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Analytical method for simultaneous estimation of ranolazine and metformin hydrochloride by validated RP-HPLC-DAD method

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ABSTRACT

Background: In the present study, a simple, sensitive, rapid, accurate, and precise reverse-phase high-performance liquid chromatography (RP-HPLC) method with diode array detection was developed and validated for simultaneous estimation of Metformin hydrochloride (MET) and Ranolazine (RANO) in a synthetic mixture.

Material and Method: The method was developed using isocratic elution mode on a reversed-phase. Chromatographic separation was performed on ACE CN C₁₈ (250 mm, 4.5 mm, 5 μm) column and a mobile phase consisting of methanol: acetonitrile:ammonium formate (45:45:10% v/v/v) with 10% formic acid to adjust the pH to 6.0, at a flow rate of 1.0 ml/min. Detection and quantification of all the analytes were carried out at 230 nm using a photodiode array detector.

Result: The method was found to be linear in the range of 1–50 μg/ml for RANO and 2–100 μg/ml for MET. Percentage recovery was found in the range of 99.07–101.53% for RANO and 99.59–100.45% for MET. The RSD of precision and repeatability was found lower than 1% for each drug. Validation of the method was carried out as per International Council on Harmonization guideline Q2 (R1).

Conclusion: The proposed RP-HPLC method can be highly suitable for the analysis of RANO and MET without interference.

Keywords: International Council on Harmonization guideline Q2 (R1), Metformin hydrochloride, Ranolazine, reverse-phase high-performance liquid chromatography, validation

INTRODUCTION

Ranolazine (RANO) is a blocker of the late sodium current and is approved to treat chronic angina as first-line treatment and Metformin hydrochloride (MET), an oral antidiabetic drug in the biguanide class, is the most widely used as first-line treatment for type 2 diabetes mellitus (T2DM).¹,² However, nowadays, coronary artery disease (CAD) and diabetes mellitus (DM) commonly coexist. It is estimated that 26% of subjects with CAD also have DM as a comorbid condition. It is, therefore, anticipated that RANO and MET could be coadministered in subjects with T2DM.³ This fixed-dose combination undergoing a clinical trial phase three and in this conventional tablet used in the strength of 1:2 dosing ratio of RANO and MET, respectively. Coadministration of RANO and MET was well tolerated in these T2DM subjects, with no serious adverse event.⁴

RANO is N- (2, 6-dimethylphenyl)-2- (4- [2-hydroxy-3- (2-methoxyphenoxy) propyl] piperazin-1-yl) acetamide, which is class of antianginal drug. MET is 3- (diaminomethylidene) 1, dimethylguanidine; hydrochloride is a hypoglycaemic agent. The chemical structures of RANO and MET are shown in Figure 1.⁵

The literature review reveals that MET is official in IP⁶ USP⁷ JP⁸ and BP⁹ and RANO is not official in any pharmacopoeia. First derivative UV spectrophotometric and reverse-phase
Preparation of Synthetic Mixture

The synthetic mixture was prepared by mixing 20 mg of MET and 10 mg of RANO with the spiking of common tablet excipients such as microcrystalline cellulose, lactose, sodium starch glycolate, and starch as a gliding agent, diluent, disintegrating agent, and binder, respectively, in 100 ml volumetric flask, about 50 ml of methanol was added and sonicated to dissolve completely and made up to the mark to get 200 μg/ml and 100 μg/ml for MET and RANO, respectively. From the above solution pipette out 2 ml appropriately, then dilute with methanol to get a final concentration of 40 μg/ml and 20 μg/ml for MET and RANO, respectively.

System Suitability Parameter

The system suitability of the chromatographic system was tested before each stage of validation. Six replicate injection of standard preparation was injected into the system and retention time, tailing factor, numbers of theoretical plates, and relative standard deviation of each were determined.

METHOD VALIDATION

Linearity

A standard stock solution was, further, diluted with methanol to get a concentration in series of 1–50 μg/ml of RANO and 2–100 μg/ml of MET, respectively.

Accuracy

Accuracy was performed using a drug to drug spiking at three different amounts of analytes at levels of 50%, 100%, and 150%. Concisely, a recovery study was performed by spiking of 10 μg/ml, 20 μg/ml, 30 μg/ml of RANO, and 20 μg/ml, 40 μg/ml, 60 μg/ml of MET to the prepared mixture containing 20 μg/ml of RANO and 40 μg/ml of MET.

Precision

Repeatability was performed under six replicates at a concentration of 20 μg/ml of RANO and 40 μg/ml of MET. Intraday and interday variations of both drugs were performed in triplicate at three different concentration levels 50%, 100%, and 150% (20 μg/ml, 40 μg/ml, and 60 μg/ml for MET and 10 μg/ml, 20 μg/ml, and 35 μg/ml for RANO). Results from the determination of repeatability and intermediate precision are expressed in the form of RSD.

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

LOD and LOQ were computed to establish method sensitivity. LOD and LOQ were determined from the standard deviation of intercept and the slope of the calibration curve using the equation LOD = 3.3*σ/C and LOQ = 10*σ/C, respectively.

Specificity

Specificity was performed under six replicates at a concentration of 20 μg/ml of RANO and 40 μg/ml of MET, with and without the addition of excipients to check the
interference of excipients. The specificity of the method was evaluated by calculating percentage interference.

Robustness
The robustness of the method was evaluated by deliberate variation in the method parameter such as flow rate variation by ±0.1 ml/min, mobile phase ratio by ±2.0 ml organic solvent, and pH of Mobile Phase ±0.5.

RESULTS AND DISCUSSION
Optimize Chromatographic Condition
The optimal composition of the mobile phase was methanol: acetonitrile:ammonium formate (pH: 6.0) (45:45:10% v/v/v) using isocratic elution mode with ACE CN C18 (250 mm, 4.5 mm, 5 μm) column. The flow rate was set to 1 ml/min and UV detection was carried out at 230 nm. The mobile phase was filtered through a nylon 0.22 mm membrane filter and was degassed before use. Chromatogram of optimizing condition is shown in Figure 2 and system suitability parameters are expressed in Table 1.

Analytical Method Validation
Linearity
Linearity for RANO and MET was found in the range of 1–50 μg/ml and 2–100 μg/ml, respectively. The correlation coefficient of RANO and MET was found to be 0.9994 and 0.9998, respectively. Linearity overlay and a calibration curve are given in Figures 3 and 4, respectively.

Specificity
Percentage interference was calculated, and it was should be found <0.5%. Thus, the method is specific.

Accuracy
Percentage recovery was found in the range 99.07–101.53% and 99.59–100.45% for RANO and MET, respectively. Thus, the percentage recoveries of drugs are acceptable and data for accuracy study are shown in Table 2.

Precision
RSD was found to be <2 that shows satisfactory precision of the method. Data for both are given in Tables 3 and 4.

LOD and LOQ
LOD and LOQ were determined using the formula given in ICH Q2 (R1) guideline. LOD and LOQ were found to be 0.136 μg/ml and 0.413 μg/ml for RANO and 0.0091 μg/ml and 0.0276 μg/ml for MET, respectively.

Robustness
This study revealed that the method was remaining unaffected by the deliberate changes in flow rate, mobile phase composition, and pH of the mobile phase. RSD was found to be <2, which shows that the proposed method was robust.

Table 1: System suitability parameter of optimized condition

<table>
<thead>
<tr>
<th>Drug</th>
<th>Retention time±SD</th>
<th>Theoretical plate±SD</th>
<th>Tailing factor±SD</th>
<th>Resolution±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>RANO</td>
<td>3.06±0.1</td>
<td>5005±100</td>
<td>1.0±0.1</td>
<td>-</td>
</tr>
<tr>
<td>MET</td>
<td>6.56±0.1</td>
<td>7574±110</td>
<td>1.0±0.1</td>
<td>14.93±0.1</td>
</tr>
</tbody>
</table>

RANO: Ranolazine, MET: Metformin hydrochloride

Table 2: Recovery data of metformin hydrochloride and ranolazine analyzed by the developed RP-HPLC method

<table>
<thead>
<tr>
<th>% Recovery level</th>
<th>Target Conc. (μg/ml)</th>
<th>Spiked Conc. (μg/ml)</th>
<th>% Recovery range (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RANO</td>
<td>MET</td>
<td>RANO</td>
</tr>
<tr>
<td>50</td>
<td>20</td>
<td>40</td>
<td>10</td>
</tr>
<tr>
<td>100</td>
<td>20</td>
<td>40</td>
<td>20</td>
</tr>
<tr>
<td>150</td>
<td>20</td>
<td>40</td>
<td>30</td>
</tr>
</tbody>
</table>

RANO: Ranolazine, MET: Metformin hydrochloride

Table 3: Repeatability study data Metformin hydrochloride and Ranolazine

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration (μg/ml) (n=6)</th>
<th>Concentration found (μg/ml) Mean±SD</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>RANO</td>
<td>20</td>
<td>19.96±0.10</td>
<td>0.513</td>
</tr>
<tr>
<td>MET</td>
<td>40</td>
<td>39.95±0.08</td>
<td>0.223</td>
</tr>
</tbody>
</table>

RANO: Ranolazine, MET: Metformin hydrochloride
Table 4: Intraday and interday precisions of Metformin hydrochloride and Ranolazine

<table>
<thead>
<tr>
<th>Precision</th>
<th>Drug</th>
<th>Interday (n=3)</th>
<th>Intraday (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Area (Mean±SD)</td>
<td>RSD</td>
</tr>
<tr>
<td>Level (%)</td>
<td>Ranolazine</td>
<td>50</td>
<td>27717.7±89.80</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>541142±3734.16</td>
<td>0.6901</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>782261±1946.9</td>
<td>0.2489</td>
</tr>
<tr>
<td>Metformin hydrochloride</td>
<td>50</td>
<td>916952±2199.29</td>
<td>0.2398</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>1844284±2430.56</td>
<td>0.2294</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>2754548±17645.5</td>
<td>0.6406</td>
</tr>
</tbody>
</table>

RANO: Ranolazine, MET: Metformin hydrochloride

Table 5: Results of robustness study of Metformin hydrochloride and Ranolazine

<table>
<thead>
<tr>
<th>Conc. (μg/ml)</th>
<th>Different Flow rate (RANO)</th>
<th>Different Flow Rate (MET)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.9 ml/min</td>
<td>1 ml/min</td>
</tr>
<tr>
<td>Ranolazine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>27717.7±89.80</td>
<td>27678.8±75.85</td>
</tr>
<tr>
<td>100</td>
<td>541142±3734.16</td>
<td>540824±1051</td>
</tr>
<tr>
<td>150</td>
<td>782261±1946.9</td>
<td>780943±4132.06</td>
</tr>
<tr>
<td>50</td>
<td>916952±2199.29</td>
<td>920196±1603.41</td>
</tr>
<tr>
<td>100</td>
<td>1844284±2430.56</td>
<td>1843165±5875.03</td>
</tr>
<tr>
<td>150</td>
<td>2754548±17645.5</td>
<td>2732908±11973</td>
</tr>
</tbody>
</table>

RANO: Ranolazine, MET: Metformin hydrochloride

Figure 3: Linearity overlay chromatogram of Metformin hydrochloride and Ranolazine
20 mg
100.77±0.33

20.17 mg

The result was found satisfactory. The result is expressed in Table 6.

CONCLUSION

A simple and economical RP-HPLC method has been developed for simultaneous estimation of RANO and Metformin HCl in a synthetic mixture. Linearity was found to be 1–50 μg/ml and 2–100 μg/ml for RANO and MET, respectively. Percentage recovery was found in the range 99.07–101.53% and 99.59–100.45% for RANO and MET, respectively, RSD was found <1 for precision and repeatability study. Hence, this method is less time-consuming, simple, specific, economical, robust, and accurate and can be successfully applied for the analysis of dosage forms with no interference by the pharmaceutical industry or research laboratories.

REFERENCES


