A) DEVELOPMENT AND VALIDATION OF HPTLC METHOD FOR THE
ESTIMATION OF METFORMIN HYDROCHLORIDE AND
PIOGLITAZONE IN PHARMACEUTICAL DOSAGE FORM

Selection of Plate

Precoated silica gel 60F_{254} on aluminium sheets was selected for study

Selection of Solvent

Ideally, a solvent employed for HPTLC should have the following properties

a) The drug should be soluble in the solvent used

b) The drug should show stability in the solvent used

c) The solvent should be volatile

d) The drug should not react with the solvent used

Methanol was selected as the solvent

Selection of Wavelength

UV spectra of Metformin Hydrochloride and Pioglitazone on pre-coated plate were recorded. At 230 nm Metformin Hydrochloride and Pioglitazone gave satisfactory separation and good resolution peaks. Hence 230 nm was selected for detection, Fig.6.1.

Development of Optimum Mobile Phase

An ideal mobile phase should give dense compact spots and acceptable degree of separation of drugs from each other. Such mobile phase is selected for the study. For this purpose different mobile phases were tried and results were given in the Table.
### Mobile Phases Used for Experiment

<table>
<thead>
<tr>
<th>Mobile Phase</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol : Chloroform (8 : 2 % v/v)</td>
<td>Poor separation</td>
</tr>
<tr>
<td>Methanol : Toluene (3 : 7 % v/v)</td>
<td>Poor separation</td>
</tr>
<tr>
<td><strong>Toluene: Methanol: Triethylamine (8 : 2 : 0.1 % v/v/v)</strong></td>
<td>Good separation with symmetric peak</td>
</tr>
<tr>
<td>Methanol : Ethyl acetate : Chloroform (3 : 6 : 1 % v/v/v)</td>
<td>Poor separation</td>
</tr>
<tr>
<td>Methanol : Toluene : n-Hexane (2 : 7 : 1 % v/v/v)</td>
<td>Poor separation</td>
</tr>
</tbody>
</table>

Among these mobile phase systems, Toluene: Methanol: Triethylamine was selected, because it showed good, compact and dense spots.

**Ratio of Mobile Phase**

With different ratios of **Toluene: Methanol: Triethylamine** (4:6:0.1, 5:5:0.1, 8:2:0.1, 6:4:0.1, 7:3:0.1) etc. were tried. The ratio of **8:2:0.1 % v/v/v** was selected because it gave required degree of compact spots with good resolution between analytes and excellent separation from solvent front and sample application positions.
<table>
<thead>
<tr>
<th>Parameters Used for the HPTLC Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stationary phase : Pre-coated silica gel 60F_{254} on aluminium sheets.</td>
</tr>
<tr>
<td>Mobile phase : Toluene: Methanol: Triethylamine (8:2:0.1% V: V: V)</td>
</tr>
<tr>
<td>Chamber saturation time : 20 minutes</td>
</tr>
<tr>
<td>Migration distance : 80 mm</td>
</tr>
<tr>
<td>Band width : 6 mm</td>
</tr>
<tr>
<td>Slit dimension : 5 X 0.45 mm</td>
</tr>
<tr>
<td>Source of radiation : Deuterium lamp</td>
</tr>
<tr>
<td>Wavelength scanning : 230 nm</td>
</tr>
</tbody>
</table>

R_f values of

- Metformin Hydrochloride : 0.25±0.03
- Pioglitazone : 0.47±0.04
Fig. 6.1: UV Spectrum of Standard Metformin Hydrochloride and Pioglitazone on TLC Plate
VALIDATION OF THE METHOD

The validation of the method was carried out in terms of linearity, limit of detection (LOD), limit of quantification (LOQ), inter and Intra day precision, repeatability of sample application, Robustness and selectivity.

**Linearity and Range**

Linear regression data showed a good linear relationship over a concentration range of 50 - 300 ng/spot for Metformin Hydrochloride and 1.5 - 9.0 ng/spot for Pioglitazone. The slope, intercept and correlation coefficient values for Metformin Hydrochloride were found to be, 0.8063, 55.8666, and 0.998 respectively, (Fig. 6.2). The slope, intercept and correlation co-efficient values for Pioglitazone were found to be 16.4990, 39.4333, and 0.998 respectively, (Fig. 6.3).

**Limit of Detection (LOD) and Limit of Quantification (LOQ)**

The LOD and LOQ of the drugs were determined by applying decreasing amounts of the drugs in triplicate on the plate. The lowest concentration at which the peak is detected is called the ‘Limit of Detection’, which was found to be 10 and 0.5 ng/spot for Metformin Hydrochloride and Pioglitazone respectively. The lowest concentration at which the peak is quantified is called ‘Limit of Quantification’, which was found to be 50 ng/spot and 1.5 ng/spot for Metformin Hydrochloride and Pioglitazone respectively.

**Recording of the Chromatogram for Standards**

With the fixed chromatographic conditions, 1-6 µl from standard stock solution of 50 µg/ml of Metformin Hydrochloride and 1.5 µg/ml of Pioglitazone solution were applied on Precoated TLC plate. The plate was analyzed photo metrically and chromatograms were recorded, Fig. 6.4-6.9.
Precision

Precision of the method was demonstrated by

i) Intra day precision

ii) Inter day precision

iii) Repeatability
    a) Repeatability of measurement
    b) Repeatability of sample application

i) Intra day Precision

Intra day precision was found out by carrying out the analysis of the standard drugs at three different concentrations in the linearity range of drugs, for three times on the same day. Each concentration was applied in duplicate and % RSD was calculated, Table 6.1

ii) Inter day Precision

Inter day precision was found out by carrying out the analysis of the standard drugs at three different concentrations in the linearity range of drugs for three days over a period of one week and % RSD was calculated, Table 6.2

iii) Repeatability

a) Repeatability of Sample Application

Repeatability of sample application was assessed by spotting 4 µl of drug solution six times on pre-coated TLC plate followed by development of plate and %RSD was calculated, Table 6.3.

b) Repeatability of Measurement

Repeatability of measurement of peak area was determined by spotting 4 µl of standard drug solutions on pre-coated TLC plate. After development of the plate, the
separated spots were scanned six times without changing position of the plate and %RSD was calculated and given in Table 6.4.

**Stability Studies**

When the developed chromatographic plate was exposed to atmosphere, the analytes were likely to decompose. Hence it is necessary to conduct stability studies.

Stability of the analytes on the plate was studied at different time intervals and peak areas were compared with the peak area of freshly scanned plate. The developed plate was found to be stable for about 5 hours as the reduction in peak areas was within the limits, Table 6.5.

**Specificity**

The peak purity of both Metformin Hydrochloride and Pioglitazone was assessed by comparing their respective spectra at peak start, peak apex and peak end positions of the spot. The ideal correlation among spectra acquired at start (s), apex (m) and end (e) of the peaks indicatives of peak purity for both Metformin Hydrochloride \{correlation r(s, m) = 0.9999, r(m, e) =0.999 \} and Pioglitazone \{correlation r(s, m) = 0.9998, r (m, e) = 0.999 \}. It can be concluded that no impurities or degradation products migrated with the peaks obtained from standard solutions of the drugs.
Fig. 6.2: Calibration Graph of Metformin Hydrochloride (50 - 300 ng/spot)

Fig. 6.3: Calibration Graph of Pioglitazone (1.5 - 9 ng/spot)
Table 6.1: Intra day Precision

<table>
<thead>
<tr>
<th>Volume applied (µl)</th>
<th>Peak area</th>
<th>%* RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MET</td>
<td>PIO</td>
</tr>
<tr>
<td></td>
<td>1220.4</td>
<td>792.5</td>
</tr>
<tr>
<td>3</td>
<td>1235.2</td>
<td>799.3</td>
</tr>
<tr>
<td></td>
<td>1226.3</td>
<td>801.2</td>
</tr>
<tr>
<td></td>
<td>1632.6</td>
<td>1042.3</td>
</tr>
<tr>
<td>4</td>
<td>1645.3</td>
<td>1039.2</td>
</tr>
<tr>
<td></td>
<td>1630.2</td>
<td>1056.2</td>
</tr>
<tr>
<td></td>
<td>2062.7</td>
<td>1255.8</td>
</tr>
<tr>
<td>5</td>
<td>2075.1</td>
<td>1258.3</td>
</tr>
<tr>
<td></td>
<td>2060.3</td>
<td>1264.5</td>
</tr>
</tbody>
</table>

MET- Metformin Hydrochloride and PIO- Pioglitazone
### Table 6.2: Inter day Precision

<table>
<thead>
<tr>
<th>Volume applied (μl)</th>
<th>Day</th>
<th>Peak area</th>
<th>%* RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MET</td>
<td>PIO</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1220.4</td>
<td>792.5</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1231.2</td>
<td>801.3</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1228.3</td>
<td>798.2</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1632.6</td>
<td>1042.3</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1642.2</td>
<td>1051.2</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1638.3</td>
<td>1048.2</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2062.7</td>
<td>1255.8</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2072.1</td>
<td>1262.2</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2068.3</td>
<td>1270.1</td>
</tr>
</tbody>
</table>

MET- Metformin Hydrochloride and PIO- Pioglitazone
Table 6.3: Repeatability of Sample Application

<table>
<thead>
<tr>
<th>Volume applied (μl)</th>
<th>Peak area</th>
<th>% RSD</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MET</td>
<td>PIO</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1645.3</td>
<td>1048.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1652.4</td>
<td>1050.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1639.4</td>
<td>1051.0</td>
<td>0.447</td>
</tr>
<tr>
<td></td>
<td>1648.6</td>
<td>1042.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1660.2</td>
<td>1035.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1655.2</td>
<td>1032.3</td>
<td></td>
</tr>
</tbody>
</table>

MET- Metformin Hydrochloride and PIO- Pioglitazone

Table 6.4: Repeatability of Measurement

<table>
<thead>
<tr>
<th>Volume applied (μl)</th>
<th>Peak area</th>
<th>% *RSD</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MET</td>
<td>PIO</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1632.6</td>
<td>1042.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1642.1</td>
<td>1028.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1639.2</td>
<td>1032.2</td>
<td>0.442</td>
</tr>
<tr>
<td></td>
<td>1643.4</td>
<td>1026.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1629.2</td>
<td>1030.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1649.1</td>
<td>1040.6</td>
<td></td>
</tr>
</tbody>
</table>

MET- Metformin Hydrochloride and PIO- Pioglitazone
Table 6.5: Stability of the Plate

<table>
<thead>
<tr>
<th>Volume applied (μl)</th>
<th>Time (hrs)</th>
<th>Peak area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MET</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>1632.6</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1620.1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1583.4</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1564.3</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1542.0</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1520.6</td>
</tr>
</tbody>
</table>

MET- Metformin Hydrochloride and PIO- Pioglitazone
CHROMATOGRAMS OF STANDARDS

Fig. 6.4: Metformin Hydrochloride (50 ng/spot) and Pioglitazone (1.5 ng/spot)

Fig. 6.5: Metformin Hydrochloride (100ng/spot) and Pioglitazone (3.0 ng/spot)
Fig. 6.6: Metformin Hydrochloride (150ng/spot) and Pioglitazone (4.5 ng/spot)

Fig. 6.7: Metformin Hydrochloride (200ng/spot) and Pioglitazone (6.0 ng/spot)
Fig. 6.8: Metformin Hydrochloride (250 ng/spot) and Pioglitazone (7.5 ng/spot),

Fig. 6.9: Metformin Hydrochloride (300 ng/spot) and Pioglitazone (9 ng/spot)
ANALYSIS OF FORMULATION

Preparation of Standard Solution: Stock solution of Metformin Hydrochloride 50 µg/ml and Pioglitazone 1.5 µg/ml were prepared in Methanol.

Preparation of Sample Solution

Twenty tablets, each tablet containing 500 mg of Metformin Hydrochloride and 15 mg of Pioglitazone were weighed and average weight was calculated. Quantity equivalent to 5 mg of Metformin Hydrochloride and 0.15 mg of Pioglitazone were weighed and transferred into a 100 ml volumetric flask, extracted with methanol and finally made up to 100 ml with the same solvent and this solution was filtered and used for further study.

Accuracy

Recovery studies of the drugs were carried out for determining accuracy parameter. It was done by mixing known quantity of standard drugs with the analyzed sample formulation and the contents were reanalyzed by the proposed method. Recovery studies carried out at 80, 100 and 120% levels. The percentage recovery and its % RSD were calculated, Table 6.6.

Recording the chromatogram of formulation

With the fixed chromatographic conditions, 2 µl from sample stock solution containing 50 µg/ml of Metformin Hydrochloride and 1.5 µg/ml of Pioglitazone were applied on Precoated TLC plate. The plate was analyzed photo metrically and chromatograms were recorded by application of sample solution obtained from the formulation, Fig. 6.10.

Peak areas of sample chromatograms were compared and amount of Metformin Hydrochloride and Pioglitazone in formulations were calculated, Table 6.7.
### Table 6.6: Recovery Studies

<table>
<thead>
<tr>
<th>Level</th>
<th>% Recovery</th>
<th>% RSD*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MET</td>
<td>PIO</td>
</tr>
<tr>
<td>80%</td>
<td>99.56</td>
<td>98.97</td>
</tr>
<tr>
<td>100%</td>
<td>101.23</td>
<td>100.89</td>
</tr>
<tr>
<td>120%</td>
<td>98.92</td>
<td>101.93</td>
</tr>
</tbody>
</table>

*RSD of six observations, MET - Metformin Hydrochloride, PIO - Pioglitazone

### Table 6.7: Analysis of Formulation

<table>
<thead>
<tr>
<th>Drug</th>
<th>Labeled</th>
<th>Estimated</th>
<th>% Label claim</th>
<th>% RSD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>MET</td>
<td>500</td>
<td>494.3</td>
<td>98.86</td>
<td>0.326</td>
</tr>
<tr>
<td>PIO</td>
<td>15</td>
<td>14.67</td>
<td>97.81</td>
<td>0.513</td>
</tr>
</tbody>
</table>

*RSD of six observations, MET - Metformin Hydrochloride, PIO - Pioglitazone
Fig. 6.10: Chromatogram of Metformin Hydrochloride (100 ng/spot) and Pioglitazone (3ng/spot) Formulation
B) DEVELOPMENT AND VALIDATION OF HPLC METHOD FOR THE
ESTIMATION OF METFORMIN HYDROCHLORIDE AND PIOGLITAZONE IN
PHARMACEUTICAL DOSAGE FORM

1. Selection of Chromatographic Method for Separation

Reverse phase chromatographic techniques was selected, since both the drugs are polar in nature.

2. Selection of Wavelength

Selectivity of HPLC method that uses UV detector depends on proper selection of wavelength. A wavelength which gives desirable response for the drugs to be detected is to be selected. From the UV spectra obtained for both drugs, 230 nm was selected as the wavelength for study.

Initial Conditions

Stationary phase : PhenomenaxC\textsubscript{18} column (250x4.6) mm i.d., 5\textmu m, particle size
Flow rate : 1ml/minute
Operating temperature : Room temperature
Selected wavelength : 230 nm
Optimization of Separation Condition

Solvent selectivity (solvent type), solvent strength (percentage of organic solvent in the mobile phase), strength and pH of buffer, flow rate etc. were varied to determine the chromatographic conditions that gave the best separation.

1. Effect of Ratio of Mobile Phase

In a mobile phase system consisting water and Acetonitrile in different ratios like 70:30, 60:40 and 80:20 %v/v, a mixture of Metformin Hydrochloride and Pioglitazone was injected. Symmetrical peaks with desirable resolution and more number of theoretical plates was obtained with a ratio of 60:40 %v/v and hence selected for further studies.

<table>
<thead>
<tr>
<th>Water: Acetonitrile(%v/v)</th>
<th>Resolution</th>
<th>No. of theoretical plates</th>
<th>Figure No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MET</td>
<td>PIO</td>
</tr>
<tr>
<td>70:30</td>
<td>12.98</td>
<td>2699</td>
<td>3000</td>
</tr>
<tr>
<td><strong>60:40</strong></td>
<td><strong>17.43</strong></td>
<td><strong>8000</strong></td>
<td><strong>7000</strong></td>
</tr>
<tr>
<td>80:20</td>
<td>15.57</td>
<td>4000</td>
<td>3250</td>
</tr>
</tbody>
</table>
Fig. 6.11: Water: Acetonitrile 70:30 (%v/v)

Fig. 6.12: Water: Acetonitrile 60:40 (%v/v)
Fig. 6.13: Water: Acetonitrile 80:20 (%v/v)

2. Selection of Strength of Sodium Dihydrogen Phosphate Buffer

Different ionic strengths of sodium dihydrogen phosphate buffer such as 15, 25, 30 mM etc. adjusted to pH 3.65 in the ratio 60:40%v/v were tried. In case of 15 and 30 mM solutions, Metformin peak had tailing and hydrochlorothiazide peaks had fronting. Gaussian peaks characteristics were observed for strength of 25 mM and hence selected for further study.

<table>
<thead>
<tr>
<th>Strength (mM)</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>Tailing</td>
</tr>
<tr>
<td>25</td>
<td>Good peak shape</td>
</tr>
<tr>
<td>30</td>
<td>Tailing</td>
</tr>
</tbody>
</table>
3. Effect of pH

Keeping the ratio of mobile phase constant (60:40%v/v), the chromatograms were recorded with different pH’s ranging from 3 - 4. In higher and lower pH’s resolution was not as desired. But for a pH of 3.65, good resolution and symmetrical peaks were obtained and hence selected for further studies.

<table>
<thead>
<tr>
<th>pH</th>
<th>Resolution</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>16.12</td>
<td>Tailing</td>
</tr>
<tr>
<td>3.65</td>
<td>17.43</td>
<td>Sharp peaks are eluted</td>
</tr>
<tr>
<td>3</td>
<td>15.87</td>
<td>Tailing</td>
</tr>
</tbody>
</table>

4. Effect of Flow Rate

Keeping all the other parameters of mobile phase system constant, the chromatograms were recorded with different flow rates like 0.8, 1.0 and 1.2 ml/ min. With a flow rate of 1.0 ml/min, it gave good symmetrical peaks and hence selected for further studies.

<table>
<thead>
<tr>
<th>Flow rate (ml/ min)</th>
<th>Retention time</th>
<th>Observation</th>
<th>Figure No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Metformin Hydrochloride</td>
<td>Pioglitazone</td>
<td>Observation</td>
</tr>
<tr>
<td>0.8</td>
<td>2.0</td>
<td>3.6</td>
<td>Broad peaks</td>
</tr>
<tr>
<td>1.0</td>
<td>2.1</td>
<td>6.2</td>
<td>Good peak shape</td>
</tr>
<tr>
<td>1.2</td>
<td>1.3</td>
<td>2.9</td>
<td>Tailing</td>
</tr>
</tbody>
</table>
Fig. 6.14: Flow Rate 0.8 ml/min

Fig. 6.15: Flow Rate 1.0 ml/min
Fig. 6.16: Flow Rate 1.2 ml/min
### Parameters Used for RP-HPLC Method

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stationary phase</td>
<td>PhenomenaxC$_{18}$ column (250x4.6) mm i.d., 5µm, particle size</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>25 mM Sodium dihydrogen Phosphate (pH 2.5 with Orthophosphoric acid): Acetonitrile</td>
</tr>
<tr>
<td>Solvent ratio</td>
<td>60: 40% v/v</td>
</tr>
<tr>
<td>PH</td>
<td>2.5</td>
</tr>
<tr>
<td>Detection wavelength</td>
<td>230 nm</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1.0 ml/ minute</td>
</tr>
<tr>
<td>Operating pressure</td>
<td>181 kgf</td>
</tr>
<tr>
<td>Temperature</td>
<td>Room temperature</td>
</tr>
</tbody>
</table>

**Retention Time**

- Metformin Hydrochloride: 2.17 min
- Pioglitazone: 6.21 min
VALIDATION OF RP-HPLC METHOD

The validation of the method was carried out in terms of linearity, limit of detection (LOD), limit of quantification (LOQ), inter and Intra day precision, repeatability of sample application, Robustness and selectivity.

1. Linearity and Range

Metformin Hydrochloride and Pioglitazone were found to be linear in the range of 5 to 45 µg/ml and 0.15 to 0.9 µg/ml respectively. Calibration graphs were plotted using peak areas of standard drugs verses concentration of standard drug solutions. The slope, intercept and correlation coefficient values for Metformin Hydrochloride were found to be 10151.54, 49424.2 and 0.998 respectively, Fig.6.17. The slope, intercept and correlation coefficient values for Pioglitazone were found to be 28205.6667, 6532.30 and 0.999 respectively, (Fig. 6.18). Record the chromatogram of standards from Fig.6.19-6.24.

2. Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were determined by injecting progressively lower concentrations of two drugs. The LOD of Metformin Hydrochloride and Pioglitazone were found to be 0.005 and 0.01µg/ml and LOQ of Metformin Hydrochloride and Pioglitazone were found to be 0.015 and 0.030 µg/ml respectively.

3. Precision

Precision of method was demonstrated by

a) Intra day precision

b) Inter day precision
a) Intra day Precision

Intra day precision was done by carrying out analysis of standard drug solutions at three different concentrations in the linearity range for three times on the same day and %RSD was calculated, Table 6.8.

b) Inter day Precision

Inter day precision was done by carrying out the analysis of standard drug solutions at three different concentrations in the linearity range for three days over a period of one week and %RSD was calculated, Table 6.9.

4. System Suitability Studies

System suitability parameters like number of theoretical plates (N), peak asymmetry factor (As), resolution (Rs) etc., were studied, and results are given in Table 6.10.

5. Specificity

Specificity of the method was determined from Peak purity index values. Peak purity index values close to one. (Metformin Hydrochloride - 0.9999 and Pioglitazone - 1.0000) shows the peak purity for both drugs. It can be concluded that no impurities or degradation products migrated with the peak obtained from standard solutions of the drugs.
Fig. 6.17: Calibration Graph of Metformin Hydrochloride (5 – 45µg/ml)

\[ (10151.54) \times \text{Conc} + (49424.20) \times r = 0.994848 \]

Fig. 6.18: Calibration Graph of Pioglitazone (0.15 - 0.9µg/ml)

\[ (2820.66) \times \text{Conc} + (6532.300) \times r = 0.999980 \]
### Table 6.8: Intra day Precision

<table>
<thead>
<tr>
<th>Day</th>
<th>Concentration (µl/ml)</th>
<th>Peak area</th>
<th>%RSD</th>
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<tbody>
<tr>
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MET- Metformin Hydrochloride and PIO- Pioglitazone

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### Table 6.9: Inter day Precision

<table>
<thead>
<tr>
<th>Day</th>
<th>Concentration (µl/ml)</th>
<th>Peak area</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
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<td>MET</td>
<td>PIO</td>
<td>MET</td>
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MET- Metformin Hydrochloride and PIO- Pioglitazone

### Table 6.10: System Suitability Studies

<table>
<thead>
<tr>
<th>Drug</th>
<th>Rₜ</th>
<th>N</th>
<th>Aₜ</th>
<th>Tailing factor</th>
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<tr>
<td>MET</td>
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<td>2687</td>
<td>1.06</td>
<td>1.26</td>
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<tr>
<td>PIO</td>
<td>7056</td>
<td>1.00</td>
<td>1.33</td>
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</table>

MET- Metformin Hydrochloride and PIO- Pioglitazone
Fig. 6.19: Standard Solution 1

(Metformin Hydrochloride (5µg/ml) + Pioglitazone (0.15 µg/ml))

Fig. 6.20: Standard Solution 2

Metformin Hydrochloride (10µg/ml) + Pioglitazone (0.3 µg/ml)
Fig. 6.21: Standard Solution 3

Metformin Hydrochloride (15µg/ml) + Pioglitazone (0.45µg/ml)

Fig. 6.22: Standard Solution 4

Metformin Hydrochloride (20µg/ml) + Pioglitazone (0.6 µg/ml)
Fig. 6.23: Standard Solution 5

Metformin Hydrochloride (25μg/ml) + Pioglitazone (0.75 μg/ml)

Fig. 6.24: Standard Solution 6

Metformin Hydrochloride (30μg/ml) + Pioglitazone (0.9 μg/ml)
ANALYSIS OF FORMULATION

Fixed chromatographic conditions were made use of for the analysis of formulation.

**Preparation of Sample Solution**

Twenty tablets, each containing quantity equivalent to 500 mg of Metformin Hydrochloride and 15 mg of Pioglitazone were weighed and average weight was calculated. Quantity equivalent to 5 mg of Metformin Hydrochloride and 0.15 mg of Pioglitazone were weighed and transferred to a 100 ml volumetric flask, extracted with methanol and finally made up to 100 ml with the same solvent and this solution was filtered through whatman filter paper and suitable aliquots of formulation solutions were prepared with mobile phase.

**Accuracy**

Recovery studies of the drugs were carried out for determining accuracy parameter. It was done by mixing known quantity of standard drugs with the analyzed sample formulation and the contents were reanalyzed by the proposed method. This analysis was carried out at 80, 100 and 120% levels. Results of recovery are shown in Table 6.11.

**Recording the Chromatogram of Formulation**

A steady baseline was recorded with the fixed chromatographic conditions. Sample solution (15 µg/ml of Metformin Hydrochloride and 0.45 µg/ml of Pioglitazone) was injected and chromatogram was recorded Fig. 6.25. This was followed by injection of sample solution obtained from the formulation.

Peak areas of sample chromatograms were compared and amount of Metformin Hydrochloride and Pioglitazone in formulations were calculated, Table 6.12.
### Table 6.11: Recovery Studies

<table>
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<th>Level</th>
<th>% Recovery</th>
<th>% RSD*</th>
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<td>MET</td>
<td>PIO</td>
</tr>
<tr>
<td>80%</td>
<td>100.02</td>
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<tr>
<td>100 %</td>
<td>99.87</td>
<td>99.22</td>
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<tr>
<td>120%</td>
<td>99.62</td>
<td>100.31</td>
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</table>

*RSD of six observations, MET- Metformin Hydrochloride, PIO- Pioglitazone

### Table 6.12: Analysis of Formulation

<table>
<thead>
<tr>
<th>Drug</th>
<th>Amount (mg/tablet)</th>
<th>% Label claim</th>
<th>% RSD*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Labeled</td>
<td>Estimated</td>
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<tr>
<td>MET</td>
<td>500</td>
<td>499.8</td>
<td>99.96</td>
</tr>
<tr>
<td>PIO</td>
<td>15</td>
<td>14.89</td>
<td>99.26</td>
</tr>
</tbody>
</table>

*RSD of six observations, MET- Metformin Hydrochloride, PIO- Pioglitazone
Fig. 6.25: Chromatogram of Formulation

Metformin Hydrochloride (15µg/ml) + Pioglitazone (0.45 µg/ml)