Original Research article

First Order Derivative Spectrophotometric Estimation of Ranolazine and Metformin Hydrochloride in Synthetic Mixture


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A B S T R A C T

This research study aimed at developing and validating a simple, precise, accurate, specific and economical spectrophotometric method for simultaneous estimation of Ranolazine (RANO) and Metformin hydrochloride (MET) in synthetic mixture. This method is based on first order derivative spectroscopy. Ranolazine and Metformin hydrochloride exhibited absorbance at working wavelength 232.86 nm (zero crossing point of metformin hydrochloride) and 249.29 nm (zero crossing point of ranolazine) respectively using water as a diluent. This method was validated as per ICH guideline. Linearity was established over the concentration range of 2-35 μg/mL both of ranolazine and metformin hydrochloride with correlation coefficients 0.999 and 0.998, respectively. Accuracy was obtained between 98.48-101.85% and 98.25-100.88% for ranolazine and metformin hydrochloride respectively. LOD and LOQ were found to be 0.08 μg/mL and 0.25 μg/mL for ranolazine and 0.25 μg/mL and 0.77 μg/mL for metformin hydrochloride, respectively. The results revealed that, the developed method was suitable for the routine analysis of determination of Ranolazine and Metformin hydrochloride in combine dosage form.

KEYWORDS
Ranolazine
Metformin hydrochloride
First order derivative
UV spectrophotometry
ICH guideline

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Graphical Abstract

Introduction

Combination of Ranolazine (RANO) and Metformin hydrochloride (MET) is used for treatment of patient suffering from chronic angina and co-morbid type 2 diabetes mellitus (T2DM). RANO increased the concentration of MET [1]. Both drugs are gave in combine dose ratio 1:1 [2]. RANO is $N$-(2,6-dimethylphenyl)-2-{4-[2-hydroxy-3-(2-methoxyphenoxy) propyl] piperazin-1-yl} acetamide, is class of antianginal drug. It’s act by inhibiting sodium and potassium ion channel is currents. This effect is obtain because of the inhibition of peak and late sodium channel, which in order increases myocardial function [3]. MET is 3-(diaminomethylidene) 1, dimethylguanidine, hydrochloride, is a hypoglycaemic agent. It may reduce the blood glucose levels by decreasing the hepatic glucose production, declining the intestinal absorption of glucose, and increasing the insulin sensitivity by increasing the peripheral glucose uptake and utilization [4]. The chemical structures of the RANO and MET are illustrated in Figure 1.

![Chemical structures of RANO and MET](image)

Figure 1. Structure of RANO [5]· MET [6]
Ranolazine and Metformin hydrochloride combination study running in phase-3 clinical trial. Co-administration of ranolazine and metformin hydrochloride was well tolerated in these T2DM subject, with no serious adverse event [2]. MET is official in IP [6], USP [7], JP [8], and BP [9] and RANO is not official in any pharmacopoeias. Several analytical methods reported such as UV spectrophotometry [10-14], HPLC [15-24], stability study [25-26] and UPLC [27] for the estimation of RANO and MET individually and it's combination with other drug. However, still no one any analytical method has been developed for the simultaneous estimation of these two drugs. So the present work discusses the spectrophotometric method development and validation for the simultaneous estimation of RANO and MET by first order derivative spectrophotometric method in combine dosage form. The proposed method was validated as per the ICH Q2 [R1] guideline [28].

Experimental

Chemicals and reagents
Ranolazine, Metformin hydrochloride and water were provided by B.K. Mody government pharmacy college. UV visible spectrophotometer (UV-1800 Shimadzu) used and data were processed using UV probe (version 2.6) software.

Preparation of standard stock solution
Standard stock solution of the RANO and MET 100 μg/mL was separately prepared in water. Further dilute to make concentration 10 μg/mL for RANO and MET.

Selection of wavelength
By appropriate dilutions from the standard stock solution, 1 μg/mL of RANO and MET were separately prepared and scanned in the UV range 200–400 nm. The overlain zero-order absorption spectra of both drugs were obtained. These absorbance spectra were converted to 1st order derivative spectra. After observing overlay first order derivative spectra with Δλ 4 and scaling factor 1 for RANO and MET, zero crossing points of drugs were selected. The first wavelength selected was 232.86 nm (zero crossing point of MET), where RANO showed considerable absorbance. The second wavelength selected was 249.29 nm (zero crossing point of RANO), where MET showed considerable absorbance. Selection of the wavelength spectra is demonstrated in Figure 2.
Figure 2. Wavelength selection spectrum of ranolazine and metformin HCL

Assay of combine dosage form
Combine dosage form was prepared by equivalent to take 15 mg for both RANO and MET with common tablet excipients in appropriate amount. This mixture was diluted with water to make concentration 15 μg/mL for both drugs.

Method validation
Linearity
The standard stock solution was diluted appropriately to obtain each concentration of 1, 2, 5, 10, 15, 20, 25, 30, 35 μg/mL of RANO and MET in 10 mL different volumetric flask.

Specificity
Specificity was performed under 6 replicates at concentration 15 μg/mL for both RANO and MET with and without addition of excipients to check the interference of excipient.

LOD/LOQ
The limit of detection (LOD) and limit of quantification (LOQ) were calculated using the Equation. For limit of detection (LOD) =3.3 σ/S, limit of quantification (LOQ) =10 σ/S, where σ = the standard deviation of the response, S = the slope of the calibration curve.

Accuracy
The accuracy of the method was carried out in triplicate at three different concentration levels of 80, 100, and 120% (12, 15 and 18 μg/mL) for RANO and MET by spiking in to placebo. The accuracy of method was evaluated by calculating the percentage of the recovery range.
Precision
Repeatability was performed in 6 replicates at concentration of 15 μg/mL for both RANO and MET. Intra-day and inter-day variations of RANO and MET were performed in triplicate at three different concentration levels 80, 100, 120% (12, 15 and 18 μg/mL). Precision was assessed by calculating the RSD.

Robustness
The robustness of method was established by introducing small change in experimental condition like wavelength. The changes made in wavelength ± 0.5 nm (232.36, 232.86, 232.91 nm for RANO and 248.79, 249.29, 249.79 nm for MET), respectively. The robustness of the method was evaluated by calculating the RSD.

Results and discussion
Linearity
The calibration curve obtained for RANO and MET was at the range of 2-35 μg/mL. The correlation coefficient of the RANO and MET was found to be 0.999 and 0.998, respectively. Thus, the method was found to be linear. Spectra and linearity graph are given in Figures 3 and 4.

Figure 3. Linearity spectra of (A) Metformin hydrochloride, (B) Ranolazine, (C) combined spectra of Ranolazine and Metformin hydrochloride
Specificity

Excipient interference was found to be less than 0.5% at the working wavelength of 232.86 nm for RANO and 249.29 nm for MET. Thus method is specific.

LOD and LOQ

LOD and LOQ of RANO and MET were determined by formula. LOD and LOQ were found to be 0.08 μg/mL and 0.25 μg/mL for ranolazine and 0.25 μg/mL and 0.77 μg/mL for metformin hydrochloride, respectively.

Accuracy

Accuracy of the recommend method was evaluated by adding known amount of standard drug into the placebo at three levels in three replicates. Accuracy was obtained between 98.48% - 101.85% and 98.25% - 100.88% for RANO and MET respectively. The results of the recovery study are shown in Table 1. Thus method is accurate as per recovery study.

Table 1. Accuracy study for Ranolazine and Metformin hydrochloride

<table>
<thead>
<tr>
<th>%Recovery level</th>
<th>Conc (μg/ml) + Placebo (n=3)</th>
<th>% Recovery range</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RONO</td>
<td>MET</td>
</tr>
<tr>
<td>80</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>100</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>120</td>
<td>18</td>
<td>18</td>
</tr>
</tbody>
</table>

Precision

Repeatability and intermediate precision express in term of RSD. Absorbance was determined and results found satisfactory as RSD<2 for both the intra-day and inter-day precision and including repeatability study. The results of the precision are presented in Tables 2 and 3, and the method was found to be precise.

Table 2. Repeatability study for Ranolazine and Metformin hydrochloride

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration (μg/ml) (n=6)</th>
<th>Absorbance (mean ± SD)</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>RANO</td>
<td>15</td>
<td>0.057 ± 0.0003</td>
<td>0.90</td>
</tr>
</tbody>
</table>
Table 3. Intermediate study for Ranolazine and Metformin hydrochloride

<table>
<thead>
<tr>
<th>Drug</th>
<th>Level (%)</th>
<th>Absorbance (mean ± SD)</th>
<th>RSD</th>
<th>Absorbance (mean ± SD)</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>RANO</td>
<td>80</td>
<td>0.0233 ± 0.0003</td>
<td>1.42</td>
<td>0.0233 ± 0.0003</td>
<td>1.42</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.0293 ± 0.0005</td>
<td>1.99</td>
<td>0.0296 ± 0.0003</td>
<td>1.13</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>0.0336 ± 0.0003</td>
<td>1.19</td>
<td>0.0338 ± 0.0005</td>
<td>1.49</td>
</tr>
<tr>
<td>MET</td>
<td>80</td>
<td>0.0443 ± 0.0007</td>
<td>1.71</td>
<td>0.0443 ± 0.0006</td>
<td>1.48</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.0563 ± 0.0001</td>
<td>0.34</td>
<td>0.0546 ± 0.0010</td>
<td>1.91</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>0.0746 ± 0.0001</td>
<td>0.25</td>
<td>0.0746 ± 0.0008</td>
<td>1.13</td>
</tr>
</tbody>
</table>

Robustness

Making a deliberate change in wavelength was taken place and RSD of absorbance found to be less than 2, specify that the method is robust. The Results are demonstrated in Table 4.

Table 4. Robustness study for Ranolazine and Metformin hydrochloride

<table>
<thead>
<tr>
<th>Conc (μg/mL)</th>
<th>Absorbance at different wavelength (RANO)</th>
<th>Absorbance at different wavelength (MET)</th>
</tr>
</thead>
<tbody>
<tr>
<td>232.36 nm</td>
<td>232.86 nm</td>
<td>233.06 nm</td>
</tr>
<tr>
<td>248.79 nm</td>
<td>249.29 nm</td>
<td>249.79 nm</td>
</tr>
<tr>
<td>15</td>
<td>0.029 ± 0.01</td>
<td>0.060 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>0.030 ± 0.01</td>
<td>0.059 ± 0.01</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>0.029 ± 0.001</td>
<td>0.058 ± 0.001</td>
</tr>
<tr>
<td>RSD</td>
<td>1.968 ± 0.001</td>
<td>1.968 ± 0.001</td>
</tr>
</tbody>
</table>

Assay of synthetic mixture

% Drug content of RANO and MET in combine dosage form was found 100.39 and 100.58% respectively. Results are given in Table 5.

Table 4. Assay of combine dosage form

<table>
<thead>
<tr>
<th>Conc (μg/mL)</th>
<th>RANO: MET (n=5)</th>
<th>Absorbance (n=5)</th>
<th>Conc. Found</th>
<th>% Drug content</th>
</tr>
</thead>
<tbody>
<tr>
<td>RANO</td>
<td>MET</td>
<td>Mean±SD</td>
<td>RANO</td>
<td>MET</td>
</tr>
<tr>
<td>15</td>
<td>0.033 ± 0.01</td>
<td>0.059 ± 0.01</td>
<td>15.56 ± 0.551</td>
<td>15.70 ± 0.451</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>15.56 ± 0.551</td>
<td>15.70 ± 0.451</td>
<td>100.58</td>
<td>100.39</td>
</tr>
</tbody>
</table>

Conclusions

The developed first order derivative method for simultaneous determination of Ranolazine (RANO) and Metformin hydrochloride (MET) in synthetic mixture. This method was validated as per ICH guideline. Linearity was established over the concentration range of 2-35 μg/mL for both of the Ranolazine and Metformin hydrochloride with the correlation coefficients of 0.999 and 0.998,
respectively. Accuracy was obtained at the range of 98.48-101.85% and 98.25-100.88% for the Ranolazine and Metformin hydrochloride, respectively. LOD and LOQ were found to be 0.08 μg/mL and 0.25 μg/mL for the Ranolazine and 0.25 μg/mL and 0.77 μg/mL for Metformin hydrochloride, respectively. The results revealed that, the developed method was suitable for determining the Ranolazine and Metformin hydrochloride in combine dosage form.

References
