

SPECTROPHOTOMETRIC SIMULTANEOUS DETERMINATION OF VALSARTAN AND AMLODIPINE BESYLATE IN COMBINED CAPSULE DOSAGE FORM BY RATIO DERIVATIVE METHOD

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ABSTRACT

A simple, economical, precise and accurate method for simultaneous determination of valsartan (VAL) and amlodipine besylate (AML) in combined capsule dosage form has been developed. The method is based on ratio spectra derivative spectrophotometry. The amplitudes 229.48 nm and 299.10 nm in the first derivative of the ratio spectra were selected to determine valsartan (VAL) and amlodipine besylate (AML) respectively in combined formulation. Beer's law is obeyed in the concentration range of 8-40 $\mu\text{g mL}^{-1}$ for VAL and 0.5-2.5 $\mu\text{g mL}^{-1}$ for AML for the methods. The % assay for commercial formulation was found to be 98.56% $\pm$ 0.27 for VAL and 98.91% $\pm$ 0.51 for AML by the proposed methods. The methods were validated with respect to linearity, precision and accuracy. Recovery by both the methods was found in the range of 98.89 – 99.60 % for VAL and 98.58 – 99.63 % for AML.

Keywords: Valsartan and Amlodipine besylate; Ratio Spectra Derivative Spectrophotometry; Capsule Dosage Form.

INTRODUCTION

Valsartan (VAL) Chemically, *N*-(1-Oxopentyl)-*N*-[[2'-(1*H*-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]-L-valine, is an orally active specific angiotensin II blocker effective in lowering blood pressure in hypertensive patients¹.

Analytical methods have been reported for the individual determination of VAL in biological fluids and pharmaceutical formulations which include methods like HPLC²⁻⁵ and LC-MS⁶. Simultaneous UV spectrophotometric methods⁷⁻⁹ have also been reported for estimation of VAL alone or in combination with other agents.

Amlodipine besylate (AMLB), 2-[(2-Aminoethoxy)-methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridine dicarboxylic acid 3-ethyl-5-methyl ester, benzosulfonate, is a potent dihydro calcium channel blocker. Several analytical methods for AML have been reported for its individual analysis which includes liquid chromatography¹⁰⁻¹⁴ and liquid chromatography coupled with spectrofluorimetry¹⁵. The aim of the study was the development and validation of ratio derivative method for VAL and AML in capsule dosage form. The proposed method was optimized and validated as per the International Conference on Harmonization (ICH) guidelines¹⁶.

MATERIAL AND METHODS

Instrumentation

An UV-Visible double beam spectrophotometer (Varian Cary 100) with 10MM matched quartz cells was used. All weighing were done on electronic balance (Model Shimadzu AUW-220D).

Reagents and chemicals

Pure drug sample of VAL, % purity 99.86 and AML, % purity 99.92 was kindly supplied as a gift sample by Cipla Ltd. Mumbai. The commercial formulation of AML and VAL available in ratio of 1:32 and 2.5/80 mg .were procured from market.

Preparation of Standard Stock Solutions and calibration Curve

The standard solutions of both the components (1mg/ml) were prepared in methanol. The working standard solutions of these drugs were obtained by dilution of the respective stock solution in methanol, using the above mentioned procedure, were used to prepare calibration curves for both the drugs.

Preparation of Sample Stock Solution and Formulation analysis

Twenty capsules were weighed accurately and a quantity of capsule powder equivalent to 320 mg of VAL (10 mg of AML) was weighed and dissolved in 80 mL of methanol with the aid of ultrasonication for 5 min and solution was filtered through Whatman paper No. 41 into a 100 mL volumetric flask. Filter paper was washed with methanol, adding washings to the volumetric flask and volume was made up to the mark with methanol. Solution was suitably diluted further to get required final concentration of VAL (24 $\mu\text{g mL}^{-1}$) and AML (1.5 $\mu\text{g mL}^{-1}$).

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Recovery studies

The accuracy of the proposed method was checked by recovery studies, by addition of standard drug solution to preanalysed sample solution at three different concentration levels (50 %, 100 % and 150%) 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 12 $\mu\text{g mL}^{-1}$ of VAL and 0.75 $\mu\text{g mL}^{-1}$ of AML.

Precision of the Method

To study intraday precision, method was repeated 5 times in a day and the average % RSD was found to be 2.36 for VAL and 1.37 for AML. Similarly the method was repeated on five different days and average % RSD was found to be 2.35 for VAL and 1.23 for AML. These values confirm the intra and inter day precision.

RESULTS AND DISCUSSION

The method involves dividing the spectrum of mixture by the standardized spectra of each of the analyte and deriving the ratio to obtain spectrum that is dependent of concentration of analyte used as a divisor. Using appropriate dilutions of standard stock solution, the two solutions were scanned separately. Under experimental conditions described, calibration curve, assay of capsules and recovery studies were performed. The ratio and ratio derivative spectra of different VAL standards at increasing concentrations were obtained by dividing each with the stored spectrum of the standard solution of AML (1.5 $\mu\text{g mL}^{-1}$) as shown in (Fig. 1 (A) and (B) respectively).

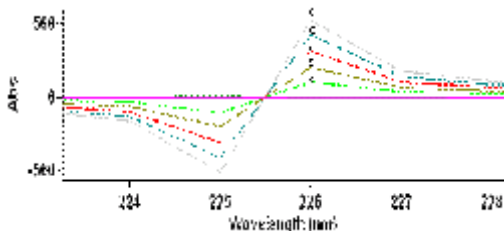


Fig. 1(A): Ratio spectra of VAL using 1.5 $\mu\text{g mL}^{-1}$ solution of AML as divisor

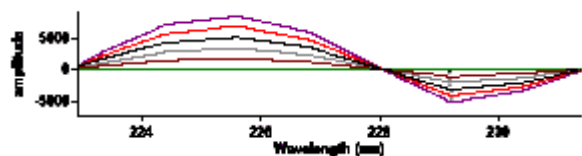


Fig. 1(B): First derivative of the ratio spectra of (a) 8, (b) 16, (c) 24, (d) 32 and (e) 40 $\mu\text{g mL}^{-1}$ solution of VAL when 1.5 $\mu\text{g mL}^{-1}$ solution of AML is used as divisor.

Wavelength 229.48 nm was selected for the quantification of VAL in VAL+AML mixture. The ratio and ratio derivative spectra of the solutions of AML at different concentrations were obtained by dividing each with the stored standard spectrum of the VAL (24 $\mu\text{g mL}^{-1}$) (Fig. 2 (A) and (B) respectively).

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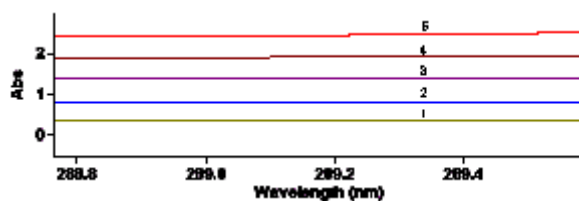


Fig 2 (A): Ratio spectra of AML using 24 $\mu\text{g mL}^{-1}$ solution of VAL as divisor

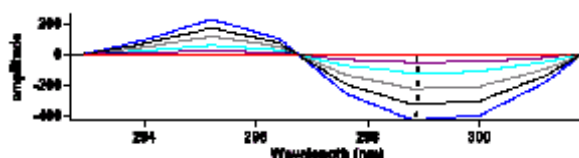


Fig. 2 (B): First derivative of the ratio spectra of (1) 0.5, (2) 1, (3) 1.5, (4) 2 and (5) 2.5 $\mu\text{g mL}^{-1}$ solution of AML when 24 $\mu\text{g mL}^{-1}$ solution of VAL is used as divisor.

Wavelength 299.10nm was selected for the quantification of AML in VAL+AML mixture. Measured analytical signals at these wavelengths were proportional to the concentrations of the drugs. Calibration curves were prepared from the measured signals at the selected wavelength and concentration of the standard solutions. The amount of VAL and AML in capsules was calculated by using following equations ¹⁷

$$\text{At nm: } C_{\text{VAL}} = \frac{d/d\lambda [A_{\text{VAL}}/A_{\text{AML}}] - \text{Intercept (C)}}{\text{Slope (m)}} \dots (1)$$

$$\text{At nm: } C_{\text{AML}} = \frac{d/d\lambda [A_{\text{AML}}/A_{\text{VAL}}] - \text{Intercept (C)}}{\text{Slope (m)}} \dots (2)$$

The zero order overlain spectra is shown in Fig 3.

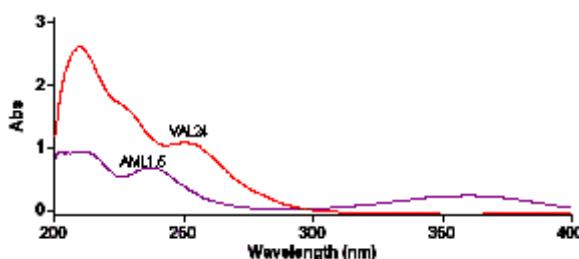


Fig. 3 : Overlain zero order absorbance spectra of VAL (24 $\mu\text{g/mL}$) and AML (1.5 $\mu\text{g/mL}$)

A critical evaluation of proposed method was 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 were linearity, accuracy and precision. Beer's law obeyed in the concentration range 8-40 $\mu\text{g mL}^{-1}$ for VAL and 0.5-2.5 $\mu\text{g mL}^{-1}$ for AML with correlation coefficient of > 0.999 for both the drugs. The proposed methods were also evaluated by the assay of commercially available capsules containing VAL and AML (n = 5). The results of formulation analysis are presented in Table 1. For VAL, the recovery study

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Table 1: Optical characteristics of method and results of formulation analysis.

Parameter		VAL	AML
λ (nm)		239.48	251.38
Beer's Law Limit (µg/mL)		8.40	0.525
Molar absorptivity		1532.721	307.525
Regression Equation ($y = mx + c$)	Slope (m)	122.47	9.47
	Intercept (c)	-48.808	-3.736
Correlation coefficient		0.9995	0.9998
Precision (% R.S.D.)	Intra-day	2.36	1.37
	Inter-day	2.36	1.23
Formulation Analysis (Assay $\pm$ SD)		98.96% $\pm$ 0.27	98.91% $\pm$ 0.51

results ranged from 98.89-99.60% with % RSD values ranging from 0.85-1.72 % and for AML, the recovery results ranged from 98.58-99.63 %, with % RSD values ranging from 0.75-1.45 %.

CONCLUSION

The validated spectrophotometric methods employed here proved to be simple, economical, precise and accurate. The method is reproducible and thus it can be used as IPQC test and for routine simultaneous determination of VAL and AML in capsule dosage form.

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