Original Article

VALIDATED RP- HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF METFORMIN HYDROCHLORIDE AND BENFOTIAMINE IN BULK DRUG AND IN PHARMACEUTICAL DOSAGE FORM

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Abstract
A new simple, precise, accurate, selective and economical RP-HPLC method has been developed and validated for simultaneous estimation of Metformin Hydrochloride (MET) and Benfotiamine (BEN) in tablet dosage form. The method was carried out on a Waters column C-18 (250 mm x 4.6 mm, 5 µm) with a mobile phase consisting of water (pH 3.2 adjusted with ortho-phosphoric acid) and acetonitrile (75:25 v/v); at a flow rate of 0.8 mL min⁻¹ with run time of 10 min. Detection was carried out at 254 nm. The retention time for MET and BEN was found to be 2.125 and 3.881 min, respectively. The MET and BEN followed linearity in the concentration range of 200- 600 µg mL⁻¹ and 30- 90 µg mL⁻¹ with r²= 0.999, respectively. The amounts of both drugs estimated by proposed method were found to be in good agreement with label claim. The developed method was validated for precision, accuracy, sensitivity, robustness and ruggedness. The LOD and LOQ were found to be 0.49 and 0.19 µg for MET and 1.6 and 0.65 µg for BEN respectively. The developed method can be used for routine analysis of titled drugs in tablet formulation.

Keywords: Metformin Hydrochloride; Benfotiamine; RP-HPLC, Validation.

Introduction
Metformin hydrochloride (MET) is an oral anti-diabetic drug and is chemically N,N dimethylimidodicarbonimidic diamide hydrochloride (1,1-dimethylbiguanide hydrochloride) [1] which acts by suppressing excessive hepatic glucose production and improving glucose clearance, its predominant effect is to decrease fasting plasma glucose [2]. It is official in Indian Pharmacopoeia [3], British Pharmacopoeia [4], European Pharmacopoeia [5] and United States Pharmacopeia [6]. A literature survey revealed spectrophotometry [7-9], HPLC [10-14], LC-MS/MS [15] and Ion-pairing HPLC [16] methods for estimation of MET in pharmaceutical formulation. Benfotiamine (BEN) is a synthetic derivative of thiamine (vitamin B-1) and is chemically N-(4-amino-2-methyl-5-pyrimidinyl) methyl)-N-(4-hydroxy-2- mercapto-1-methyl-1 butenyl)formamide- S-benzoate dihydrogen phosphate [1] which show beneficial effects on general nerve health, neuropathy, retinopathy, peripheral neuropathy, general ageing [17-21] A literature survey revealed RP-HPLC [22-23] method for estimation of benfotiamine in pharmaceutical formulation. Combination product of metformin hydrochloride (MET) and benfotiamine (BEN) is Benforce M tablet which used as a nutraceuticals [24]. The chemical structures of both drugs are shown in (Figure 1, 2).

To the best of our knowledge, few literature methods were reported for estimation of MET and BEN individually. but no literatures have been found for simultaneous determination of MET and BEN in pharmaceutical preparations. The present paper describes a precise, accurate, specific, sensitive and cost effective RP-HPLC method for the simultaneous determination of MET and BEN in the tablet dosage form and validation of the same as per the ICH guidelines [25-27].

Materials and Methods

Chemicals
Metformin hydrochloride and Benfotiamine were obtained from Rajat Pharmachem. Pvt. ltd, Ankleshwar and Akhil...
Preparation of Standard Stock Solutions

Standard stock solutions of 2000 µg mL⁻¹ of MET and 300 µg mL⁻¹ of BEN were prepared separately using diluent. The stock solution of MET was diluted with diluent to give working standard solutions containing 200 - 600 µg mL⁻¹ concentrations, similarly the BEN stock solution was diluted with diluent to give working standard solutions in the range 30 - 90 µg mL⁻¹. These standard solutions were injected into HPLC column and calibration curves were plotted by taking drug peak areas vs concentrations.

Analysis of marketed tablet formulation

To determine the content of MET and BEN in tablets (Brand name: Benforce M, label claim: MET 500 mg, BEN 75 mg per tablet), twenty tablets were weighed, their mean weight determined and finally powdered. An accurately weighed tablet powder equivalent to 500 mg of MET and 75 mg BEN was transferred into 100 mL volumetric flask containing 50 mL of diluent, sonicate for 10 minute and volume was made up to mark with diluent, the resulting solution was filtered using 0.45 µm filter (Mill filter, Milford, MA). From filtrate, 8 mL of solution was transferred into 100 mL volumetric flask and volume was made up to mark with diluent to obtain the concentration of 400 µg mL⁻¹ MET and 60 µg mL⁻¹ of BEN was subjected to propose method and the amount of MET and BEN were determined.

Validation of Method

The HPLC method was validated in accordance with ICH guidelines.

**Precision**

The precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample. Precision was estimated by repeatability and the repeatability was assessed by analyzing six injection of a homogeneous sample of 400 µg mL⁻¹ of MET and 60 µg mL⁻¹ of BEN. The value of precision of repeatability along with intra-day and inter day using three different concentrations 300 µg mL⁻¹, 400 µg mL⁻¹ and 500 µg mL⁻¹ of MET and 45 µg mL⁻¹, 60 µg mL⁻¹ and 75 µg mL⁻¹ of BEN respectively, in triplicate for three consecutive days.

**Accuracy**

The different between theoretical added amount and practically achieved amount is called accuracy of analytical method. Accuracy was determined at three different level 80 %, 100 % and 120 % of the target concentration 400 µg mL⁻¹ of MET and 60 µg mL⁻¹ of BEN in triplicate.

**Specificity**

The specificity of an analytical method may defined as the ability to detect the analyte peak in the presence of the analyte by product, or other inactive components, such as dosage form excipient or impurities.

**Limit of detection (LOD) and Limit of quantification (LOQ)**

Limit of detection and limit of quantification were estimated from signal to noise ratio. LOD is the lowest concentration resulting in a peak area of three times the baseline noise and the equation is LOD = 3.3 x ASD/S. LOQ is the lowest concentration that provide signal to noise ratio more than 10 and the equation is LOQ = 10 x ASD/S, where ‘ASD’ is the average standard deviation and ‘S’ is the slope of the line.

**Robustness**

Robustness was performed by deliberately changing the chromatographic conditions. The important parameter to be studied was the resolution factor between two peaks. Robustness of the method was carried out by deliberately made small variation in the flow rate, pH of mobile phase, organic phase ratio and column oven temperature by using 400 µg mL⁻¹ of MET and 60 µg mL⁻¹ solution of BEN, respectively.
Ruggedness
Ruggedness was determined between two different labs, different analyst, different instrument and columns. Ruggedness of the method was performed by injecting 400 µg mL⁻¹ of MET and 60 µg mL⁻¹ solution of BEN, respectively.

Results and Discussion
Selection of Chromatographic Conditions and Optimization of Mobile Phase
Mobile phase was optimized to separate MET and BEN using Waters column C₁₈, 5 µ (250 X 4.6 mm). Initially, phosphate buffer pH 3.5 and acetonitrile in the various proportions were tried as mobile phase but the broad peaks for both the drugs were observed. Therefore, we select Water (pH 3.2 adjusted ortho-phosphoric acid) and acetonitrile as a mobile phase composition in 75:25 % v/v ratio. Good resolution and symmetric peaks were obtained for both drugs when the pH of the mobile phase was adjusted to 3.2. The flow rate of the mobile phase was 0.8 mL min⁻¹. Under optimum chromatographic conditions, the retention time for MET and BEN was found to be 2.125 and 3.881 respectively when the detection was carried out at 254 nm. A typical chromatogram of two drugs is shown in Figure 3.

Linearity
The linearity was determined at five levels over the range of 50 % to 150 % of standard concentration in a diluent and calibration curve constructed by plotting peak area against the respective concentrations. The linearity of MET and BEN followed in the concentration range of 200 - 600 µg mL⁻¹ and 30 - 90 µg mL⁻¹, respectively. Each sample solution was chromatographed in triplicate and the mean peak area for MET and BEN calculated. The results are shown in Table 1, Figures 4,5.

Precision
The precision of this method was evaluated by calculating the % RSD of the peak area of six replicate injections. The RSD for repeatability of MET and BEN was found to be 0.55 and 1.27 %, respectively. The RSD values for intra-day precision for MET and BEN were found to be in the range 0.11-0.31 and 0.75 -1.29 %, respectively and the inter-day precision for MET and BEN was found to be in the range 0.18 - 0.25 and 0.67- 1.3 %, respectively. The assay method precision acceptance criteria set in the validation was RSD ≤ 2.0%. Results of precision study are shown in Table 2.

Table 1: Linearity Studies
<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>MET</th>
<th>BEN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity [µg mL⁻¹]</td>
<td>200 – 600</td>
<td>30 – 90</td>
</tr>
<tr>
<td>Linearity Equation</td>
<td>Y = 5481X +33007</td>
<td>Y = 26543X-10859</td>
</tr>
<tr>
<td>Slope</td>
<td>5481</td>
<td>26543</td>
</tr>
<tr>
<td>Intercept</td>
<td>33007</td>
<td>10859</td>
</tr>
<tr>
<td>Correlation Coefficient</td>
<td>0.999</td>
<td>0.999</td>
</tr>
</tbody>
</table>

Table 2: Results of Precision studies

<table>
<thead>
<tr>
<th>Conc. [µg/mL]</th>
<th>MET</th>
<th>Conc. [µg/mL]</th>
<th>BEN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amount Found</td>
<td>% RSD</td>
<td>Amount Found</td>
</tr>
<tr>
<td></td>
<td>in µg mL⁻¹ [n= 3] ± SD</td>
<td></td>
<td>in µg mL⁻¹ [n= 3] ± SD</td>
</tr>
<tr>
<td>Intra – day Precision</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>300.27 ± 0.51</td>
<td>0.17</td>
<td>45</td>
</tr>
<tr>
<td>400</td>
<td>400.40 ± 0.45</td>
<td>0.11</td>
<td>60</td>
</tr>
<tr>
<td>500</td>
<td>499.85 ± 0.59</td>
<td>0.31</td>
<td>75</td>
</tr>
<tr>
<td>Inter – day Precision</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>299.44 ± 0.65</td>
<td>0.21</td>
<td>45</td>
</tr>
<tr>
<td>400</td>
<td>399.99 ± 0.74</td>
<td>0.18</td>
<td>60</td>
</tr>
<tr>
<td>500</td>
<td>499.18 ± 1.26</td>
<td>0.25</td>
<td>75</td>
</tr>
</tbody>
</table>

Figure 3: Typical Chromatogram of MET and BEN

Figure 4: Calibration curve of MET Correlation Coefficient = 0.999, Slope = 5481, Intercept = 33007

Figure 5: Calibration curve of BEN Correlation Coefficient = 0.999, Slope = 26543, Intercept = 10859
System Suitability

Test according to USP 2009, system suitability tests were an integral part of liquid chromatographic methods in the course of optimizing the conditions of the proposed method. System suitability tests were used to verify that the resolution and reproducibility were adequate for the performed. The parameter of these tests is column efficiency (number of theoretical plate), tailing factor, resolution, peak asymmetry and capacity factor were calculated for standard solutions. The results obtained from validation of the methods and system suitability studies are summarized in Table 5.

Table 5: Summary of system suitability study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MET</th>
<th>BEN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention time [tR]</td>
<td>2.125</td>
<td>3.881</td>
</tr>
<tr>
<td>Theoretical plates [N]</td>
<td>2736</td>
<td>7742</td>
</tr>
<tr>
<td>Resolution [Rs]</td>
<td>-</td>
<td>7.93</td>
</tr>
<tr>
<td>Asymmetry [T]</td>
<td>0.93</td>
<td>0.94</td>
</tr>
</tbody>
</table>

Conclusion

The proposed new RP-HPLC method provide simple, fast, accurate, precise, reproducible and economical approach for the simultaneous identification and quantification that can be used for the quality control of the metformin hydrochloride and benfotiamine in tablet formulation in routine quality control laboratories and the method was validated as per ICH guidelines.

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References
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