Chapter 5

Simultaneous Determination of Metformin and Glimepiride in Pharmaceutical Dosage Form by Reverse Phase Liquid Chromatography
5.1 Drug profile

5.1.1 Metformin Hydrochloride

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5.1.2 Glimepiride [1-2]

a. Structure:

![Chemical Structure of Glimepiride]

b. Chemical name: \((1-[4 -[2 -(3-ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamido)ethyl ]-phenylsulfonyl ]-3-(4-methylcyclohexyl)urea )\)

c. Molecular formula: \(C_{24}H_{34}N_{4}O_{5}S\)

d. Molecular weight: 490.62

e. Description: White to almost white powder.

f. Solubility: Soluble in DMF, slightly soluble in methanol, methylene chloride, practically insoluble in water. It also dissolves in dilute alkali hydroxides and in dilute acids.

g. Melting point: 207°C

h. Identification: Compare the Infrared absorption spectrum with reference spectrum of glimepiride.

i. Storage: Store in well closed containers, at a temperature not exceeding 25°C.

j. Therapeutic category: Antidiabetic

k. Dosage forms: Tablets of combination of Metformin HCl and Glimepiride are available in the following strengths [3]

1. Metformin HCl (1000 mg) + Glimepiride(1mg) Tablets
2. Metformin HCl (1000 mg) + Glimepiride (2 mg) Tablets
3. Metformin HCl (500 mg) + Glimepiride (1 mg) Tablets
4. Metformin HCl (500 mg) + Glimepiride (2 mg) Tablets
5.2 Introduction
A combination of Metformin (1,1-dimethyl biguanide hydrochloride) and Glimepiride (1-[4 -(3-ethyl-4 -methyl -2-oxo -3 -pyrroline -1-carboxamido)ethyl ] - phenylsulfonyl ] -3-(4-methylcyclohexyl)urea ) is available commercially as tablets. Glimepiride is a sulphonylurea targeting insulin secretion and is most commonly used to treat patients with type 2 diabetes. Metformin is an oral agent that targets insulin resistance. Clinical studies have indicated that the combination provides better hyperglycemic control than when given alone, with added advantages of patient compliance [4].

5.2.1 Reported methods for the analysis of metformin and glimepiride
Literature survey revealed that few methods are reported for the individual estimation of metformin, some for the estimation of metformin in tablets [5-7] or in plasma [8-11]. One method is published for determination of glimepiride isomers [13], but no single method has been published for simultaneous determination of metformin and glimepiride in tablets.

In the present research work attempts have been made to develop a method for the simultaneous determination of metformin and glimepiride in tablets.

For the analyses of the active ingredients of the combined dosage form sometimes it requires two separate sample preparations due to large differences in the label claims. Though large differences are there in the label claims of the active ingredients of this dosage form, the analyses have been done with the same sample preparation.

The reported methods [5,6] are the pharmacopoeial methods for the estimation of metformin in tablets. While the other method [7] is for the estimation of metformin in combination with glipizide and gliclazide in tablets by HPLC.

5.3 Experimental

5.3.1 Materials and Reagents
Metformin HCl and Glimepiride were obtained from Wockhardt Research Centre (Aurangabad, Maharashtra State (M.S.), India). Sodium dodecyl sulphate (SDS), and disodium hydrogen phosphate were obtained from E. Merck (India) Ltd. Worli, Mumbai. Orthophosphoric acid, and acetonitrile (HPLC grade) was obtained from Qualigens Fine Chemicals, Dr. Annie

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Besant Road, Mumbai, India. The 0.45-µm nylon filter was obtained from Advanced Microdevices Pvt. Ltd. Ambala Cantt, India. The tablets of glimepiride in combination with metformin were purchased from the Indian market. Double distilled water was used throughout the experiment. Other chemicals were of analytical or HPLC grade.

5.3.2 Chromatographic conditions

A HPLC instrument (Thermoseparation Products) was utilized consisting of a pump (Constametric 3500), an autosampler (AS 3000) and a uv-d detector (UV 1000). A reversed –phase column (Agilent Zorbax XDB C18, 5 µm, 4.6 x 150 mm ) was used. The mobile phase flow rate was 1 mL /min, the column oven temperature was 40ºC, and the detection of the analytes was done at a wavelength of 226 nm. The injection volume was a 25 µL. Data acquisition was done with commercial software (PC 1000, Thermo Separation Products, Illinois, USA). The peak purity was checked with a photodiode array detector (Thermo Separation Products, UV6000 LP, Illinois, USA).

5.3.3 Preparation of solutions

a. Mobile phase

The mobile phase consisted of an aqueous buffer and acetonitrile in the ratios of (68:32, v/v). The buffer consisted of 10 mmol/L disodium hydrogen phosphate and 10 mmol/L sodium dodecyl sulfate (SDS) in doubly distilled water adjusted to pH 7.5 with orthophosphoric acid. The mobile phase was premixed, filtered through a 0.45- µm nylon filter, and degassed.

b. Standard solutions

Standard solutions were prepared by dissolving the drugs in the diluents and diluting them to the desired concentration. Diluents used for the standard preparation and sample preparation were prepared as follows

i. Buffer: 10 mmol/L disodium hydrogen phosphate in doubly distilled water, pH adjusted to 8.0 with 10% orthophosphoric acid.

ii. Diluent: composed of buffer and acetonitrile in the ratios of (60:40, v/v)

iii. Metformin standard

A 125 mg sample of metformin HCl was accurately weighed, transferred in a 50 mL volumetric flask, and dissolved with the diluent.
iv. Glimepiride standard
A 20 mg sample of glimepiride was accurately weighed, transferred in a 100 mL volumetric flask, 70 mL of acetonitrile and 20 mL of buffer was then added, and sonication was performed for 5 min. After sonication the solution was cooled to room temperature and diluted to volume with diluent. This solution was further diluted by transferring 2.5 mL of this solution in a 50 mL volumetric flask and diluted with the diluent.

v. Mixed standard preparation
A mixed standard solution was prepared from these stock solutions by transferring 2 mL of a metformin standard solution, 2 mL of glimepiride standard solution in a 50 mL volumetric flask and diluted with diluent. This solution contains 100 µg/mL of metformin HCl and 0.4 µg/mL of glimepiride.

vi. Assay preparation
Ten tablets were weighed and finely powdered. A quantity equivalent to one tablet containing 500 mg of metformin HCl and 2 mg of glimepiride was transferred to a 100 mL volumetric flask, 70 mL of acetonitrile and 20 mL of diluent was then added and sonication was performed for 30 min with intermittent shaking. After sonication the solution was cooled to ambient temperature and diluted to volume with diluent and mixed well. The solution was centrifuged at 10000 rpm for 5 min. From the centrifuged solution 1 mL of clear solution was transferred into a 50 mL volumetric flask and diluted with diluent.

5.4 Results and Discussion
5.4.1 Optimisation of chromatographic conditions
A reversed-phase column procedure was proposed as a suitable method for the simultaneous determination of metformin and glimepiride in combined dosage form. The chromatographic conditions were optimized by changing the mobile phase composition, pH and buffers used in the mobile phase. Different experiments were performed, to optimize the mobile phase. Ion pair reagent, 1-octane sulphonic acid sodium salt, was also used in the mobile phase in different concentrations but the adequate retention of metformin was not achieved. Finally, SDS was used in the mobile phase, and it was observed
that the peak of metformin eluted at 5 min. To optimize retentions and the resolution, effects of the mobile phase components, changes in ionic strength, SDS concentration, and pH were studied. From the previously mentioned studies, it was determined that at 10 mM SDS concentration and 10 mM disodium hydrogen phosphate in doubly distilled water and acetonitrile in the ratios of (68:32,v/v) after mixing the mobile phase components pH was adjusted to 7.5 with orthophosphoric acid, was found to be an appropriate mobile phase allowing adequate separation of active substances of the combined dosage form.

Typical chromatograms obtained by using the aforementioned mobile phase, from a 25 µL of the mixed standard preparation is illustrated in Figure 1. The retention factors of metformin and glimepiride were 14.27 and 8.14 respectively.

5.5 Validation of the method
5.5.1 Specificity
The specificity of the method was checked by peak purity test of the sample preparation done by the software of the photodiode array detector. The peak purity for metformin and glimepiride was observed to be 997 and 999. The results of the peak purity analysis shows that the peaks of the analytes were pure and also the formulation excipients were not interfering with the analyte peaks.

5.5.2 Linearity
The linearity of the method was tested from 25-150% of the targeted level of the assay concentration (metformin HCl 100 µg/mL and Glimepiride 0.4 µg/mL) for both the analytes. For linearity the mixed standard solutions containing 25 µg/mL - 150 µg/mL of metformin HCl and 0.1 µg/mL - 0.6 µg/mL of glimepiride were prepared. Linearity solutions were injected in triplicate. The calibration graphs were plotted by using peak area of the analyte against the concentration of the drug in µg/mL.

In the simultaneous determination, the calibration graphs were found to be linear for both the analytes in the mentioned concentrations. The regression equations for metformin and glimepiride were found to be $y=75839x+117487$ and $y=60679x-31.357$ and the correlation coefficients for the
regression line were 0.9968 and 0.9947 respectively. The tabulated results and related linearity graphs are shown in Table-I and Figure-2A and 2B respectively.

5.5.3 Accuracy
Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts of the drug to the placebo. The recovery was done at three levels 80%, 100% and 120% of the label claim per tablet (500 mg of metformin HCl and 2 mg of glimepiride). Three samples were prepared for each recovery level. The recovery values for metformin HCl and glimepiride ranged from 98.9-100.5 and 98.8-99.8 respectively (Table II A and II B). The average recovery of three levels (nine determinations) for metformin and glimepiride were 99.6 and 99.4 respectively.

5.5.4 Precision
The precision (repeatability and intermediate precision) of the method was determined from one lot of combined dosage form.

a. Repeatability
For repeatability of the method six determinations of the amounts of each active ingredient in the tablets were done. The results are shown in the Table III.

b. Intermediate precision
For intermediate precision of the method multiple execution of the analysis by different chemists and on different instruments and on different days, preferentially using also different columns was performed. The percentage assay was calculated using the area of the mixed standard preparation. The assay results are shown in Table IV.

5.5.5 Determination of LOD and LOQ
For determining LOD and LOQ the method based on the residual standard deviation of a regression line and slope was adopted. To determine the LOD and LOQ, a specific calibration curve was studied using samples containing the analytes in the range of detection limit and quantitation limit. The limit of detection for metformin, glimepiride was 0.013 µg/mL, 0.007 µg/mL and
The limit of quantitation was 0.039 µg/mL, 0.022 µg/mL respectively. The results are shown in Table-V.

5.5.6 Solution stability

The stability of the standard solutions and the sample solutions were performed at intervals of 24 hr, 48 hr and 72 hr. The stability of solution was determined in terms of the assay of the drugs in standard solutions and sample solutions against the freshly prepared standard solutions. The relative standard deviation for the assay values determined up to 72 hr for metformin and glimepiride in sample preparation were 0.59% and 0.78% respectively. The assay values were within ± 2% after 72 hr. The results indicate that the solutions were stable for 72 hr at ambient temperature. The results are tabulated in Table-VI.

5.5.7 System suitability

For system suitability studies, five replicate injections of mixed standard solutions were injected and the parameters like % RSD of peak area, column efficiency, resolution, and tailing factor of the peaks were calculated. Results are shown in Table-VII.

5.6 Application of the method for analysis of tablets

The optimized and validated chromatographic conditions and sample procedure was applied for the analysis of tablets containing metformin and glimepiride. The assays were done (six times) and precision was established in two sets one for repeatability and intermediate precision. The results are shown in Table-III and IV.

5.7 Conclusion

The recovery values for metformin and glimepiride by the proposed method are 99.6% and 99.4 % with a relative standard deviation (RSD) less than 1.0%. The proposed method is rapid, accurate, precise and less expensive and can be used in the quality control departments for the assay of the tablets containing metformin and glimepiride.
### 5.8 : Result tables

**Table-I**

**Linearity**

<table>
<thead>
<tr>
<th>Level</th>
<th>Concentration (ppm)</th>
<th>Metformin</th>
<th>Glimepiride</th>
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<td>1973188</td>
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<td>50</td>
<td>0.2</td>
<td>3806448</td>
</tr>
</tbody>
</table>

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### Table-IIA Recovery (Accuracy study)
#### Analyte : Metformin HCl

<table>
<thead>
<tr>
<th>Level of addition (%)</th>
<th>Sample preparation</th>
<th>Amount added (mg)</th>
<th>Amount found (mg)</th>
<th>%Recovery</th>
<th>Mean ± SD</th>
<th>%RSD</th>
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<tbody>
<tr>
<td>80%</td>
<td>1</td>
<td>401.1</td>
<td>403.06</td>
<td>100.5</td>
<td>100.5+0.28</td>
<td>0.28</td>
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<td></td>
<td>2</td>
<td>399.5</td>
<td>400.39</td>
<td>100.2</td>
<td>100.8</td>
<td></td>
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<tr>
<td></td>
<td>3</td>
<td>400.4</td>
<td>403.57</td>
<td>100.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100%</td>
<td>1</td>
<td>501.5</td>
<td>498.68</td>
<td>99.4</td>
<td>98.9+0.46</td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>504.2</td>
<td>497.88</td>
<td>98.7</td>
<td>100+0.91</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>503.4</td>
<td>496.15</td>
<td>98.6</td>
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<td></td>
</tr>
<tr>
<td>120%</td>
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<td>601.2</td>
<td>597.8</td>
<td>99.4</td>
<td>100+0.91</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>603.4</td>
<td>600.6</td>
<td>99.5</td>
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<tr>
<td></td>
<td>3</td>
<td>598.7</td>
<td>605.1</td>
<td>101.1</td>
<td></td>
<td></td>
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<td><strong>Average Recovery (%)</strong></td>
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<td></td>
<td></td>
<td><strong>99.8+0.88</strong></td>
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<td>0.88</td>
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</table>

### Table-II B : Recovery (Accuracy study)
#### Analyte : Glimepiride

<table>
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<tr>
<th>Level of addition (%)</th>
<th>Sample preparation</th>
<th>Amount added (mg)</th>
<th>Amount found (mg)</th>
<th>%Recovery</th>
<th>Mean ± SD</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>80%</td>
<td>1</td>
<td>1.6</td>
<td>1.59</td>
<td>99.3</td>
<td>99.5+0.34</td>
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<td>1.6</td>
<td>1.59</td>
<td>99.2</td>
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<td>1.6</td>
<td>1.60</td>
<td>99.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100%</td>
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<td>1.96</td>
<td>98.2</td>
<td>98.8+1.39</td>
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<td>2</td>
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<td>2.01</td>
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Table-III
Repeatability
Analysis of tablets containing Metformin HCl 500mg and Glimepiride 2mg

<table>
<thead>
<tr>
<th>Sample preparation</th>
<th>Metformin HCl Found (mg)</th>
<th>Glimepiride Found (mg)</th>
<th>% Assay of Metformin HCl</th>
<th>% Assay of Glimepiride</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>496.47</td>
<td>1.93</td>
<td>99.3</td>
<td>96.5</td>
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<td>2</td>
<td>494.14</td>
<td>1.95</td>
<td>98.8</td>
<td>97.5</td>
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<td>3</td>
<td>491.13</td>
<td>1.95</td>
<td>98.2</td>
<td>97.5</td>
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<tr>
<td>4</td>
<td>489.50</td>
<td>1.96</td>
<td>97.9</td>
<td>98.2</td>
</tr>
<tr>
<td>5</td>
<td>498.75</td>
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<td>99.8</td>
<td>95.3</td>
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<tr>
<td>6</td>
<td>508.35</td>
<td>1.87</td>
<td>101.7</td>
<td>93.5</td>
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<tr>
<td>Mean</td>
<td>496.5</td>
<td>1.93</td>
<td>99.3</td>
<td>96.4</td>
</tr>
<tr>
<td>SD</td>
<td>6.76</td>
<td>0.04</td>
<td>1.35</td>
<td>1.76</td>
</tr>
<tr>
<td>%RSD</td>
<td>1.36</td>
<td>1.82</td>
<td>1.36</td>
<td>1.82</td>
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</tbody>
</table>

Table-IV: Intermediate precision study

<table>
<thead>
<tr>
<th>Sample preparation</th>
<th>Metformin HCl Found (mg)</th>
<th>Glimepiride Found (mg)</th>
<th>% Assay of Metformin HCl</th>
<th>% Assay of Glimepiride</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>506.55</td>
<td>1.91</td>
<td>101.3</td>
<td>95.7</td>
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<td>493.84</td>
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<td>491.83</td>
<td>1.97</td>
<td>98.4</td>
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<td>495.98</td>
<td>1.93</td>
<td>99.2</td>
<td>96.7</td>
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<td>491.52</td>
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<td>494.50</td>
<td>1.95</td>
<td>98.9</td>
<td>97.3</td>
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</tbody>
</table>
### Table-V

#### Calibration curve for determination of LOD & LOQ

<table>
<thead>
<tr>
<th>Calibration level</th>
<th>Mixed standard (conc in ppm)</th>
<th>Avg response peak height (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Metformin HCl</td>
<td>Glimepiride</td>
</tr>
<tr>
<td>1</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>2</td>
<td>0.02</td>
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<tr>
<td>3</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>4</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>5</td>
<td>0.08</td>
<td>0.08</td>
</tr>
<tr>
<td>6</td>
<td>0.10</td>
<td>0.10</td>
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<tr>
<td>7</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>8</td>
<td>0.30</td>
<td>0.30</td>
</tr>
</tbody>
</table>

Slope from the calibration curve (S): 7071, 5658
Standard deviation of residuals (σ): 27.244937, 12.394954
LOD: 0.013, 0.007
LOQ: 0.039, 0.022

### Table-VI

#### Solution stability

<table>
<thead>
<tr>
<th>Sample</th>
<th>Initial (%)</th>
<th>24 hrs (%)</th>
<th>48 hrs (%)</th>
<th>72 hrs (%)</th>
<th>Mean (%)</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>Metformin</td>
<td>100.0</td>
<td>99.2</td>
<td>98.6</td>
<td>99.0</td>
<td>99.2</td>
</tr>
<tr>
<td></td>
<td>Glimepiride</td>
<td>100.0</td>
<td>99.4</td>
<td>99.0</td>
<td>98.3</td>
<td>99.2</td>
</tr>
<tr>
<td>Sample</td>
<td>Metformin</td>
<td>101.1</td>
<td>100.3</td>
<td>99.7</td>
<td>100.0</td>
<td>100.3</td>
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<td></td>
<td>Glimepiride</td>
<td>98.0</td>
<td>96.4</td>
<td>96.5</td>
<td>97.1</td>
<td>97.0</td>
</tr>
</tbody>
</table>

### Table-VII

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## System suitability

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Parameter</th>
<th>Metformin</th>
<th>Glimepiride</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Theoretical plates(^1)</td>
<td>9700</td>
<td>4150</td>
</tr>
<tr>
<td>2</td>
<td>Retention factors</td>
<td>14.27</td>
<td>8.14</td>
</tr>
<tr>
<td>3</td>
<td>Tailing factor</td>
<td>1.02</td>
<td>1.05</td>
</tr>
<tr>
<td>4</td>
<td>%RSD</td>
<td>0.54</td>
<td>1.16</td>
</tr>
</tbody>
</table>

\(^1\) per column length.
Figure 2A: Linearity graph for Glimepiride
Graph-I
Linearity plot for glimepride

\[ y = 60679x - 31.357 \]
\[ R^2 = 0.9947 \]

Figure 2 B : Linearity graph for metformin

Graph-II
Linearity plot for metformin

\[ y = 75839x + 117487 \]
\[ R^2 = 0.9968 \]

5.10 References: