Simultaneous Determination of Metformin in Combination with Rosiglitazone by Reversed-Phase Liquid Chromatography

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Abstract
A simple, rapid, and precise reversed-phase liquid chromatographic method is developed for the simultaneous determination of metformin in combination with rosiglitazone. This method uses a Zorbax XDB C18 15-cm analytical column, a mobile phase of acetonitrile and buffer containing 10mM disodium hydrogen phosphate, and 5mM sodium dodecyl sulphate in the ratio of 34:66 (v/v), and pH is adjusted to 7.1 with orthophosphoric acid. The instrumental settings are a flow rate of 1 mL/min, column temperature at 40°C, and detector wavelength of 226 nm. The internal standard method is used for the quantitation of metformin and rosiglitazone. Methylparaben is used as an internal standard. The method is validated and shown to be linear. The correlation coefficients for metformin and rosiglitazone are 0.9996 and 0.9997, respectively. The relative standard deviation for six replicate measurements in two sets of each drug in the tablets is always less than 2%.

Introduction
Metformin HCl is chemically 1,1-dimethyl biguanide hydrochloride (Figure 1A). Rosiglitazone is 5-[[4-[(methyl-2-pyridinylamino)ethoxy]phenyl]methyl]-2,4-thiazolidinedione (Figure 1B). A combination of 500 mg of metformin HCl and 4 mg of rosiglitazone is available commercially as tablets. The complementary actions of the antidiabetic agents metformin hydrochloride and rosiglitazone maleate improve glycemic control, insulin sensitivity, and beta cell function more effectively than treatment with metformin alone (1).

Literature survey shows that few methods are reported for the individual estimation of metformin and rosiglitazone. The methods for metformin (2–9) and rosiglitazone (10–13) are reported for the estimation of these drugs in tablets and plasma. However, 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 were achieved using the mobile phase containing 5mM sodium dodecyl sulphate (SDS) and 10mM disodium hydrogen phosphate in double-distilled water and acetonitrile in the ratio of 66:34 (v/v), and the pH was adjusted to 7.1 with orthophosphoric acid.

Sometimes it requires two sample preparations because of large differences in the label claims of the ingredients. Although there is a large difference in the label claims of the metformin and rosiglitazone per tablet, the analysis of these two ingredients has been done in the same sample preparation.

Experimental
Materials and reagents
Metformin HCl and rosiglitazone maleate were obtained from Wockhardt Research Center (Aurangabad, Maharashtra State, India). SDS, disodium hydrogen phosphate, and 1-octanesul-
phoric acid sodium salt were obtained from E. Merck, Ltd. (Worli, Mumbai, India). Orthophosphoric acid, hydrochloric acid, and acetonitrile [high-performance liquid chromatographic (HPLC) grade] was obtained from Qualigens Fine Chemicals (Mumbai, India). Methylparaben was obtained from HiMedia Laboratories, Ltd. (Mumbai, India). The 0.45-µm nylon filter was obtained from Advanced Microdevices, Pvt., Ltd. (Ambala Cantt, India). The tablets of rosiglitazone 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.

**Chromatographic conditions**

A Thermoseparation Products HPLC (San Jose, CA) was utilized consisting of the following components: Constametric 3500 pump, AS 3000 autosampler, and UV 1000 detector. A Zorbax XDB C18 (5 µm, 4.6 × 150 mm) column was used. The instrumental settings were a flow rate of 1 mL/min, a column temperature at 40°C, and a detector wavelength of 226 nm. The injection volume was 25 µL. Data acquisition was made with the software PC 1000 (ThermoSeparations Products, Riviera Beach, FL). The peak purity was checked with the photodiode array detector of ThermoSeparation Products, UV6000 LP.

**Mobile phase**

The mobile phase consisted of buffer and acetonitrile in the ratio 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.

**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 and sample preparations were prepared as follow: diluent-A, composed of 5 mM disodium hydrogen phosphate and acetonitrile in the ratio of 50:50 (v/v), pH adjusted to 2.0 with HCl and diluent-B, composed of 5 mM disodium hydrogen phosphate and acetonitrile in the ratio of 70:30 (v/v), pH adjusted to 2.3 with HCl.

**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.

**Rosiglitazone maleate**

A 26.5-mg sample of rosiglitazone maleate (equivalent to 20 mg of rosiglitazone) was accurately weighed, transferred into a 50-mL volumetric flask, and dissolved with diluent-A; a 2.5-mL volume of this solution was transferred into a 50-mL volumetric flask and diluted with diluent-A.

**Methylparaben**

A 25-mg sample of methylparaben was accurately weighed, transferred in a 100-mL volumetric flask, and dissolved and diluted with diluent-A.

**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 into a 50-mL volumetric flask and diluted with diluent-B. This solution contained 100 µg/mL of metformin HCl, 0.8 µg/mL of rosiglitazone, and 5 µg/mL of methylparaben.

**Calibration curve solutions**

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

**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, sonication was performed for 15 min with shaking the flask, the solution was cooled to ambient temperature, diluted to volume with diluent-A, and mixed well. The solution was centrifuged at 10,000 rpm for 5 min. From the centrifuged solution, 1 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.

**Results and Discussion**

**Optimization of 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 eluted at 1.27 min, but at this composition, the peak of rosiglitazone was much retained. Different experiments were performed to achieve the adequate retention 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 eluted at 1.92 min, but the peak of rosiglitazone eluted up to 25 min. Finally, SDS was used in the mobile phase, and it was observed that the peak of metformin eluted at 5 min. To set the adequate retentions and resolution, the effects of the mobile phase components, changes in ionic strength, SDS concentrations, and pH of the mobile phase were studied. The ionic strength in the mobile phase was adjusted by adding disodium hydrogen phosphate and varied from 2.5–15 mM by keeping other components of the mobile phase constant. From these studies, it was observed that from 10 to 15 mM ionic strength concentration in the mobile phase, the effect on the retentions of the analytes was not significant. However, at 2.5 mM ionic strength concentration, the elution order of the rosiglitazone and metformin peaks changed; the peak of rosiglitazone eluted before the metformin peak, and without sufficient resolu-
tion between these two peaks and at 5mM concentration, the peaks merged with each other. There was not much effect on the retention of methylparaben peak. From the pH effects study, it was observed that the resolution increased 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 7.5mM, the peak of rosiglitazone merged with the peak of metformin, and at 10mM concentration of SDS, the rosiglitazone peak eluted before the peak of metformin.

From the described studies, it was observed that at 5mM SDS and 10mM disodium hydrogen phosphate concentrations 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 min.

Validation of the method

Specificity

The specificity of the method was checked by a 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 the analytes were pure and, also, the formulation excipients were not interfering with the analyte peaks.

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 25–150 µg/mL of metformin HCl, 0.2–1.2 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 correlation coefficients are shown in Table I).

Precision (reproducibility)

The precision of the method was studied by determining the concentrations of each ingredient in the tablets six times. The results of the precision study (Table I) indicate that the method is reliable [relative standard deviation (RSD) percentage < 2].

Accuracy (recovery test)

The accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts 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 II). The average recovery of three levels (nine determinations) for metformin and rosiglitazone were 101.20 and 100.11, respectively.

Intermediate precision

Intermediate precision of the method was done by analyzing the samples six times on different days, by different chemists, 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 III.

Determination of the limits of detection and quantitation

For determining the limits of detection (LOD) and quantitation (LOQ), the method based on the residual standard deviation (SD) 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 the detection and quantitation limits. The LODs for metformin and rosiglitazone were 0.023 and 0.004 µg/mL, and the LOQs were 0.069 and 0.013 µg/mL, respectively.

Solution stability

The stability of the standard and sample solutions was performed at intervals of 24, 48, and 72 h. The stability of solution was determined in terms of the assay of the drugs in standard and sample solutions against the freshly prepared standard solutions. The RSD for the assay values determined up to 72 h for metformin HCl, 0.023 and 0.004 µg/mL, and the LOQs were 0.069 and 0.013 µg/mL, respectively.

Table I. Results of the Linearity and Precision Study (n = 6)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Precision (%RSD)</th>
<th>Linearity (µg/mL)</th>
<th>Coefficient of correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metformin HCl</td>
<td>1.01</td>
<td>25–150</td>
<td>0.9996</td>
</tr>
<tr>
<td>Rosiglitazone</td>
<td>1.58</td>
<td>0.2–1.2</td>
<td>0.9997</td>
</tr>
</tbody>
</table>

Table II. Results of the Recovery Tests for the Drugs (n = 3)

<table>
<thead>
<tr>
<th>Level of addition (%)</th>
<th>Ingredient</th>
<th>Amount added (mg)</th>
<th>Recovery (%)*</th>
<th>Average recovery†</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>Metformin HCl</td>
<td>400.0</td>
<td>101.45 (0.23)</td>
<td>101.20 (0.28)</td>
</tr>
<tr>
<td></td>
<td>Rosiglitazone</td>
<td>3.2</td>
<td>100.85 (0.86)</td>
<td>100.11 (0.79)</td>
</tr>
<tr>
<td>100</td>
<td>Metformin HCl</td>
<td>500.0</td>
<td>101.20 (0.25)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rosiglitazone</td>
<td>4.0</td>
<td>99.47 (0.24)</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>Metformin HCl</td>
<td>600.0</td>
<td>100.94 (0.02)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rosiglitazone</td>
<td>4.8</td>
<td>100.03 (0.47)</td>
<td></td>
</tr>
</tbody>
</table>

* RSD shown in parenthesis.
† Average recovery = the average of three levels, nine determinations.

Table III. Assay Results of Active Ingredients in Tablets

<table>
<thead>
<tr>
<th>Set</th>
<th>Ingredient</th>
<th>Label value (mg)</th>
<th>Found (mg)*</th>
<th>%Label claim</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precision</td>
<td>Metformin HCl</td>
<td>500</td>
<td>517.83</td>
<td>103.57</td>
</tr>
<tr>
<td>Rosiglitazone</td>
<td></td>
<td></td>
<td>3.92</td>
<td>97.92</td>
</tr>
<tr>
<td>Intermediate</td>
<td>Metformin HCl</td>
<td>500</td>
<td>518.95</td>
<td>103.79</td>
</tr>
<tr>
<td>Rosiglitazone</td>
<td></td>
<td></td>
<td>3.94</td>
<td>98.56</td>
</tr>
</tbody>
</table>

* Average of six analyses.
formin, rosiglitazone in sample preparation, and methylparaben standard were 0.90%, 0.69%, and 0.29%, respectively. The assay values were within ±2% after 72 h. The results indicate that the solutions were stable for 72 h at ambient temperature.

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. Results are shown in Table IV.

Determination of active ingredients in tablets
The contents of two drugs in tablets were determined by the proposed method using a 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 III. The chromatogram of the tablet sample is shown in Figure 2.

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.

Acknowledgments
The authors are grateful to the Wockhardt Research Center, Aurangabad, India and Head-Department of Chemical Technology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, Maharashtra State, India for providing the facilities for this research work.

References

Table IV. System Suitability Parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Methylparaben</th>
<th>Metformin</th>
<th>Rosiglitazone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Theoretical plates*</td>
<td>10,072</td>
<td>7567</td>
<td>10,010</td>
</tr>
<tr>
<td>Resolution</td>
<td>9.55</td>
<td>8.96</td>
<td></td>
</tr>
<tr>
<td>Tailing factor</td>
<td>1.15</td>
<td>1.59</td>
<td>1.01</td>
</tr>
<tr>
<td>%RSD</td>
<td>0.31</td>
<td>0.93</td>
<td></td>
</tr>
</tbody>
</table>

* Per column length.

Figure 2. A typical chromatogram of the tablet: methylparaben (3.30), metformin (5.04), and rosiglitazone (7.36).