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Research Article

DEVELOPMENT AND VALIDATION OF SIMULTANEOUS EQUATION METHOD FOR DETERMINATION OF METOPROLOL AND AMLODIPINE IN COMBINED DOSAGE FORM

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ABSTRACT

A simple, rapid, precise, accurate and economical spectrophotometric method requiring no prior separation has been developed for the simultaneous estimation of amlodipine and metoprolol in tablet dosage form. Amlodipine and Metoprolol exhibit absorption maxima at 237.5 and 223 nm respectively. The developed Simultaneous equation method obeyed Beer's Law in the concentration range of 5-25 µg/ml for both the drugs. The proposed method is recommended for routine analysis of amlodipine and metoprolol as it is rapid, precise, accurate and reproducible. The results of the tablet analysis were validated with respect to recovery (accuracy), linearity, limit of detection and limit of quantization according to ICH guidelines and found to be satisfactory.

Keywords: Simultaneous Equation Method, Amlodipine, Metoprolol, ICH guidelines.

INTRODUCTION

Amlodipine (AML), chemically (*RS*)-3-ethyl-5-methyl 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1, 4-dihydropyridine-3, 5-dicarboxylate (Fig.1), is a long-acting calcium channel blocker used in the management of hypertension by inhibiting the inward movement of calcium by binding to L-Type calcium channels in the heart and in smooth muscle of the coronary and peripheral vasculature relaxing the smooth muscle and dilating arterioles thereby decreasing peripheral resistance and hence improve blood pressure and in angina pectoris by improving blood flow to the myocardium[1-2] whereas Metoprolol (MET), chemically (*RS*)-1-(Isopropylamino)-3-[4-(2-methoxy ethyl) - phenoxy] propan-2-ol (Fig. 2) is a selective β_1 receptor blocker used in the treatment of several diseases of cardiovascular system especially hypertension. It is a beta adrenergic blocking agent, which reduces chest pain and lowers high blood pressure.[3]

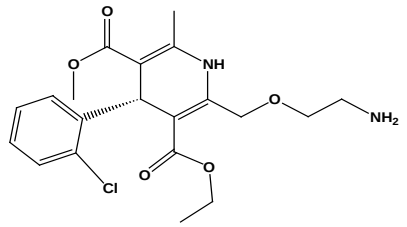

Fig. 1: Structure of Amlodipine

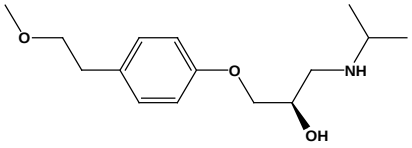

Fig. 2: Structure of Metoprolol

Literature survey revealed the estimation of AML and MET with other drugs using UV[4-14], HPLC [15-22] and HPTLC[23]. So far this combination has only been analysed using HPLC[24]and no spectrophotometric method has been reported. So the present study is focussed on a successful attempt to estimate AML and MET using UV spectroscopy.

MATERIALS AND METHODS

Standard bulk drug samples of amlodipine and metoprolol were provided as gift samples. Tablets of combined dosage form were procured from the local market. All other reagents used were of analytical grade.

A double-beam Shimadzu UV-visible spectrophotometer, 1800 with a pair of 1 cm matched quartz cells were used to measure the absorbance of the solutions.

Preparation of Standard Solutions

10 mg of MET was accurately weighed and transferred to a 10 ml volumetric flask containing small amount of methanol. The drug was dissolved with sonication and the final volume was adjusted with methanol up to the mark to get a solution of 1000 µg/ml and then further diluted to get 10µg/ml. Solution of AML was also prepared in a similar way to get a concentration of 10µg/ml.

Determination of Absorption maxima

By scanning standard solutions in the UV-VIS spectrophotometer in the wavelength range of 200-400 nm, an overlain spectrum was recorded (Fig.3). Using the overlain spectra of AML and MET in methanol, the wavelength maxima of both drugs, i.e., 237.5 nm (λ_1) and 223.0 nm (λ_2), were selected as two sampling wavelengths for this method. The prepared stock solutions were diluted to get solutions of concentrations of 5-25 µg/ml. The absorbance of these solutions were measured at the selected wavelengths and absorptivities were determined. The concentrations of the drugs were obtained by using following equations:

$$C_x = \frac{A_1 a_{y_2} - A_2 a_{y_1}}{a_{x_1} a_{y_2} - a_{x_2} a_{y_1}} \quad \text{.....Eq. 1}$$

$$C_y = \frac{A_1 a_{x_2} - A_2 a_{x_1}}{a_{y_1} a_{x_2} - a_{y_2} a_{x_1}} \quad \text{.....Eq. 2}$$

Where, A_1 and A_2 are absorbance of mixture at 237.5 nm and 223 nm respectively, a_{x_1} and a_{x_2} are absorptivities of AML at λ_1 and λ_2 respectively and a_{y_1} and a_{y_2} are absorptivities of MET at λ_1 and λ_2 respectively. C_x and C_y are the concentrations of AML and MET respectively. The results of analysis are given in Table-1.

Application of the developed method on tablet dosage form

Twenty tablets were weighed, crushed and an accurately weighed sample equivalent to 20 mg of MET and 2 mg of AML was transferred to a 10 ml measuring flask and then by standard addition method 18 mg of pure AML was added in order to bring MET and AML in ratio of 1:1. The drug powder was dissolved in methanol with sonication, filtered through Whatman filter paper and then volume was made up to 10 ml with methanol to get stock solution of 1000 µg/ml of each drug and then further diluted to get 10µg/ml. All determinations were carried out three times at 237.5 and 223 nm and then the concentration of both the drugs was calculated using Equation 1 and 2 and the results are given in Table 1.

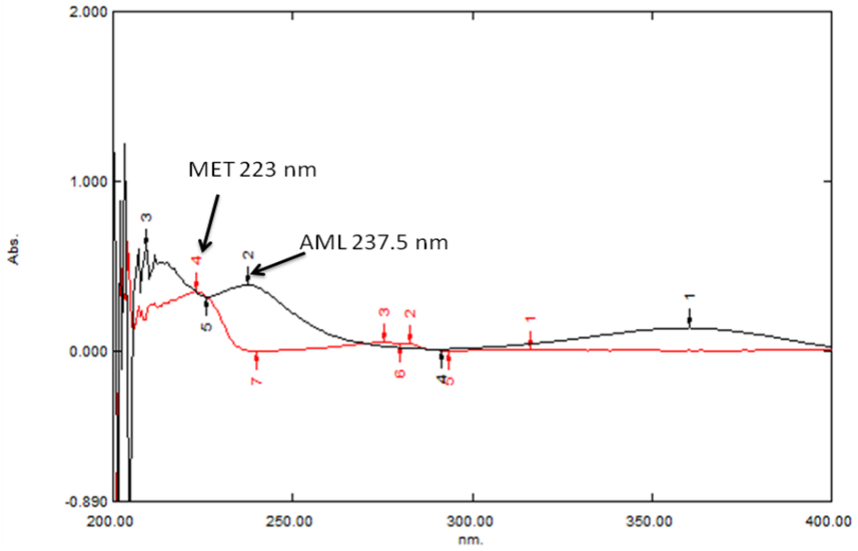

Fig. 3: Overlain spectrum of AML and MET showing λ_{max} of AML at 237.5 nm and λ_{max} of MET at 223 nm

Table 1: Result of Analysis in Marketed Formulation.

Drug	Label claim	Amount found (mg)	% drug found ± SD
AML	5 mg	5.003	100.06±0.740
MET	50 mg	50.15	100.3±1.267

Values expressed mean ± SD (n=3)

Statistical Validation

The described method has been validated for the assay of both the major components of bulk drug using following parameters according to ICH guidelines.[25-26]

Limit of detection and limit of quantification

Limit of detection (LOD) is the minimum concentration of the analyte that can be detected by the instrument. Limit of quantification (LOQ) is the concentration of analyte that can be quantified. These parameters were calculated for the proposed method based on the standard deviation (SD) of the y-intercept and the slope (S) of the calibration curves. LOD = 3.3 (SD/S) and LOQ = 10 (SD/S) and the values obtained are given in Table 2.

Linearity

Linearity was studied by preparing solutions at different concentration levels. Calibration curve was plotted using standard solutions of 5µg/ml to 25µg/ml and regression analysis was carried out. Regression coefficients are reported in Table 2 and linearity graph is shown in Fig.4.

Precision

Precision of the method was studied by intra- and inter-day variations in the test method of AML and MET. Intra-day precision was run in triplicate on the same day and inter-day precision for three consecutive days. Precision and accuracy data is shown in Table 3 and 4.

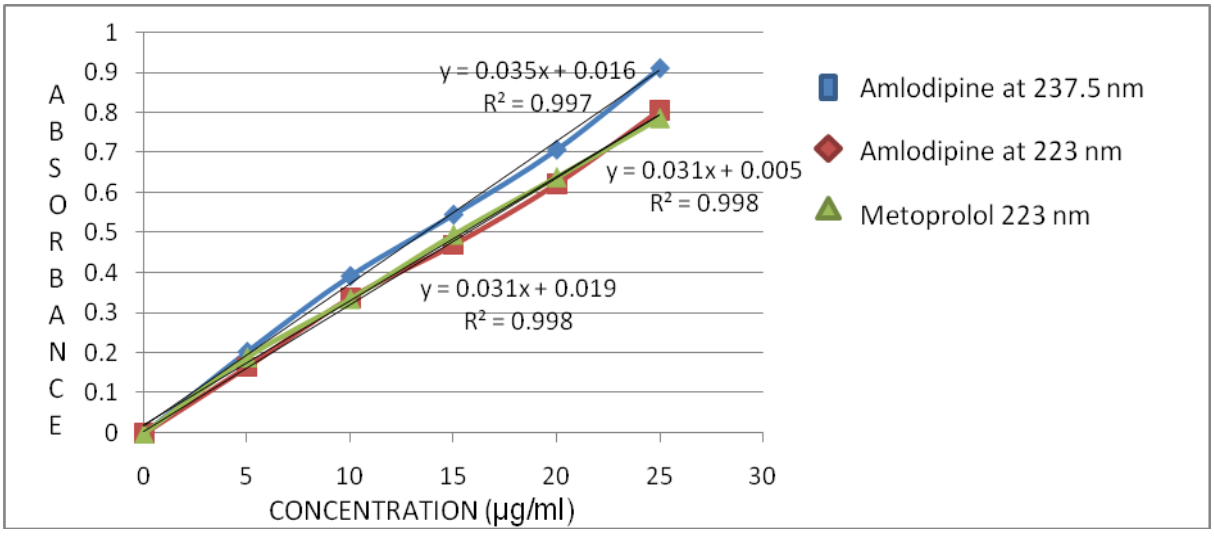

Fig. 4: Linearity graphs of AML and MET plotted between absorbance on X-axis and concentration (µg/ml) on Y-axis

Table 2: Optical Characteristics

Parameter	Amlodipine		Metoprolol	
	237.5 nm	223 nm	237.5 nm	223 nm
Beer's law limit(µg/ml)	5-25	5-25	5-25	5-25
Regression equation	Y= mX + c			
Slope (m)	0.031	0.035	0.031	-
Intercept (c)	0.005	0.015	0.019	-
Correlation coefficient(R^2)	0.998	0.998	0.998	
Standard deviation SD	0.0047	0.0013	0.0009	-
Limit of detection (µg/ml)	0.5	0.12	0.095	-
Limit of quantification (µg/ml)	1.51	0.37	0.29	-

Table 3: Intraday Precision

Drug	Conc. (µg/ml)	Simultaneous Equation Method	
		Accuracy± SD	%RSD
AML	10	100.06± 0.740	0.74
MET	10	98.26±0.309	0.3141

Values expressed mean ± SD (n=3)

Table 4: Interday Precision

Drug	Conc. (µg/ml)	Simultaneous Equation Method	
		Accuracy± SD	%RSD
AML	10	100.23±0.057	0.057
MET	10	99.98±0.520	0.52

Values expressed mean ± SD (n=3)

Table 5: Recovery Study

Drug	Amount added (µg/ml)	Amount recovered (µg/ml)	%Recovery ± SD	% RSD
AML	80% (9µg/ml)	9.036	100.4±0.081	0.081
	100% (10µg/ml)	10.07	100.7±0.082	0.081
	120% (11µg/ml)	11.154	101.4±0.326	0.321
MET	80%(9µg/ml)	9.108	101.2±0.244	0.241
	100%(10µg/ml)	10.03	100.3±0.205	0.204
	120%(11µg/ml)	10.813	98.3±0.244	0.248

Values expressed mean ± SD (n=3)

Recovery

It is a measure of closeness between actual value and the analytical value and is calculated by applying test procedure for a number of times at three different levels viz. 80%, 100% and 120%. Samples of concentration 10µg/ml were prepared and analyzed using the proposed method. Percentage recovery was calculated using the equation for the method and the results are given in Table 5.

RESULTS & DISCUSSION

The method discussed in the present work provides a convenient, simple, rapid, accurate, precise and economical way to estimate AML and MET in pharmaceutical dosage form. Linearity of both the drugs was analysed on selected wavelengths (237.5 nm (λ_1) and 223.0 nm (λ_2)) and was found to be 5-25µg/ml for both drugs as shown in Table 2. Regression analysis was made for the slope (m), intercept (c) and correlation coefficient (r^2) as shown in Table 2. Higher values of correlation coefficient (r^2) indicate good linearity of the calibration curve for both the drugs as shown in Fig. 4.

Sensitivity of the method was determined by calculating limit of detection (LOD) and limit of quantification (LOQ). Precision of the proposed method was determined by inter- and intra-day precision methods and the results range from 99.98-100.23% in case of inter-day precision and 98.26-100.06% in intra-day precision. % RSD calculated was less than equal to 2 which indicate the accuracy and reproducibility of the method. Results are shown in Table 3 and 4.

Drug content in the tablet was directly calculated from the given equations and the results ranges from 100.06-100.3% as shown in Table 1. The proposed method was validated according to the ICH guidelines. Standard deviation (SD) and % relative standard deviation (%RSD) is calculated in Table 2 & 3. Low values of standard deviation show the accuracy, repeatability and reproducibility of the method. The accuracy of the method was proved by performing recovery studies on the commercial formulation at 80, 100 and 120% level. Recovery ranges from 98.3-101.4% (Table 5). The results of recovery study indicate that these drugs could be quantified simultaneously and that there is no interference of the excipients present in the formulation.

From statistical data it is clear that the method is repeatable and specific for the analysis of two drugs in combination and since none of the methods is reported for simultaneous estimation of metoprolol and amlodipine from combined dosage form, the developed method can be used for routine analysis of two components without prior separation.

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