Simultaneous Estimation of Metformin Hydrochloride and Gliclazide in Bulk and Tablet Dosage form by RP-HPLC Method

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Abstract— A precise, accurate , rapid, selective, and economic reversed phase high-performance liquid chromatography (RP-HPLC) method has been established for simultaneous analysis of Metformin hydrochloride and Gliclazide in Bulk and Pharmaceutical dosage form on a Phenomenex C18 (250x4.6mm i.d) chromatographic column equilibrated with mobile phase containing acetonitrile & Mixture of Phosphate buffer (2.954g of potassium dihydrogen orthophosphate and 0.545g of dipotassium hydrogen orthophosphate in 1000 ml water). Experimental conditions such as pH of mobile phase, organic phase ratio, flow rate, selection of wavelength, etc. were critically studied and the optimum conditions were selected. Efficient chromatographic separation was achieved with mobile phase containing combination of acetonitrile & phosphate buffer pH 4.7 in ratio of 75:25 v/v and mixture was adjusted to pH 4.7 at flow rate of 1.0 ml/min and eluent was monitored at 240 nm. The sample was injected using a 20 μl fixed loop, and the total run time was 6 min. The retention time for Metformin and Gliclazide were 3.380 min and 4.137 min respectively. The method was linear in the range of 5-15 μg/ml and 0.8-2.4 μg/ml for Metformin and Gliclazide respectively. The proposed method was successfully applied to the analysis of Metformin & Gliclazide in their Bulk and Pharmaceutical dosage form without interference from other additives. As per ICH guidelines the developed method was validated. Linearity, regression value, recovery, % RSD of method precision, LOD, LOQ values were found within the limits and the method was found to be satisfactory. This validated HPLC procedure is economic, sensitive, user-friendly & less time consuming than other chromatographic procedures.

Keywords-component; RP-HPLC, Metformin, Gliclazide, and validation

I. INTRODUCTION

Metformin drug (molecular formula c4h11n5) is 1-carbamimidamidono-n,ndimethylmethanimidamide meformin is that the compound belongs to the category of organic compounds called biguanides. These ar organic compounds containing 2 n-linked guanidines. Antidiabetic drug may be a biguanide antihyperglycemic agent .it is used for treating niddm mellitus (niddm). It improves glycemic management by decreasing vicus aldohexose production, decreasing aldohexose absorption and increasing insulin-mediated aldohexose uptake. Antidiabetic drug is that the solely oral antihyperglycemic agent.. For weighty ketosis-resistant diabetes mellitus patients metformin may be a drug of alternative . Once used alone, antidiabetic drug doesn't cause hypoglycemia; but, it's going to raise the symptom effects of sulfonylureas and hormone. Its main aspect effects ar indigestion, nausea and looseness of the bowels.

Gliclazide: 
Gliclazide (molecular formula c15h21n3o3s) Gliclazide: may be a opposing hyperglycemic agent that is much insoluble in water; slightly soluble in alcohol; meagerly soluble in dissolve ; freely soluble in dichloro alkane series. Gliclazide is instantly absorbed from the dirty dog. It's extensively certain to plasma proteins and includes a 0.5 – lifetime of or so ten to twelve hours. Gliclazide is extensively metabolized within the liver to metabolites that don't have any vital symptom activity. Metabolites and atiny low quantity of unchanged drug ar excreted within the excreta. Its temperature is 180-182 °c and physiological condition, severe urinary organ or viscus failure, severe acetonemia, acidosis, diabetic precoma and coma.

II. Instruments and chemicals

A. Chemicals agents

The chemicals agents used for the for concurrent analysis of antidiabetic drug complex and gliclazide in bulk and pharmaceutical indefinite quantity type ar hplc grade k dihydrogen phosphate , phosphoric acid , acetointrile , dipotassium atomic number 1 phosphate , hplc grade water , operating customary used ar antidiabetic drug complex with the efficiency ninety nine.6 and gliclazide with efficiency ninety nine.8 .

B. Equipments

The equipments concerned for concurrent analysis of antidiabetic drug complex and gliclazide in bulk and pharmaceutical indefinite quantity type ar agilent model hplc of agilent company , sendero luminoso 159 model uv- spectrum analysis of elico company , 11615 model hydrogen ion concentration meter of elico company , ax two hundred model balance of shimadzu company and sonicator of pci company , vacuum filter of promivac company.
### III. Method Development

#### A. Preparation of Standard and Sample Solutions

Several trials were performed for selection of column and mobile phase pH for the development of reliable method for simultaneous estimation of Metformin hydrochloride and Gliclazide.

Preparation of buffer:

Accurately 2.954g of potassium dihydrogen orthophosphate and 0.545g of dipotassium hydrogen orthophosphate were weighed and transferred into a 1000 ml volumetric flask and dissolved in small amount of water (hplc grade) and volume was made up to 1000 ml with hplc grade water. The pH of the buffer was adjusted to 4.7 using dilute orthophosphoric acid.

Preparation of Standard stock solution of Metformin and Gliclazide:

- Accurately weighed and transferred 10 mg of Metformin hydrochloride and 1.6 mg of Gliclazide standards into a 10 ml clean dry volumetric flask and added 3/4th volume of diluent, sonicated for 5 min and make up to the final volume with diluent.(5ml of water and 5ml of acetonitrile is used as diluent).

- Preparation of working standard solution of 10 µg Metformin and 1.6 µg Gliclazide:

  - Working standards solution of Metformin hydrochloride and Gliclazide was prepared by taking 0.1 ml from the above stock solution into a 10 ml volumetric flask and made up to 10 ml with diluent.(mobile phase used as diluent).

Preparation of Mobile phase:

Prepared a mixture of acetonitrile and Phosphate buffer in the ratio of 60: 40% v/v and pH of mobile phase was adjusted to 4.8 using dilute orthophosphoric acid.

Diluent: Acetonitrile: phosphate buffer pH 4.7 (75:25% v/v), and mixture was adjusted to pH 4.7 using dilute orthophosphoric acid was used as diluents

#### B. Preparation of Standard stock solution of Metformin and Gliclazide:

- Preparation of working standard solution of 10 µg Metformin and 1.6 µg Gliclazide:

- Working standards solution of Metformin hydrochloride and Gliclazide was prepared by taking 0.1 ml from the above stock solution into a 10 ml volumetric flask and made up to 10 ml with diluent.(mobile phase used as diluent).

Preparation of Mobile phase:

Prepared a mixture of acetonitrile and Phosphate buffer in the ratio of 60: 40% v/v and pH of mobile phase was adjusted to 4.8 using dilute orthophosphoric acid.

- Diluent: Acetonitrile: phosphate buffer pH 4.7 (75:25% v/v), and mixture was adjusted to pH 4.7 using dilute orthophosphoric acid was used as diluents

### C. Instrumentation and Chromatographic Conditions

The developed method HPLC system with UV detector data were acquired and processed by Elico. The separation was carried out at ambient temperature by using a (4.6 x 250mm, 5µm Phenomenex C18 (250x4.6 mm, 5.0 µm). The mobile phase consisting of acetonitrile and phosphate buffer in the ratio of 75:25 % v/v and ph of mobile phase was adjusted to 4.8 adjusted with orthophosphoric acid. The flow rate was 1 ml/min. The injection volume was 20µL and detection at a wavelength of 240nm.

### D. Sample Preparation

- Weighed 20 tablets and determined the average weight and crushed to fine powder. Weighed accurately tablet powder equivalent to 10mg of Metformin hydrochloride and 1.6 mg of Gliclazide, transferred into 10 ml volumetric flask, added 3ml of diluent, sonicated for 30 min in cold water and volume was made up to the mark with diluent and filtered. From the filtered solution 0.1 ml was pipetted out into a 10 ml volumetric flask and made up to 10 ml with diluent.

Assay for Marketed formulation

Injected 20 µl of filtered portion of the sample preparation and standard preparation into the chromatograph. The responses for the major peaks were recorded and the content of Metformin hydrochloride and Gliclazide per each tablet was calculated from the following expression.

- Calculation:

  \[
  \text{Assay} \% = \frac{\text{Spl area x Std dil x Avg wt x P}}{\text{Std area x Spl dil x L.C x 100}}
  \]

- Where,

  - Spl area = Sample area
  - Std area = Standard area
  - Std dil = Standard dilution
  - Spl dil = Sample dilution
  - Avg wt = Average weight of tablets
  - P = Percentage purity of working standard
  - L.C = Label claim

- Acceptance criteria

The percentage purity of Metformin hydrochloride & Gliclazide should be not less than 95 % and not more than 105 %.

### Table 1: Results of Standard & Test

<table>
<thead>
<tr>
<th>S. No</th>
<th>PEAK</th>
<th>METFORMIN</th>
<th>GLICLAZIDE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RT (min)</td>
<td>AREA (mAU)</td>
<td>% Purity</td>
</tr>
<tr>
<td>1</td>
<td>3.380</td>
<td>77738</td>
<td>99.6 %</td>
</tr>
<tr>
<td>2</td>
<td>3.377</td>
<td>77619</td>
<td>99.2 %</td>
</tr>
</tbody>
</table>

**Figure 1 Chromatogram of Metformin and Gliclazide**

**Figure 2 Chromatograms for Assay**
IV. Method validation / Results and discussion
Method validation was performed as per the ICH pointers. The developed technique was valid for the subsequent parameters.

- Linearity
- Range
- Accuracy
- Precision
- Limit of detection
- Limit of quantification
- Robustness

A Linearity:
The dimensionality of associate degree analytical technique was administered to visualize its ability to elicit take a look at results that area unit directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples inside a given vary. completely different levels of ordinary solutions were ready and injected into the HPLC and therefore the chromatograms were recorded.

Procedure:
Preparation of ordinary stock resolution (10 µg antidiabetic & one.6 µg Gliclazide)
Accurately weighed quantities of ten mg of antidiabetic coordination compound and one.6 mg of Gliclazide were transferred into ten cc clean dry volumetrical flask, and 3/4th volume of dilutant was additional, sonicated for half-hour and created up to the ultimate volume with dilutant. From the on top of stock resolution, one cc was pipetted go into to a 10ml volumetrical flask then created up to the ultimate volume with dilutant and used for any dilutions.

C Preparation of serial dilutions
Different levels of commonplace resolution were ready by pipetting out famous volume of stock resolution and created up to volumes with the dilutant to urge completely different analyte concentrations. on top of solutions were injected into the activity system (Injected higher levels 5 times for precision), the realm response for every level was recorded and therefore the slope, intercept, coefficient of correlation and parametric statistic (R2) were calculated. A graph of concentration (µg) on coordinate axis versus space on coordinate axis was premeditated.

Acceptance criteria : coefficient of correlation shouldn't be but zero.995.11

D. RANGE:
The vary of associate degree analytical procedure is that the interval between the higher and lower concentrations (amounts) of analyte within the sample (including these concentrations) that it's been incontestible that the analytical procedure includes a appropriate level of preciseness, accuracy and dimensionality.

The vary for antidiabetic and Gliclazide was found to be 5-15 µg/ml and zero.8-2.4 µg/ml severally.

(E)ACCURACY:
The accuracy of associate degree associate degree analytical technique is that the closeness of agreement between worth[the worth] that is accepted either as a standard true worth or an accepted reference worth and therefore the value found.

Accuracy was performed by following direct comparison technique. The study was performed by creating 3 completely different commonplace concentrations at five hundredth, 100% and one hundred and fiftieth levels of famous amounts of studied medicine. The accuracy of associate degree analytical technique ought to be established across its vary. Finally, the ultimate volume created up with dilutant (acetonitrile:phosphate buffer) and mixed well. The ensuing mixtures were analyzed by the planned HPLC technique at 240 nm. the superb mean recoveries and variance advised sensible accuracy results of the propose technique.

Preparation of ordinary stock resolution
Accurately weighed quantities of ten mg of antidiabetic coordination compound and one.6 mg of Gliclazide were transferred into ten cc clean dry volumetrical flask, and 3/4th volume of dilutant was additional, sonicated for half-hour and created up to the ultimate volume with dilutant. From the on top of stock resolution, one cc was pipetted go into to a 10ml volumetrical flask then created up to the ultimate volume with dilutant and used for any dilutions.

Preparation of sample stock resolution
Weighed twenty tablets and determined the typical weight and crushed to fine powder. Weighed accurately pill powder akin to ten mg of antidiabetic coordination compound and one.6 mg of Gliclazide transferred into ten cc volumetrical flask, 3ml of dilutant was additional and sonicated for fifteen min in cold water, created up with dilutant and filtered. From the filtered resolution one cc was pipetted out into [a ten] cc volumetrical flask and created up to 10 cc with dilutant and used for any dilutions.

Preparation of 50% stock resolution
Sample resolution akin to 5µg of antidiabetic coordination compound and zero.8µg Gliclazide was taken. to the present aliquots akin to a pair of.5 µg of antidiabetic coordination compound and zero.4µg of Gliclazide commonplace stock resolution was additional to urge five hundredth level resolution

Preparation of 100% Sample stock resolution
Sample resolution akin to 5µg of antidiabetic coordination compound and zero.8µg Gliclazide was taken. to the present aliquots akin to five.8µg of antidiabetic coordination compound and zero.8µg of Gliclazide commonplace stock resolution was additional to urge 100% level resolution

Preparation of a 100 and 50 % sample stock resolution
Sample resolution akin to 5µg of antidiabetic coordination compound and zero.8µg Gliclazide was taken. to the present aliquots akin to seven.5µg of antidiabetic coordination compound and one.2µg of Gliclazide commonplace stock resolution was additional to urge one hundred and fiftieth level resolution

Procedure:
Sample solutions ready were injected thrice into the activity system and recorded the chromatograms.

Acceptance criteria: The % of recovery ought to be between ninety five to a hundred and fifth
(F) PRECISION

The preciseness of associate degree analytical technique could be a live of the random error and is outlined because the agreement between replicate measurements of a similar sample. it's expressed because the proportion constant of variation (%CV) or relative variance (RSD) of the replicate measurements.

Method precision:

Intraday precision

Preparation of sample resolution

Weighed twenty tablets and determined the typical weight and crushed to fine powder. Weighed accurately pill powder akin to ten mg of antidiabetic and one.6 mg of Gliclazide, transferred into one hundred cc volumetric flask, thirty cc of diluant additional and sonicated for fifteen min in cold water, created up to needed volume with diluant and filtered. From the filtered resolution one cc was pipetted out into a ten cc volumetric flask and created up to 10 cc with diluant.

Procedure

For preciseness studies six replicate injections of antidiabetic and Gliclazide commonplace were performed. %RSD was resolute for peak areas of antidiabetic and Gliclazide.

Acceptance criteria

The % RSD for the realm of six commonplace injections results shouldn’t be a pair of.

Interday precision

Preparation of sample solution

Weighed 20 tablets and determined the average weight and crushed to fine powder. Weighed accurately tablet powder equivalent to 10 mg of Metformin and 1.6 mg of Gliclazide, transferred into 100 ml volumetric flask, 30 ml of diluant added and sonicated for 15 min in cold water, made up to required volume with diluant and filtered. From the filtered solution 1 ml was pipetted out into a 10 ml volumetric flask and made up to 10 ml with diluant.

Procedure

For inter day method precision studies 6 replicate injections of Metformin and Gliclazide sample were performed. %RSD was determined for peak areas of Metformin & Gliclazide.

Acceptance criteria

The % RSD for the area of six standard injections results should not be more than 2%.

G) Limit of Detection (LOD):

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated. The detection limit can be calculated based on the Standard Deviation of the Response and the Slope.

The parameter LOD was determined on the basis of response and slope of the regression equation. The Detection Limit (DL) may be expressed as:

\[ \text{LOD} = 3.3 \times \text{F/S} \]

where, F = Residual Standard deviation of the response, \( S = \text{Slope of the calibration curve.} \)

The LOD for this method was found to be 1.68 µg/ml and 0.28 µg/ml for Metformin hydrochloride and Gliclazide respectively

H) Limit of Quantification (LOQ):

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantification limit can be calculated based on the Standard Deviation of the Response and the Slope.

The parameter LOQ was determined on the basis of response and slope of the regression equation. The Quantitation Limit (QL) may be expressed as:

\[ \text{LOQ} = 10 \times \text{F/S} \]

where, F = Residual Standard deviation of the response, \( S = \text{Slope of the calibration curve.} \)

The LOQ for this method was found to be 4.95 µg/ml and 0.79 µg/ml for Metformin and Gliclazide respectively.

I) ROBUSTNESS:

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

The robustness of the proposed method was determined by analysis of aliquots from homogenous lots by differing physical parameters like flow rate, mobile phase pH and wavelength which may differ but the responses were still within the specified limits of the assay. The standard solution and sample solution were injected into the chromatograph at varied conditions of flow ± 20% ml/min, mobile phase buffer pH ± 0.5 units and wavelength by ± 5nm.

a) Effect of variation of flow rate

A study was conducted to determine the effect of variation in flow rate. Standard solution was prepared and injected into the HPLC system by keeping variation in flow rate ± 0.2ml. The effect of variation of flow rate was evaluated.

b) Effect of variation in the mobile phase pH

A study was conducted to determine the effect of variation in mobile phase pH. Standard solution was prepared and injected into the HPLC system by keeping variation in mobile phase pH. The effect of variation of mobile phase pH was evaluated.

c) Effect of variation of detection wavelength

A study was conducted to determine the effect of variation in detection wavelength. Standard solution was prepared and injected into the HPLC system by keeping variation in detection wavelength ± 5 nm. The effect of variation of detection wavelength was evaluated.

Table: 2 Linearity Data

Table 2.1 Linearity data of Metformin
<table>
<thead>
<tr>
<th>S.No</th>
<th>Concentration (µg/ml)</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>46403977</td>
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<tr>
<td>2</td>
<td>7.5</td>
<td>61615273</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
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<td>4</td>
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<tr>
<td>5</td>
<td>15</td>
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</table>

**Table 2.2 Linearity data for Gliclazide**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Concentration (µg/ml)</th>
<th>Area</th>
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<tr>
<td>5</td>
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</table>

**Table 3.1 The accuracy results for Gliclazide**

<table>
<thead>
<tr>
<th>%Concentration (at specification Level)</th>
<th>Sample Area Avg</th>
<th>Standard Area Avg</th>
<th>Amount Added (mg)</th>
<th>Amount Found (mg)</th>
<th>% Recovery</th>
<th>Mean Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 %</td>
<td>151 974 2</td>
<td>1615 539</td>
<td>1.2</td>
<td>1.21</td>
<td>100.8</td>
<td>98.5</td>
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<tr>
<td>100 %</td>
<td>211 592 6</td>
<td>2110 004</td>
<td>1.6</td>
<td>1.59</td>
<td>99.3</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3.4 The accuracy results for Metformin**

<table>
<thead>
<tr>
<th>%Concentration (at specification Level)</th>
<th>Sample Area Avg</th>
<th>Standard Area Avg</th>
<th>Amount Added (mg)</th>
<th>Amount Found (mg)</th>
<th>% Recovery</th>
<th>Mean Recovery</th>
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<tbody>
<tr>
<td>50 %</td>
<td>618 755 588</td>
<td>616 7.5</td>
<td>7.1</td>
<td>95.4%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>150 %</td>
<td>346 716 9</td>
<td>341 790 0</td>
<td>2.0</td>
<td>1.91</td>
<td>95.5</td>
<td></td>
</tr>
</tbody>
</table>
This developed and validated method for simultaneous analysis of Metformin and Gliclazide in pharmaceutical preparations is very rapid, accurate, and precise. The method was successfully applied for determination of MET and GLI in its Bulk and Pharmaceutical dosage form. The proposed method was simple and did not involve laborious time-consuming sample preparation. Moreover it has advantages of low costs of reagents used, short run time and the possibility of analysis of a large number of samples. Hence this method can be conveniently used for routine analysis and quality control of pharmaceutical preparations containing these drugs either as such or in combination.

REFERENCES


