DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF S(-) METOPROLOL SUCCINATE AND CLOPIDOGREL BISULPHATE IN BULK AND TABLET DOSAGE FORM

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Received: 14 Jun 2013, Revised and Accepted: 06 Jul 2013

ABSTRACT

The objective of present work was to develop an UV spectrophotometric method for simultaneous determination of S(-) Metoprolol Succinate (S(-) MET) and Clopidogrel Bisulphate (CLOP) in bulk and tablet dosage form. Methanol was used as a common solvent for both drugs. This method was based on generation of simultaneous equations at 224 nm and 219 nm. Linearity for both drugs was found in the concentration range of 5-30 μg/ml. This method was validated as per International Conference on Harmonization (ICH) guidelines. Low values of %RSD for intra- and inter-day precision suggested reproducibility of the method. Satisfactory values of percent recovery indicated accuracy of the method. Sensitivity of the method was proved by low value of Limit of Detection and Limit of Quantification. Assay results of marketed formulation were found to be 101.90% and 101.11% for S(-) MET and CLOP, respectively. Results suggest that the proposed method can be applied in routine quality control studies for assay of S(-) MET and CLOP in bulk and tablet dosage forms.

Keywords: S(-) Metoprolol Succinate, Clopidogrel Bisulphate, Determination, UV spectrophotometric, Simultaneous equation method.

INTRODUCTION

Metoprolol is widely used in the treatment of hypertension, angina and congestive heart failure [1]. Metoprolol is a racemic mixture of R- and S-isomers. S-isomer exhibits beta-2 adrenergic receptor blocking activity (cardio selectivity) while R-isomer exhibits beta-2 adrenergic receptor blocking activity [2]. The cardiac therapeautic effect of metoprolol is due to S-isomer whereas side effects are due to R-isomer. Therefore, S(-) Metoprolol, active enantiomer is preferred due to its specificity, less dose (half of the racemate dose); devoid of beta-2 receptor mediated side effects [1, 3]. S(-) Metoprolol Succinate (S(-) MET) is chemically, (2S)-1-[4-(2-Methoxyethyl) phenoxyl]-3-[1-methylthethyl] amino)-2-propanol Succinate (Figure 1).

Clopidogrel Bisulphate (CLOP) is widely used to prevent myocardial infarction and ischaemic stroke [4]. CLOP is an anti thrombotic agent which is an analogue of ticlopidine [5]. It selectively and irreversibly inhibits the binding of adenosine diphosphate (ADP) to its platelet receptors thus prevents ADP induced platelet aggregation through an active metabolite [4]. Clopidogrel Bisulphate (CLOP) is chemically, methyl (S)-α-(6-chlorophenyl) - 6, 7 dihydrossothio [3, 2-c] pyridine-5-(H)-acetate sulphate (Figure 2). Clopidogrel Bisulphate is official drug in Indian Pharmacopoeia and United State Pharmacopeia [6, 7].

S(-) MET and CLOP combination is useful for treatment of hypertension in patients who need antplatelet therapy. A number of analytical methods have been reported for estimation of racemic Metoprolol succinate in single component dosage form and in combination with other drugs, including UV spectrophotometry [8-10], HPLC [11-12], HPTLC [13] and chromatography-tandem mass spectrometry [14-15]. Several methods have been reported for CLOP in single form and in combination with other drugs including spectrophotometry [16], HPLC [17-21], HPTLC [22-23]. However, no UV spectrophotometric method for simultaneous determination of S(-) MET and CLOP in combined dosage form has been reported so far. Such determination plays an important role in routine analysis of the formulation, especially in pharmaceutical industry. Therefore, present study comprises the development and validation of UV spectrophotometric method for determination of S(-) MET and CLOP in bulk and tablet dosage form.

MATERIALS AND METHODS

Chemicals and Reagents

Active pharmaceutical ingredients of S(-) MET and CLOP were received as a gift samples from Emcure Pharmaceuticals Limited Bhosari, Pune (India). Commercially available tablets (Label Claim: 50 mg of S(-) Metoprolol succinate and 75 mg of clopidogrel Bisulphate) of the combined dosage form were procured from local market. The solvent (methanol) used was of analytical grade. It was purchased from Merck India Ltd.

Instruments

Shimadzu UV 1800 (Japan) double beam spectrophotometer with 1 cm matched quartz cells and connected to computer loaded with UV Probe Software was employed for this work Shimadzu AX200 (Japan) digital balance and Spectra lab UCB 40 (Germany) ultrasonicator, were also used.

Preparation of Standard Solutions

The standard stock solution of S(-) MET was prepared by transferring, accurately weighed, 100 mg of API to 100 mL of volumetric flask. The drug was suitably dissolved with sonication in 40 mL of methanol and volume was made up to the mark by using methanol. This standard stock solution was further diluted with the same solvent to obtain 10 μg/mL of S(-) MET. Similarly, Solution of CLOP was prepared in methanol to get a concentration of 10 μg/mL.

Simultaneous equation method

This method was based on absorption of drugs (S(-) MET and CLOP) at the wavelength maximum of both drugs. Wavelength maximum of these drugs was selected by scanning the standard solutions of pure single drug within 400-200 nm after baseline correction and an overlay spectrum was obtained (Figure 3). Here, 224 nm (λ1) and 219 nm (λ2) were selected as sampling wavelengths for this method. The calibration curves were prepared in the concentration range of 5-30 μg/ml on these wavelengths for both drugs. The absorptivity values were calculated for both drugs at these wavelengths [24-25]. The concentrations of the drugs were obtained by using following equations [26].

\[
C_x = \frac{A_2 ay_1 - A_1 ay_2}{ax_2 ay_1 - ax_1 ay_2} \quad \text{Eq. 1}
\]

\[
C_y = \frac{A_1 ax_2 - A_2 ax_1}{ay_1 ax_2 - ay_2 ax_1} \quad \text{Eq. 2}
\]
Interday precision was 1.775 and 1.620, respectively and accuracy of the method was determined by assaying six different sample preparations on the same day. Interday precision was performed by assaying six different sample preparations on different days at different time intervals. The percentage relative standard deviation (%RSD) was calculated (Table 2 and 3).

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness [27]. The method was applied to drug sample and accuracy of the method was determined by calculating recovery of S (-) MET and CLOP at 80%, 100% and 120% level of label claim. Percentage recovery was calculated using equation for the method and the results are presented in Table 4.

Limit of Detection (LOD) and Limit of Quantitation (LOQ):

Six sets of known concentrations (5-30 µg/ml) were prepared. Calibration curves were plotted for each set. LOD and LOQ were calculated using the formulae as

\[
LOD = \frac{3.3 \times SD}{S}
\]

\[
LOQ = \frac{10 \times SD}{S}
\]

Where, S is value of slopes of calibration plot and SD is calculated using values of y intercepts of regression equations. The results of LOD and LOQ are presented in Table 5.

RESULTS AND DISCUSSION

The summary of validation parameters for the proposed analytical spectrophotometric method is given in Table 5. Here, value of \( R^2 \) was very close to 1 (Figure 4 and Figure 5), which suggest that the developed method is following linearity in the concentration range of 5-30 µg/ml for both drugs. %RSD values of S (-) MET and CLOP for the intra-day precision were 1.775 and 1.620, respectively and %RSD values of S (-) MET and CLOP for the inter-day precision were 1.893 and 1.930, respectively. Results of %RSD were within limits (≤ 2%). This indicates good precision of developed method. Percent recovery ranges from 98.15-99.97% for S (-) MET and 98.30-100.42% for CLOP. The results of recovery study proved that the developed method is accurate. Sensitivity of the method was determined by calculating limit of detection (LOD) and limit of quantitation (LOQ). Limit of detection for S (-) MET and CLOP was 0.097 µg/ml and 0.243 µg/ml, respectively. Limit of quantitation for S (-) MET and CLOP was found 0.294 µg/ml and 0.736 µg/ml, respectively with suitable precision and accuracy. Results of assay of tablets range from 101.11-101.90%, which suggest no interference from the excipients of formulation.

**Fig. 1: Chemical structure of S (-) Metoprolol Succinate [Chiral Center is indicated by (\*)]**

**Fig. 2: Chemical structure of Clopidogrel Bisulphate**
Fig. 3: Overlain spectrum of S (−) MET (λmax 224 nm) and CLOP (λmax 219 nm) in Methanol

Fig. 4: Calibration Curves of S (−) MET at 224 nm and 219 nm

Absorbance vs. Concentration (µg/mL)

- S (−) MET at 224 nm:
  \[ y = 0.0364x + 0.0101 \]
  \[ R^2 = 0.9991 \]

- CLOP at 219 nm:
  \[ y = 0.031x + 0.0095 \]
  \[ R^2 = 0.9986 \]
### Table 1: Assay of Tablet dosage form

<table>
<thead>
<tr>
<th>Drug</th>
<th>Label Claim (mg)</th>
<th>Amount Found (mg)</th>
<th>Mean % Drug Recovered ±SD*</th>
<th>% RSD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>S(-)MET</td>
<td>50</td>
<td>50.95</td>
<td>101.90 ± 1.0</td>
<td>0.981</td>
</tr>
<tr>
<td>CLOP</td>
<td>75</td>
<td>75.83</td>
<td>101.11 ± 1.293</td>
<td>1.279</td>
</tr>
</tbody>
</table>

*n=3

### Table 2: Intraday Precision

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration(µg/ml)</th>
<th>Mean % Assay ± SD*</th>
<th>% RSD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>S(-)MET</td>
<td>10</td>
<td>101.41 ± 1.80</td>
<td>1.775</td>
</tr>
<tr>
<td>CLOP</td>
<td>15</td>
<td>100.97 ± 1.650</td>
<td>1.620</td>
</tr>
</tbody>
</table>

*n=6

### Table 3: Interday Precision

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration(µg/ml)</th>
<th>Mean % Assay ± SD*</th>
<th>% RSD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>S(-)MET</td>
<td>10</td>
<td>101.55 ± 1.922</td>
<td>1.893</td>
</tr>
<tr>
<td>CLOP</td>
<td>15</td>
<td>101.80 ± 1.965</td>
<td>1.930</td>
</tr>
</tbody>
</table>

*n=6

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**Fig. 5: Calibration Curves of CLOP at 219nm and 224 nm**

- **Top Diagram:**
  - Absorbance vs. Concentration(µg/mL)
  - Equation: $y = 0.0351x + 0.012$
  - $R^2 = 0.9989$

- **Bottom Diagram:**
  - Absorbance vs. Concentration(µg/mL)
  - Equation: $y = 0.0327x - 0.001$
  - $R^2 = 0.9969$
CONCLUSION

From statistical data it is clear that the developed method is simple, rapid, precise, accurate and economical for simultaneous estimation of S(-) MET and CLOP in combined dosage form. This method was validated as per ICH guidelines. Results suggest that the proposed method can be used for routine quality control studies for assay of S(-) MET and CLOP in bulk and combined tablet dosage form.

ACKNOWLEDGEMENT

The authors would like to convey regards to Emcure Pharmaceuticals Ltd., Bhosari, Pune (India) for providing gift sample of S(-) MET and CLOP for research work. The authors also thank to Dr. K. N. Gujjar, Principal, Sinhgad college of Pharmacy, Vadgaon (Bk), Pune for providing the necessary facilities to carry out this research work.

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