Chapter 2

Simultaneous Determination of Metformin in Combination with Rosiglitazone by Reverse Phase Liquid Chromatography
2.1 Drug profile

2.1.1 Metformin Hydrochloride \([1-3]\)

a. Structure:

![Metformin Structure](image)

b. Chemical name: 1,1-dimethyl biguanide hydrochloride
c. Molecular formula: \(C_4H_{11}N_5\cdot HCl\)
d. Molecular weight: 162.62
e. Description: White crystalline powder.
f. Solubility: Freely soluble in water, slightly soluble in alcohol; practically insoluble in acetone and in methylene chloride.
g. Melting point: 222-226°C
h. Identification:
   a) Compare the Infrared absorption spectrum with reference spectrum of metformin hydrochloride.
   b) Dissolve about 5 mg of metformin hydrochloride in water and dilute to 100 ml with the same solvent. To 2 ml of the solution add 0.25 ml of strong sodium hydroxide solution and 0.10 ml of alpha-naphthol solution. Mix and allow to stand in ice cool water for 15 minutes. Add 0.5ml of sodium hypobromite solution and mix. A pink color develops.
i. Storage: Preserve in well closed containers. Store at room temperature.
j. Therapeutic category: Antidiabetic

2.1.2 Rosiglitazone Maleate [3]
a. Structure:

b. Chemical name: 5-[(4-[(2-(methyl-pyridin-2-yl-amino) ethoxy)phenyl]methyl] thiazolidin-2,4-dione

c. Molecular formula: Rosiglitazone Maleate C_{18}H_{19}N_{3}O_{3}S.C_{4}H_{4}O_{4}

d. Molecular weight: 473.51

e. Description: White to off white powder.

f. Solubility: Readily soluble in ethanol and in buffered aqueous solution with pH of 2.3; solubility decreases with increasing pH in the physiological range.

g. Melting point: 122-123°C

h. Therapeutic category: Antidiabetic.

i. Dosage forms: Tablets of combination of Metformin HCl and Rosiglitazone maleate are available in the following strengths [4].

1. Metformin HCl (500 mg)+ Rosiglitazone (2 mg) Tablets.
2. Metformin HCl (500 mg)+ Rosiglitazone (4 mg) Tablets.
3. Metformin HCl (1000 mg)+ Rosiglitazone (4 mg) Tablets.
4. Metformin HCl (500 mg)+ Rosiglitazone (2 mg) Tablets.

2.2 Introduction

Metformin HCl is chemically 1,1-dimethyl biguanide hydrochloride. Rosiglitazone is 5-[(4 – [2 – (Methyl –2 – pyridinylamino) ethoxy] phenyl] methyl] –2, 4 –thiazolidinedione. A combination of 500 mg of metformin HCl and 4 mg of rosiglitazone is available commercially as tablets [5].

2.2.1 Reported methods for the analysis of metformin and rosiglitazone
Literature survey shows that few methods are reported for the estimation of metformin in tablets [6-8] and in plasma [9-13]. Also methods are reported for the estimation of rosiglitazone in tablets [14, 15] and in plasma [16, 17]. But yet there is no single method reported for the simultaneous determination of metformin and rosiglitazone.

In the present research paper attempts have been made to develop a method for the simultaneous determination of metformin and rosiglitazone. An internal standard method was used for the quantitation of rosiglitazone and metformin. Methylparaben was used as an internal standard. The adequate retention and resolution of metformin, rosiglitazone and methylparaben peaks was achieved by using the mobile phase containing 5 mM sodium dodecyl sulphate (SDS) and 10 mM disodium hydrogen phosphate in double distilled water and acetonitrile in the ratios of (66:34, v/v), pH adjusted to 7.1 with orthophosphoric acid.

Sometimes it requires two sample preparations due to large differences in the label claims of the active ingredients. Though there is a large differences in the label claims of the metformin and rosiglitazone per tablet, the analysis of these two ingredients have been done in the same sample preparation.

2.3 Experimental

2.3.1 Materials and Reagents
Metformin HCl and rosiglitazone maleate were obtained from Wockhardt Research Centre (Aurangabad, Maharashtra State (M.S.), India). Sodium dodecyl sulphate (SDS), disodium hydrogen phosphate and 1-Octanesulphonic acid sodium salt were obtained from E. Merck (India) Ltd. Worli, Mumbai. Orthophosphoric acid, hydrochloric acid and acetonitrile (HPLC grade) was obtained from Qualigens Fine Chemicals, Dr. Annie Besant Road, Mumbai, India. Methylparaben was obtained from HiMedia Laboratories Ltd. Mumbai. The 0.45-µm nylon filter was obtained from Advanced Microdevices Pvt. Ltd. Ambala Cantt, India. The tablets of metformin in combination with rosiglitazone were purchased from the Indian market. Double distilled water was used throughout the experiment. Other chemicals were of analytical or HPLC grade.

2.3.2 Chromatographic conditions
A Thermoseparation products HPLC was utilized consisting of the following components, Constametric 3500 pump, AS 3000 autosampler, UV 1000 detector. A Zorbax XDB C18 (5 µm, 4.6 x 150 mm) column was used. The instrumental settings were, flow rate of 1 mL/min, column temperature at 40º C and detector wavelength of 226 nm. The injection volume was 25 µL. Data acquisition was made with the software PC 1000. The peak purity was checked with the photodiode array detector of Thermo separation products, UV6000 LP.

2.3.3 Mobile phase
Mobile phase was consisted of buffer and acetonitrile in the ratios of (66:34, v/v). The pH of the mobile phase was adjusted to 7.1 with orthophosphoric acid. The buffer used in the mobile phase consisted of 10 mM disodium hydrogen phosphate and 5 mM SDS in double distilled water. The mobile phase was premixed and filtered through a 0.45-µm nylon filter and degassed.

2.3.4 Standard stock 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
Diluent-A: composed of 5 mM disodium hydrogen phosphate and acetonitrile in the ratios of (50:50, v/v) pH adjusted to 2.0 with HCl.
Diluent-B: composed of 5 mM disodium hydrogen phosphate and acetonitrile in the ratios of (70:30, v/v) pH adjusted to 2.3 with HCl.

a) Metformin HCl
A 125-mg sample of metformin HCl was accurately weighed, transferred in a 50-mL volumetric flask and dissolved with the diluent-A.

b) Rosiglitazone Maleate
A 26.5-mg sample of rosiglitazone maleate (equivalent to 20-mg of rosiglitazone) was accurately weighed, transferred in a 50-mL volumetric flask and dissolved with diluent-A. 2.5-mL volume of this solution was transferred in a 50-mL volumetric flask and diluted with the diluent-A.
c) Methylparaben
A 25- mg sample of methylparaben was accurately weighed, transferred in a 100- mL volumetric flask and dissolved and diluted with diluent-A.

d) Mixed standard solution
A mixed standard solution was prepared from these stock solutions by transferring 2 mL of a metformin standard solution, 2 mL of rosiglitazone standard solution and 1 mL of methylparaben standard solution in a 50- mL volumetric flask and diluted with diluent-B. This solution contains a 100 µg/mL of metformin HCl, 0.8 µg/mL of rosiglitazone and 5 µg/mL of methylparaben.

2.3.5 Calibration curve solutions
From the above stock solutions of metformin, rosiglitazone and methylparaben the calibration curve solutions containing 25 µg/mL –150 µg/mL of metformin HCl, 0.2 µg/mL – 1.2 µg/mL of rosiglitazone and 5 µg/mL of methylparaben in each calibration level were prepared.

2.3.6 Preparation of sample solutions
Ten tablets were weighed and finely powdered. A quantity equivalent to one tablet containing 500- mg of metformin HCl and 4-mg of rosiglitazone was transferred in a 100- mL volumetric flask. To this flask 70- mL of diluent-A was added and sonication was done for 15 min with intermittent shaking the flask and the solution was cooled to ambient temperature and diluted to volume with diluent-A and mixed well. The solution was centrifuged at 10000 rpm for 5 min. From the centrifuged solution 2- mL of clear solution was transferred into a 50-mL volumetric flask and 1-mL of the internal standard stock solution was added to it and diluted with diluent-B.

2.4 Results and Discussion

Optimisation of the chromatographic conditions
The chromatographic method was optimized by changing the various variable parameters of the mobile phase. It was observed that at 80% aqueous composition containing the disodium hydrogen phosphate and 20% acetonitrile in the mobile phase the peak of metformin elutes at 1.27 min but at
this composition the peak of rosiglitazone was much retained. Different experiments were done to achieve the adequate retentions and resolution for the peaks of metformin and rosiglitazone. The ion pair reagent, 1-Octanesulphonic acid sodium salt was used in the mobile phase at different concentrations and at 20 mM concentration, the metformin peak elutes at 1.92 min but the peak of rosiglitazone elutes up to 25 min. Finally SDS was used in the mobile phase and it was observed that the peak of metformin elutes at 5 min. To set the adequate retentions and the resolution, effects of the mobile phase components, changes in ionic strength, SDS concentrations and pH of the mobile phase were studied. The ionic strength was varied in the mobile phase from 2.5 mM –15 mM by keeping other components of the mobile phase constant. From these studies, it was observed that from 10mM –15mM ionic strength concentration in the mobile phase, the effect on the retentions of the analytes was not significant. But at 2.5 mM ionic strength concentration, the elution order of the rosiglitazone and metformin peaks changes, the peak of rosiglitazone elutes before metformin peak without sufficient resolution between these two peaks and at 5 mM concentration the peaks merge with each other. There was no much effect on the retention of methylparaben peak. From the pH effects study, it was observed that the resolution increases with decreasing the pH of the mobile phase and at pH 7.5, the peaks of metformin and rosiglitazone merge with each other. When the SDS concentration was increased in the mobile phase it was observed that at 7.5 mM the peak of rosiglitazone merges with the peak of metformin and at 10 mM concentration of SDS, the rosiglitazone peak elutes before the peak of metformin. From the above studies, it was observed that at 5 mM SDS and 10 mM disodium hydrogen phosphate concentration in double distilled water and acetonitrile in the composition of (66:34, v/v) at pH 7.1 the peaks of methylparaben, metformin and rosiglitazone gave adequate retentions and resolution and the chromatographic run was less than 15 minutes. (Fig 2)

2.5 Validation of the method

2.5.1 Specificity

The specificity of the method was checked by peak purity test of the sample preparation done by photodiode array detector. The peak purity for
methylparaben, metformin and rosiglitazone was observed to be 999, 996 and 999 respectively. The results of the peak purity analysis shows that the peaks of analytes were pure and also the formulation excipients were not interfering with the analyte peaks.

2.5.2 Calibration and linearity
An internal standard method was used for quantitative determinations. Linearity of the method was tested from 25-150% of the targeted level of the assay concentration (metformin HCl 100 µg/ mL and rosiglitazone 0.8 µg/ mL) for both the analytes. The mixed standard solutions containing 25 µg/ mL - 150 µg/ mL of metformin HCl, 0.2 µg/ mL –1.2 µg/ mL of rosiglitazone and 5 µg/ mL of methylparaben in each linearity level were prepared. Linearity solutions were injected in triplicate. In the simultaneous determination, the calibration graphs were found to be linear for both the analytes in the mentioned concentrations. The results and correlation coefficients are shown in Table-I and the linearity graphs for metformin HCl and rosiglitazone are shown in Figure -1A and 1B respectively.

2.5.3 Precision (Reproducibility)
The precision of the method was studied by determining the concentrations of each active ingredient in the tablets six times. The results of the precision study (Table- II) indicate that the method is reliable (RSD <2.0%)

2.5.4 Accuracy (Recovery test)
Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding a known amount of the drugs in 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 4- mg of rosiglitazone). Three samples were prepared for each recovery level. The recovery values for metformin HCl and rosiglitazone ranged from 100.92-101.70 and 99.21-101.60 respectively (Table IIIA & IIIB). The average recovery of three levels (nine determinations) for metformin and rosiglitazone were 101.20 and 100.11 respectively.

2.5.5 Intermediate precision
Intermediate precision of the method was done by analyzing the samples six times on different days, by different chemist, by using different analytical
columns of the same make and different HPLC systems. The percentage assay was calculated using the calibration curve. The assay results are shown in Table IV.

**2.5.6 Determination of the limit of detection and quantitation**

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, rosiglitazone was 0.023 µg/mL, 0.004 µg/mL and limit of quantitation was 0.069 µg/mL, 0.013 µg/mL respectively. The results are tabulated in Table-V.

**2.5.7 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 upto 72 hr for metformin, rosiglitazone in sample preparation and methylparaben standard were 0.90%, 0.69% and 0.29% 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 shown in Table-VI.

**2.5.8 System suitability**

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

**2.6 Determination of active ingredients in tablets**

The contents of two drugs in tablets were determined by the proposed method using calibration curve. The determinations were done in two sets, one for precision and the second for intermediate precision and six samples were
prepared for each set. The results are shown in Table II and IV. The chromatogram of the tablet sample is shown in Figure 2.

2.7 Conclusion

This method can be used for the simultaneous determination of metformin and rosiglitazone in the pharmaceutical dosage form. The method is validated and shown to be accurate and precise. It can be used in the quality control departments for the assay and dissolution of tablets of metformin in combination with rosiglitazone.

2.8 Tables

Table-I
Linearity
Table-II : Precision study
Analysis of tablets containing Metformin HCl (500mg) and Rosiglitazone (4mg)

<table>
<thead>
<tr>
<th>Sample preparation</th>
<th>Metformin Found (mg)</th>
<th>Rosiglitazone Found (mg)</th>
<th>% Assay of Metformin HCl</th>
<th>% Assay of Rosiglitazone</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 25 0.2</td>
<td>521.79</td>
<td>3.92</td>
<td>104.36</td>
<td>97.99</td>
</tr>
<tr>
<td>2 50 0.4</td>
<td>518.63</td>
<td>3.96</td>
<td>103.73</td>
<td>98.96</td>
</tr>
<tr>
<td>3 75 0.6</td>
<td>518.47</td>
<td>3.91</td>
<td>103.69</td>
<td>97.67</td>
</tr>
<tr>
<td>4 100 0.8</td>
<td>517.13</td>
<td>3.91</td>
<td>103.43</td>
<td>97.84</td>
</tr>
<tr>
<td>5 125 0.1</td>
<td>512.94</td>
<td>3.89</td>
<td>102.59</td>
<td>97.26</td>
</tr>
<tr>
<td>6 150 1.2</td>
<td>517.99</td>
<td>3.91</td>
<td>103.60</td>
<td>97.78</td>
</tr>
<tr>
<td>Mean</td>
<td>517.83</td>
<td>3.92</td>
<td>103.57</td>
<td>97.92</td>
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<tr>
<td>SD</td>
<td>2.87</td>
<td>0.02</td>
<td>0.57</td>
<td>0.57</td>
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<tr>
<td>%RSD</td>
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<td>0.58</td>
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Table-III A: Recovery (Accuracy study)
Analyte : Rosiglitazone

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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>
</table>

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47
### Table-III B
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>400</td>
<td>406.78</td>
<td>101.70</td>
<td>101.45+0.23</td>
<td>0.23</td>
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<tr>
<td></td>
<td>2</td>
<td>400</td>
<td>405.67</td>
<td>101.42</td>
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<tr>
<td></td>
<td>3</td>
<td>400</td>
<td>404.92</td>
<td>101.23</td>
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<tr>
<td>100%</td>
<td>1</td>
<td>500</td>
<td>505.12</td>
<td>101.02</td>
<td>101.20+0.26</td>
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<tr>
<td></td>
<td>2</td>
<td>500</td>
<td>507.49</td>
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<tr>
<td></td>
<td>3</td>
<td>500</td>
<td>505.44</td>
<td>101.09</td>
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<tr>
<td>120%</td>
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<td>605.73</td>
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<tr>
<td></td>
<td>2</td>
<td>600</td>
<td>605.55</td>
<td>100.92</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>600</td>
<td>605.66</td>
<td>100.94</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Average Recovery (%)</strong></td>
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<td></td>
<td></td>
<td></td>
<td><strong>101.20+0.28</strong></td>
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### Table-IV
Intermediate precision study

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<tr>
<th>Sample preparation.</th>
<th>Metformin HCl Found (mg)</th>
<th>Rosiglitazone Found (mg)</th>
<th>% Assay of Metformin HCl</th>
<th>% Assay of Rosiglitazone</th>
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<tbody>
<tr>
<td>1</td>
<td>516.47</td>
<td>3.93</td>
<td>103.29</td>
<td>98.17</td>
</tr>
<tr>
<td>2</td>
<td>513.44</td>
<td>3.94</td>
<td>102.69</td>
<td>98.45</td>
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<tr>
<td>3</td>
<td>514.24</td>
<td>3.91</td>
<td>102.85</td>
<td>97.63</td>
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</table>

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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>
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</thead>
<tbody>
<tr>
<td></td>
<td>Metformin HCl</td>
<td>Rosiglitazone ppm</td>
</tr>
<tr>
<td>1</td>
<td>0.02</td>
<td>0.02</td>
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<tr>
<td>2</td>
<td>0.04</td>
<td>0.04</td>
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<tr>
<td>3</td>
<td>0.06</td>
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<tr>
<td>4</td>
<td>0.08</td>
<td>0.08</td>
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<tr>
<td>5</td>
<td>0.1</td>
<td>0.1</td>
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<tr>
<td>6</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>7</td>
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<td>8</td>
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Table-VI
Solution stability

<table>
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<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>
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<td>Standard</td>
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<tr>
<td>Metformin</td>
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<td>101.02</td>
<td>99.90</td>
<td>100.39</td>
<td>100.33</td>
<td>0.51</td>
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<tr>
<td>Rosiglitazone</td>
<td>100</td>
<td>99.88</td>
<td>100.69</td>
<td>100.17</td>
<td>100.19</td>
<td>0.36</td>
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<tr>
<td>Methylparaben (IS)</td>
<td>100</td>
<td>99.87</td>
<td>99.57</td>
<td>99.35</td>
<td>99.70</td>
<td>0.29</td>
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<tr>
<td>Sample</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Metformin</td>
<td>101.9</td>
<td>100.83</td>
<td>100.10</td>
<td>99.91</td>
<td>100.69</td>
<td>0.90</td>
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Table-VII
System suitability

<table>
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<tr>
<th>Sr.No.</th>
<th>Parameter</th>
<th>Methylparaben</th>
<th>Metformin</th>
<th>Rosiglitazone</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Theoretical plates(^1)</td>
<td>10072</td>
<td>7567</td>
<td>10010</td>
</tr>
<tr>
<td>2</td>
<td>Resolution</td>
<td>9.55</td>
<td></td>
<td>8.96</td>
</tr>
<tr>
<td>3</td>
<td>Tailing factor</td>
<td>1.15</td>
<td>1.59</td>
<td>1.01</td>
</tr>
<tr>
<td>4</td>
<td>%RSD</td>
<td>0.31</td>
<td>0.93</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) per column length.

2.9 Figures

Figure- 1A  Calibration graph for Rosiglitazone
Figure 1B  Calibration graph for Metformin HCl
Figure-2: A typical chromatogram of the tablet: methylparaben (3.30), Metformin (5.04), and rosiglitazone (7.36).
2.10 References

5. Sanjiv Malik (Edt.), Indian drugs review (IDR), Mediworld publications Pvt.Ltd., New Delhi. 2003, 11, 513


