DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF METFORMIN HCl AND GLICLAZIDE IN DOSAGE FORMS

Pravalika Ammineni*, Ramalingam Peraman, Sasikala Mallikarjuna, Padmanabha Reddy Yeragamreddy

Division of Pharmaceutical Analysis and Quality Assurance, Raghavendra Institute of Pharmaceutical Education and Research, Anantapur, Andra pradesh, India -515 721

ABSTRACT

A new stability-indicating high performance liquid chromatographic method was developed for simultaneous analysis of Metformin hydrochloride (MET) and Gliclazide (GLI) in pharmaceutical dosage forms. Chromatographic separation was performed on C8 column (Qualisil BDS 250 mm x 4.6 mm, 5µm) with 15:15:70 (v/v) mixture of Methanol, Acetonitrile and water containing 0.2 % v/v n-heptanesulfonicacid, 0.2 % v/v Triethyl amine and the pH was adjusted to 6.4 with ortho phosphoric acid, as mobile phase. The flow rate was 1mL/minute and the photo diode array detection wavelength was 227 nm. The linear regression coefficient for Metformin and Gliclazide were 0.9993 and 0.9990 in the concentration range of 48-192 µg/mL, and 8-32 µg/mL, respectively. The relative standard deviations for intra and interday precision were lower than 1.5%. Drugs were subjected to various stress conditions of acidic and basic hydrolysis, oxidative, photolytic, neutral and thermal degradation. Chromatographic peak purity data of Metformin and Gliclazide indicated no co-eluting peaks with the main peaks. The method revealed 14 degradants, out of which gliclazide was found to more sensitive for all stress conditions whilst metformin produced more degradants in basic conditions. 

Key words: Metformin, Gliclazide, simultaneous, stability-indicating assay, RP-HPLC.

INTRODUCTION

The International Conference on Harmonization (ICH) guidelines Q1AR2 require the use of a validated stability-indicating assay method (SIAM) for stability testing of a drug substance or product. It also emphasizes on the conduct of a forced degradation study on the drug substance to generate information on degradation products that can form under the influence of hydrolytic, oxidative, dry heat and photolytic conditions. Gliclazide is an antidiabetic agent used as a single dose or with metformin hydrochloride in patients with Type 2 diabetes mellitus and it acts by stimulating the pancreatic b-cells to secrete insulin. Chemically, it is 1-(3-azabicyclo[3.3.0]oct-3-yl)-3-(p-tolylsulfonyl) urea Figure 1 (a). Metformin hydrochloride (MET) is the drug of choice for the treatment of type II diabetes, particularly in overweight, obese people and individuals with normal
kidney function. Chemically, it is 3-(diaminomethylidene)-1,1-dimethylguanidine hydrochloride is an antidiabetic agent Figure 1(b). In combination these are available in 80/500 mg and 60/500 mg of GLI and MET, respectively.

MET is official in IP, BP and USPNF where as GLI is official in BP and USPNF. The literature reveals that there are various methods like UV¹, HPTLC²,³, HPLC⁴-¹¹ and ion-pair HPLC¹² method have been reported for the estimation of MET and Gliclazide alone, along with other drugs in various dosage forms. As far as we are aware, few stability indicating RP-HPLC¹³,¹⁴ method is reported for GLI alone and in combination of MET with Repaglinide. To date, no simultaneous stability assay method available for MET and GLI, the main objective of this present work is to develop and validate a stability indicating reverse phase high performance liquid chromatographic method for the simultaneous estimation of GLI and MET that could demonstrate all possible impurities in combined dosage forms. Advantages of simultaneous stability studies are, to demonstrate new impurities apart from impurities occurred in stability assay of MET/ GLI alone, to understand the mutual induction or /and inhibition in the rate of degradation and to identify common impurities of both the drugs in combined dosage forms.

Figure 1(a): Chemical structure of Gliclazide (GLI)

Figure 1(b): Chemical structure of Metformin (MET)
EXPERIMENTAL

Reagents and Chemicals
Samples of MET and GLI were obtained as gift sample from HeteroLabs, Hyderabad Pvt. Ltd (India). Tablet formulation of Gliclazide and Metformin hydrochloride (GLICLAMET tablet) were procured from commercial market. All the solutions were protected from light and were analyzed on the day of preparations. Glass wares used in each procedure were soaked overnight in a mixture of chromic acid and sulphuric acid rinsed thoroughly with double distilled water and dried in hot air oven. Water and Methanol, Acetonitrile (HPLC grade) were purchased from Merck, India.

HPLC Instrumentation and Chromatographic Conditions
HPLC analysis for method development, forced degradation studies, and method validation was performed with a Agilent-1200 binary pump plus manual sampler and a Agilent photo diode-array detector (PDA). The output signal was monitored and processed using Ezchrome elite software resident in a Pentium computer (Digital Equipment). Compounds were separated on a 250 mm-4.6 mm, 5µm particle, Agilent C₈ column with Methanol, Acetonitrile and water (15:15:70, v/v) with 0.2 (%w/v) n-heptanesulfonic acid (HSA), 0.2%(v/v) Triethyl amine (TEA) and pH was adjusted to 6.4 with ortho phosphoric acid (OPA) as mobile phase. The injection volume was 20 µL, the mobile phase flow rate was 1.0 mL. min⁻¹ and the detection wavelength was 227 nm.

Forced Degradation Studies
Forced degradation of MET and GLI drug substance was carried out under neutral, acidic, basic, oxidative, thermal and photolytic stress conditions. In stress study, aliquots of stress sample were diluted with mobile phase and achieved a concentration of 100 µg/mL. pH of stress sample was adjusted to 3-4 and injected in the optimized condition with appropriate blank. The samples from acid hydrolysis were neutralized with 0.1N NaOH and the samples from base hydrolysis were neutralized with 0.1N HCl. Blank solutions for each hydrolysis were prepared at the same time of preparation of stock solutions.

Preparation of Stock Solution for stress studies
An accurately weighed quantity of 10 mg of each drug substance was carefully transferred in to 10 mL volumetric flask, dissolved completely in water and the volume
was made up to the mark to get 1000 μg mL⁻¹. The same procedure was used to prepare stress solutions of acid hydrolysis, base hydrolysis and oxidation respectively with 0.1N HCl, 0.1N NaOH and 0.3% H₂O₂. Thermal degradation was carried out for solid state by means of heating the samples over a period in hot air oven, at 105 ºC. Photo degradation studies were conducted by exposing the solution sample in sunlight. At various time intervals, 10 mg of the samples were weighed, suitably dissolved and diluted with mobile phase to get a concentration of 100 μg/mL and injected into the system. In all stress studies, stress was carried out for both MET and GLI alone (control) as well as in combined form. Result of degradation studies for MET and GLI alone was compared with degradation profile of combined studies.

RESULTS AND DISCUSSION

Method Development and Optimization of the chromatographic conditions

The objectives of the present chromatographic method were to separate both MET and GLI from their all possible degradants and to elute them as symmetrical peak. Agilent Qualisil BDS column C₈ (250mm×4.6mm, 5μm) was used as stationery phase. The flow rate was 1 mL/minute and the photo diode array detection wavelength was 227 nm. Various trials with methanol and water as mobile phase (80 : 20 v/v to 50 : 50 v/v) were performed, MET was not retained and shoulder for GLI was observed in between 5-20 min with tailing factor more than 2.5. Use of Methanol, Acetonitrile and water (15:15:70 v/v) as mobile phase and heptane sulfonic acid (HSA) at 0.2% in aqueous part of mobile phase retained MET but tailing was observed for both the peaks. pH changes have drastic effect on retention time and peak shape. It was found that use of Triethyl amine (TEA) between 0.1 – 0.25% in aqueous adjust the pH to 6.4 with ortho phosphoric acid (OPA) reduced the tailing and enabled separation of MET and GLI with adequate resolution, theoretical plate and retention for stability indicating assay method. 0.2% of TEA and HSA was used in optimized condition. The retention time of MET and GLI was 5.00 ±0.35 min and 16.06 ± 0.3 min, respectively. The optimized chromatogram was shown in Figure 2. The method has proven specificity by separating the degradants in various stress conditions. It was observed that fourteen major degradants were formed with retention times of 2.48min (D₁), 2.8±0.1 min (D₂), 3.2 (D₃), 3.5±0.16 min (D₄), 4.05±0.05 min (D₅), 6.7 min (D₆), 8.02 min (D₇), 8.94 min (D₈), 9.12±0.06 min (D₉),
10.6±0.2 min (D10), 12.04±0.6 min (D11), 13.96 min (D12), 14.09 min (D13), 20.2±0.1 min (D14). The degradants formed in different stress conditions were shown in Fig 3-8.

Figure 2. Optimised RP-HPLC Chromatogram of MET (Rt : 5.0) and GLI (Rt: 16.0) on C8 Column

Validation of the method

Method was validated as per ICH (Q2) guidelines with respect to specificity, linearity and range, accuracy, precision, robustness, limit of detection and limit of quantification.

Specificity

Forced degradation studies were performed on MET and GLI to support the specificity of the stability-indicating method. The study was employed on degradation of MET and GLI by exposing to sun light (for 48 hr), heat (105°C for 6 hr), acid hydrolysis (0.1 N HCl, kept at RT for 48 hr), base hydrolysis (0.1 N NaOH, kept at RT for 72 hr), water hydrolysis (kept at RT for 48 hr) and oxidation (0.3% H2O2, kept at RT for 60hr). All degradants adequately separated from MET and GLI, thus the specificity of the method was proven.

Linearity and range

The linearity of detector response to different concentrations of MET and GLI was studied in the range from 48-192 μg/mL and 8-32 μg/mL, respectively. Samples were analyzed in triplicate at seven different concentrations. The correlation coefficient (r² value) obtained was 0.9993 for MET and 0.9990 for GLI.
Accuracy

Accuracy was performed by recovery studies using standard addition method. Standard drugs in the range of 80, 100 and 120% of the sample concentration were added into the sample solution as given in the Table 1. Each concentration was analyzed in triplicate. Results of recovery studies were found to be in between 98.87% to 101.40% for MET and 99.81% to 101.15% for GLI.

**TABLE 1: RESULTS OF ACCURACY**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Amount (μg/mL)</th>
<th>Recovery Level</th>
<th>Amount added (μg/mL)</th>
<th>Amount recovered (μg/mL) (Mean±SD)</th>
<th>% Recovery (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MET</td>
<td>84</td>
<td>80 %</td>
<td>151.2</td>
<td>149.86 ±0.782</td>
<td>99.12</td>
</tr>
<tr>
<td></td>
<td>84</td>
<td>100 %</td>
<td>168</td>
<td>170.35±1.150</td>
<td>101.40</td>
</tr>
<tr>
<td></td>
<td>84</td>
<td>120 %</td>
<td>184.8</td>
<td>182.71±0.607</td>
<td>98.87</td>
</tr>
<tr>
<td>GLI</td>
<td>14</td>
<td>80 %</td>
<td>25.2</td>
<td>25.28±0.060</td>
<td>100.32</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>100 %</td>
<td>28</td>
<td>27.94±0.158</td>
<td>99.81</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>120 %</td>
<td>30.8</td>
<td>31.15±0.500</td>
<td>101.15</td>
</tr>
</tbody>
</table>

Precision

Data for intraday and interday precision studies were obtained from three different concentrations (72, 120, 192 μg/mL for MET and 12, 20, 32 μg/mL for GLI) in the linearity range. The % RSD values for intraday and interday precision were below 1.5 %, indicating that the method was sufficiently precise and the result is shown in Table 2.

**TABLE 2: RESULTS OF PRECISION STUDIES.**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Amount (μg/mL)</th>
<th>Interday (n=3)</th>
<th>Intraday (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Amount found Mean ±SD</td>
<td>% RSD</td>
</tr>
<tr>
<td>MET</td>
<td>72</td>
<td>71.20±0.71</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>120.10±0.73</td>
<td>0.61</td>
</tr>
<tr>
<td></td>
<td>192</td>
<td>192.25±2.51</td>
<td>1.30</td>
</tr>
<tr>
<td>GLI</td>
<td>12</td>
<td>11.98±0.15</td>
<td>1.25</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>19.54±0.23</td>
<td>1.17</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>32.06±0.35</td>
<td>1.09</td>
</tr>
</tbody>
</table>

Robustness

The robustness of the developed method was determined by analyzing the samples under a variety of conditions of the method parameters, such as change in flow rate (± 0.1 mL/minute), Wavelength (±2 nm) and Organic phase (±2 %). The method was robust for all the parameters tested.
Limit of detection and limit of quantification

LOD and LOQ were determined based on signal to noise ratio. The S/N ratio of 3:1 was taken as LOD and S/N of 10:1 was taken as LOQ. LOD was found to be 0.216 µg/mL, 0.422 µg/mL while LOQ was 0.654 µg/mL , 1.281 µg/mL for MET and GLI, respectively.

Analysis of Metformin and Gliclazide in tablet dosage form

Twenty tablets of marketed formulation Gliclamet tablet containing MET 500 mg and GLI 80 mg were weighed, and finely powdered. Tablet powder equivalent to 100 mg MET with relevant quantities of GLI was weighed and transferred to a 100 mL volumetric flask, extracted for 30mins with water and volume was made up to 100 mL with diluent. 0.8 ml of above solution was taken in 10 mL volumetric flask and volume was made up to 10 mL with mobile phase, and final solution was filtered through 0.45 µ syringe filter and it was analyzed. The results of the assay were shown in Table 3.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Drugs</th>
<th>Labelled Claim (in mg)</th>
<th>Amount Found (Mean ±SD)</th>
<th>Assay (in %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gliclamet</td>
<td>MET</td>
<td>500</td>
<td>501.34±1.75</td>
<td>100.26</td>
</tr>
<tr>
<td></td>
<td>GLI</td>
<td>80</td>
<td>79.04±0.97</td>
<td>98.80</td>
</tr>
</tbody>
</table>

Forced Degradation

Degradation behavior of MET as well as GLI was studied individually and in combination. Different stress conditions were employed as stated in experimental section. The percentage degradation and number of degradants formed in various stress conditions was listed in the Table 4. GLI was more labile than MET in all stress conditions except basic stress. In the presence of 0.1N HCl (48 h, RT) MET alone as well as in combination was not found to be degraded, whereas GLI was degraded almost to same extent and the amount of degradants in control as well as in combined product was same. In basic stress (0.1N NaOH) MET was found to be degraded to higher extent than GLI in both control and combined stress. In case of Oxidative stress studies a total 6 impurities formed for GLI out of which four degradants were observed as new degradation product (3.5min (D4), 12min (D11).13.9 min (D12) and 20.2min (D14)) which were not formed in control. Degradants occurred in photolytic is similar to that of neutral hydrolytic products, but the rate of degradation was high and % degradation was
found to 11% for GLI (control) and 13% for GLI in presence of MET. Interestingly it was noted that a photolytic impurity was detected for MET in presence of GLI but not in control. The photolytic impurity of at 3.1 min may possess oxidative nature.

**TABLE 4: COMPARATIVE STUDY OF STABILITY DATA OF MET AND GLI:**

<table>
<thead>
<tr>
<th>Conditions (Duration)</th>
<th>% degradation</th>
<th>No. of Impurities (D x) (x = 1-14)</th>
<th>Alone</th>
<th>Combined</th>
<th>Alone</th>
<th>Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>MET</td>
<td>GLI</td>
<td>MET</td>
<td>GLI</td>
</tr>
<tr>
<td>Water (48 hr)</td>
<td>-- 3.5</td>
<td>-- D4, D9, D10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1N HCl (48 hr)</td>
<td>-- 24.6</td>
<td>-- D4, D8, D10</td>
<td></td>
<td></td>
<td>D2</td>
<td>D4, D9, D10, D12</td>
</tr>
<tr>
<td>0.1N NaOH (72 hr)</td>
<td>12.8 3.5</td>
<td>D2, D3, D8, D10</td>
<td></td>
<td></td>
<td>D1, D2, D8</td>
<td></td>
</tr>
<tr>
<td>0.3% H2O2 (60 hr)</td>
<td>1.42 5.5</td>
<td>D1 D9, D10</td>
<td></td>
<td></td>
<td>D1</td>
<td>D4, D9, D10, D11, D12, D14</td>
</tr>
<tr>
<td>Thermal 105ºC (6 hr)</td>
<td>2.35 18.2</td>
<td>D3, D5</td>
<td>D4, D7, D9, D13, D14</td>
<td></td>
<td>D3, D5</td>
<td>D4, D7, D9, D11, D13, D14</td>
</tr>
<tr>
<td>Photolytic (48 hr)</td>
<td>-- 11.7</td>
<td>-- D4, D8</td>
<td></td>
<td></td>
<td>D3</td>
<td>D4, D6, D9, D10</td>
</tr>
</tbody>
</table>

The peak purity was checked with PDA detector. The drug peaks were well resolved from the degrading peaks and the peak purity factor was within the threshold limit for all stressed samples indicative of the homogeneity of the analyte peaks. The study was not intended to identify degradation products but merely to show they would not interfere if and when present. To conclude, the results of stress testing studies indicate a high degree of specificity of this method for both MET and GLI.
Figure 3. Neutral degradation (in Water for 48hr) chromatogram of MET and GLI

Figure 4. Acid degradation (0.1N HCl for 48 hr) chromatogram of MET and GLI
Figure 5. Base degradation (0.1N NaOH for 72 hr) chromatogram of MET and GLI

Figure 6. Oxidative degradation (0.3 % H₂O₂ for 60hr) Chromatogram of MET and GLI
Figure 7. Thermal degradation (Solid - 105º C for 6 hr) chromatogram of MET and GLI

Figure 8. Photolytic degradation (In Sun light for 48hr) chromatogram of MET and GLI

REFERENCES

1. Ketan PD, Parthika A and Yadendra KA: Development and Validation of Spectrophotometric Method for Simultaneous Estimation of Gliclazide and Metformin Hydrochloride in Bulk and Tablet Dosage form by Simultaneous


9. Pawar S, Meshram GA, Phadke M and Jadhav RK: Simultaneous High Performance Liquid Chromatographic Determination of Gliclazide and


For Correspondence:
Pravalika ammineni
Email: pravallikaammineni@gmail.com