### 3.1 MATERIALS:

**Table 3.1: List of materials used with manufacturer details**

<table>
<thead>
<tr>
<th>SL. No.</th>
<th>Materials Used</th>
<th>Grade</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Glipizide</td>
<td>LR</td>
<td>Dr. Reddy’s labs, Hyderabad, India.</td>
</tr>
<tr>
<td>2.</td>
<td>Glimepiride</td>
<td>LR</td>
<td>Dr. Reddy’s labs, Hyderabad, India.</td>
</tr>
<tr>
<td>7.</td>
<td>Span 80</td>
<td>LR</td>
<td>S.D. Fine Chemicals Ltd., Mumbai, India.</td>
</tr>
<tr>
<td>10.</td>
<td>Acetone</td>
<td>LR</td>
<td>Ranbaxy Fine Chemicals Ltd., Delhi, India.</td>
</tr>
<tr>
<td>11.</td>
<td>Ethanol</td>
<td>LR</td>
<td>Ranbaxy Fine Chemicals Ltd., Delhi, India.</td>
</tr>
</tbody>
</table>
15. Potassium di hydrogen orthophosphate LR Ranbaxy Fine Chemicals Ltd., Delhi, India.
17. *Aloe barbadensis miller* leaves Local Plants, Anantapur, India.
18. *Ficus bengalensis* fruits Local Plants, Anantapur, India.
19. *Ficus carica* fruits Local Plants, Anantapur, India.
20. *Ficus glomerata* fruits Local Plants, Anantapur, India.
23. Male Rabbits weighing 1.5-2.0 kg -- Shri. Venkateshwara Enterprises, Bangalore, India.

### 3.2 EQUIPMENT:

**Table 3.2: List of equipment used with manufacturer details**

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Equipment</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.</td>
<td>FTIR 200 Spectrometer</td>
<td>Perkin Elmer, spectrum-100, Japan</td>
</tr>
<tr>
<td>3.</td>
<td>HPLC</td>
<td>Shimadzu Corporation, Japan.</td>
</tr>
</tbody>
</table>
6. Digital pH meter | Elico India, Ahmedabad, India.
7. Roche type Friability Test Apparatus | Campbell Electronics, Mumbai, India.
8. Viscometer | Brookfield engineering labs Inc., USA.
9. Monsanto Hardness Tester | Campbell Electronics, Bombay, India.
10. Disintegration test apparatus | Electro lab, Mumbai, India
12. Petri dish | National Scientifics, Guntur, India.
13. Dissolution Apparatus TDT-06 P;USP XXIII | Electro lab, Mumbai, India
14. Hot Air Oven | P.S.M. Industries, Bangalore, India.

3.3 ANALYSIS OF DRUGS USED

3.3.1 Estimation of Glipizide and Glimepiride:

Preparation of phosphate buffer pH 7.4:

Dissolve 2.38 g of di-sodium hydrogen phosphate and 0.19 g of potassium di-hydrogen phosphate in 1000 mL of distilled water and to it add 8.0 g of sodium chloride and adjust the pH to 7.4.

Preparation of Standard Calibration Curve of Glipizide:

10 mg of Glipizide was dissolved in small amount of phosphate buffer saline pH 7.4 and volume was made up to 100 mL using the same. From this stock solution serial dilutions were done to obtain solutions in the concentration ranging from 2.0 – 20.0 µg/mL. The
absorbance of the solutions was measured at 223 nm using UV-visible spectrophotometer. The absorbance values of Glipizide standard curve was represented in table 3.25 and a graph was plotted of concentration vs. absorbance which was shown in Fig. 3.37

**Preparation of Standard Calibration Curve of Glimepiride**

10 mg of Glimepiride was dissolved in small amount of phosphate buffer saline pH 7.4 and volume was made up to 100 mL using the same. From this stock solution serial dilutions were done to obtain solutions in the concentration ranging from 2.0 – 20.0 µg/mL. The absorbance of the solutions was measured at 230 nm using UV-visible spectrophotometer. The absorbance values of Glimepiride standard curve was represented in table 3.26 and a graph of concentration vs. absorbance was plotted which was shown in Fig 3.38.

### 3.4 EXTRACTION OF MUCILAGES

#### 3.4.1 Extraction of *Aloe barbadensis miller* leaves mucilage/ *Ficus bengalensis* fruits/ *Ficus carica* fruits/ *Ficus glomerata* fruit mucilages:

Fresh *Aloe barbadensis miller* leaves mucilage/*Ficus bengalensis* fruits/*Ficus carica* fruits/*Ficus glomerata* fruits were procured from the local plants of Anantapur and authenticated by the Botany department, Sri Krishnadevaraya University, Anantapur. The seeds do not contain any mucilage and were removed prior to extraction. The fruits were sliced (In case of *Aloe barbadensis miller* leaves sliced), homogenized
with five times its weight of water, centrifuged at 4000 rpm for 15 min and the clear, viscous solution decanted. The mucilage was precipitated with three volumes of ethanol and washed with more ethanol followed by acetone. The mucilage so obtained was dried under vacuum (less than 1 Torr at 25°C for 12 h).

**3.4.2 Purification of the Mucilage:**

General methods of isolation of gums from food were used. The crude mucilage (1 %) was homogenized (Potter homogenizer) with cold dilute tri chloro acetic acid solution (5%). The solution was centrifuged (3500 rpm for 20 min), neutralized with sodium hydroxide by drop wise addition and then dialyzed for 30 h against distilled water. The mucilage was precipitated with ethanol (in the quantities of three times the volumes) and washed successively with ethanol, acetone and diethyl ether\(^{202}\).

**3.4.3 Characterization of Mucilage:**

The collected mucilage was evaluated for physicochemical characteristics viz., morphological characteristics, identification by chemical tests, Solubility\(^{203}\), melting range, pH, Swelling index\(^{204}\), Ash values\(^{205}\), presence of foreign organic matter\(^{206}\), test for lead and arsenic\(^{207}\), Loss on drying\(^{208}\), Density, compressibility index and angle of repose\(^{209}\) and etc. (Table 4.1, 4.2 and 4.3) The evaluation was carried out as per procedures describe in official books.
3.5 PRE FORMULATION STUDIES:

Identification of Glipizide, Glimepiride and their compatibility with Aloe barbadensis miller leaf mucilage, Guar gum, Ficus bengalensis, Ficus carica, Ficus glomerata and Povidone K-30 were subjected to Pre formulation studies\textsuperscript{210-214}.

3.5.1 Identification of Pure Drugs:

This study involves

I. Solubility analysis:

Pre formulation solubility analysis was done to select a suitable solvent system to dissolve the drug well as excipients used for the design of matrix tablets and transdermal patches.

II. Determination of Melting point:

Glipizide and Glimepiride’s melting points were determined by open capillary method.

III. Determination of $\lambda_{\text{max}}$ using UV spectrophotometer:

10 mg of Glipizide/Glimepiride was accurately weighed and dissolved in 10 mL of pH 7.4 phosphate buffer (1000µg/mL) separately. 1 mL of this solution was taken in 100 mL volumetric flask and volume was made up to the mark with pH 7.4 (10 µg/mL) buffer and obtained solution was scanned on a UV Scanner between 200 to 400nm. The maximum obtained in the graph was considered as $\lambda_{\text{max}}$ for the pure drug.
3.5.2 Compatibility Studies:

3.5.2.1 Ultra Violet/ Visible spectral analysis:

Glipizide and Glimepiride content in the matrix tablets was estimated by an UV spectrophotometric method based on the measurement of absorbance at 223 nm and 230 nm respectively in pH 7.4 phosphate buffer. The linearity, accuracy and precision of the method were validated. The method obeyed Beer's law in the concentration range 5-30 µg/mL. When a standard drug solution was assayed repeatedly (n=6), the mean error (accuracy) and relative standard deviation (precision) were found to be 0.6% and 0.8% respectively. The spectrums were shown in Fig. 4.3 to Fig 4.6.

3.5.2.2 Differential Scanning Calorimetry:

Differential Scanning Calorimetry of pure drugs and polymers used were studied to investigate any changes in melting points of the drug after combining it with the excipients.

Differential Scanning Calorimetry (DSC) curves were obtained by a differential scanning calorimeter (Schimadzu DSC-50, Tokyo, Japan) at a heating rate of 10°C/min from 25°-250°C in nitrogen atmosphere (20 mL/min) with a sample weight of 3mg. The thermo grams were shown in Fig.4.7 to 4.14.

3.5.2.3 Fourier Transform Infra-Red (FT-IR) spectral analysis:

Fourier–Transformed Infrared (FT–IR) spectrums of Glipizide and Glimepiride with leaf mucilage of Aloe barbadensis miller, Guar gum, Povidone K-30, Ficus bengalensis, Ficus carica and Ficus glomerata were
obtained individually and in combinations on a Fourier Transform Infrared (FTIR) spectrophotometer, (Perkin Elmer, spectrum-100, Japan using the KBr disk method (2 mg sample in 200 mg KBr). The scanning range was 400 to 4000 cm\(^{-1}\) and the resolution was 1 cm\(^{-1}\). This spectral analysis was employed to check the compatibility of drugs with the polymers used. The spectrums were shown in Fig. 4.15 to 4.31.

3.5.2.4 Scanning Electron Microscopy (SEM) studies:

The Scanning Electron Microscopy (MERLIN Field Emission Scanning Electron Microscope (FE-SEM), Carl Zeiss, Germany) of the selected Glipizide matrix tablets and transdermal patches were shown in Fig. 4.32 to 4.37.

3.6 FORMULATIONS OF MATRIX TABLETS: 215, 216

3.6.1 Formulations of Glipizide Matrix Tablets

3.6.1.1 Formulations of Glipizide Aloe barbadensis miller leaf mucilage/ Guar gum/Povidone matrix tablets

Controlled release matrix tablets of Glipizide with Aloe barbadensis miller leaves mucilage/Guar gum/Povidone were prepared by using different drug: mucilage ratios viz. 1:0.4, 1:0.8, 1:1.2, 1:1.6 and 1:2.0. Aloe barbadensis miller leaves mucilage/Guar gum/Povidone was used as matrix forming material while microcrystalline cellulose as a diluent and Magnesium stearate as a lubricant. All ingredients used were passed through a # 100 sieve, weighed and blended. The above formulations were compressed by a direct compression technique using
8 mm flat faced punches. The compositions of formulations were showed in Table 4.6 to 4.8.

The matrix tablets prepared using *Aloe barbadensis miller* and Povidone shown satisfactory results. So, combinations of both polymers were also formulated.

### 3.6.1.2 Formulations of Glipizide *Aloe barbadensis miller*-Povidone Matrix Tablets

Controlled release matrix tablets of Glipizide with *Aloe barbadensis miller* leaf mucilage Povidone were prepared by using different drug: mucilage ratios viz. 1:0.4, 1:0.8, 1:1.2, 1:1.6 and 1:2.0. Both *Aloe barbadensis miller* and Povidone were used as matrix forming material while microcrystalline cellulose as a diluent and Magnesium stearate as a lubricant. All ingredients used were passed through a #100 sieve, weighed and blended. The above formulations were compressed by a direct compression technique using 8 mm flat faced punches. The compositions of formulations were showed in Table 4.9.

### 3.6.2 Formulations of Glimepiride Matrix Tablets

#### 3.6.2.1 Formulations of Glimepiride *Aloe barbadensis miller* leaf mucilage/Guar gum/Povidone matrix tablets

Controlled release matrix tablets of Glimepiride with *Aloe barbadensis miller* leaves mucilage/Guar gum/Povidone were prepared by using different drug: mucilage ratios viz. 1:2, 1:4, 1:6, 1:8 and 1:10. *Aloe barbadensis miller* leaves mucilage/Guar gum/Povidone was used as matrix forming material while microcrystalline cellulose as a diluent and
Magnesium stearate as a lubricant. All ingredients used were passed through a # 100 sieve, weighed and blended. The above formulations were compressed by a direct compression technique using 8 mm flat faced punches. The compositions of formulations were showed in Table 4.10 to 4.12.

The matrix tablets prepared using *Aloe barbadensis miller* and Povidone shown satisfactory results. So, combinations of both polymers were also formulated.

### 3.6.2.2 Formulations of *Aloe barbadensis miller* leaf mucilage-Glimepiride Povidone Matrix Tablets

Controlled release matrix tablets of Glimepiride with *Aloe barbadensis miller* leaves mucilage Povidone were prepared by using different drug: mucilage ratios viz. 1:2, 1:4, 1:6, 1:8 and 1:10. (Both *Aloe barbadensis miller* and Povidone was used as matrix forming material while microcrystalline cellulose as a diluent and Magnesium stearate as a lubricant. All ingredients used were passed through a # 100 sieve, weighed and blended. The above formulations were compressed by a direct compression technique using 8 mm flat faced punches. The compositions of formulations were showed in Table 4.13.
3.7 FORMULATIONS OF TRANSDERMAL PATCHES

3.7.1 Formulations of Glipizide Transdermal Patches

3.7.1.1 Formulations of Glipizide *Ficus bengalensis*/ *Ficus carica*/ *Ficus glomerata* fruit mucilage/Povidone Transdermal patches

Various proportions of *Ficus bengalensis*/ *Ficus carica*/ *Ficus glomerata* fruits mucilage/ Povidone was taken in a beaker, Propylene glycol, Span-80, Propyl paraben, Methyl paraben and Glipizide (20 mg) were added with continuous stirring using Teflon-coated magnetic bead placed in magnetic stirrer for 0.5h at 500 rpm. The above mixture was poured within the glass bangles (6.1 cm diameter) kept on mercury surface in a Petri dish. The rate of evaporation was controlled by inverting a funnel over the Petri dish\(^{217, 218}\). After 24 h the dried patches were taken out and stored in desiccator till use. The formulae of various patches were showed in table 4.14 to 4.17.

The matrix transdermal patches prepared using *Ficus glomerata* and Povidone shown satisfactory results. So, combinations of both polymers were also formulated.

3.7.1.2 Formulations of Glipizide *Ficus glomerata* fruit mucilage and Povidone Transdermal patches

Various proportions of *Ficus glomerata* mucilage and Povidone was taken in a beaker, Propylene glycol (plasticizer), Span-80 (penetration enhancer) Propyl paraben, Methyl paraben (preservatives) and Glipizide (20 mg) were added with continuous stirring using Teflon-coated magnetic bead placed in magnetic stirrer for 0.5h at 500 rpm. The above
mixture was poured within the glass bangles (6.1 cm diameter) placed on mercury surface in a Petri dish. The rate of evaporation was controlled by inverting a funnel over the Petri dish. After 24 h the dried patches were taken out and stored in desiccator till use. The formulae of various patches were showed in table 4.18.

3.7.2 Formulations of Glimepiride Transdermal Patches:

3.7.2.1 Formulations of Glimepiride *Ficus bengalensis// Ficus carica/ Ficus glomerata* fruit mucilage/Povidone Transdermal patches

Various proportions of *Ficus bengalensis/ Ficus carica/ Ficus glomerata* fruit mucilage/Povidone was taken in a beaker, Propylene glycol, Span-80, Propyl paraben, Methyl paraben and Glimepiride (8 mg) were added with continuous stirring using Teflon-coated magnetic bead placed in magnetic stirrer for 0.5h at 500 rpm. The above mixture was poured within the glass bangles (6.1 cm diameter) placed on mercury surface in a Petri dish. The rate of evaporation was controlled by inverting a funnel over the Petri dish. After 24 h the dried patches were taken out and stored in desiccator till use. The formulae of various patches were showed in table 4.19 to 4.22.

The matrix transdermal patches prepared using *Ficus glomerata* and Povidone shown satisfactory results. So, combinations of both polymers were also formulated.
3.7.2.2 Formulations of Glimepiride *Ficus glomerata*-Povidone Transdermal Patches

Various proportions of *Ficus glomerata*-Povidone was taken in a beaker, Propylene glycol, Span-80, Propyl paraben, Methyl paraben and Glimepiride (4 mg) were added with continuous stirring using Teflon-coated magnetic bead placed in magnetic stirrer for 0.5h at 500 rpm. The above mixture was poured within the glass bangles (6.1 cm diameter) placed on mercury surface in a Petri dish. The rate of evaporation was controlled by inverting a funnel over the Petri dish. After 24 h the dried patches were taken out and stored in desiccator till use. The compositions of various patches were shown in table 4.23.

3.8 EVALUATION

3.8.1 Evaluation of Matrix tablets

A) Pre compression Parameters/ Evaluation of Granules:

1) Angle of Repose: \(^{219}\)

The frictional forces in a loose powder or granules can be measured by the angle of repose. This is the maximum angle possible between the surface of a pile of powder or granules and the horizontal plane. Different ranges of flow ability in terms of angle of repose are given in Table No. 3.3.

**Table 3.3: Flow ability of powders and its relative Angle of Repose**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Flow ability</th>
<th>Angle of Repose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Excellent</td>
<td>25-30°</td>
</tr>
<tr>
<td>2.</td>
<td>Good</td>
<td>30-35°</td>
</tr>
<tr>
<td>3.</td>
<td>Fair</td>
<td>35-37°</td>
</tr>
<tr>
<td>4.</td>
<td>Poor</td>
<td>37-45°</td>
</tr>
</tbody>
</table>
Method:

A funnel was filled to the brim and the test sample was allowed to flow smoothly through the orifice under gravity. From the cone formed on a graph sheet was taken to measure the area of pile(r), thereby evaluating the flow ability of the granules. Height of the pile (h) is then measured. Angle of repose can be calculated by eq. 9 and 10.

\[ r = \left( \frac{\text{area}}{\pi} \right)^{\frac{1}{2}} \] \hspace{1cm} (9)

\[ \theta = \tan^{-1} \left( \frac{h}{r} \right) \] \hspace{1cm} (10)

2) Bulk Density:

Bulk density is defined as the mass of a powder divided by the bulk volume. The bulk density of a powder depends primarily on particle size distribution, particle shape and the tendency of the particles to adhere to one another\textsuperscript{220}. 

Method:

Both loose bulk density (LBD) and tapped bulk density (TBD) were determined. A quantity of 2g of powder from each formula, previously lightly shaken to break any agglomerates formed, was introduced into a 10 mL measuring cylinder. After the initial volume was observed, the cylinder was allowed to fall under its own weight onto a hard surface from the height of 2.5 cm at 2-second intervals. The tapping was continued until no further change in volume was noted. LBD and TBD were calculated using eq. 11 and 12. The bulk densities of polymers used were mentioned in Table 4.25 and 4.26.
LBD = \frac{\text{Weight of the Powder}}{\text{Volume of the packing}} \quad --- \ (11)

TBD = \frac{\text{Weight of the powder}}{\text{Tapped volume of the packing}} \quad --- \ (12)

3) Compressibility Index:

The compressibility index of the granules was determined by Carr’s compressibility index and it has shown in eq. 13 and grading of the powders for their flow properties was shown in Table 4.27.

\[
\text{Carr’s Index} \ (% ) = \frac{\text{TBD} - \text{LBD}}{\text{TBD}} \times 100 \quad ---- \ (13)
\]

Table 3.4: Grading of the powders for their flow properties according to Carr’s Index 322:

<table>
<thead>
<tr>
<th>Carr’s Index (%)</th>
<th>Flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 – 15</td>
<td>Excellent</td>
</tr>
<tr>
<td>12 – 16</td>
<td>Good</td>
</tr>
<tr>
<td>18 – 21</td>
<td>Fair to passable</td>
</tr>
<tr>
<td>23 – 35</td>
<td>Poor</td>
</tr>
<tr>
<td>33 – 38</td>
<td>Very poor</td>
</tr>
<tr>
<td>&gt;40</td>
<td>Very, very poor</td>
</tr>
</tbody>
</table>

4) Hausner ratio: 221

Hausner found that the ratio TBD: LBD was related to inter particle friction and could be used to predict powder flow properties. Hausner ratio greater than 1.25 is considered to be an indication of poor flowability. The Hausner ratio of polymers used was mentioned in Table 4.28.
B) Post-Compression Parameters / Evaluation of Tablets:

1) General appearance of Tablets:

Randomly selected tablets from each batch examined under lens for the shape and color of the tablet, its overall elegance, uniformity, consistency, surface texture, odour, etc.

2) Thickness and Diameter Test: 222

Thickenss and diameter test permits accurate measurement and provides information on the variation between tablets. Ten tablets were taken and the thickness and diameter was measured using a dial-caliper. The tablet thickness and diameter should be controlled within a ±5% variation of a standard value.

3) Uniformity of Weight Test: 223, 224

Ten tablets were selected randomly from each formulation and weighed individually to check for weight variation. The average weight of tablet with % deviation as per IP was represented in Table 3.5.

Table 3.5: Average weight of tablet with % deviation as per IP

<table>
<thead>
<tr>
<th>Avg. Wt. of tablet</th>
<th>% deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>80 mg or less</td>
<td>10</td>
</tr>
<tr>
<td>More than 80 mg and less than 250mg</td>
<td>7.5</td>
</tr>
<tr>
<td>250 mg or more</td>
<td>5</td>
</tr>
</tbody>
</table>

4) Hardness Test: 225

Hardness indicates the ability of a tablet to withstand mechanical shocks while handling. It expressed. The hardness of the tablets was
determined using Monsanto Hardness Tester. The force needed to disrupt tablet by crushing in kg/cm² expresses it. Three tablets were randomly picked from each formulation and the mean and standard deviation values were calculated. The hardness of the tablets should be more than 4 kg/cm².

5) **Friability Test:** 226, 227

It is the phenomenon whereby tablet surfaces are damaged and/or show evidence of lamination or breakage when subjected to mechanical shock or attrition. The friability of tablets was determined using Roche Friabilator. Ten tablets were initially weighed (W_{initial}) and transferred into friabilator. The friabilator was operated at 25 rpm for 4 minutes (run up to 100 revolutions and tablets weight was taken (W_{final}). The % friability was then calculated by eq. 14.

\[
F = \frac{W_{initial} - W_{final}}{W_{initial}} \times 100 \quad \text{.......................... (14)}
\]

% friability of tablets less than 1% are considered to be acceptable.

6) **Drug Content Uniformity Test:**

The uncoated tablets were tested for their drug content.

**Procedure:**

Ten tablets were randomly selected, accurately weighed and average weight per tablet calculated. Tablets were ground individually to fine powder. Accurately weighed tablet powder, equivalent to 5 mg of
Glipizide and Glimepiride were separately transferred to 100 mL volumetric flask. These powders were dissolved in 85 mL of 0.1 N Sodium Hydroxide and sonicated well to ensure complete solubility of the drugs. Then the volume was made up to 100 mL with 0.1N sodium hydroxide. Applying vacuum later filtered this solution. From this 1 mL of solution was withdrawn and volume made up to 100 mL by using 0.1 N sodium hydroxide solution. Absorbance of the sample solution was measured at 223±2 and 230±2 nm respectively and concentration of drug in sample was calculated using standard calibration curve.

### 7) Swelling Behavior of Formulated Matrix Tablets

The extent of swelling was measured in terms of % weight gain by the tablet. The swelling behaviors of all matrix tablet formulations were studied. One tablet from each formulation was kept in a Petri dish containing phosphate buffer pH 7.4. At the end of 2 h, the tablet was withdrawn, kept on tissue paper and weighed. Then for 2 h and every 2 h, weights of the tablet were noted. This process was continued till the end of 12 h. % weight obtained by the tablet was calculated by eq. 15.

The swelling behaviors of different matrix tablet formulations were showed in Fig. 4.39 to 4.41.

\[
S.I = \frac{(Mt-M0)}{M0} \times 100 \quad \text{--------- (15)}
\]

Where,

- S.I = swelling index, Mt = weight of tablet at time (t) and Mo = weight of tablet at time t = 0.
8) In vitro Dissolution Study:

Dissolution testing was carried out in USP dissolution apparatus II (paddle) in phosphate buffer of pH 7.4. All the solutions were degassed (to remove dissolved air) for 20 minutes before use. Table No. 3.6 summarizes the general conditions in this study. Drug releases from different matrix tablet formulations were showed in Table 4.41 to 4.45.

Table 3.6: Summary of Dissolution Conditions

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Parameter</th>
<th>Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Dissolution medium</td>
<td>Phosphate buffer (pH 7.4)</td>
</tr>
<tr>
<td>2.</td>
<td>Temperature</td>
<td>37.0 ±0.5 °C</td>
</tr>
<tr>
<td>3.</td>
<td>Initial Volume</td>
<td>900mL</td>
</tr>
<tr>
<td>4.</td>
<td>Rotation speed</td>
<td>100rpm</td>
</tr>
<tr>
<td>5.</td>
<td>Drawn Volume</td>
<td>5mL</td>
</tr>
<tr>
<td>6.</td>
<td>Running time</td>
<td>12 h</td>
</tr>
</tbody>
</table>

Estimation of Glipizide/ Glimepiride from formulated matrix tablets

The release of Glipizide/Glimepiride from GPA-1, GPA-2, GPA-3, GPA-4, GPA-5, GPG-1, GPG-2, GPG-3, GPG-4, GPG-5, GPP-1, GPP-2, GPP-3, GPP-4, GPP-5, GPAP-1, GPAP-2, GPAP-3, GPAP-4, GPAP-5, GMA-1, GMA-2, GMA-3, GMA-4, GMA-5, GMG-1, GMG-2, GMG-3, GMG-4, GMG-5, GMP-1, GMP-2, GMP-3, GMP-4, GMP-5, GMAP-1, GMAP-2, GMAP-3, GMAP-4 and GMAP-5 matrix tablets were studied in 900mL phosphate buffer (pH 7.4) using an United States Pharmacopoeia (USP) 6-station Dissolution Rate Test Apparatus (Model Electro lab, TDT-06T, Mumbai, India) with a rotating paddle stirrer at 50 rpm and 37° ± 0.5°C as prescribed for Glipizide tablets in USP XXIII. A sample of Glipizide matrix tablets equivalent to 10 mg of Glipizide was used in
each test. Samples of dissolution fluid were withdrawn through a filter (0.45 μm) at different time intervals and were assayed at 223 nm for Glipizide content using a UV/visible single-beam spectrophotometer-117 (Systronics Corporation, Mumbai, India). *In-vitro* drug release studies were conducted in triplicate (n = 3).

Formulations GPAP-5 and GMAP-5 were found to be optimum for further investigation, so are only considered for further evaluations.

9) **Comparison of *In Vitro* Release Profile of Optimized Formulation with Marketed Formulation**

Dissolution testing was carried out on USP dissolution apparatus II (paddle) for GPAP-5 matrix tablets (optimized formulation) with marketed tablets, which was showed Fig. 4.64.

10) **In Vivo Pharmacodynamic Studies**

The selected GPAP-5 and GMAP-5 matrix tablets were tested in rabbits for their hypoglycemic actions and release patterns, which were showed in Table 4.55 and 4.56.

11) **Pharmacokinetic Evaluation of Formulated Matrix Tablets**

*In vitro* studies of Glipizide *Aloe barbadensis miller* leaf mucilage shown better release pattern compared to other formulations with Guar gum and Povidone, hence Glipizide *Aloe barbadensis miller* leaf mucilage matrix tablets were selected for *In vivo* studies.

*In vivo*, pharmacokinetic and pharmacodynamic evaluation was done on Glipizide *Aloe barbadensis miller* leaf mucilage matrix tablets in
comparison to Glipizide pure drug in Rabbits. Both Glipizide pure drug and Glipizide *Aloe barbadensis miller* leaf mucilage matrix tablets were tested at a dose equivalent to 800 µg/kg body weight of Rabbits. Approval from Institutional animal ethical committee was taken before commencing the animal studies (Reg. no. 470/01/a/CPCSEA dt. 24 August, 2001).

**Estimation of Glipizide in Serum by Reversed Phase HPLC Method:**

A reversed phase HPLC method was used for the estimation of Glipizide concentration in serum.

**Instrumentation:** The following instrumentation system was used

The HPLC system (make: M/s Shimadzu Corporation, Japan.) consisted of UV – visible detector (Shimadzu, model: SPD – 10 AVP), Altima C – 18 column (Phenomenex, DESC: Gemini 5µ C18 110A, Size: 250 mm x 4.6 mm, S/No: 315984 – 17), 2 pumps (Model: LC – 10 ATVP) and a micro syringe of capacity 25 µl (Model: Microliter® # 702, Mfd. by: M/s Hamilton). The mobile phase consists of a mixture of methanol-phosphate buffer in 70:30 ratio (pH 3.0; 0.01 mol/L). The mobile phase was filtered through 0.45µ membrane filter before use and was run at a flow rate of 1mL/min.

**Detection:** The column effluent was monitored at 223 nm.

**Estimation of Glipizide in serum:**
For the estimation of Glipizide in serum samples, a calibration curve was constructed initially by analyzing serum samples containing different amounts of Glipizide as follows.

To a series of tubes containing 0.5mL of serum in each, 0.1 mL drug solution containing 1, 2, 4 and 6 µg of Glipizide were added and mixed. To each tube 1 mL of Methanol was added, mixed thoroughly and centrifuged at 5000 rpm for 20 min. The organic layer (0.5 mL) was taken into a dry tube and the Methanol was evaporated. To the dried residue 0.5mL of mobile phase [a mixture of methanol–phosphate buffer (pH 3.0; 0.01 mol/L) (70:30, v/v)] was added and mixed for reconstitution. Subsequently 20 µl were injected into the column for HPLC analysis.

The Serum concentration of Glipizide and the corresponding peak areas are given in Table 4.59 and shown in Fig.4.67. This calibration curve was used for the estimation of Glipizide in the serum samples collected in the pharmacokinetic evaluation. Serum (0.5 mL) was analyzed for Glipizide using calibration curve.

Serum Glipizide concentrations estimated following the oral administration of Glipizide and its matrix tablets are given in Table 4.60 and shown in Fig.4.68. From the time Vs serum concentration data various pharmacokinetic parameters such as peak concentration (C_max), time at which peak occurred (T_max), Area Under the Curve (AUC), elimination rate constant (K_e), biological half-life (t_1/2), percent absorbed to various times and absorption rate constant (K_a) were calculated in
each case as per known standard methods. The results were represented in Table 4.61.

**Determination of Pharmacokinetic Parameters** 235, 236

**Determination of C<sub>max</sub> and T<sub>max</sub>:**

From the time versus serum concentration curves, peak serum concentration (C<sub>max</sub>) and time at which peak occurred (T<sub>max</sub>) were recorded.

**Determination of Elimination Rate Constant (K<sub>el</sub>) and Biological half-life (t<sub>1/2</sub>):**

Time versus serum concentration data was plotted on a semi logarithmic graph paper. The elimination rate constant (K<sub>el</sub>) was calculated from the slope of the linear line in the elimination phase (the ‘best fit’ linear regression line for the points in the elimination phase was drawn by the method of least squares). The corresponding biological half-life was calculated using the equation 16

\[
t_{1/2} = \frac{0.693}{K_{el}} \quad \text{(16)}
\]

**Determination of Percentages Absorbed to Various Times and Absorption Rate Constant (Ka):**

Percentages absorbed to various times and absorption rate constant (Ka) were calculated from serum concentration data by the described by Wagner and Nelson2, 3. The equation developed for the determination of absorption rate from blood data can be expressed as equation 17.
\[
\frac{dA}{dt} = V_d \frac{dC_b}{dt} + K_{el} C_b
\]

(17)

Where, \( \frac{dA}{dt} \) = absorption rate, \( V_d \) = apparent volume of distribution,

\( \frac{dC_b}{dt} \) = rate of change of blood concentration (\( C_b \)) with respect to time \( t \) and \( K_{el} \) = elimination rate constant.

The equation may be integrated between the limits of \( t = 0 \) and \( t = T \) and divided by \( V_d \) to give Eq 18 and 19.

\[
\frac{A_T}{V_d} = C_T + K_{el} \int_{t=0}^{t=T} C_b dt
\]

(18)

\[
\frac{A_T}{V_d} = C_T + K_{el} \left[ AUC \right]_{t=0}^{t=T}
\]

(19)

Where \( A_T \) = amount of drug absorbed to time \( T \), \( C_T \) = blood concentration at time \( T \) and the quantity under the integral sign is the area under the blood concentration versus time curve between the indicated limits. When the successive values of \( A_T / V_d \) are calculated, a maximum asymptotic value \( [A_T / V_d]_{\infty} \) is obtained. The maximum asymptomatic value is divided into successive values of \( A_T / V_d \) to yield percentage absorbed data i.e., Eq 20.

\[
\left( \frac{A_T / V_d}{[A_T / V_d]_{\infty}} \right) \times 100 \text{ as a function of time}
\]

(20)

A graph of log percent unabsorbed vs time was a linear plot, the slope of which is equal to \( -K_a / 2.303 \) from which \( K_a \) was calculated.

**Estimation of Area under the Curve [AUC]:**
The area under the time versus serum concentration curve (AUC) for 12 h period was estimated, from an arithmetic plot of time versus serum concentration by applying trapezoidal rule. The remaining area from 12 h to $\infty$ time was calculated using the following eq. 21 and 22.

$$[AUC]_{12}^\infty = \frac{\text{Concentration at 12th h}}{K_{el}} \quad \ldots \ldots \quad (21)$$

Then,

$$[AUC]_0^\infty = [AUC]_{12}^\infty + [AUC]_{12}^0 \quad \ldots \ldots \ldots \ldots \quad (22)$$

**Determination of Mean Residence Time:**

The tendency of drugs and metabolites to remain in the body can be assessed by measuring the mean residence time (MRT). Assuming that the drug in the organs and elimination is always in equilibrium with drug in serum, the mean residence time can be defined as the average amount of time spent by drug molecules in the body before being eliminated (under constant clearance conditions). If one considers time course of drug concentration in serum as statistical distribution curve, it can be shown that (e q. 23)

$$\text{MRT} = \frac{\text{AUMC}}{\text{AUC}} \quad \ldots \ldots \ldots \quad (23)$$

Where the AUMC is the area under the ‘first movement curve’ and is obtained from a plot of the product of drug concentration in serum and time versus time from zero to infinity (e q. 24).

$$\text{AUMC} = \int_0^\infty tC(t)dt \quad \ldots \ldots \ldots \quad (24)$$

AUC is the area under the ‘zero movement curve’ and is obtained by plotting the drug concentration in serum versus time (C vs. t) from zero to infinity (e q. 25).
The MRT is considered as the statistical movement analogy to the half-life ($t_{1/2}$). Plots of time versus serum concentration ($t$ vs. $C$) and time versus the product of concentration and time ($t$ vs. $C\cdot t$) were plotted and the area under the corresponding curves i.e. AUC and AUMC respectively were computed. The mean residence time (MRT) in each case calculated as eq. 26

$$MRT = \frac{AUMC}{AUC} \quad \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots (26)$$

12) Accelerated Stability Studies:

Accelerated Stability studies were conducted according to the International Conference on Harmonization (ICH) guidelines for the selected formulations at 40 ± 0.5°C and 75 ± 5 % RH for the period of 3 months using programmable environmental test chamber (Remi, India).

In the present study, accelerated stability studies of GPAP-5 matrix tablets were carried out at a temperature of 40 ± 2°C and relative humidity of 75 ± 5 % for a period of 90 days. All the preparations were divided into 3 sets and were stored at a temperature of 40 ± 2°C and 75 ± 5 % relative humidity. After 30, 60 and 90 days drug content in the formulation was determined. The GPAP-5 matrix tablets were evaluated for physicochemical parameters viz. general appearance, thickness, uniformity of weight, Hardness, Friability and drug content before and after accelerated stability studies, which were tabulated in Table 4.62, 4.63 and showed in Fig. 4.69 to 4.71.

The drug content was determined by withdrawing the sample at 0, 30, 60 and 90 days and analyzed for the drug content by HPLC (Waters, USA). The chromatographic conditions were as follows: column
Lichrospher RP-5µm (125×4 mm); mobile phase: methanol: 0.01M disodium hydrogen phosphate (60:40); flow rate: 1.5 mL min⁻¹; injection volume: 20 µL; detector: UV (at 223 nm for Glipizide and 230 nm for Glimepiride); Retention time: 5.5 min.

3.8.2 Evaluation of Transdermal Patches:

The evaluation includes:

- Physicochemical
- *In vitro*
- *In vivo*

**Physicochemical Evaluation:**

1. **Thickness:**

The purpose of the present study was to check the uniformity of thickness of the formulated transdermal patches. The thickness was measured at five different points of the transdermal patches. Using BAKER Digital caliper, the mean of three readings was calculated, which were shown in Table 4.64 and 4.65.

2. **Uniformity of weight:**

The tests were performed on patches which were dried at 60°C for 4 h prior to testing. Three different patches from individual batches were weighed individually and the average weight was calculated; 20 patches from each formulation were weighed using an electronic balance (Sartorius, 2434, Germany) and mean and relative standard deviations of the weight were determined based on an official method, which were shown in Table 4.66 and 4.67.
3. Moisture content:

The patches were weighed and kept in a desiccator at 40°C and dried for at least 24 h (containing calcium chloride). The patches were weighed until it showed a constant weight. The moisture content was the difference between the constant weight gained and the initial weight and was reported in terms of % (by weight) moisture content\textsuperscript{238, 240}, which were shown in Table 4.68 and 4.69.

4. Flatness and elongation brake:

Longitudinal strips were cut out from the prepared transdermal patches. The flatness of prepared patches was resolute at various points by using vernier calipers\textsuperscript{239, 241}. The percentage elongation brake was determined by noting the length just before the break point and substituted in the eq. 27, which were shown in Table 4.70 and 4.71.

\[
\text{Elongation (\%) = } \frac{L_1 - L_2 \times 100}{L_2} \quad \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots
measured periodically to constant weights, which were shown in Table 4.72 and 4.75.

6. Determination of Tensile Strength:

A 1 X 1cm patch was taken and subjected to studies. Tensile strength was determined using a computerized Precisa bottom-loading balance, with necessary modifications\textsuperscript{242}, which were shown in Table 4.76 and 4.77.

7. Folding Endurance:

Folding endurance of patches was determined by repeatedly folding a small strip of film (2 X 2 cm) at the same place till it broke. The number of times the film could be folded at the same place without breaking was the folding endurance value\textsuperscript{242}, which were shown in Table 4.78 and 4.79.

8. Drug Content Determination of Patches:

Five pieces of 1 cm\textsuperscript{2} each (1 x 1 cm) were cut from different parts of the prepared transdermal patch. Each was taken in separate stoppered conical flasks containing 100 mL of suitable dissolution medium (0.1-N HCL: CH\textsubscript{3}OH mixture) and stirred vigorously for 6 h using magnetic stirrer\textsuperscript{243}. The above solutions were filtered and suitable dilutions were made. Absorbance was observed using UV-Visible spectrophotometer (Systronics 117, Mumbai, India) at their respective wavelengths, against a blank solution. The values were shown in Table 4.80 and 4.81.
9. **In vitro Skin Permeation Test:**

**In Vitro Permeation profile of Glipizide/Glimepiride from transdermal patches**

The GPFB-1, GPFB-2, GPFB-3, GPFB-4, GPFB-5, GPFC-1, GPFC-2, GPFC-3, GPFC-4 GPFC-5, GPFG-1, GPFG-2, GPFG-3, GPFG-4, GPFG-5, GPPT-1, GPPT-2, GPPT-3, GPPT-4, GPPT-5, GPFGP-1, GPFGP-2, GPFGP-3, GPFGP-4, GPFGP-5, The GMFB-1, GMFB-2, GMFB-3, GMFB-4, GMFB-5, GMFC-1, GMFC-2, GMFC-3, GMFC-4 GMFC-5, GMFG-1, GMFG-2, GMFG-3, GMFG-4, GMFG-5, GMPT-1, GMPT-2, GMPT-3, GMPT-4, GMPT-5, GPFGP-1, GPFGP-2, GPFGP-3, GPFGP-4 and GPFGP-5 transdermal patches were subjected to *In vitro* evaluation across rat dorsal skin. After the removal of epidermal hair, skin was cleaned and any adhering subcutaneous tissue and blood vessels were removed. The skin was mounted overnight (12 h) on receptor phase to remove any water soluble (UV absorbing) material. The *In vitro* skin permeation of Glipizide from various transdermal patches was studied using locally fabricated Keshary-Chien type of diffusion cell. The permeability studies were carried out across rat skin. Samples (1.0 mL) were withdrawn and replaced with the same volume of fresh receptor solution, through the sampling port of the diffusion cell at predetermined time intervals till 48 h. Absorbance of the withdrawn samples were measured at 223 nm. The experiments were done in triplicates, simultaneously blanks were also
run and the average values reported\textsuperscript{57, 58}. The values were shown in Table 4.82 to 4.86 and 4.89 to 4.93.

10. \textit{In vivo} Blood Glucose Level Estimation for Selected Formulations:

The \textit{in vitro} studies can be fully explored by \textit{in vivo} studies. \textit{In vivo} evaluation of TDDS was carried out using animal models\textsuperscript{229} (rabbits). The values were shown in Table 4.96, 4.97 and showed in Fig. 4.98, 4.99. The selected GPFGP-5 and GMFGP-5 were tested \textit{in vivo} using rabbits\textsuperscript{341}.

11. Skin Irritation Tests:

The rabbits were divided into 5 groups and each group contain 6 animals (n=6). The hair present on the back of rabbits was removed on one day before starting the study. Group-I animals were considered as normal (without any drug treatment). Group-II animals were considered as control) were applied with official adhesive tape-USP. Transdermal blank patch (placebo-without drug) and transdermal patch with drug were applied onto open skin of animals of Group-III & IV. Group-IV were applied with Formalin solution (0.8\% v/v) as a standard irritant. The animals were applied with new transdermal patch/formalin solution each day up to 7 days. The application sites were graded (visual scoring scale-always by the same person). The erythema scale as 0, 1, 2, 3 and 4 indicates none, slight, well defined, moderate and scar formation respectively. The edema scale as 0, 1, 2, 3 and 4 represents none, slight,
well defined, moderate and severe. The values were tabulated in Table 4.100.

12. Accelerated Stability Studies:

The optimized transdermal patches (GPFGP-5) were divided into 3 sets and were stored at a temperature of 40±2°C and 75±5% relative humidity. After 30, 60 and 90 days drug content in the formulation was determined. At the end of 90 days the physicochemical parameters like thickness, flatness, folding endurance, tensile strength, moisture content, moisture uptake, drug content and drug permeation were studied. The values were tabulated in Table 4.101, 4.102 and showed in 4.93 to 4.95.

4.1 ANALYSIS OF DRUGS USED

4.1.1 Standard Calibration Curves of Glipizide/ Glimepiride:

Table 4.1: Glipizide concentration and its relative absorbance