6.1 Drug profile

6.1.1 Metformin Hydrochloride

Refer Chapter 2, Page No. 39
6.1.2 Glipizide [1-3]

a. Structure:

![Glipizide Structure Image]

b. Chemical name: 1 cyclohexyl –3- [[P-[2-(5- methylpyrazine-carboxamido ) ethyl ] –phenyl] sulfonyl urea

c. Molecular formula: $C_{21}H_{27}N_5O_4S$

d. Molecular weight: 445.54

e. Description: A white or almost white crystalline powder.


g. Melting point: Crystals from ethanol, melting point 208-209°C. Also reported as melting point 200-203°C.

h. Identification:

a). Compare the Infrared absorption spectrum with reference spectrum of Glipizide

b) Dissolve about 2 mg in methanol and dilute to 100 ml with the same solvent. Examine between 220 nm to 350 nm, the solution shows two absorption maxima; at 226 nm and 274 nm. The ratio of the absorbance measured at the maximum of 226 nm to that at 274 nm is 2.0 to 2.4.

i. Therapeutic category: Antidiabetic

j. Storage: Preserve in light containers, protected from light, store at room temperature.

6.1.3 Gliclazide [1-2]

a. Structure:
b. **Chemical name:** 1-(3-azabicyclo [3.3.0] oct-3-yl) –3-(p-tolylsulphonyl)-urea

c. **Molecular formula:** $C_{15}H_{21}N_{3}O_{3}S$

d. **Molecular weight:** 323.42

e. **Description:** A white or almost white powder.

f. **Solubility:** Practically insoluble in water, freely soluble in methylene chloride, sparingly soluble in acetone, slightly soluble in alcohol.

g. **Identification:**
   a) Compare the Infrared absorption spectrum with reference spectrum of Gliclazide.

h. **Dosage forms:** Tablets of Combined dosage forms are available in the following strengths [4]

1. **Combination-I (Metformin and glipizide)**
   a. Metformin (500mg) + Glipizide (5mg) Tablet
   b. Metformin (250mg) + Glipizide (2.5mg) Tablet
   c. Metformin (400mg) + Glipizide (2.5mg) Tablet

2. **Combination-II (Metformin and gliclazide)**
   a. Metformin (500 mg) + Gliclazide (40mg) Tablet
   b. Metformin (500 mg) + Gliclazide (80mg) Tablet
   c. Metformin (400 mg) + Gliclazide (40mg) Tablet
   d. Metformin (500 mg) + Gliclazide (60mg) Tablet
   e. Metformin (250 mg) + Gliclazide (40mg) Tablet

---

**6.2 Introduction**
Metformin HCl is chemically 1,1-dimethyl biguanide hydrochloride. Glipizide is 1 cyclohexyl –3- [[P-[2-(5- methylpyrazine-carboxamido ) ethyl ] –phenyl] sulfonyl urea. Gliclazide is chemically 1-(3-azabicyclo [3.3.0] oct-3-yl ) –3-(p-tolylsulphonyl)-urea. These three drugs are oral hypoglemic agents. A combination of 500 mg of metformin and 5 mg of glipizide (Combination I), 500 mg of metformin and 80 mg of gliclazide (Combination II) are available commercially as tablets [5]. These two combinations are used in the treatment of non insulin dependent diabetes mellitus (NIDDM).

6.2.1 Reported Methods for estimation of Metformin, glipizide and gliclazide

Literature survey reveals that several methods are there for the estimation of metformin, glipizide and gliclazide, individually [6-13]. But there is only one method [14] for the simultaneous estimation of these two combinations (I and II) in which ion pair liquid chromatographic technique has been used. In the present research work an attempt has been made to develop a method for the simultaneous estimation of these two combinations (I and II) by using micellar liquid chromatography.

The separation of analytes and their quantitation in multicomponent dosage forms has become a very important part of analytical chemistry. Sometimes it becomes very difficult to separate and quantitate the drugs in the multicomponent dosages by conventional analytical methodologies. Therefore, new analytical strategies and techniques are required to separate and quantitate the drugs in the multicomponent dosages of pharmaceutical preparations.

Several separation schemes have been shown to be useful for separating complex molecules and include HPLC, capillary zone electrophoresis and to a much lesser extent gas chromatography and supercritical fluid chromatography. Problems have been associated with these separation techniques and although each holds promise, none have been found to be acceptable for the routine analysis for all types of complex molecules. An alternative to these analytical techniques would be micellar HPLC (MLC).

Micellar liquid chromatography (MLC) is a technique where a micellar agent is added to a mobile phase that contains a buffer and a small amount of organic modifier. Several advantages are apparent with MLC when compared to reversed phase liquid chromatography. MLC uses a much lower amount of
organic modifier and is therefore less toxic and gradient MLC is done without
the need for long column re-equilibration.

In 1980, Armstrong and Henry first demonstrated that aqueous micellar
solutions could be used as a mobile phases in reverse phase liquid
chromatography (RPLC). They called this technique pseudophase or micellar
liquid chromatography.

Micellar mobile phases have certain advantages over traditional hydro-organic
mobile phases in RPLC, e.g. direct injection of biologicals, resolution of
optical isomers via chiral micelles, and unusual selectivity to name a few.
However, there is a problem with MLC, it tends to be less efficient than
conventional RPLC.

Dorsey et al [15] were the first to address this problem. They believed the
reduction in column efficiency was due to slow mass transfer, which arises
principally from poor wetting of the stationary phase. Dorsey demonstrated
that chromatographic efficiency in MLC can be improved by adding a small
amount of propanol, 3 % by volume to the mobile phase. Yarmchuck and
Cline–Love [16] on the other hand attributed the reduced efficiency associated
with ionic micellar mobile phases to poor mass transfer between the micelle
and the stationary phase, with the micelle exit rate constant being the limiting
factor for hydrophobic solutes. Borgerding and Hinze [17] concluded that poor
mass transfer within the stationary phase itself, resulting from adsorption of
surfactant onto the alkyl bonded phase, is responsible for the low efficiencies
observed in MLC. They demonstrated that addition of an alcohol, such as
isopropanol (IPA) to a nonionic micellar solution reduces the amount of
surfactant adsorbed on the stationary phase, resulting in a more efficient
separation. In contrast to what has been reported by other workers, Cassidy
[18] in a recent study on band broadening in MLC concluded that
improvement in solute mass transfer which can occur upon addition of
propanol to an SDS micellar solution is due to changes in the structure of the
micelles and not mass transfer effects related to the loading of surfactant on
the bonded phase.

Several interesting separations have been accomplished using MLC. Cline
Love and co-workers [19-21] reported the direct injection of serum and urine
into a reversed phase column with no protein precipitation or pressure build up problems. This method was used for therapeutic drug monitoring without the requirement of sample cleanup prior to injection. MLC has been shown to be useful for the separation of amino acids, peptides [22] and proteins [23]. One study found that small changes in the conc. of surfactant produced tremendous changes in the retention of different proteins[23]. MLC have been applied for the estimation of diuretics, [24-25] anabolic steroids [26] and catecholmines [27]theophylline [28] in pharmaceuticals and for the analysis of ampicillin, cloxacillin and their related substances in pharmaceuticals[29].

The purpose of this research was to determine the effects that each mobile phase variable had on the retention and resolution of metformin, glipizide and gliclazide using MLC. The mobile phase variables that were studied include, the concentration of micellar agent, mobile phase ionic strength, concentration of organic modifier and mobile phase pH. The results from these studies are discussed. From these studies, the mobile phase which gives adequate retention times and separation of metformin, glipizide and gliclazide was selected for the analysis of these two combinations (I and II).

6.3 Experimental

6.3.1 Chromatographic conditions:

The instrumentation consisted of Thermo separation products constametric 3500 pump, Thermo separation products AS 3000 autosampler, Thermo separation products UV 1000 detector. Data acquisition was made with PC 1000 software version 3.5.1. The Zorbax XDB C18 column (4.6 x 150 mm, 5 µm) was used for the analysis. The mobile phase flow rate was 1.2 ml/min. The detection was performed in UV at 226 nm. All the experiments were carried at temperature 30 °C.

6.3.2 Chemicals and Reagents

Disodium hydrogen phosphate, Ortho-phosphoric acid (A.R. grade), Isopropyl alcohol and acetonitrile (HPLC grade) were obtained from Qualigens Fine Chemicals, Dr. Annie Besant Road, Mumbai, Maharashtra State, India. Sodium dodecyl sulphate (SDS) was obtained from E. Merck (India) Limited,

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artment of Chemical Technology, Dr. Babasaheb Ambedkar Marathwada University Aurangabad

Worli, Mumbai. Glipizide, Gliclazide and metformin standards were obtained from Wockhardt Research Centre, Aurangabad, Maharashtra State, India. The tablets of combination I and combination II were purchased from the market. Nylon membrane filter (0.45 µm) was obtained from (Advanced Microdevices Pvt. Ltd. 21, Industrial Area, Ambala Cantt, India). Whatman 41 filter paper was obtained from Whatman International Ltd Maidstone England. Double distilled water was used throughout the procedure. Micellar mobile phase was prepared by using 2.5 mM disodium hydrogen phosphate and 50 mM SDS and 5 % IPA, pH adjusted to 7.2 with 10 % ortho-phosphoric acid. The mobile phase was vacuum filtered through 0.45 µm Nylon membranes. Diluent for standard and sample preparations was prepared containing of 10 mM disodium hydrogen phosphate (pH 9.3) and acetonitrile in the ratio of 50:50 (v/v).

6.3.3 Preparation of standard solutions
Stock standard solutions of metformin (5 mg/ml), glipizide (1 mg/ml) and gliclazide(0.8 mg/ml) was first prepared in diluent. The standards were dissolved using sonication for 5 min. The stock standard solution of glipizide was further diluted in the mobile phase to obtain 0.05 mg/ml. Working standard solutions for calibration curves were prepared by the subsequent dilution of the stock standard solutions with mobile phase.

6.3.4 Preparation of sample solutions
For the analysis of tablets, ten tablets were weighed and finely ground in a mortar. For combination I the portion equivalent to 5 mg of glipizide and 500 mg of metformin was transferred in a 100 ml volumetric flask, 50 ml of diluent was then added and sonication was done for 15 min with swirling. After sonication, the volume was made up to the mark with the diluent, and mixed well. The solution was filtered through Whatman filter paper 41. The first 5 ml portion of the filtrate was rejected and then 2 ml of the filtered solution was transferred into a 50 ml volumetric flask and diluted with the mobile phase.

For combination II the portion equivalent to 40 mg of gliclazide and 250 mg of metformin was taken and transferred in 100 ml volumetric flask, 50 ml of diluent was then added and sonication was done for 15 min. with swirling.
After sonication, the volume was made with the diluent, and mixed well. The solution was filtered through Whatman filter paper 41. The first 5 ml portion of the filtrate was rejected and then 2 ml of the filtered solution was transferred into a 25 ml volumetric flask and diluted with the mobile phase. For both the combinations, six determinations were performed.

6.4 Discussion and optimization of chromatographic conditions

MLC mobile phases consist of a surfactant, a buffer, and a low concentration of organic modifier. A major advantage of MLC is that the mobile phases contain a much lower concentration of organic modifier than a reversed phase system and is therefore less toxic. The surfactants used in MLC consist of two portions that contain distinctly different properties, a polar head group and a hydrocarbon tail. These properties allow the surfactant to adsorb at interfaces (stationary phase) where both the hydrophobic and hydrophilic character can be satisfied. The formation of micelles is the result of opposing forces, hydrophilic and hydrophobic. When the critical micelle concentration is achieved, the surfactant molecules arrange in such a way that the hydrophobic tails are oriented towards the centre of the aggregate and the polar heads point outwards [30]. The repulsion between the polar head groups is the controlling force that determines the size and shape of the micelles.

The separation mechanism in MLC is similar to RPLC in that the primary equilibrium of the analyte is between the mobile phase and the stationary phase. In MLC a secondary equilibrium is also involved in the separation, this equilibrium is the partitioning of the analyte between the mobile phase and the micelles [30-31]. Various mobile phase parameters will have an effect on the retention and separation of organic analytes. The parameters that were studied include concentration of surfactant, ionic strength of mobile phase, concentration of organic modifier and mobile phase pH.

6.4.1 Effect of Ionic Strength of Mobile Phase

In MLC, electrostatic interactions are involved between a charged analyte and the micelle in the diffuse secondary layer while hydrophobic interactions take place in the hydrophobic inner portion of the micelle. Armstrong and Stine [32] have shown that the thickness of the double layer decreases with increasing ionic strength, which allows hydrophobic interactions to take place.
between the analyte and the micelle. Anti-binding analytes (compounds that are strongly excluded or repelled from a micelle) have been found to have increased retention with higher ionic strength mobile phases[36]. For the transition from anti-binding to non-binding to binding to occur, the analyte ion must have enough hydrophobic character to associate with the nonpolar portion of the micelle, overcoming electrostatic repulsion. Bromophenol blue has been shown to change an anti-binding to a binding analyte with a corresponding increase in retention using SDS in mobile phase with 0.02 M NaCl added [32].

The results are shown in Table-1A and Figure 1A shows how the ionic strength affects the retention of metformin, glipizide and gliclazide. It was observed that when the ionic strength increased in the mobile phase, metformin showed reduction in retention time, whereas glipizide and gliclazide were not affected. The reduction in retention time for metformin may be because of its binding characteristics. The lack of effect on the retentions of glipizide and gliclazide could be attributed to their non-binding character.

6.4.2 Effect of Micellar Concentration

When the concentration of a micellar agent was increased in the mobile phase, a corresponding decrease in analyte retention was usually observed [33]. The rate at which the retention of the analyte changes varies with the charge and hydrophobicity of solutes as well as the length of the alkyl chain, charge and concentration of the micelles[34]. A study done by Bailey and Cassidy[35] showed that the efficiency of the micellar system improved for hydrophobic analytes but not for polar analytes as the micellar concentration was increased. The results are shown in Table-1B and Figure 1B shows how the concentration of SDS influenced the retention of metformin, glipizide and gliclazide. As the concentration of SDS was increased in mobile phase the retention of metformin, glipizide and gliclazide were decreased. This would be expected since at low concentration of micellar agent, the chromatographic system resembles conventional reversed phase liquid chromatography. As the concentration of micellar agent is increased the number of micelles in the
system increases and binding between the analyte and the micelles increases[36].

Change in elution order was observed for metformin at and above 0.1 M concentration of SDS and may be due to differences in the binding constants of the micelle and the analyte. Selectivity between analytes may be change due to the contribution of electrostatic and hydrophobic interactions, which is dependent on the structure of the compound. From this study, it was observed that the concentration of 50 mM SDS is adequate for the retention and separation of metformin, glipizide and gliclazide.

6.4.3 Effect of Mobile Phase pH

The micellar mobile phase pH will have a dramatic effect on the retention of weak organic acids and bases. Partition coefficients for the micelle - analyte interactions are different for the associated and unassociated forms. Several studies have shown that small changes in the mobile phase pH will have an effect on retention especially when the mobile phase pH is close to the analyte’s pkₐ value[37-39]. Adsorption of anionic surfactant monomers on the surface of a C₈ stationary phase cause protonated organic bases to be retained for a longer period of time than the neutral free base form due to electrostatic attraction. Research has also shown that the dependence of k’ on pH at a constant concentration of micellar agent is sigmoidal if there is no electrostatic repulsion between any of the acid base forms and surfactant molecules [40]. It was observed that the effect of pH on retention of metformin and gliclazide is more pronounced than that on the retention of glipizide. The results are shown in Table-1C and Figure 1C.

6.4.4 Effect of Organic Modifier Concentration

The amount of organic modifier present in the mobile phase will have an effect on analyte retention. Khaledi and co-workers [41] have shown that elution strength increased with an increase in the organic solvent concentration. A corresponding enhancement in the separation selectivity was also observed. The selectivity enhancement was found to occur systematically and was observed for a large number of ionic and nonionic compound with different functional groups, and also for two different surfactants, one anionic and one cationic. The selectivity, enhancement was credited to competing
partitioning equilibria in micellar HPLC systems and/or to the characteristics of micelles to compartmentalize solutes and organic solvents [41]. Some concern has been expressed that micellar mobile phases would act like a hydro-organic system at higher concentration of organic modifier. This however, was shown not to be the case, it has been demonstrated that a micellar eluent that contains up to 20 % IPA does not change to a hydro-organic system [22]. The addition of an organic modifier actually enhances the solvent strength and selectivity for some ionic and nonionic analytes. Retention characteristics for a solvent – water – micellar system were also found to be similar to a purely aqueous micellar eluent [42-43]. It was concluded, from these studies that the micelle influences the role of an organic modifier in the mobile phase.

The results are shown in Table-1D and figure 1D shows the effect of IPA on the retention of metformin, glipizide and gliclazide. When the concentration of IPA in the mobile phase was very low upto 1.5 %, retention of metformin, glipizide and gliclazide was extremely high, but the retentions decreased with increasing concentration of IPA.

**6.4.5 Optimized Chromatographic Conditions:**

From the above studies, by changing various variable parameters of mobile phase, it was observed that the separation of the analytes in combination I and II was accomplished with the mobile phase consisting 50 mM SDS, 2.5 mM disodium hydrogen phosphate and 5 % IPA, pH adjusted to 7.2 with 10 % orthophosphoric acid. This mobile phase gave adequate retention and good resolution for the components and was therefore selected for further analysis of combination I and combination II.

**6.5 Results**

**6.5.1 System Suitability**

The mixed standard solution for combination I was prepared containing 2 µg/ml glipizide and 200 µg/ml of metformin in mobile phase. Similarly, for combination II, a mixed standard solution containing 32 µg/ml of gliclazide and 200 µg/ml of metformin was prepared. Five replicate injections of the mixed standard solutions were injected and the parameters were evaluated.
The system suitability test was done on three different days. The observed system suitability parameters are shown in Table 2A and 2B.

6.5.1 Analytical data

The calibration curve of glipizide and metformin for the analysis of combination I was obtained by triplicate injections of standard solutions with varying concentrations of glipizide and metformin in the range of 0.5 µg/ml – 3.0 µg/ml and 50 µg/ml – 300 µg/ml respectively. From the regression of calibration curve, the correlation coefficients (r) for peak area, peak heights of glipizide and metformin were 0.9998, 0.9999 and 1.00, 0.9954 respectively. The calibration curve of gliclazide and metformin for the analysis of combination II was obtained by triplicate injections of standard solutions with varying concentrations of gliclazide and metformin in the range 8 µg/ml – 48 µg/ml and 50 µg/ml – 300 µg/ml respectively. From the regression of calibration curve, the correlation coefficients (r) for peak area, peak heights of gliclazide and metformin were 0.9999, 0.9999 and 0.9999, 0.9969 respectively. The results are tabulated in the tables 3A & 3B for combination –I and for combination –II in tablets 4A and 4B and graphical presentation are shown in figures 2A.1 to 2A.4 and 3A.1 to 3A.4 respectively.

6.5.2 Reproducibility

The reproducibility was evaluated from two series of six aliquots of combination I and II. The coefficient of variation was 1.30 % for glipizide and 1.39 % for metformin in combination I. The coefficient of variation was 1.75 % for gliclazide and 1.28 % for metformin in combination II.

6.6 Analysis of Tablets of Combination I and II

The procedure was applied to the determination of glipizide, metformin (Combination I) and gliclazide, metformin (Combination II) tablets obtained in Indian market. Figure 4A shows the chromatogram of combination I (glipizide and metformin) and Figure 4B shows the chromatogram of combination II (gliclazide and metformin). The procedure was applied for the tablets of combination I and II by different manufacturers. The results are shown in Table 5A and 5B. The glipizide and metformin content was determined by taking six aliquots of each formulation and injected into the chromatographic system. The results were reproducible and the recoveries with respect to the values declared.
by the manufacturers for glipizide and metformin (combination I) were in the range 96.1 - 103.4 %, 95.0 - 100.0 % using peak areas and 96.4 - 103.6 %, 97.3 - 102.5 % using peak heights respectively.

The recoveries for the gliclazide and metformin (combination II) were in the range 94.7 - 100.8 %, 96.2 - 101.2 % using peak areas and 93.1 - 101.5 %, 92.2 - 102.5 % using peak heights respectively. The proposed procedure for the determination of metformin and glipizide in combination I and gliclazide and metformin in combination II is rapid and reliable.

6.7 Conclusion

The MLC technique described herein provides a simple, rapid and reproducible determination of metformin in combination with glipizide and gliclazide dosage forms which makes it potentially valuable in quality control.

6.8 Tables

Table 1A : Effect of Ionic Strength on K’

<table>
<thead>
<tr>
<th>Ionic strength (M)</th>
<th>K’</th>
<th>Glipizide</th>
<th>Gliclazide</th>
<th>Metformin</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0025</td>
<td>7.22</td>
<td>19.95</td>
<td>26.33</td>
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</tr>
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<td>0.005</td>
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<td>27.00</td>
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<td>0.01</td>
<td>7.76</td>
<td>21.29</td>
<td>25.91</td>
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<tr>
<td>0.02</td>
<td>6.62</td>
<td>18.56</td>
<td>21.26</td>
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</tr>
<tr>
<td>0.05</td>
<td>6.75</td>
<td>19.43</td>
<td>17.00</td>
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</tr>
<tr>
<td>0.1</td>
<td>6.66</td>
<td>19.2</td>
<td>13.22</td>
<td></td>
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<td>0.2</td>
<td>6.57</td>
<td>19.57</td>
<td>9.31</td>
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</tbody>
</table>
Table 1B: Effect of Micellar concentration on $K'$

<table>
<thead>
<tr>
<th>Micellar concentration (M)</th>
<th>Glipizide</th>
<th>Gliclazide</th>
<th>Metformin</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>13.75</td>
<td>28.2</td>
<td>144.9</td>
</tr>
<tr>
<td>0.025</td>
<td>11.17</td>
<td>26.05</td>
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<td>0.05</td>
<td>8.19</td>
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<td>29.12</td>
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<tr>
<td>0.1</td>
<td>6.22</td>
<td>18.67</td>
<td>14.85</td>
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<tr>
<td>0.2</td>
<td>4.11</td>
<td>13.65</td>
<td>8.4</td>
</tr>
</tbody>
</table>

Table 1C: Effect of Mobile phase pH on $K'$

<table>
<thead>
<tr>
<th>Mobile phase pH</th>
<th>Glipizide</th>
<th>Gliclazide</th>
<th>Metformin</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>18.25</td>
<td>110.69</td>
<td>109.52</td>
</tr>
<tr>
<td>3</td>
<td>17.97</td>
<td>104.38</td>
<td>110.823</td>
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<td>4</td>
<td>18.08</td>
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<td>5</td>
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Table 1D: Effect of IPA on $K'$

<table>
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<th>IPA (%)</th>
<th>Glipizide</th>
<th>Gliclazide</th>
<th>Metformin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glipizide</td>
<td>Gliclazide</td>
<td>Metformin</td>
</tr>
</tbody>
</table>

Department of Chemical Technology, Dr. Babasaheb Ambedkar Marathwada University Aurangabad
### Table-2A : System suitability Test Results (Product : Combination I)

<table>
<thead>
<tr>
<th>Day</th>
<th>Analyte</th>
<th>RSD (%)</th>
<th>RSD (%)</th>
<th>Theoretical Plates</th>
<th>K’</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Glipizide</td>
<td>0.40</td>
<td>1.22</td>
<td>2094</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>Metformin</td>
<td>0.36</td>
<td>1.28</td>
<td>5969</td>
<td>22.8</td>
</tr>
<tr>
<td>2</td>
<td>Glipizide</td>
<td>1.01</td>
<td>1.33</td>
<td>1775</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td>Metformin</td>
<td>0.32</td>
<td>1.47</td>
<td>5591</td>
<td>23.5</td>
</tr>
<tr>
<td>3</td>
<td>Glipizide</td>
<td>1.20</td>
<td>1.27</td>
<td>1730</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td>Metformin</td>
<td>0.37</td>
<td>1.56</td>
<td>5194</td>
<td>22.7</td>
</tr>
</tbody>
</table>

### Table-2B : System suitability Test Results (Product : Combination II)

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<thead>
<tr>
<th>Day</th>
<th>Analyte</th>
<th>RSD (%)</th>
<th>RSD (%)</th>
<th>Theoretical Plates</th>
<th>K’</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Gliclazide</td>
<td>0.33</td>
<td>1.13</td>
<td>3180</td>
<td>12.4</td>
</tr>
<tr>
<td></td>
<td>Metformin</td>
<td>0.36</td>
<td>1.38</td>
<td>5518</td>
<td>22.9</td>
</tr>
<tr>
<td>2</td>
<td>Gliclazide</td>
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<td>1.15</td>
<td>3170</td>
<td>12.6</td>
</tr>
<tr>
<td></td>
<td>Metformin</td>
<td>0.32</td>
<td>1.47</td>
<td>5591</td>
<td>23.5</td>
</tr>
<tr>
<td>3</td>
<td>Gliclazide</td>
<td>0.28</td>
<td>1.14</td>
<td>3108</td>
<td>12.8</td>
</tr>
<tr>
<td></td>
<td>Metformin</td>
<td>0.37</td>
<td>1.56</td>
<td>5194</td>
<td>22.7</td>
</tr>
</tbody>
</table>

### Table-3A : Calibration curve (Combination –I)

<table>
<thead>
<tr>
<th>Calibration level</th>
<th>Concentration of Glipizide in ppm</th>
<th>Average peak height (n=3)</th>
<th>Average peak area (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5</td>
<td>4439</td>
<td>43595</td>
</tr>
<tr>
<td>2</td>
<td>1.0</td>
<td>8952</td>
<td>87808</td>
</tr>
<tr>
<td>3</td>
<td>1.5</td>
<td>13172</td>
<td>129094</td>
</tr>
<tr>
<td>4</td>
<td>2.0</td>
<td>17694</td>
<td>174733</td>
</tr>
</tbody>
</table>
### Table-3 B: Calibration curve (Combination –I)

<table>
<thead>
<tr>
<th>Calibration level</th>
<th>Concentration of Metformin in ppm</th>
<th>Average peak height (n=3)</th>
<th>Average peak area (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>237586</td>
<td>5432615</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>457585</td>
<td>10477513</td>
</tr>
<tr>
<td>3</td>
<td>150</td>
<td>673117</td>
<td>15512621</td>
</tr>
<tr>
<td>4</td>
<td>200</td>
<td>882573</td>
<td>20642250</td>
</tr>
<tr>
<td>5</td>
<td>250</td>
<td>1091183</td>
<td>25659636</td>
</tr>
<tr>
<td>6</td>
<td>300</td>
<td>1220256</td>
<td>30648653</td>
</tr>
</tbody>
</table>

### Table-4A: Calibration curve Combination –II

<table>
<thead>
<tr>
<th>Calibration level</th>
<th>Concentration of Gliclazide in ppm</th>
<th>Average peak height (n=3)</th>
<th>Average peak area (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>28616</td>
<td>540629</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>59332</td>
<td>1093233</td>
</tr>
<tr>
<td>3</td>
<td>24</td>
<td>90389</td>
<td>1647298</td>
</tr>
<tr>
<td>4</td>
<td>32</td>
<td>118926</td>
<td>2169638</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>148766</td>
<td>2724222</td>
</tr>
<tr>
<td>6</td>
<td>48</td>
<td>178294</td>
<td>3265710</td>
</tr>
</tbody>
</table>

### Table-4 B: Calibration curve

<table>
<thead>
<tr>
<th>Calibration level</th>
<th>Concentration of Metformin in ppm</th>
<th>Average peak height (n=3)</th>
<th>Average peak area (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>227426</td>
<td>5428757</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>449684</td>
<td>10800199</td>
</tr>
<tr>
<td>3</td>
<td>150</td>
<td>668145</td>
<td>16210798</td>
</tr>
</tbody>
</table>
### Table- 5A : Analysis of Tablets (Combination- I)

<table>
<thead>
<tr>
<th>Product</th>
<th>Label claim</th>
<th>Amount found (mg)</th>
<th>% Assay</th>
<th>Amount found (mg)</th>
<th>% Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Product-1</td>
<td>Glipizide- 5mg</td>
<td>5.05±0.07</td>
<td>101.0</td>
<td>5.08±0.06</td>
<td>101.5</td>
</tr>
<tr>
<td>Product-1</td>
<td>Metformin HCl 500mg</td>
<td>483.52±8.41</td>
<td>96.70</td>
<td>495.54±8.63</td>
<td>99.1</td>
</tr>
<tr>
<td>Product-2</td>
<td>Glipizide- 5mg</td>
<td>4.88±0.06</td>
<td>97.5</td>
<td>4.90±0.08</td>
<td>98.0</td>
</tr>
<tr>
<td>Product-2</td>
<td>Metformin HCl 500mg</td>
<td>479.88±6.15</td>
<td>96.0</td>
<td>491.0±6.46</td>
<td>98.2</td>
</tr>
</tbody>
</table>

### Table- 5B : Analysis of Tablets Combination- II

<table>
<thead>
<tr>
<th>Product</th>
<th>Label claim</th>
<th>Amount found (mg)</th>
<th>% Assay</th>
<th>Amount found (mg)</th>
<th>% Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Product-1</td>
<td>Gliclazide- 80mg</td>
<td>78.75 ± 1.31</td>
<td>98.4</td>
<td>78.55 ± 1.71</td>
<td>98.2</td>
</tr>
<tr>
<td>Product-1</td>
<td>Metformin HCl 500mg</td>
<td>498.04 ± 5.34</td>
<td>99.6</td>
<td>502.07 ± 6.32</td>
<td>100.4</td>
</tr>
</tbody>
</table>
### Product-2

<table>
<thead>
<tr>
<th>Component</th>
<th>Formula</th>
<th>Retention Factor</th>
<th>Efficiency</th>
<th>Peak Area</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gliclazide</td>
<td>80mg</td>
<td>78.45 ± 0.62</td>
<td>98.1</td>
<td>78.04 ± 1.05</td>
<td>97.6</td>
</tr>
<tr>
<td>Metformin HCl</td>
<td>500mg</td>
<td>499.09 ± 4.25</td>
<td>99.8</td>
<td>503.09 ± 4.23</td>
<td>100.6</td>
</tr>
</tbody>
</table>

### Product-3

<table>
<thead>
<tr>
<th>Component</th>
<th>Formula</th>
<th>Retention Factor</th>
<th>Efficiency</th>
<th>Peak Area</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gliclazide</td>
<td>80mg</td>
<td>78.18 ± 1.80</td>
<td>97.7</td>
<td>77.80 ± 1.84</td>
<td>97.2</td>
</tr>
<tr>
<td>Metformin HCl</td>
<td>500mg</td>
<td>492.95 ± 9.69</td>
<td>98.6</td>
<td>497.76 ± 8.79</td>
<td>99.6</td>
</tr>
</tbody>
</table>

### 6.9 Figures

Figure 1A. Effect of Ionic Strength on the retention of Glipizide, Gliclazide and Metformin. Mobile phase : 2.5 mM disodium hydrogen phosphate, 50 mM SDS, 5 % IPA, pH 7.2.
Figure 1B. Effect of Concentration of SDS on the retention of Glipizide, Gliclazide and Metformin.

Figure 1C. Effect of Mobile Phase pH on the retention of Glipizide, Gliclazide and Metformin.

Figure 1D. Effect of Organic Modifier Concentration on retention of Glipizide, Gliclazide and Metformin.
Fig 2A.1
Calibration curve for glipizide (combination-I)

Fig 2A.2
Calibration curve for Metformin (combination-I)
Fig 2A.3
Calibration curve for glipizide (combination-I)

Fig 2A.4 Calibration curve for Metformin (combination-I)
**Fig 3A.1**
Calibration curve for gliclazide (combination-II)

**Fig 3A.2**
Calibration curve for Metformin (combination-II)
Combination-II
Linearity graph for Metformin HCl in peak height

\[ y = 4116.5x + 25866 \]
\[ R^2 = 0.9969 \]

---

Fig 3A.3
Calibration curve for gliclazide (combination-II)

Linearity graph for Gliclazide

\[ y = 68039x + 1455.6 \]
\[ R^2 = 0.9999 \]

---

Fig 3A.4
Calibration curve for metformin (combination-II)
Figure 4A. Chromatogram of combination I showing the peaks of Glipizide (I) and Metformin (II).
Figure 4B. Chromatogram of combination II showing peaks of Gliclazide (I) and Metformin (II).

6.10 References


