradant.\textsuperscript{[28]} HPLC and spectrophotometric methods have been developed and validated for determination of MET and PGZ in a combined pharmaceutical-dosage form.\textsuperscript{[6]}

No detailed stability-indicating report for the determination of PGZ and MET in combined dosage form as tablets did not investigated and published.\textsuperscript{[29]} The aim of the present study is to develop a simple stability indicating method for the determination of MET and PGZ in bulk and tablet dosage form under the mild stress conditions is available to resolve the drug from its potential impurities and degradation products. There is no interference found by the impurities and degradants during the method development. Chemical structures of PGZ and MET are shown in Figure 1. The aim of the present study was the development and validation of a simple, accuracy, precise, stability-indicating and reliable and cost effective LC method for the simultaneous estimation of PGZ and MET in a combined commercial tablet form. Stability tests were performed on both drug substances as per International Conference on Harmonization (ICH) norms.

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{chemical_structures.png}
\caption{Chemical structures of PGZ and MET}
\end{figure}

**MATERIALS AND METHODS**

**Chemicals**

PGZ and MET were generously provided by Dr. Reddy’s Laboratories Limited, Hyderabad, India. Methanol, water, acetonitrile are of HPLC grade and hydrochloric acid, ortho-
phosphoric acid were purchased from SD Fine Chemicals Ltd., Mumbai, India. All the other chemicals and reagents are of analytical reagent (AR) grade.

**Chromatographic Conditions**

The chromatographic system consists of HPLC system (Shimadzu Separation Module LC-10Atvp) with UV-detector, SIL-10ADvp auto sampler, CTO-10Avp Column temperature oven. All the components of the system are controlled using SCL-10Avp system controller. Data acquisition was carried out using LC solutions version 1.23 SP 1 software. The chromatographic analysis was performed on Agilent Variant C<sub>18</sub> column (150mm×4.6mm, 5µm).

Mobile phase consisting of Methanol-0.1%v/v Ortho-phosphoric acid (45:55) was used in isocratic mode and the mobile phase was filtered through nylon membrane filter of 0.22µm (Millipore) and sonicated for 5 min before use. The flow rate was 1 ml/min and the injection volume was 20 µl. Detection was performed at 230 nm and the separation was achieved at ambient temperature.

**Preparation of Stock Solutions**

Stock solutions of both the drugs were separately taken such that their concentrations are approximately 1 mg/ml. The concentration of the free form for the individual stocks is corrected for its purity, salt forms and actual amount weighed. For preparation of linear calibration standards and quality control samples, suitable volumes of individual stocks were taken and transferred into a 10 ml volumetric flask and made up to volume with diluents solution (Methanol: Acetonitrile: Water (25:25:50 v/v)). The final concentration of the drugs in each calibration standard and quality control sample is given in Table 1.

**Table 1: Final concentrations of calibration standards and quality control samples.**

<table>
<thead>
<tr>
<th>ID of the sample</th>
<th>Concentration of MET (µg/ml)</th>
<th>Concentration of PGZ (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC 1</td>
<td>5</td>
<td>5.02</td>
</tr>
<tr>
<td>CC 2</td>
<td>10</td>
<td>10.04</td>
</tr>
<tr>
<td>CC 3</td>
<td>20</td>
<td>20.08</td>
</tr>
<tr>
<td>CC 4</td>
<td>30</td>
<td>30.11</td>
</tr>
<tr>
<td>CC 5</td>
<td>40</td>
<td>40.15</td>
</tr>
<tr>
<td>CC 6</td>
<td>50</td>
<td>50.19</td>
</tr>
<tr>
<td>50% Recovery Solution</td>
<td>12.5</td>
<td>12.55</td>
</tr>
<tr>
<td>100% Recovery Solution</td>
<td>25</td>
<td>25.10</td>
</tr>
<tr>
<td>150% Recovery Solution</td>
<td>37.5</td>
<td>37.64</td>
</tr>
</tbody>
</table>
RESULTS AND DISCUSSION

Method development and optimization

All determinations were performed at room temperature. The mobile phase was (45:55 v/v) methanol: 0.1% v/v ortho-phosphoric acid, which was run isocratic. Flow rate was 1 ml/min, Agilent variant C18 (150×4.6 mm, 5μm) analytical column, maintained at room temperature, was used for the separation of PGZ and MET. The method was validated for the determination of PGZ and MET in Pioglit-Mf 15® tablets. Mobile phase optimization was initiated by using Methanol: Water (50:50 v/v) at 1 ml/min flow rate using Phenomenex C18 column (150×4.6mm, 5μm); the MET was shown significant tailing and PGZ eluted at 3.0 min with poor resolution. In other trail, using MeOH: PDP (50:50 v/v) at 1 ml/min flow rate using Phenomenex C18 column (150×4.6mm, 5μm), the MET and PGZ peaks eluted as a straight line; ACN:MeOH:0.1% OPA (25:25:50 v/v) using Agilent variant (150×4.6mm), both the drugs eluted unsymmetrical, with poor resolution and the another trail using the mobile phase MeOH:0.1% OPA (40:60 v/v) using Agilent variant (150×4.6mm), the peaks showed broadening. Finally, good peak shapes and resolution were obtained with a mobile phase composition of Methanol: 0.1% OPA (45:55 v/v) at flow rate of 1ml/min using Methanol: Acetonitrile: Water (25:25:50 v/v) as diluent; the MET was eluted at 1.63 min and PGZ at 3.8 min and tailing factors for both drugs were within the limits and the peaks were eluted within 5 min run time. For quantitative analytical purpose, wavelength was set at 230 nm, which provided better reproducibility with minimum or no interference (Figure 2).

Method validation

The method was validated in accordance with the ICH requirements[30] which involved system suitability, specificity, accuracy, precision, linearity, limit of detection, limit of quantification.
System Suitability

System Suitability tests are an integral part of liquid chromatographic methods in the course of optimizing the conditions of the proposed method. They are used to verify that the resolution and reproducibility were adequate for the analysis performed. The parameters of these tests are column efficiency (number of theoretical plates), tailing factor of chromatographic peak, repeatability as % RSD of peak area for six injections and reproducibility of retention time of a solution.

System suitability testing is an integral part of the analytical procedure. System suitability studies were carried out by injecting mixed standard concentration 6 times, 25µg/ml of PGZ and 25µg/ml for MET. The RSD (%) values for system suitability test parameters like retention time \( t_R = 3.8 \pm 0.26 \) for PGZ and \( t_R = 1.63 \pm 0.61 \) for MET, tailing factor \( T_f = 1.43 \pm 0.70 \) for PGZ and \( T_f = 1.93 \pm 1.04 \) for MET and theoretical plate number \( \# = 6461 \pm 0.49 \) for PGZ and \( \# = 2711 \pm 1.90 \) for MET were found to be less than 2% except MET theoretical plates %RSD, indicating that the present conditions were suitable for the analysis of PGZ and MET in tablet dosage form. The results of these tests for the proposed method are listed in Table 2.

Table 2: System Suitability Data of the both drugs.

<table>
<thead>
<tr>
<th>Drug name</th>
<th>Retention Time (min)</th>
<th>Peak Area (µg/ml)</th>
<th>Theoretical Plates (#)</th>
<th>Tailing Factor (T_f)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pioglitazone Hydrochloride</td>
<td>3.8</td>
<td>1796433.83</td>
<td>6461.00</td>
<td>1.43</td>
</tr>
<tr>
<td>Metformin Hydrochloride</td>
<td>1.63</td>
<td>4648171.83</td>
<td>2711.83</td>
<td>1.93</td>
</tr>
</tbody>
</table>

Specificity

Specificity is the ability of the analytical method to measure the analyte response in the presence of interferences including degradation products and related substances. Specificity of the developed method was also assessed by performing forced degradation studies. Specificity of the method was observed by injecting the blank, working standard solutions of PGZ, MET and the mixture. The specificity chromatogram was shown in Figure 3 (a-d). Where the retention times of PGZ and MET were does not interfere with the other.
Fig 3. Typical specificity chromatograms. (a) Blank sample, (b) MET, (c) PGZ, (d) Both MET and PGZ.

Linearity
A linear relationship was evaluated across the range of the analytical procedure with minimum of 6 concentrations. A series of combination standard dilutions were prepared over a concentration range of 5.02 to 50.19µg/ml for PGZ and 5-50µg/ml for MET from final stock solutions of both drugs and injected on to the column. Linearity is evaluated by a plot of peak areas as a function of analyte concentration, and the test results were evaluated by appropriate statistical methods where by slope, intercept, and regression (R²), correlation coefficient (r) were calculated.

Linear relationships were observed at the concentrations of 5.02-50.19µg/ml for PGZ and 5-50µg/ml for MET and were analyzed. Peak areas and concentrations were subjected to least square regression analysis to calculate regression equation. The peak areas of PGZ and MET were found to be linear in the range concentrations. Calibration curves for PGZ and MET were shown in Figure 4 and 5. The data from the calibration curve are given in Table 3.
Precision

Precision is the measure of closeness of the data values to each other for a number of measurements under the same analytical conditions. Repeatability of the method was assessed by six determinations for the concentrations representing 50%, 100% and 150% respectively. The standard deviation and the relative standard deviation (RSD %) were reported for precision.

Precision studies were carried out in terms of repeatability. Repeatability of standard application was carried out by injecting six replicates of the mixed standard at concentrations of 12.55/12.50, 25.10/25.0, 37.64/37.50µg/ml of PGZ and MET (Table 3). The RSD (%) was found to be below 2 for peak areas of PGZ and MET; this shows the closeness of the data values to each other, indicating the precision of the method.
Accuracy

Accuracy was established across the specified range of the analytical procedure. To ascertain the accuracy of the proposed method, recovery studies were performed. The percentage recovery and RSD % were calculated for both drugs.

Accuracy of the developed method was indicated by studying recovery with three different concentrations (50%, 100%, 150%) by standard addition technique and these solutions were analyzed in triplicate in each level of addition. The results of percentage recovery were found to be 93.98-96.73 for PGZ and 96.4-98.72 for MET and the RSD (%) were within the acceptable limits in all cases. It is evident from the results of accuracy study given in Table 3, that the proposed method enables very accurate quantitative simultaneous estimation of PGZ and MET in tablet dosage form.

Table 3: Linearity, Precision and Accuracy data of the two drugs.

<table>
<thead>
<tr>
<th></th>
<th>Validation data of PGZ and MET</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PGZ</strong></td>
<td><strong>MET</strong></td>
</tr>
<tr>
<td><strong>Linearity</strong></td>
<td>Range 5.02-50.19 µg/ml</td>
</tr>
<tr>
<td></td>
<td>Y = 35419x+30960</td>
</tr>
<tr>
<td></td>
<td>R² = 0.9991</td>
</tr>
<tr>
<td><strong>Average peak area of the standard sample (%RSD)</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Precision</strong></td>
<td>881918.67 (1.51)</td>
</tr>
<tr>
<td><strong>Accuracy</strong></td>
<td>Mean percentage recovery (%RSD)</td>
</tr>
<tr>
<td>50%</td>
<td>92.75 (0.7)</td>
</tr>
<tr>
<td>100%</td>
<td>95.72 (1.58)</td>
</tr>
<tr>
<td>150%</td>
<td>99.46 (0.53)</td>
</tr>
</tbody>
</table>

Limit of Detection and Limit of Quantification

Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated based on calibration curves. They were expressed as LOD = (3.3× σ)/S; LOQ = (10× σ)/S (where σ is the standard deviation of the y-intercepts of the regression line and m is mean of the slope of the calibration curve).

LOD and LOQ were determined from the average slope and standard deviation of y-intercept from the calibration curve. The LOD for PGZ and MET were found to be 0.00554 and 0.00927µg/ml, respectively. The LOQ for PGZ and MET were found to be 0.01678 and 0.0281µg/ml, respectively.
**Robustness and ruggedness**

To determine the robustness of the method developed, the experimental conditions were deliberately altered and the chromatographic parameters, viz., tailing factor, number of theoretical plates and percentage assay were recorded. The flow rate of the mobile phase was 1 ml/min. To study the effect of flow rate (± 0.1 ml) and the effect of mobile phase variation (± 5 %) was evaluated.

Method robustness was determined by analyzing the same sample at normal operating condition and also by changing the operating analytical conditions like flow rate variation, mobile phase composition. Percentage assay values were also estimated under these changed conditions, the results given in Table 4. Changes in the flow rate slightly affected the retention times of the PGZ and MET. However, the parameters like theoretical plate number, tailing factor and were within the limits. Similar results were obtained with the changed mobile phase ratio within the acceptable range. These results indicated that the method is robust in terms of changed flow rate and mobile phase ratio.

Ruggedness is the degree of reproducibility of test results under normal operational conditions such as laboratory to laboratory and analyst to analyst. The ruggedness of the assay was studied by analysis of the same sample in triplicate under a variety of test conditions such as different days, analysts and instruments.

Method ruggedness was determined by analyzing the same sample in the triplicate under a variety of test conditions like different analysts and results obtained were shown in Table 4.

**Table 4: Robustness and Ruggedness data for PGZ and MET.**

<table>
<thead>
<tr>
<th>Chromatographic parameters</th>
<th>Retention time (min)</th>
<th>Peak area</th>
<th>Theoretical plates (♯)</th>
<th>Tailing factor (Tf)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PGZ</td>
<td>MET</td>
<td>PGZ</td>
<td>MET</td>
</tr>
<tr>
<td>Flow rate (ml/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.9</td>
<td>4.89</td>
<td>1.8</td>
<td>966649</td>
<td>2803816.67</td>
</tr>
<tr>
<td>1.1</td>
<td>3.82</td>
<td>1.44</td>
<td>827338.67</td>
<td>2170227</td>
</tr>
<tr>
<td>Mobile phase (v/v)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40:60</td>
<td>6.33</td>
<td>1.60</td>
<td>856500</td>
<td>2353025.67</td>
</tr>
<tr>
<td>50:50</td>
<td>3.13</td>
<td>1.59</td>
<td>912001.33</td>
<td>2406275.67</td>
</tr>
<tr>
<td>Analyst variation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Analyst-1</td>
<td>3.79</td>
<td>1.61</td>
<td>878150</td>
<td>2409516</td>
</tr>
<tr>
<td>Analyst-2</td>
<td>3.81</td>
<td>1.62</td>
<td>881918</td>
<td>2330743</td>
</tr>
</tbody>
</table>
Stress testing Studies

Generation of Stressed Samples

Stability indicating studies of both drugs were carried out under mild stress conditions of hydrolysis (acidic, basic), oxidation and photolytic conditions.

Acidic and Basic Hydrolysis: Hydrolytic degradation studies were carried out in acidic (0.1% HCl) and basic (0.1% NaOH) media. Stock solutions of PGZ and MET were prepared and kept at room temperature for 1 day and analyzed after suitable dilution.

Oxidative Degradation: Oxidative degradation studies were carried out in 3% (v/v) H2O2. Stock solutions of PGZ and MET were prepared and were kept at room temperature for 1 day and analyzed after suitable dilution.

Photolytic Degradation: For the photolytic degradation was carried out in UV- radiation (overall illumination of ≥ 210 Wh/m² at room temperature with UV radiation). Stock solutions of PGZ and MET were taken and the prepared solutions were exposed to UV light for 6 hours.

For the HPLC analysis, all the degraded sample solutions of PGZ and MET were diluted with diluent and injected individually and analyzed under the same chromatographic analysis conditions.

Intentional degradation was attempted to various stress conditions such as acid hydrolysis (using 0.1% HCl), base hydrolysis (using 0.1% NaOH), oxidative hydrolysis (using 3% H2O2) and photolytic degradation (overall illumination of ≥ 210 Wh/m² at room temperature with UV radiation), to evaluate the ability of the proposed method to separate PGZ and MET from their degradation products. Under alkaline hydrolysis conditions, the peak of MET and PGZ were eluted at 1.95 min and 4.2 min was obtained respectively. The percentage remained were found to be 96.83 and 96.09 for PGZ and MET respectively. The percentage degradation were found to be 3.17 and 3.91 for PGZ and MET respectively. The results are shown in Figure 6 (a,b) and given in Table 5. Under acid hydrolysis conditions, degradation peak of MET and PGZ were eluted at 1.6 min and 4.1 min was obtained respectively. The percentage remained were found to be 92.18 and 93.61 for PGZ and MET respectively. The percentage degradation were found to be 7.82 and 6.39 for PGZ and MET respectively. The results are shown in Figure 6 (c,d) and given in Table 5. Under oxidative stress conditions,
degradation peak of MET and PGZ were eluted at 1.59 min and 4.05 min was obtained respectively. The percentage remained were found to be 94.8 and 98.6 for PGZ and MET respectively. The percentage degradation were found to be 5.2 and 1.4 for PGZ and MET respectively. The results are shown in Figure 6 (e,f) and given in Table 5. Under photolytic stress conditions, degradation peak of MET and PGZ were eluted at 1.63 min and 4.06 min was obtained respectively. The percentage remained were found to be 95.31 and 99.38 for PGZ and MET respectively. The percentage degradation were found to be 4.96 and 0.62 for PGZ and MET respectively. The results are shown in Figure 6 (g,h) and given in Table 5.

Table 5: Forced degradation study data for PGZ and MET

<table>
<thead>
<tr>
<th>Degradation condition</th>
<th>Peak Area</th>
<th>Peak area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PGZ</td>
<td>MET</td>
</tr>
<tr>
<td>Acidic hydrolysis (0.1% HCl)</td>
<td>999586.5</td>
<td>2555182.5</td>
</tr>
<tr>
<td>Basic hydrolysis (0.1% NaOH)</td>
<td>956481.5</td>
<td>2495518</td>
</tr>
<tr>
<td>Oxidation (3% H₂O₂)</td>
<td>975264.5</td>
<td>2368147.5</td>
</tr>
<tr>
<td>Photolytic degradation (UV light)</td>
<td>970563</td>
<td>2386851</td>
</tr>
<tr>
<td>Control sample</td>
<td>927067.5</td>
<td>2401726.5</td>
</tr>
</tbody>
</table>

Fig 6. HPLC chromatograms for stressed samples. (a) PGZ in 0.1N NaOH at 25°C after 24 hr, (b) MET in 0.1N NaOH at 25°C after 24 hr, (c) PGZ in 0.1N HCl at 25°C after 24 hr, (d) MET in 0.1N HCl at 25°C after 24 hr, (e) PGZ in 3% H₂O₂ at25°C after 24 hr, (f) MET in 3% H₂O₂ at25°C after 24 hr, (g) PGZ in UV light (210 nm) after 24 hr, (h) MET in UV light (210 nm) after 24 hr.
Assay of Pioglit Mf 15® Tablets

Accordingly, twenty tablets of Pioglit Mf 15® were weighed separately and the average weight of each tablet was calculated. The tablets were then finely powdered in a mortar and pestle. The assay of MET and PGZ was carried out separately, tablet powder equivalent to one tablet weight containing approximately 15 mg of PGZ and 500 mg of MET as per label claim was accurately weighed and transferred into 100 ml volumetric flask and dissolved in 90% aqueous acetonitrile and vortexed for 5 min and volume was adjusted up to the mark with 90% aqueous acetonitrile to get of 0.15 mg/ml PGZ and 5 mg/ml of MET (solution-1). 0.1 ml of this solution-1 was taken in 20 ml volumetric flask and made up to the volume with diluents (solution-2). This solution is injected for assay of MET. For the assay of PGZ, 2 ml of solution-1 was taken in a 25 ml volumetric flask and made up to volume to get Solution 3. This solution is injected for assay of PGZ.

Assay of PGZ and MET combination formulation was performed by the proposed method and the percentage assay of the formulation was calculated as an average of three determinations, which was about 95.75±0.5 for PGZ and 97.32±1.00 for MET. These results indicate that the present HPLC method can be successfully used for the analysis of PGZ and MET in bulk and dosage forms.

CONCLUSION

In this work, a simple, efficient, LC-MS compatible and stability-indicating RP-HPLC method has been developed for the simultaneous determination of PGZ and MET from bulk and tablet dosage form. The method was validated fully as per ICH guidelines and validation acceptance criteria were met in all cases. Application of this method for simultaneous determination of PGZ and MET from tablet dosage form showed that neither the degradation products nor the excipients interfered in the estimation of both the drugs; therefore this method was specific and stability indicated and can be employed successfully for the simultaneous estimation of PGZ and MET in commercial tablet dosage form.

ACKNOWLEDGEMENT

The authors are thankful to Mr. Santosh Tata, Corpuscle Research Solutions, Visakhapatnam, India, for providing samples, and also providing the necessary facilities to carry out this research work.
REFERENCES


