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Spectrophotometric Estimation And Validation Of Metoprolol Succinate And Amlodipine Besylate By Different Method From Pure And Tablet Dosage Form

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Abstract: A simple, validated, accurate, economic and reproducible UV-Spectrophotometric method has been developed for the Simultaneous Estimation of Amlodipine Besylate and Metoprolol Succinate in both bulk and tablet formulation. Amlodipine Besylate and Metoprolol Succinate in combined tablet formulation were estimated by using the Simultaneous Equation Method and Absortion Ratio Method at 223 nm for Metoprolol Succinate and 240nm for Amlodipine Besylate in their solution in distilled water. The Beer's law obeyed the concentration range of 5-25 μ g/ml for Metoprolol Succinate and 5-25 μ g/ml for Amlodipine Besylate. Mean recovery of 98.78% for Metoprolol Succinate and 102.82% for Amlodipine Besylate respectively signifies the accuracy of the method. This methods can be used for the routine estimation of Metoprolol Succinate and Amlodipine Besylate in industries and other analytical laboratories.

Key words: Amlodipine Besylate, Metoprolol Succinate, Simultaneous Equation Method, Absorption Ratio Method.

INTRODUCTION:

Metoprolol is the prototype of cardio selective adrenoceptor antagonist. It is official in IP (Indian Pharmacopoeia, 1996) and chemically it is 1-[4-(2methoxyethyl) phenoxy]-3-[(1-methylethyl)amino]-2- propanol. Amlodipine is the calcium channel blocker, pharmacokinetically, the most distinct dihydropyridines. Chemically it is 2-[(2-Aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4-

dihydro-6- methyl-3,5-pyridine dicarboxylic acid- 3ethyl 5-methyl ester. A fixed combination of Metoprolol Succinate (25 mg) and Amlodipine Besylate (5 mg) are marketed as tablet formulation (Met XL AM 25/5). This combination is used in treatment of angina pectoris, cardiac arrhythmia and hypertension. However, there is no analytical method reported for the simultaneous estimation and Absorbance ratio method of Metoprolol Succinate and Amlodipine Besylate in a combined dosage formulation. Present work describes simple, accurate, reproducible, rapid and economical methods for simultaneous estimation of Metoprolol Succinate and Amlodipine Besylate in tablet formulation¹⁻⁴.





Figure 2: Structure of Amlodipine Besylate:



MATERIALS AND METHODS:

Chemicals & Reagents: Analytically pure Metoprolol Succinate and Amlodipine Besylate were obtained as gift samples. Commercial tablet formulations were purchased from the local market. All chemicals and reagents used were of Analytical Grade, obtained from Merck.

Instrument: A JASCO double beam UV/Visible recording spectrophotometer (Model: V-630) with 2 nm spectral bandwidth was employed for all spectrophotometric measurement using 10mm matched quartz cell and Borosil glass wares were used for the study. Calibrated electronic single pan balances Shimadzu AY 220, Ultrasonic Bath Sonicator were also used during the analysis.

Standard Stock Solution:

The standard stock solutions of Metoprolol Succinate and Amlodipine Besylate were prepared by dissolving accurately weighed 10 mg of drug in 100 ml of 0.1N NaOH in two separate 100 ml volumetric flasks to get a concentration of 100 mg/ml (Stock Solution).

Determination of max:

The standard solution of both Metoprolol Succinate and Amlodipine Besylate (10 μ g /ml) were scanned in the wavelength region of 200-400nm and the max was found to be 223nm and 240nm respectively^{5,6}.

Preparation of calibration curve:

For each drug, appropriate aliquots were pipetted out from each standard stock solution into a series of 10 ml volumetric flasks. The volume was made up to mark with 0.1N NaOH to get set of solutions having concentration range 5-25 µg/ml for each drug. The prepared working solutions of Metoprolol Succinate and Amlodipine Besylate were scanned at 223nm and 240nm respectively. The respective absorbances were recorded and absorbances were plotted against concentrations to obtain the respective the calibration curves. The absorbance maxima of Metoprolol Succinate (223nm) and Amlodipine Besylate (240nm) were used for the analysis of Metoprolol Succinate and Amlodipine Besylate respectively by Simultaneous Equation Method and Absorbance Ratio Method. Five different mixed standards of Metoprolol Succinate and Amlodipine Besylate were prepared from stock solution of 100 µg/ml of Metoprolol Succinate and 100 µg/ml of Amlodipine Besylate. Before analyzing the selected tablet formulations the method was validated by analyzing the physical admixtures of Metoprolol Succinate and Amlodipine Besylate in the ratio of

each component as in the formulation in consideration 7 .

Assay of tablet dosage form:

Two batches of tablet formulations containing 25 mg and 5 mg of Metoprolol Succinate and Amlodipine Besylate respectively were used for analysis by the proposed method. The tablets were triturated with the help of mortar and pestle and made fine powder. Then the residue equivalent to average weight of a tablet was dissolved in 0.1N NaOH and sonicated for 10 minutes in the sonicator. This sonicated solution was filtered by using Whatmann filter paper and the volume was made to 100 ml by 0.1N NaOH. Different solutions were prepared and analyzed as per procedure. The results were validated statistically. Preanalyzed samples of tablet were taken to which different amount of standard solution of Metoprolol Succinate and Amlodipine Besylate were added and analyzed tostudy recovery of drugs by proposed method⁸.

Simultaneous Equation Method:

Two wavelengths selected for the method are 223 nm and 240 nm. The stock solutions of both the drugs were further diluted separately with 0.1N NaOH to get a series of standard solutions of 5-25µg /mL concentrations of Amlodipine Besylate and 5-30ug /mL concentrations of Metoprolol Succinate. The absorbance were measured at the selected wavelengths and absorptivities (A 1%, 1 cm) for both the drugs at both wavelengths were determined as mean of six independent determinations. Concentrations in the sample were obtained by using following equations-

$$Cx = \frac{A1 \text{ ay2} - A2 \text{ a y1}}{ax1ay2 - ax2ay1}$$
$$A1 \text{ a x2} - A2 \text{ a x1}$$
$$Cy = \frac{A1 \text{ a x2} - A2 \text{ a x1}}{ay1ax2 - ay2a x1}$$

Where, A1 and A2 are absorbances of mixture at 239 nm and 223 nm respectively, ax1 and ax2 are absorptivities of Amlodipine Besylate at 1 and 2 respectively and ay1 and ay2 are absorptivities of Metoprolol Succinate at 1 and 2 respectively. Cx and Cy are concentrations of Amlodipine Besylate and Metoprolol Succinate respectively^{8,9}.

Absorption Ratio Method:

The quantitation of Amlodipine Besylate and Metoprolol Succinate by proposed method was done using the selected wavelengths, 233 nm was taken as max for Metoprolol Succinate and 234 nm, an isobestic point for estimation of Metoprolol Succinate, respectively. Series of different concentrations in range of 5-25 µg/mL for Amlodipine Besylate and Metoprolol Succinate were prepared from stock solutions. The calibration curves were constructed and regression analysis (Table I). was carried out. The absorptivities (A1%, 1 cm) of both the drugs at both the wavelengths were determined. By using the following equations, one can easily find out the concentration of the individual drug in admixture at the two wavelengths. For estimation of ADB:

$$c_{x} = \frac{Q_{m} - Q_{y}}{Q_{x} - Q_{y}} \frac{A}{ax} \qquad -----(1)$$

And for estimation of MET:

$$c_{y} = \frac{Q_{m} - Q_{y}}{Q_{y} - Q_{x}} \frac{A}{ay} \qquad -----(2)$$

where,

cx and cy are concentrations of Amlodipine Besylate and Metoprolol Succinate respectively (g/100mL in final solution),

Qx = the ratio of absorptivity of Amlodipine Besylate at 223 and 234 nm.

Qy = the ratio of absorptivity of Metoprolol Succinate at 223 and 234 nm.

Qm = the ratio of absorbance of mixture at 223 and 234 nm

A = the absorbance of mixture at isoabsorptive point.

ax = the absorptivity value of Amlodipine Besylate at isoabsorbtive point

ay =the absorptivity value of Metoprolol Succinate at isoabsorptive point¹⁰.

Validation:

Accuracy:

Accuracy was confirmed by recovery study as per ICH norms at three different concentration levels 80%, 100%, 120% by replicate analysis (n = 3). Here to a preanalysed sample solution, standard drug solutions were added and then percentage of drug content was calculated. The result of accuracy study was reported in Table 2. From the recovery study it is clear that the method is accurate for quantitative estimation of Amlodipine Besylate & Metoprolol

Succinate in tablet dosage form as the statistical parameters are within the acceptance range (S.D. < 2.0).

Precision:

Precision was determined as a repeatability and intermediate precision.

Repeatability:

Repeatability result indicates the precision under the same operating conditions over a short interval of time and inter-assay precision. The standard deviation, coefficient of variance and standard error were calculated. Repeatability was performed for six times with tablets formulation. The results of statistical evaluation are given in Table.

Linearity:

For each drug, appropriate dilutions of standard stock solutions were assayed as per the developed methods. The Beer- Lambert's concentration range is 0-25 μ g/mL for Amlodipine Besylate and Metoprolol Succinate. The linearity data for both methods are presented in Table.

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

The LOD and LOQ of Amlodipine Besylate and Metoprolol Succinate. by proposed methods were determined using calibration standards. LOD and LOQ were calculated as 3.3s/S and 10s/S respectively, where S is the slope of the calibration curve and s is the standard deviation of response^{11,12}.



Fig 1: Calibration Curve of Amplodipine Besylate



Fig 2: Calibration Curve of Metoprolol Succinate



Fig 3: Overlay Spectra of Amplodipine Besylate and Metoprolol Succinate

Method	Drug	Wavelength	Concentration	Intercept	Slope	\mathbf{r}^2
		(nm)	Range (µg/ml)			
1	Amplodipine	240	5-25	0.0001	0.012	0.999
	Besylate					
	Metoprolol	223	5-25	0.009	0.028	0.998
	Succinate					
2	Amplodipine	240	5-25	0.0001	0.012	0.999
	Besylate	234	5-25	0.0002	0.010	0.989
	Metoprolol	223	5-25	0.009	0.028	0.998
	Succinate	234	5-25	0.008	0.25	0.991

Table 1: Regression Analysis Of The Calibration Curves

Method 1= Simultaneous Equation method and Method 2= Absorbance Ratio Methodr2= Correlation Coefficient

Parameters	Amplodipine Besylate	Metoprolol Succinate
Linearity Range (µg/ml)	5-25	5-25
Correlation coefficient	0.999	0.998
Slope	0.012	0.028
Accuracy(Average)	98.78%	102.82%
Y- Intercept	0.0001	0.009
LOD	0.3513	0.0624
LOQ	1.0645	0.1893

Table 2: Summary Of Validation Parameters

Results and Discussion:

The Beer- Lambert's concentration range is 0-25 μ g/mL for Amlodipine Besylate, Metoprolol Succinate at 240 nm, 223 nm wavelengths with coefficient of correlation 0.9991, 0.99. Drugs show good regression values at their respective wavelengths and the recovery study reveals that any small change in the drug concentration in the solution could be accurately determined by the proposed methods. Percentage estimation in tablet dosage form is 80, 108 by method I where as 90, 96

by method II for Amlodipine Besylate, Metoprolol Succinate respectively with standard deviation <2. The validity and reliability of proposed methods are assessed by recovery studies. Sample recoveries for both the methods are in good agreement with their respective label claims, which suggests noninterference of formulation additives in estimation. The standard deviation, is 0.1146 and 0.2641 for Amplodipine Besylate and Metoprolol Succinate. The LOD values are 0.3513, 0.0624 and LOQ values are 1.0645, 0.1893 for Amplodipine Besylate and Metoprolol Succinate. Low LOD and LOQ indicates good sensitivity of proposed methods.

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