

Simultaneous Estimation of Aliskiren and Valsartan by Ratio Spectra Derivative Spectrophotometry Method in their Fixed Dosage Forms

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Abstract : Aliskiren is the first in a class of drugs called direct renin inhibitors and valsartan is an angiotensin II receptor antagonist (more commonly called angiotensin receptor blocker), The combination therapy targets renin angiotensin aldosterone system (RAAS). The Ratio spectra derivative spectrophotometric method was developed for the simultaneous determination of Aliskiren (ALS) and Valsartan (VAL) in their fixed dosage forms. The method depends on the use of the first derivative of the ratio-spectra obtained by dividing the absorption spectrum of binary mixtures by a standard spectrum of one of the compounds. The first derivative amplitudes at 289nm and 245nm were selected for the determination of ALS and VAL respectively. The wavelength interval was selected as $\Delta\lambda = 4$ nm. Methanol: water (50:50) was used as the diluent. Both the drugs, Aliskiren and Valsartan showed linearity in the range of 50-200 $\mu\text{g mL}^{-1}$ and 5-24 $\mu\text{g mL}^{-1}$ respectively. The method was validated statistically and recovery studies were carried out. It was found to be accurate, precise and reproducible. The method was applied to the assay of in-house formulation, which was found in the range of 98.0% to 102.0% of the labeled value for both Aliskiren and Valsartan. Hence, the method herein described can be successfully applied in quality control of combined pharmaceutical dosage forms.

Keywords: Aliskiren; Ratio Spectra Derivative Spectrophotometry; Valsartan.

1. INTRODUCTION:

Aliskiren (ALS), (2(S), 4(S), 5(S), 7(S)-N- (2-carbamoyl-2-methyl propyl)-5-amino-4-hydroxy-2,7 diisopropyl-8-[4-methoxy-3-(3-methoxy propoxy) phenyl] octanamide hemifumarate)1-3 (Figure-1).is a first oral direct renin inhibitor approved for clinical use, exhibits a novel and advantageous pharmacokinetic and pharmacodynamic profile for the long-term treatment of hypertension. Aliskiren blocks the renin system at its rate-limiting step by directly inhibiting the catalytic activity of renin, thereby reducing generation of angiotensin I and angiotensin II⁽¹⁾.

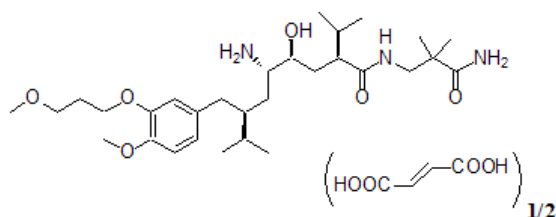


Figure-1;Chemical structure of Aliskiren

Valsartan (VAL), chemically known as (S)-3-methyl-2-(N-{{[2'-(2H-1, 2, 3, 4-tetrazol-5-yl) biphenyl-4-yl] methyl}pentanamido)butanoic acid (Figure.2), is an angiotensin II receptor antagonist and is used in the treatment of high blood pressure, congestive heart failure (CHF) or post-myocardial infarction (MI)(Saydam and Takka, 2007). It is official in United State Pharmacopoeia.

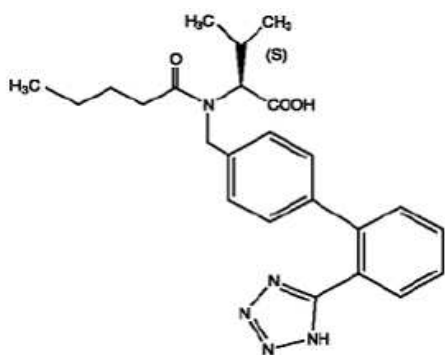


Figure 2: Chemical structure of Valsartan

Combination therapy of Aliskiren(ALS) with valsartan(VAL) is indicated for the treatment of high blood pressure in patients not adequately controlled on Aliskiren (ALS) or angiotensin receptor blocker (ARB) mono therapy and as initial therapy in patients likely to need multiple drugs to achieve their blood pressure goals.⁽²⁾ The effects of the combination of the lower doses of Aliskiren (ALS) and Valsartan (VAL) were similar to those of 300 mg Aliskiren (ALS) and larger than those of 160 mg Valsartan (VAL). Aliskiren (ALS) also blunted the valsartan (VAL)-induced rise in plasma renin activity and in plasma concentration of angiotensin I and II (Azizi et al 2004). These findings suggest that, at lower doses, renin inhibitors and angiotensin-receptor blockers might have synergistic effects on the renin system⁽³⁾.

A detailed survey of analytical literature for ALS revealed several methods based on varied techniques, viz, HPLC^(9,11), MEKC method⁽¹⁴⁾, Spectrophotometry^(5,10,13), Spectrofluorimetric Determination⁽¹⁵⁾. Similarly, a survey of the analytical literature for VAL revealed methods based on HPLC⁽¹⁶⁾ for determination in pharmaceuticals, UV-spectrophotometric determination⁽¹⁷⁻¹⁹⁾.

Literature survey reveals the availability of several methods for estimation of both Aliskiren (ALS) and Valsartan (VAL)^(4,8,12). No method has been reported for the estimation of Aliskiren (ALS) and Valsartan (VAL) in combined dosage form with comparative data using other advance method. Present work emphasizes on a spectrophotometric method based on the use of the first derivative of the ratio spectra, first developed by Salinas et al.^(6,7), for resolving binary mixtures. The objective of this work was to develop simple, precise and rapid ratio spectra derivative spectrophotometric method for combination drug products containing Aliskiren (ALS) and Valsartan (VAL).

2. EXPERIMENTAL

2.1 Materials and Reagents

Reference standards of Aliskiren hemifumarate and Valsartan were gifted by Mopren laboratories, Delhi, India and AUM research center, Ahmedabad, India, respectively. Identical placebo⁽²⁾ was prepared and used for accuracy study and test solution preparation. Methanol (HPLC grade) was purchased from Spectrochem (Mumbai, India). Nylon syringe filters (0.45 μm) were purchased from MillexHN, Millipore (Mumbai, India).

2.2 Instrumentation

A double-beam UV-Visible spectrophotometer make: Analytical Technologies Ltd, Model SPECTRO UV2080 PLUS, equipped with 1 cm quartz cells, with a fixed slit width (1 nm), with UV-VIS Analyst Version 5.2. For scanning, the wavelength range selected was from 400 nm to 200 nm.

2.3 Standard and Test Solutions

2.3.1 Preparation of Standard Solution

The standard stock solutions containing 1000 $\mu\text{g mL}^{-1}$ each of ALS and VAL were prepared separately by dissolving standards in Methanol and diluting with the diluent (methanol : Water) (50:50 v/v). Standard solutions of both the drugs were prepared individually by dilution of the standard stock solutions with diluent to obtain the concentration range of 50-200 $\mu\text{g mL}^{-1}$ for ALS and 5-24 $\mu\text{g mL}^{-1}$ for VAL.

2.3.2 Preparation of Test Solution

Identical placebo was prepared based on package insert available for Valtorna tablets 300/320mg. Powder equivalent to 100 mg each of ALS was accurately weighed and transferred to a 200 mL calibrated volumetric flask. Around 10 mL of Methanol was added, and the solution was sonicated for 30 minutes. Volume was made up to the mark with diluent. The solution was filtered through 0.45 μm nylon syringe filter. The resultant solution contained 500 $\mu\text{g mL}^{-1}$ of ALS and 533.3 $\mu\text{g mL}^{-1}$ of VAL. The solution was further diluted with diluent to get concentration of 100 $\mu\text{g mL}^{-1}$ of ALS and 10.6 $\mu\text{g mL}^{-1}$ of VAL.

2.4 Ratio Spectra Derivative Method

This method works on two mechanisms viz. (1) Ratio and (2) Derivatization. In this method, the mixture spectra are divided with the divisor and first derivative spectra of these ratio spectra are generated. The main advantage of the ratio-spectra derivative spectrophotometry is the chance of doing easy measurements in correspondence of peaks so it permits the use of the wavelength of highest value of analytical signals (a maximum or a minimum). Moreover, the presence of a lot of maxima and minima is another advantage by the fact that these wavelengths give an opportunity for the determination of active compounds in the presence of other compounds and excipients which possibly interferes the assay.

For the determination of ALS, the spectra of ALS at increasing concentrations in diluent (figure-3a) were divided by previously stored absorption spectrum standard solution of VAL (10 $\mu\text{g mL}^{-1}$) to obtain the corresponding ratio spectra. Then the first derivative of the obtained ratio spectra were traced with interval of $\Delta\lambda = 4$ nm (figure-4a). In the binary mixtures, content of ALS was determined by measuring the first derivative amplitude at 289.0 nm, where there is no contribution or interference from VAL.

On the other hand, for the determination of VAL, an analogous procedure was followed. The spectra of VAL at increasing concentrations (figure-3b) were divided by previously stored spectrum of 100 $\mu\text{g mL}^{-1}$ solution of ALS and the first derivative of the developed ratio spectra were traced with $\Delta\lambda = 4$ nm (figure-4b). In the binary mixtures, content VAL was determined by measuring the first derivative amplitude at 245 nm, where there is no contribution or interference from ALS.

First-derivative technique traced with $\Delta\lambda = 4$ nm was used to resolve the spectral overlapping. The calibration curves were checked for linearity and linear behavior was observed in the concentration range of 50-200 $\mu\text{g mL}^{-1}$ and 5-24 $\mu\text{g mL}^{-1}$, for ALS and VAL respectively.

2.5 Method Validation

The method was validated as per ICH guidelines for parameters like Linearity, Accuracy and Precision. The accuracy studies were carried out at different concentrations by spiking a known concentration of standard drug to the placebo sample and contents were reanalyzed by the developed method. Precision was studied by analyzing six replicates of sample solutions.

3. RESULTS AND DISCUSSION

Zero-order absorption spectra of $100 \mu\text{g mL}^{-1}$ and $10 \mu\text{g mL}^{-1}$ of each of ALS and VAL showed overlapping peaks that interfere with the simultaneous determination of this formulation as shown in Figure-1. So it was thought of interest to develop the ratio spectra derivative spectrophotometry method for the simultaneous estimation of ALS and VAL. 50% v/v Methanol in water was used as the solvent since both the drugs exhibit good solubility in it and no interference due to excipients of the tablet formulation were observed.

3.1. Ratio spectra Derivative Spectrophotometry Method:

The absorption spectra of ALS prepared at increasing concentrations in diluent were recorded in the spectral region of 200-400 nm and divided by the previously stored spectrum of $10.0 \mu\text{g mL}^{-1}$ VAL in the same diluent and their ratio spectra were obtained as seen in the Figure-3a. Then, the first derivatives of ratio-spectra were recorded as shown in Figure-4a which were plotted with the interval of $\Delta\lambda = 4 \text{ nm}$ and the values of the derivatives were measured at suitably selected wavelength for the determination of ALS. The influence of the obtaining the first derivative was tested and $\Delta\lambda = 4 \text{ nm}$ was considered as suitable. The concentration of divisor can be modified, and different calibration graphs are then obtained. A concentration of $10.0 \mu\text{g mL}^{-1}$ of VAL was considered as suitable. The calibration graph was established by measuring at the amplitude at 289 nm corresponding to a maximum wavelength.

For determining VAL, an analogous procedure was followed. The ratio spectra were obtained by dividing the spectra of VAL with previously stored spectrum of a $100 \mu\text{g mL}^{-1}$ ALS solution as shown in figure-3b and their first derivatives were calculated with the interval of $\Delta\lambda = 4 \text{ nm}$ as shown in figure-4b. The values of the derivatives were measured at suitably selected wavelength for the determination of VAL. A concentration of $100.0 \mu\text{g mL}^{-1}$ of ALS was considered as suitable. The calibration graph was established by measuring at the amplitude at 245 nm corresponding to a maximum wavelength.

Figure – 1 Overlaid Zero order spectra for ALS and VAL

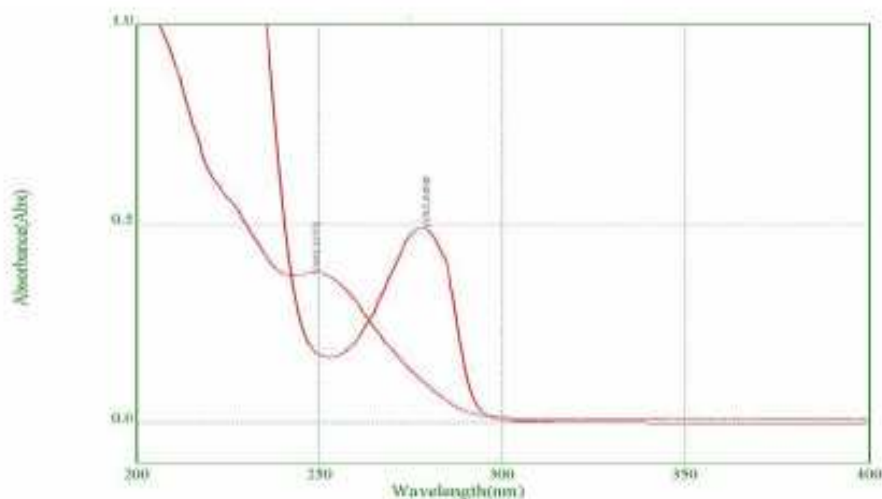


Figure – 2 Linearity study for Zero order spectra for (a) ALS and (b) VAL

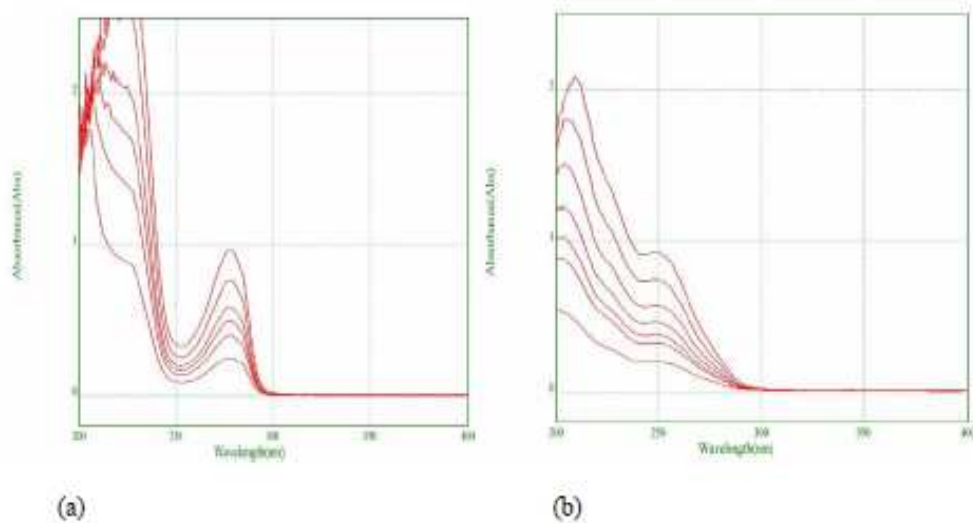


Figure – 3 Ratio spectra for (a) ALS and (b) VAL

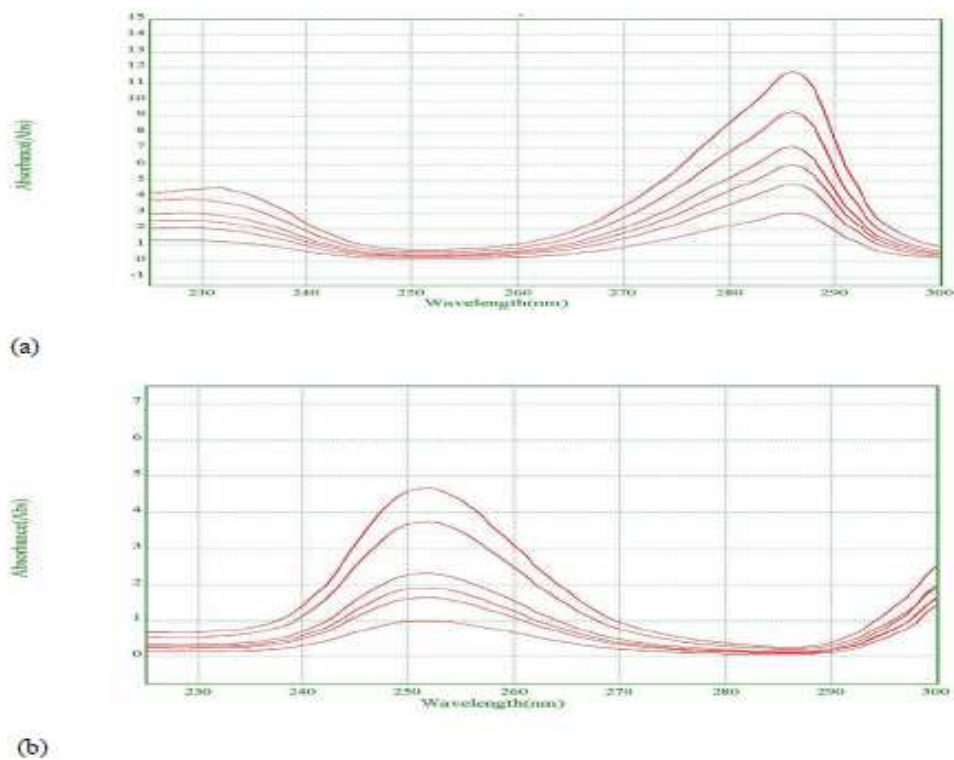
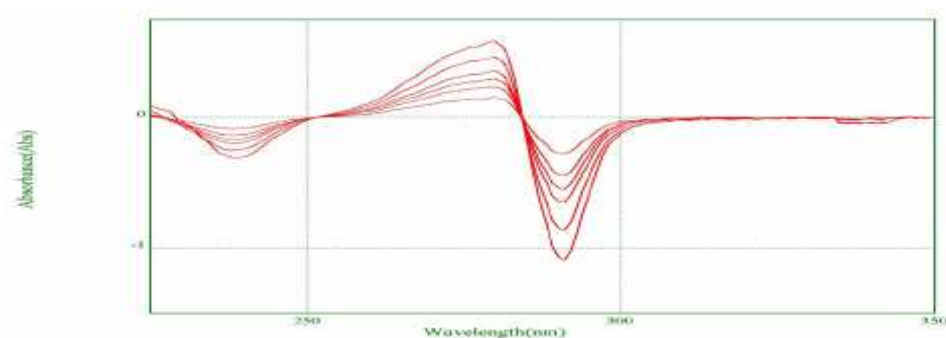
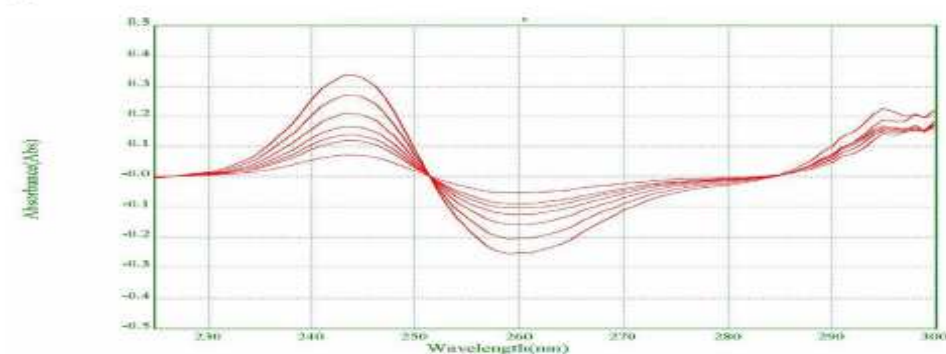


Figure – 4 First derivative ratio spectra for (a) ALS and (b) VAL



(a)



(b)

3.2 Method validation

The developed method was validated for parameters like linearity, precision and accuracy. The method was found to be linear in the range of 50-200 $\mu\text{g mL}^{-1}$ and 5-24 $\mu\text{g mL}^{-1}$ for ALS and VAL respectively with correlation coefficient of 0.998 and 0.999 for ALS and VAL respectively. The data for linearity and precision are presented in the Table-1 & 2. The data for recovery study are shown in the Table-3. The low value of %RSD indicates that the method is precise and accurate.

3.3 Correlation of method with HPLC method

The results obtained with proposed Ratio spectra derivative Spectrophotometric method were compared with HPLC method⁸. The results obtained show the high reliability and reproducibility of the method. The comparative data are presented in Table-4.

Table-1: Spectrophotometric Data

	ALS	VAL
Absorption maxima (λ max)	278 nm	245 nm
Linearity range ($\mu\text{g/ml}$)	50-200	5-24
Correlation coefficient	0.999	0.999

Table–2: Data showing linearity and precision of Ratio spectra Derivative Method

	ALS	VAL
Linearity range ($\mu\text{g/ml}$)	50-200	5-24 ¹
Correlation coefficient	0.998	0.999
Method precision (n=6)	Mean :98.3 RSD: 0.75	Mean :99.3 RSD: 0.85

Table–3: Data showing recovery of proposed Ratio spectra Derivative Method

	Accuracy level(%)	Amount added (in mg)	Amount found (in mg)	% recovery	Mean recovery(%)
ALS	80	80.5	80.1	99.5	99.1
	100	99.8	99.5	99.7	
	120	120.4	118.1	98.1	
VAL	80	85.3	83.9	98.4	98.9
	100	106.6	105.3	98.8	
	120	128	127.5	100.5	

Table–4: Data showing comparison with proposed Ratio spectra Derivative Method with HPLC method

% Assay Value	ALS	VAL
By Proposed method	99.1	98.9
By HPLC method	99.5	99.1

4. CONCLUSION

The proposed method is accurate and precise for determination of assay of ALS and VAL in their binary mixture. In this proposed method the linearity is observed in the concentration range of 50-200 $\mu\text{g mL}^{-1}$ with coefficient of correlation 0.998 for ALS and 5-24 $\mu\text{g mL}^{-1}$ with co-efficient of correlation 0.999 for VAL. The result of the analysis of formulation by the proposed method is reliable and correlate with HPLC method. The method can be used for the routine analysis of the ALK and VAL in combined dosage form without any interference of the excipients.

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