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Absorption Corrected Method & Isoabsorptive Point Method for Simultaneous Estimation of Metoprolol and Amlodipine in Their Combined Dosage Form

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ABSTRACT

Two simple, economical, precise and accurate methods are described for the simultaneous determination of Metoprolol Succinate (METO) and Amlodipine (AMLO) in combined tablet dosage form. The first method (Method A) is Absorption Corrected Method and second method (Method B) is Isoabsorptive Point Method. The absorbances at 277.017 nm and 235.62 nm in the Absorption Corrected Method and Isoabsorptive Point Method were selected to determine METO and AMLO, respectively in combined formulation. The methods were validated by following the analytical performance parameters suggested by the International Conference on Harmonization (ICH). All validation parameters were within the acceptable range. Under experimental conditions described, calibration curve, assay of tablets and recovery studies were performed. A critical evaluation of proposed methods were performed by statistical analysis of data where slope, intercept, correlation coefficient is shown in Table (1). As per the ICH guidelines, the method validation parameters checked. Beer's law is obeyed in the concentration range of 50-250µg/ml and 5-25µg/ml for Metoprolol succinate and Amlodipine by both the methods.

Keywords: Beer's law, Absorption Corrected Method, Isoabsorptive Point Method, ICH guidelines.

INTRODUCTION

Metoprolol succinate (METO) is a selective β-adrenergic antagonist, which is used in the treatment of cardiovascular disorders such as hypertension, angina pectoris, cardiac arrhythmias, congestive heart failure and myocardial infarction. Metoprolol is administered orally as tablet. Chemically Metoprolol succinate is (RS)-1-(Isopropylamino)-3-[p-(2-methoxyethyl) phenoxy] propan-2-ol succinate with molecular formula $C_{34}H_{54}N_2O_{10}$ [1-2] (Fig. 1). Amlodipine (AMLO) is calcium channel blocker, used for patients with angina pectoris and hypertension. Chemically it is 2-[(2-Aminoethoxy) methyl]-4-(2-chlorophenyl)-1, 4-dihydro-6-methyl-3, 5-pyridine dicarboxylic acid- 3-ethyl-5-methyl ester [3] having the molecular formula $C_{20}H_{25}ClN_2O_5$ [4] (Fig. 2).

A combination of METO (25) and AMLO (2.5) are marketed as tablet formulation (Met pure-AM) in the ratio 10:1. This combination is indicated for treatment of angina pectoris, cardiac arrhythmia and hypertension. Literature survey shows that few analytical techniques such as spectrophotometry [5], HPLC [6] have been reported for the combination as well as the individual drugs. Simultaneous determination of METO with other CVS acting drugs has been reported. [7-11] As far as Amlodipine is concerned, few

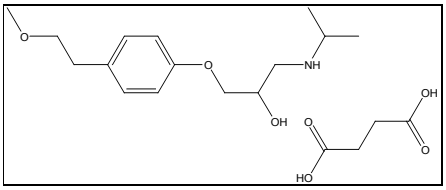

Fig. 1: Structure of Metoprolol succinate

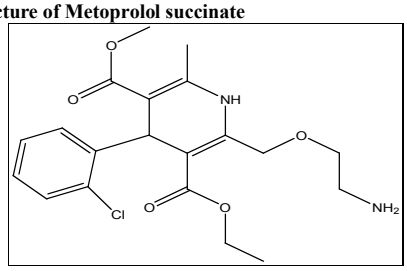

Fig. 2: Structure of Amlodipine

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reports are available for its estimation in bulk and formulation such as spectrophotometry [12-16], HPLC and bioanalytical methods. [17] The method was validated for linearity, accuracy, precision, sensitivity, robustness, etc. in accordance with International Conference on Harmonization (ICH) guidelines. [18]

In the present work an attempt has been made to develop an analytical method development for the simultaneous estimation of METO and AMLO in the combine dosage form by using Absorption Corrected Method and Isoabsorptive Point Method by UV spectroscopy.

MATERIALS AND METHODS

Instrumentation

UV-Visible double beam spectrophotometer (Varian Cary 100) with 10 mm matched quartz cells was used. Electronic balance (Model Shimadzu AUW-220D) was used for weighing.

Reagents and chemicals

Pure drug sample of METO, percentage purity 99.86% and AMLO, percentage purity 99.92% was kindly supplied as a gift sample by Cipla Ltd. These samples were used without further purification. Spectroscopy grade methanol was used throughout the study. Tablets each containing 25 mg of METO and 2.5 mg of AMLO used for analysis was Met pure-AM manufactured by Emcure Ltd.

Preparation of Standard Stock Solutions and Calibration Curve

Standard stock solutions of pure drug containing 1000 µg/ml of METO and AMLO were prepared separately in methanol. Standard stock solutions were further diluted with methanol to get working standard solutions of analytes in the concentration range of 50-250µg/ml and 5-25µg/ml of Metoprolol Succinate (METO) and Amlodipine (AMLO), respectively and scanned in the range of 200-400nm. For ratio derivative amplitudes (at interval 1.2 and filter size 9) of ratio spectra were measured at 277.017 nm and 235.62 nm for METO and AMLO, respectively. First derivative amplitudes of ratio spectra and concentrations were used to construct calibration curve. [19]

Preparation of Sample Solution and Formulation Analysis

Twenty tablets were weighed accurately and a quantity of tablet powder equivalent to 25 mg of METO and AMLO (2.5 mg) was weighed and dissolved in the 30 ml of methanol with the aid of ultrasonication for 7 min and solution was filtered through Whatman paper No. 41 into a 100 ml volumetric flask. Filter paper was washed with same solvent, adding washings to the volumetric flask and volume was made up to the mark with methanol. The solution was suitably diluted further with methanol to get required final concentration of METO (150µg/ml) and AMLO (15µg/ml) [Fig. 3].

Theoretical aspects

Method A: Absorption Corrected Method

λ max of METO and AMLO was determined by scanning the drug solution in UV Spectrophotometer in the range 200-400 nm at 0.5 band width and 600 nm/min scan speed and was found to be at 277.017 nm and 235.62 nm respectively. METO also showed absorbance at 277.017 nm, while AMLO did not show any interference at 277.017 nm [Fig. 3]. To construct Beer's plot for METO and AMLO , stock solutions of 1000µg/ml of both the drugs were prepared in methanol and working standard dilutions were made in methanol using stock solution of 1000µg/ml. Also Beer's plot was constructed for METO and AMLO in solution mixture at different concentration (50:5, 100:10, 150:15, 200:20, 250:25 µg/ml) levels. Both the drugs followed linearity individually and in mixture within the concentration range 50-250µg/ml and 5-25µg/ml for METO and AMLO, respectively.

Method B: Isoabsorptive point method

In this method, absorbances are measured at two wavelengths being the Isoabsorptive method of the two components & other being the wavelength of maximum absorbance of one of the component. From the overlain spectra of Metoprolol & Amlodipine two wavelengths one at 277.017 nm, which was Isoabsorptive point for both the drugs & other at 235.62 nm the λ max for Amlodipine.

For these methods, standard solution containing 1 mg/ml of each of the drug was prepared by dissolving 100 mg of pure drug in 100 ml methanol in separate volumetric flask. The stock solutions were further diluted separately & in combination with methanol to prepare solutions having concentration range of 50-250µg/ml & 5-25µg/ml for METO & AMLO respectively. The individual standards were scanned in the UV range & absorptivity values were calculated at selected wavelengths for both the drugs.

Concentrations of the drugs in sample solutions were determined by using the following formulae

For Metoprolol

$$Cx = \frac{Qm - Qy\ A1}{Qx - Qy\ ax1}$$

For Amlodipine

$$Cy = \frac{Qm - Qy\ A1}{Qy - Qx\ ay1}$$

Where, Cx = Concentration of metoprolol succinate

Cy = Concentration of Amlodipine

A_1 = Absorbance of sample at Isoabsorptive wavelength (235.62)

$ax_1 = ay_1$ = Absorptivity values of Metoprolol succinate & Amlodipine, respectively at Isoabsorptive wavelength.

$$Qm = \frac{\text{Absorbance of sample solution at 277.017 nm}}{\text{Absorbance of sample solution at 235.62 nm}}$$

$$Qx = \frac{\text{Absorbance of metoprolol solution at 277.017 nm}}{\text{Absorbance of metoprolol solution at 235.62 nm}}$$

$$Qy = \frac{\text{Absorbance of amlodipine solution at 277.017 nm}}{\text{Absorbance of amlodipine solution at 235.62 nm}}$$

For the analysis of the commercial formulation, 20 tablets were weighed & powdered. The amount of powder equivalent to 100 mg of each of the drugs was weighed accurately & transferred into a suitable flask. The tablet powder was dissolved in methanol & filtered through a Whatman filter paper no.41. This filtrate was diluted with methanol to 100 ml. This solution was diluted suitably to obtain the solution having concentrations equivalent to 5µg/ml & 50µg/ml of each of these drugs. Absorbances of these solutions were measured at 277.017 nm & at 235.62 nm by using multiple wavelength modes against the blank. These values were then equated in the above-mentioned equations & the concentration of each drug was calculated. [20]

Validation of Analytical method

Validation is the process of establishing documented evidence that provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality attributes. Present method has been validated as per ICH guidelines as follows.

Accuracy

Accuracy of the method was determined in terms of % recovery of standard. Recovery studies were carried out by addition of standard drug solution at the level of 80%, 100% and 120% to the pre analyzed sample. Results of the recovery study were found to be within the acceptance criteria 100±10 %, indicating a good degree of sensitivity of the method towards detection of analytes in sample. Accuracy results for METO & AMLO are shown in Table 1 & 2 respectively.

Solution stability

Method stability was checked by analyzing solution kept in refrigerator and at room temperature by both methods. Solution at room temperature was stable for 12 hours and solution in refrigerator was stable for 30 days (% RSD < 2).

Precision of the Method

Method repeatability was determined by six times repetitions of assay procedure. For intra-day precision method was repeated 5 times in a day and the average % RSD was determined. Similarly the method was repeated on five different days for inter-day precision and average % RSD was determined (Table 3).

Table 1: Accuracy Result for METO

Ingredient	Recovery Level %	Drug Amount (µg/ml)	Amount Spiked (µg/ml)	Amount Recovered (µg/ml)	% Mean Recovery, % RSD (N=3)
METO	80%	75	60	134.68	99.762, 0.96296
	100%	75	75	149.89	99.926, 0.66667
	120%	75	90	164.2	99.515, 0.915152

Table 2: Accuracy result for AMLO

Ingredient	Recovery Level %	Drug Amount (µg/ml)	Amount Spiked (µg/ml)	Amount Recovered (µg/ml)	% Mean Recovery, % RSD (N=3)
AMLO	80%	7.5	6	13.49	99.925, 0.92593
	100%	7.5	7.5	15.09	100.6, 0.850
	120%	7.5	9	16.45	99.696, 0.9697

Table 3: Determination of precision

S. No.	Reproducibility (%)	(Intra-Day Precision) (N=4) (%)	(Inter-Day Precision) (N=3) (%)
1	99.02716	99.02716	99.2057
2	99.55	100.4089	99.3763
3	100.0667	100.2095	99.02716
4	100.45	99.97441	98.02734
5	100.15	100.266	99.31679
6	100.58	100.0756	99.31679
Mean Deviation	99.9764333	99.993595	99.045013
Standard Deviation	0.584738518	0.496867626	0.513668

Linearity

The linearity of the proposed method was evaluated for each drug by analyzing a series of different concentrations of each of METO; AMLO within the range stated in Table 3. The assay was performed according to the experimental conditions previously established. The absorbance values for METO and AMLO were measured, at the specified wavelengths (Table 3), and plotted against its concentration. A straight line was obtained in each case. The statistical analysis of these graphs using least squares method was made for the slope, intercept and correlation coefficients.

The results obtained show that the linearity of calibration graphs and the compliance with Beer`s law for the both drugs i.e. METO and AMLO Fig. 5 & 6. The correlation coefficients of calibration plots for METO and AMLO were 0.998 and 0.999 as indicated in Table 4.

Specificity

Specificity is a procedure to detect quantitatively the analyte in the presence of component that may be expected to be present in the sample matrix. Commonly used excipients in tablet preparation were spiked in a pre-weighed quantity of drugs and then absorbance was measured and calculations done to determine the quantity of the drugs.

Limit of Quantification (LOQ) and Limit of Detection (LOD)

LOD and LOQ were calculated statistically from formula shown in equation No 3 & 4 respectively:

$$LOD = 3.3 \times \frac{SD}{SLOPE} \quad \text{..... (3)}$$

$$LOQ = 10 \times \frac{SD}{SLOPE} \quad \text{..... (4)}$$

Where, SD: Standard Deviation of y- intercepts of regression lines,

The LOD and LOQ of METO & AMLO by statistical and visualization methods were mentioned in Table 4.

Table 4: Optical characteristics of the proposed methods and result of linearity, sensitivity and formulation analysis

S. No.	Parameters		Metoprolol (METO)	Amlodipine (AMLO)
1	Wavelength	Nm	277.017	235.62
2	Beer's law	(µg/ml)	50-250	5-25
3	Linearity	(µg/ml)	50-250	5-25
		Regression Equation*	Y=0.004X+0.024	y = 0.012x + 0.050
		R^2	0.998	0.999
4	Formulation Analysis (% Assay, % RSD), n=6	Met pures-AM	99.56% , 0.5864	98.96%, 0.6549
5	LOD	(µg/ml)	0.07508	-0.3154
6	LOQ	(µg/ml)	0.2275	-0.9559

Table 5: Results of Robustness

S. No.	Analyst I	Analyst II
1	99.02716	99.257
2	100.51	99.3763
3	100.667	99.027116
4	100.1053	98.02734
5	100.1172	99.31679
6	100.397	99.31679
Mean	100.037227	99.0450133
Standard Deviation	0.52632396	0.51366797
RSD	0.00526128	0.51366797
% RSD	0.5261281	0.51862073

Robustness

Robustness of the method was determined by repeating the proposed method by other analyst in the same laboratory and calculating % RSD. The results are indicated in Table 5

Recovery studies

The accuracy of the proposed methods were checked by recovery studies, by addition of standard drug solution to pre analyzed sample solution at three different concentration levels (80 %, 100 % and 120 %) within the range of linearity for both the drugs. The basic concentration level of sample solution selected for spiking of the drugs standard solution was of 50 µg/ml of METO and 5 µg/ml of AMLO for both the methods.

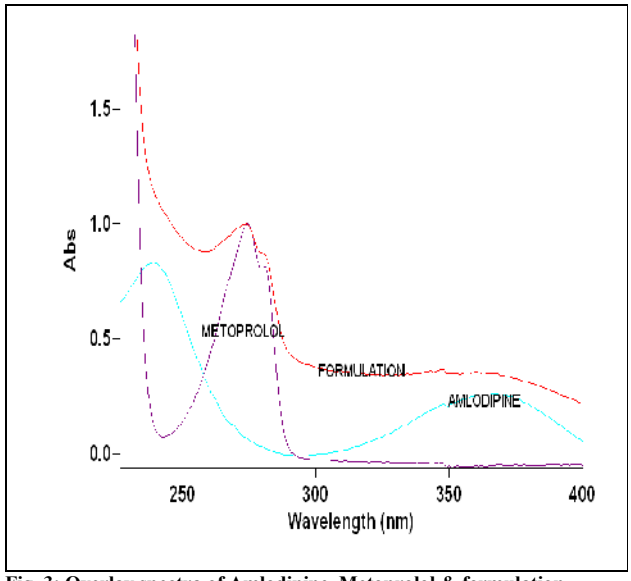

Fig. 3: Overlay spectra of Amlodipine, Metoprolol & formulation

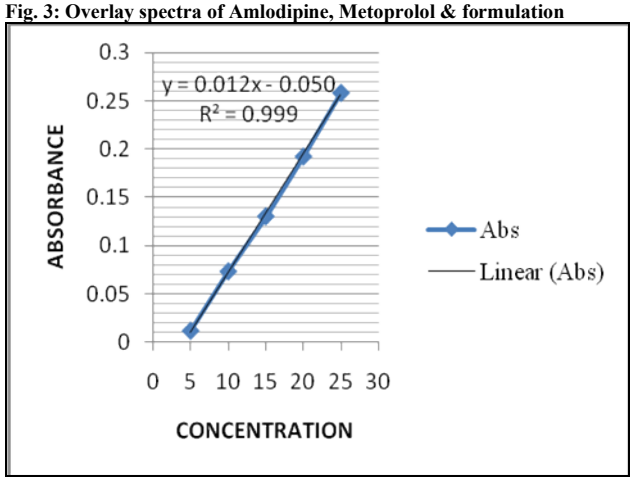

Fig. 4: Calibration curve of Metoprolol

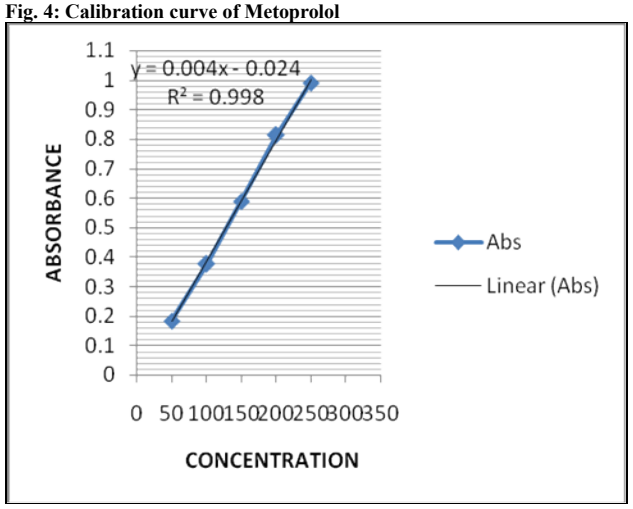

Fig. 5: Calibration curve of Amlodipine

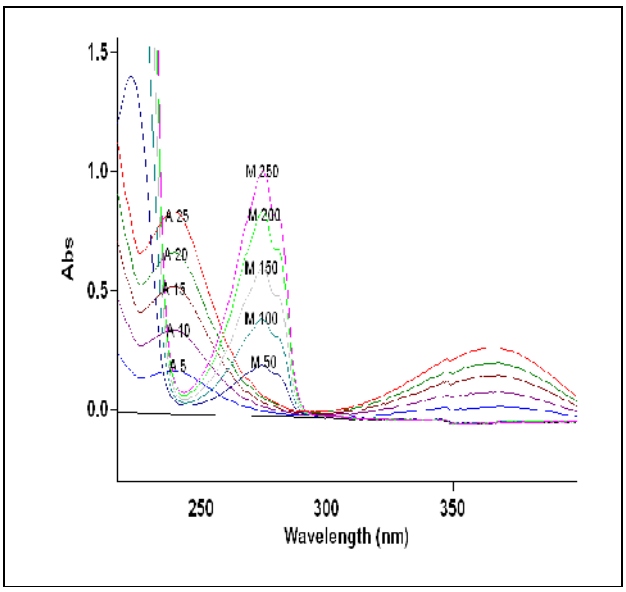

Fig. 6: Calibration curve of formulation

RESULTS & DISCUSSION

Under experimental conditions described, calibration curve, assay of tablets and recovery studies were performed. Using appropriate dilutions of standard stock solution the two solutions were scanned separately. A critical evaluation of proposed method was performed by statistical analysis of data where slope, intercept, correlation coefficient are shown in Table 3. As per the ICH guidelines, the method validation parameters checked were linearity, accuracy and precision, stability, LOD& LOQ. Beer's law is obeyed in the concentration range of 50-250 µg/ml and 5-25µg/ml for METO and AMLO, respectively. Correlation coefficient was greater than 0.999 for both the drugs. The proposed methods were also evaluated by the assay of commercially available tablets containing METO and AMLO. The results of formulation analysis are presented in Table 3. Recovery was found in the range of 99.51-99.92% for METO (Table 1) and 99.69-100.6-% for AMLO (Table 2)

The validated spectrophotometric methods employed here proved to be simple, economical, precise and accurate. The results obtained by these two methods are significant for Metoprolol & Amlodipine follows Beer's law & as per ICH guidelines the various validation parameters are found to be within the specified limits. Thus it can be used as IPQC test and for routine simultaneous determination of METO and AMLO in tablet dosage form.

In future the present method will be suitable for routine analytical work of the different marketed formulations & useful validated analytical method.

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