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Research Article

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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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 Quantitation. 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 a 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-1 adrenergic receptor blocking activity (cardio selectivity) while R-isomer exhibits beta-2 adrenergic receptor blocking activity [2]. The cardiac therapeutic 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, (2*S*)-1-[4-(2-Methoxyethyl) phenoxy]-3-[(1-methylethyl) amino]-2-propanol Succinate (Figure 1).

Clopidogrel Bisulphate (CLOP) is widely used to prevent myocardial infarction and ischaemic stroke [4]. CLOP is an antithrombotic 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-(o-chlorophenyl) - 6, 7 dihydrothieno [3, 2-c] pyridine-5-(4H)-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 antiplatelet 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 10µg/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 overlain spectrum was obtained (Figure 3). Here, 224 nm (λ_1) and 219 nm (λ_2) 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].

$$Cx = \frac{A_2\, ay_1 - A_1\, ay_2}{ax_2\, ay_1 - ax_1\, ay_2} \quad \text{Eq.1}$$

$$Cy = \frac{A_1\, ax_2 - A_2\, ax_1}{ay_1\, ax_2 - ay_2\, ax_1} \quad \text{Eq.2}$$

Where, A_1 and A_2 are absorbance of mixture at 224 nm and 219 nm, respectively; ax_1 and ax_2 are absorptivities of S (-) MET at λ_1 and λ_2, respectively and ay_1 and ay_2 are absorptivities of CLOP at λ_1 and λ_2, respectively. C_x and C_y are the concentrations of S (-) MET and CLOP, respectively.

Assay of Combined Tablet Dosage Form

Twenty tablets were accurately weighed and average weight was calculated. These tablets were crushed and powdered in glass mortar. Tablet Powder equivalent to 75 mg of CLOP was weighed accurately and transferred into a 100 ml volumetric flask. It was dissolved with about 40 ml methanol. The sample contents were sonicated for 15 minutes and volume was made up to the mark with methanol. The solution was filtered using Whatmann filter paper (No.41). This solution was further diluted with methanol to obtain final concentration of S (-) MET (10 µg/ml) and CLOP (15 µg/ml). The absorbance of final sample solution was measured against methanol as blank at 224 nm and 219 nm. All the determinations were carried out at three times and then concentrations of both drugs were calculated using simultaneous equation method. The results of analysis are given in Table 1.

Method Validation

Validation of an analytical procedure is the process by which it is established by laboratory studies that the performance characteristics of the procedure meet the requirements for the intended analytical application. The proposed method was validated for various parameters such as linearity, precision, accuracy, Limit of detection (LOD), Limit of Quantitation (LOQ) according to ICH Q2 (R1) guidelines [27].

Linearity and range

Linearity was studied by diluting stock standard solutions of S (-) MET and CLOP with methanol to give a concentration range of 5 to 30 µg/ml. Calibration curve of Absorbance vs. Concentration was plotted using standard solutions of 5µg/ml to 30µg/ml and regression line equation and correlation coefficient was determined. The range of solution has been decided according to statistical analysis of regression equation. Calibration curve for both drugs are shown in Figure 4 and Figure 5.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions [27]. Precision of the method was studied by intra- and inter-day variations in the test method of S (-) MET and CLOP. Intraday precision was evaluated 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

$$LOD = 3.3\frac{SD}{S}$$

$$LOQ = 10\frac{SD}{S}$$

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.

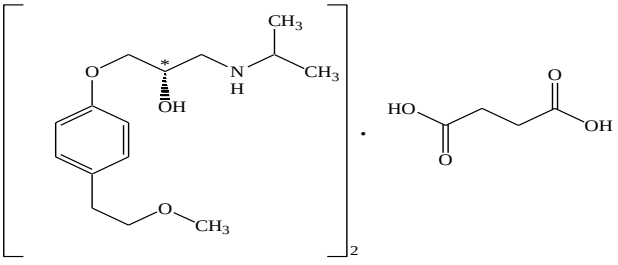

Fig. 1: Chemical structure of S (-) Metoprolol Succinate [Chiral Center is indicated by (*)]

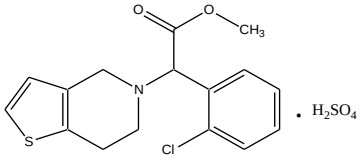

Fig. 2: Chemical structure of Clopidogrel Bisulphate

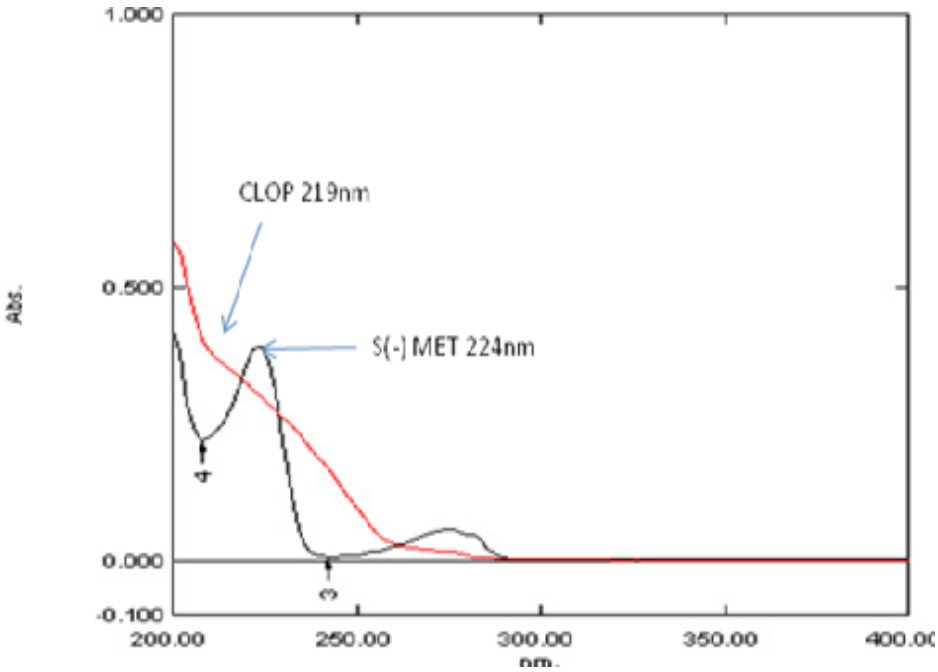

Fig. 3: Overlain spectrum of S (-) MET (λmax 224 nm) and CLOP (λmax 219 nm) in Methanol

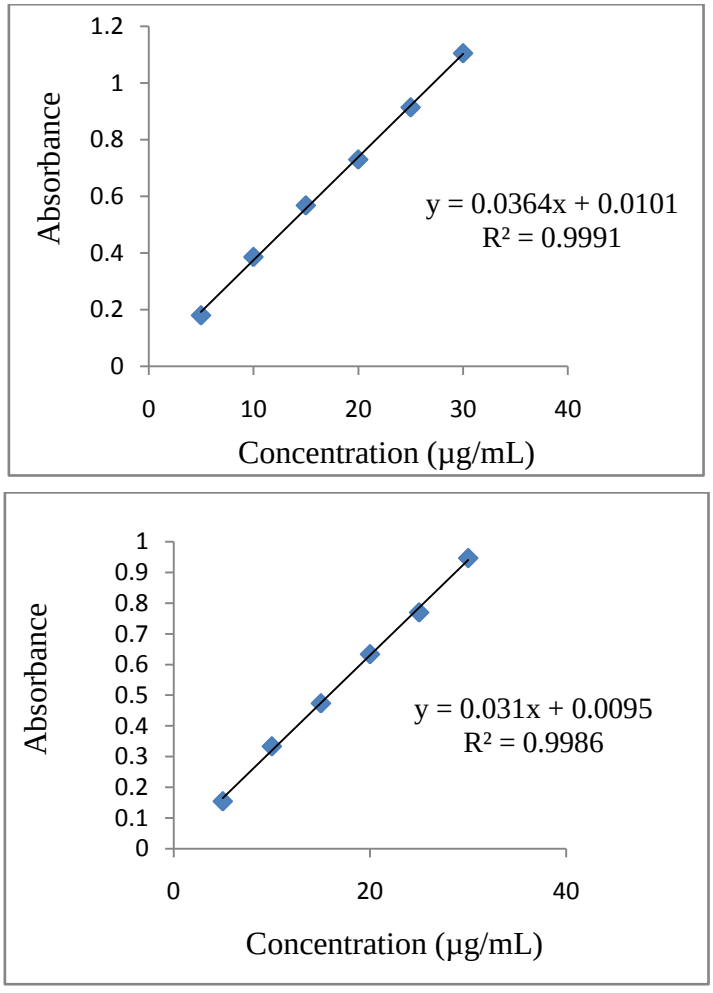

Fig. 4: Calibration Curves of S (-) MET at 224 nm and 219 nm

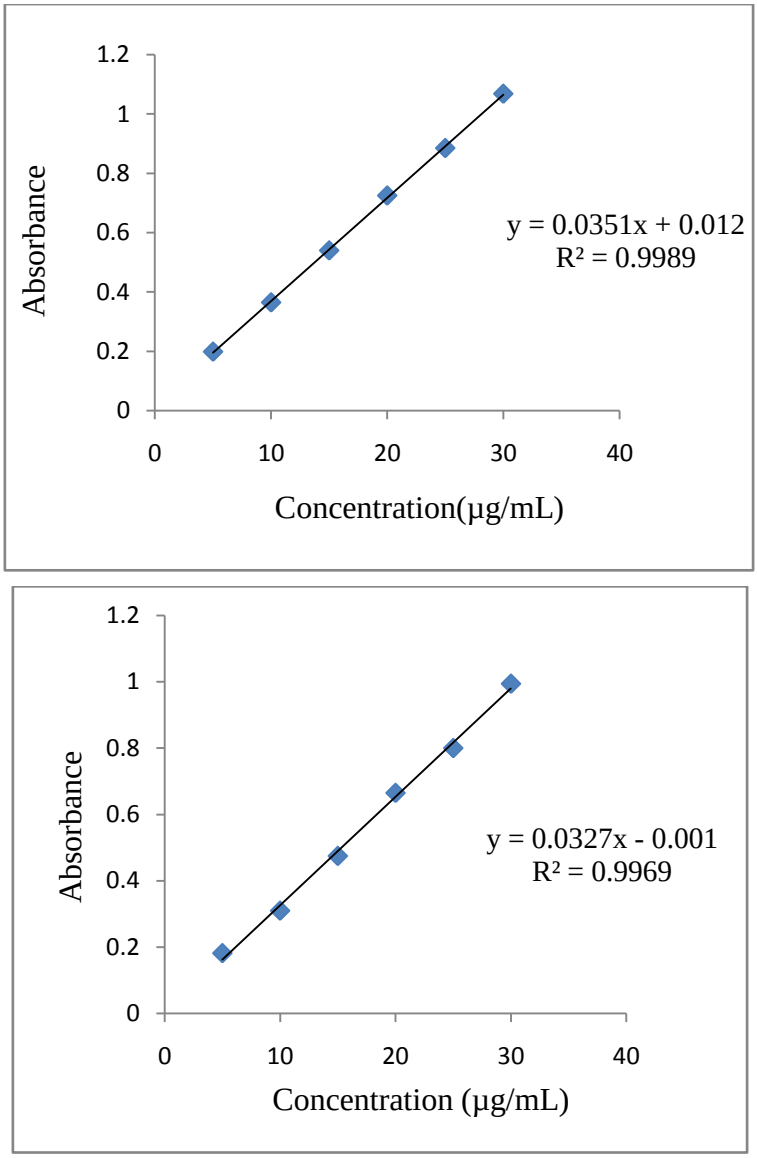

Fig. 5: Calibration Curves of CLOP at 219nm and 224 nm

Table 1: Assay of Tablet dosage form

Drug	Label Claim (mg)	Amount Found (mg)	Mean % Drug Recovered ±SD*	% RSD*
S(-)MET	50	50.95	101.90 ± 1.0	0.981
CLOP	75	75.83	101.11 ± 1.293	1.279

*n=3

Table 2: Intraday Precision

Drug	Concentration(µg/ml)	Mean % Assay ± SD*	% RSD*
S(-)MET	10	101.41 ± 1.80	1.775
CLOP	15	100.97 ± 1.650	1.620

*n=6

Table 3: Interday Precision

Drug	Concentration(µg/ml)	Mean % Assay ± SD*	% RSD*
S(-)MET	10	101.55 ± 1.922	1.893
CLOP	15	101.80 ± 1.965	1.930

*n=6

Table 4: Recovery Study

Drug	Amount Added (µg/ml)	Amount recovered (µg/ml)	Mean % Recovery ± SD*	% RSD*
S(-)MET	80% (8 µg/ml)	7.99	99.97% ± 1.20	1.200
	100% (10 µg/ml)	9.97	99.81% ± 0.535	0.533
	120% (12 µg/ml)	11.77	98.15% ± 0.147	0.150
CLOP	80% (12 µg/ml)	11.79	98.30% ± 1.863	1.895
	100% (15 µg/ml)	15.09	100.42% ± 0.214	0.210
	120% (18 µg/ml)	18.01	100.10% ± 0.531	0.529

*n=3

Table 5: Analytical Method Validation Results

Parameter	S(-)MET	CLOP
λ max (nm)	224	219
Linearity and Range (µg/ml)	5-30	5-30
Correlation Coefficient (R^2)	0.9991 at 224 nm	0.9989 at 219 nm
	0.9986 at 219 nm	0.9969 at 224 nm
Precision (% R.S.D*)		
Intraday	1.775	1.620
Interday	1.893	1.930
Accuracy (Mean % Recovery)	98.15-99.97	98.30-100.42
LOD (µg/ml)	0.097	0.243
LOQ (µg/ml)	0.294	0.736

*n=6

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.

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