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Research Article

DEVELOPMENT AND VALIDATION OF DUAL WAVELENGTH SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF AMLODIPINE BESYLATE AND ENALAPRIL MALEATE IN COMBINED DOSAGE FORM

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ABSTRACT

A simple and economical dual wavelength spectrophotometric method has been developed for the simultaneous estimation of amlodipine besylate and enalapril maleate in their combined dosage forms. The method was based on property of additivity of absorbances. From the UV absorption spectrum of amlodipine besylate, three wavelengths were selected, which were 226.6, 247.8 and 360.6 nm. From the UV absorption spectrum of enalapril maleate, 226.6 and 247.8 nm wavelengths were selected. At 360.6 nm only amlodipine besylate has reasonable absorbance, so it was selected for the estimation of it from combination drug product. At 226.6 and 247.8 nm both drugs had shown some absorbance. At these two wavelengths absorbance for amlodipine besylate was found to be same i.e. absorbance difference was zero for any concentration, while for enalapril maleate concomitantly increase in absorbance difference with increase in its concentration. So, 226.6 and 247.8 nm wavelengths were selected for the estimation of enalapril maleate from its combination drug product. The method involved solving of an equation based on measurement of absorbances at two wavelengths 226.6 and 247.8 nm. The proposed method was found to be simple, economical, accurate and reproducible for the routine analysis of both drugs in tablet dosage forms.

Key words Spectrophotometric, Dual Wavelength, Amlodipine Besylate, Enalapril Maleate.

1. INTRODUCTION

Amlodipine besylate (AML) is long-acting calcium channel blocker (dihydropyridine) used as an anti-hypertensive and in the treatment of angina while Enalapril maleate (ENA) is Competitive inhibitor of angiotensin- converting enzyme(ACE). Chemically AML is (RS)-3-ethyl-5-methyl-2-(2-aminoethoxymethyl)-4-(2-chlorophenyl) -1,4-dihydro-6-methyl 3,5pyridine dicarboxylatebenzenesulfonate(1) while ENA is (S)-1-[N-[1-(ethoxy carbonyl)-3 phenyle propyl]-L- alanyle]- L-proline maleate[1]. AML is official in IP[2], BP[3], EP[4] and USP[5] while ENA is official in IP[6], BP[7] and USP[8] but they do not involve simultaneous determination of AML and ENA. Deep survey of literature for AML revealed methods based on Spectrophotometry[9,10], RP-HPLC[11] using fluorescence detection, HPLC-tandem mass spectrometry[12,13], RP-HPLC using UV detection[14,15], HPLC[16-20] in combination with other drugs, Flow injection analysis using UV-detection[21], HPTLC[22], stability indicating HPLC[23] and stability indicating HPLC[24] in combination with benazepril hydrochloride have been reported. Similarly survey of literature for

ENA revealed methods based on Colorimetric and spectrophotometric[25-27], HPTLC[28] and HPLC[29] in combination with Hydrochlorothiazide. In the present investigation an attempt has been made to develop a simple, economic, accurate, reproducible and less time consuming spectrophotometric method for the simultaneous estimation of AML and ENA in their combined dosage forms at three different wavelengths. The method was based on dual wavelength data processing program (dual wavelength spectrophotometry or DW spectrophotometry). The proposed method was successfully applied for simultaneous determination of AML and ENA in combined dosage forms that are available in market.

2. MATERIAL AND METHOD

2.1 Instruments and Apparatus

A double beam UV-visible Spectrophotometer (Shimadzu, UV-1700, Japan), attached to a computer software UV probe 2.0, with a spectral width of 2 nm, wavelength accuracy of 0.2 nm and pair of 1 cm matched quartz cells, Analytical Balance (CP224S, Sartorius, Germany), Ultrasonic Cleaner (Frontline FS 4, Mumbai, India), Corning volumetric flasks, pipettes of borosilicate glass were used in the study, and Water Purification System (Millipore Bioscience Division Pvt.Ltd, India) was used during study.

2.2 Chemicals and Reagents

Kindly gifted reference standards of AML and ENA (Zydus cadila Healthcare Ltd, Moraiya, Ahmedabad, India), were used without further purification. Tablet preparations containing 5 mg AML and 5 mg ENA were purchase from Local pharmacy. Methanol (A.R. grade; S. D. Fine Chemical Ltd.), Triple Distilled water (Millipore Distilation Unit) prepared in laboratory, Tri sodium hydrogen phosphate (FINAR chemical Ltd.Ahmedabad), 0.1 N HCL (FINAR chemical Ltd.Ahmedabad) and Whatman filter paper no. 41 (Whatman International Ltd., England) was used for the study.

2.3. Preparation Solutions

2.3.1. Phosphate Buffer (6.8 pH)

For the preparation of phosphate buffer (6.8 pH) , 75 ml of 0.1 N HCL was mixed with 25 ml 0.2 M Tri Sodium Hydrogen Phosphate Solution in 100 ml volumetric flask.

2.3.2. Standard stock solution of AML (500 µg/ml)

An accurately weighed quantity of about 12.5 mg AML was transferred into 25 ml volumetric flask. About 10 ml of methanol was added and sonicated for 10 min. The solution was made upto volume with methanol to obtained final solution of 500 µg/ml.

2.3.4. Standard stock solution of ENA (500 µg/ml)

An accurately weighed quantity of about 12.5 mg ENA was transferred into 25 ml volumetric flask. About 10 ml of methanol was added and sonicated for 10 min. The solution was made upto volume with methanol to obtained final solution of 500 µg/ml.

2.3.5. Mixed standard stock solution of AML & ENA

Accurately weighed AML and ENA (12.5mg) were transferred to a 25 ml volumetric flask, dissolved and diluted up to the mark with methanol to get final concentration of 500 µg/ml for both drugs.

2.3.6. Sample preparation

Twenty tablets (each tablet contains 5 mg ENA and AML both) were accurately weighed, their mean weight was determined, and was ground to fine powder in a glass mortar. An amount of powdered mass equivalent to 5 mg of ENA and AML was weighed and transferred in 25ml conical flask. The drugs from powder were dissolved and extracted with methanol. To ensure complete extraction of drugs it was sonicated for 30 min. The extract was filtered through Whatmann filter paper No. 41 and residue was washed with methanol. The extract and washing were pooled and transferred to a 25 ml volumetric flask and volume was made with methanol. This solution was expected to contain 200 µg/ml

of AML and ENA both. From this solution, 1 ml aliquote was transferred to a 10 ml volumetric flask and volume was made with buffer. This solution was expected to contain 20 µg/ml for both of AML and ENA.

2.4. Selection of wavelength for estimation of AML and ENA

Absorbance spectrum of pure AML was scanned in the spectrum basic mode using the cursor function the absorbance coresponding to 360.6nm (wavelength λ_1, the wave length of reasonable absorbance for AML) was noted from the spectrum. Then the cursor function was moved along with peak curve and second wavelength 226.6 nm (λ_2) was selected, absorbance was noted at λ_2. Again cursor function was moved with peak curve until the absorbance equal to that of absorbance at 226.6 nm was found. The wavelength obtained coresponding to absorbance value at 226.6 nm was 247.8 nm (λ_3) Absorbance spectrum of pure ENA was also scanned in spectrum basic mode ENA showed some absorbance value at 226.6nm (λ_2) and 247.8nm (λ_3) while it dose not showed any absorbance at 360.6nm (λ_1), the absorbance value at 360.6nm was due to AML only in the combined mixture of both drugs and was selected for the measurement of AML. The absorbance of various dilutions of AML and ENA in diluents was measured at λ_2 and λ_3. At these two wavelength absorbance difference of AML at any concentration level was found to be zero while for ENA, absorbance difference was found to increase concomitantly as concentration was increase.

2.5. Preparation of Calibration Curve

Appropriate Aliquots from the stock solution of AML and ENA were used to prepare three different sets of dilutions, series A, B, C as follows.

Series A and B consisted of different concentration, 5-80 µg/ml for both of AML and ENA. Aliquot of the stock solutions of AML and ENA (500 µg/ml of each) was pipetted out in to a series of 10 ml volumetric flask and diluted with methanol to get final concentration in range 5-80 µg/ml for both of AML and ENA.

Series C comprised of mixture of AML and ENA having concentrations of 5-80 µg/ml.

The solution were prepared by transferring 0.1, 0.2, 0.4, 0.6, 0.8, 1.2, 1.6 ml of the mixed standard stock solution (500 µg/ml) of AML and ENA, in to a series of 10 ml volumetric flask and the volume was made up to mark with buffer.

The absorbance of resulting solution was measured at 226.6 nm, 247.8 nm and 360.6 nm. For AML calibration curve was constructed by plotting absorbance at 360.6 nm Vs concentration for AML and difference in absorbance ($A_{226.6}$-$A_{247.8}$) Vs concentration for ENA.

2.6. Application of Developed Method to Pharmaceutical Formulation

The absorbance of final sample solution was measured against blank at 226.6nm, 247.8 nm and 360.6 nm the amount of AML and ENA was computed using respective equation of straight line.

3. RESULTS AND DISCUSSION

The utility of dual wave length data processing program is its ability to calculate unknown concentration of component of interest in a mixture containing an interfering component. For elimination of the effects of interfering components, three specific wavelengths were chosen first wavelength, λ_1 at which zero absorbance of ENA and reasonable absorbance of AML was observed. Second and third wavelength λ_2 and λ_3, were the wavelength at which the absorbance of the AML was equal (Figure 1).

In the proposed procedure the absorbance of ENA alone in mixture of AML and ENA was determined using dual wavelength data processing program. To remove interference of AML, 226.6nm (λ_2) and 247.8nm (λ_3) were chosen. The absorbance at these two selected wavelength was found to be equal for AML at any concentration. At this two wavelength absorbance difference for AML at any concentration level was found to be zero while for ENA, absorbance difference was found to increase concomitantly as concentration was increase. Therefore wavelengths were employed to determine the concentration of ENA from the mixture of AML and ENA. The

difference in absorbance at these two wavelengths ($A_{226.6}$-$A_{247.8}$) cancels out the contribution of absorbance of AML in measurement of ENA at 226.6 nm and 247.8 nm, the difference in absorbance was proportional to the concentration of ENA in the mixture. In the proposed method phosphate buffer pH 6.8 was used to get stable results throughout study.

It was found that this difference in absorbance values was linear in the range of 5-80 µg/ml of ENA with correlation coefficient 0.9984 (Table 1). Further, the absorbance value at 360.6 nm was only due to AML, as ENA has zero absorbance at this wavelength. The absorbance values were found to be linear over the range of 5-80 µg/ml of AML with correlation coefficient of 0.9992 (Table 2). These results confirm the suitability of the proposed method for the simultaneous determination of ENA and AML from their mixture. Regression analysis (Table 3) for series A and C shows no difference in the equations of straight line and thus indicates that there is no interference of AML in determination of ENA. Same way for series B and C, no difference in the equations of straight line indicates that there is no interference of ENA on measurement of AML. From the series C, the limit of detection (LOD) and limit of quantification (LOQ) were determined by visual methods as suggested in ICH guidelines, which were found to be 1.7 µg/ml and 5µg/ml, respectively for ENA and 1.5 µg/ml and 5µg/ml, respectively for AML. Sensitivity parameters such as molar absorptivity (L/ mole/ cm) and Sandall's sensitivity (µg/ml/cm^2/0.001 Absorbance units) were found to be 5.4 ×10^{-1} and 9.43 ×10^{-2}, respectively for ENA and 6.8 ×10^{-1} and 8.33 ×10^{-2}, respectively for AML. Accuracy was checked by recovery study at 3 different concentration levels, i.e., a multilevel recovery study. The tablet samples were spiked with an extra 50, 100, 150 % of standard ENA and AML, and the mixtures were analyzed by proposed method. Results of the recovery study are shown in Table 4 suggested that method was accurate for the simultaneous estimation of ATV and AML from their combination drug products. The method was applied for the analysis of three marketed formulations containing ENA 5 mg and AML 5 mg per tablet. The results of analysis of tablet formulations are shown in Table 5. All of them meet pharmacopoeial requirement of ETV and AML.

Table 1: Determination of ENA alone and in presence of AML by proposed D.W. Spectrophotometry

SERIES A				SERIES C			
Composition of mixture (µg/ml)		Difference in Absorbance($A_{226.6}$ nm-$A_{247.8}$ nm) ± S.D. (n=5)	CV	Composition of mixture (µg/ml)		Difference in Absorbance($A_{226.6}$ nm-$A_{247.8}$ nm) ± S.D. (n=5)	CV
ENA	AML			ENA	AML		
5	0	0.057 ± .010	1.77	5	5	0.058 ± .008	1.38
10	0	0.096 ± .006	1.41	10	10	0.097 ± .007	1.53
20	0	0.213 ± .011	1.65	20	20	0.212 ± .010	1.47
40	0	0.459 ± .002	1.76	40	40	0.461 ± .002	1.81
60	0	0.652 ± .005	1.57	60	60	0.654 ± .006	1.59
80	0	0.910 ± .003	1.54	80	80	0.908 ± .004	1.76

n= Number of determinations.

Table 2: Determination of AML alone and in presence of ENA by proposed D.W. Spectrophotometry

SERIES B				SERIES C			
Composition of mixture (µg/ml)		Absorbance at 360.6 nm	CV	Composition of mixture (µg/ml)		Absorbance At 360.6 nm	CV
ENA	AML			ENA	AML		
0	5	0.070 ± .013	2.00	5	5	0.074 ±.011	1.73
0	10	0.105 ± .016	1.79	10	10	0.106 ±.013	1.54
0	20	0.241 ± .006	1.86	20	20	0.240 ±.020	1.68
0	40	0.468 ± .016	1.56	40	40	0.470 ±.018	1.44
0	60	0.714 ± .007	2.41	60	60	0.718 ±.007	2.26
0	80	0.966 ± .010	1.99	80	80	0.965 ±.008	1.66

n= Number of determinations.

Table 3: Regression analysis data of the calibration curve obtained using series A, B, C

Series	Composition of the sample solution		Regression equation of the curve	Correlation coefficient
	ENA (µg/ml)	AML (µg/ml)		
A	5-80	0	Y= 0.014X-0.009	0.9984
B	0	5-80	Y= 0.012X-0.003	0.9992
C	5-80	5-80	*Y= 0.014X-0.011	0.9989
			**Y=0.012X-0.009	0.9996

Y is absorbance and X is concentration in µg/ml.
*Regression equation for ENA
**Regression equation for AML

Table 4: Recovery study of AML and ENA from tablet formulations (n=3)

Label Claim (mg/tablet)		Amount of standard Added (%)		Total Amount of standard Added (µg/ml)		Amount of standard Recovered (µg/ml)		% Recovery* ± S.D.	
ENA	AML	ENA	AML	ENA	AML	ENA	AML	ENA	AML
5	5	50	50	2.5	2.5	2.48	2.48	99.2± 0.4	99.2± 0.4
5	5	100	100	5.0	5.0	4.93	4.94	98.53± 0.5	98.8± 0.1
5	5	150	150	7.5	7.5	7.45	7.48	99.39± 0.4	99.75± 0.16

* Indicates that each value is mean ± standard deviation of three determinations

Table 5: Analysis of pharmaceutical formulations

Formulation	Amount of drug taken (mg)		Amount of drug found (mg)		% Amount found (n=3) ± SD	
	AML	ENA	AML	ENA	AML	ENA
Tablet 1	5	5	5.02	4.98	100.4 ± 0.32	99.6 ± 0.56
Tablet 2	5	5	4.96	4.99	99.2 ± 1.14	99.80 ± 1.35
Tablet 3	5	5	5.05	5.07	101.0 ± 1.64	101.4 ± 1.28

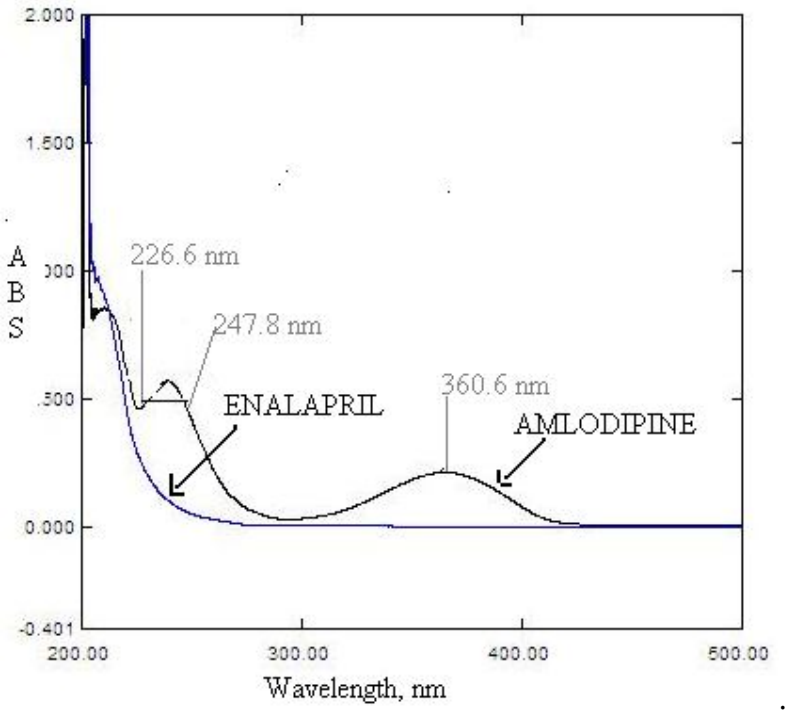

Fig. 1: Overlain Spectra of AML and ENA

4. CONCLUSION

The proposed method is based on dual wavelength data processing and only requires measurement of absorbance at selected wavelengths. Interference studies revealed that the common excipients and other additives usually present in the tablet dosage forms did not interfere in the proposed method for estimation of both drugs. The proposed method was found to be simple, rapid, economical, accurate and precise. It can be useful for routine in process quality control and simultaneous estimation of ENA and AML from their combined tablet dosage forms.

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