Spectrophotometric methods for simultaneous estimation of ramipril and valsartan in combined tablet dosage form

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ABSTRACT

Two simple, accurate and precise spectrophotometric methods for the simultaneous estimation of Valsartan (VAL) and Ramipril (RAMI) in combined dosage form have been developed and validated. First spectrophotometric method employed "Simultaneous equation method" using 210.0 nm and 249.0 nm as two wavelengths for absorbance measurement using methanol and 0.1N HCl as the solvents. Beer's law is obeyed in the concentration range of 1.25-40μg/mL and 5-40μg/mL for RAMI and VAL, respectively. Second method employed "Absorbance Correction Method". The Wavelength selected was 274.5 nm. Molar absorptivities, Limit of Detection (LOD) and Limit of Quantitation (LOQ) were calculated. Both methods have been successfully applied for the analysis of these drugs in a pharmaceutical formulation. Results of analysis were validated statistically and by recovery studies. © 2008 Trade Science Inc. - INDIA

INTRODUCTION

Valsartan (VAL) is known as N-[p-(o-1H-Tetrazol-5-ylphenyl)benzyl]-N-valeryl-L-valine. It is an Angiostensin II blocker and it is used as an antihypertensive agent. Ramipril (RAMI) is chemically (2S, 3aS, 6aS)-1-[(S)-N-[(S)-1-carboxy-3- phenylpropyl] alanyl] octahydrocyclopenta[b]pyrrole-2-carboxylic acid, 1-ethyl ester. It is an angiostensin-converting enzyme inhibitor. It inhibits the conversion of the inactive angiostensin I to the highly potent vasoconstrictor, angiostensin II[1]. Both the drugs, Valsartan and Ramipril are official in USP 2007[2]. Ramipril is also official in BP 2007[3]. Detail survey of literature of Valsartan revealed several UV Spectrophotometric methods [4-8] as a single drug and in combined dosage form. Similarly, literature survey of Ramipril revealed several methods, viz.,

HPTLC[9-10] and UV Spectrophotometric[11-15]. However there are no UV methods reported for the Simultaneous estimation of both of these drugs in combination.

The present work describes a useful method for the simultaneous determination of both of these drugs in combined dosage form.

EXPERIMENTAL

Chemicals

The working standards of Ramipril was procured from Mepro Pharmaceuticals pvt.Ltd. Wadhwan, India. and Valsartan was kindly provided by Lupin Research Park, Pune, India. Methanol AR grade was purchased from Ashonuj Chem. Pvt. Ltd. Navi Mumbai. Hydrochloric acid (HCl) was purchased from Loba

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chemie Pvt. Ltd. Mumbai.

Instrumentation

The zero order-overlain spectra were recorded in the wavelength range 200 - 400 nm using UV double beam spectrophotometer of make Jasco, model V-550 with 1cm matched quartz cells. The instrumental parameters optimized for the zero order-overlain spectrums were:

Bandwidth: 0.2nm; Scanning speed: 400 nm/min; Data pitch: 0.5 nm

PROCEDURE

Method 1

Simultaneous equation method

Preparation of working standard solution

10mg of ramipril and 10mg of Valsartan was transferred to 25 ml volumetric flask separately. To it 15 ml of methanol was added and sonicated for 10 min and then volume was made up to 25 ml with methanol so as to get the concentration of 0.4mg/ml. The two wavelengths selected for determination were 210.0nm, λ_{max} for Ramipril and 249.0nm, λ_{max} for Valsartan.

The absorptivity values for both the drugs at both the selected wavelengths were calculated by dividing the respective absorbance values by concentrations. The procedure was repeated 5 times and average absorptivity values were considered for further calculations. Refer TABLE 1 for values.

Estimation of drugs from tablet solution

Ten tablets were crushed and weight equivalent to 5mg of ramipril and 80 mg of Valsartan were taken in 25 ml volumetric flask To it, 15 ml of methanol was added and sonicated for 10 min and then volume was made up to 25ml. The resulting solution was filtered through whatmann filter paper no.41. A quantity of 62.5μl from the above solution was transferred in 10ml volumetric flask and diluted upto the mark with 0.1NHCl so as to get the solution of concentration 1.25μg/ml and 20μg/ml for ramipril and Valsartan respectively.

The absorbances A_1 and A_2 of this solution were measured at 210.0nm and 249.0nm respectively. The concentration of both the drugs was calculated by using the formula:

$$C_{VAL} = A_2\, a_{y1} - A_1\, a_{y2} / a_{x2}\, a_{y1} - a_{x1}\, a_{y2} \qquad (1)$$
$$C_{RAMI} = A_1\, a_{x2} - A_2\, a_{x1} / a_{x2}\, a_{y1} - a_{x1}\, a_{y2} \qquad (2)$$

where, A_1 and A_2 are absorbance of sample solution at 210.0nm nm and 249.0 nm respectively, a_{x1} (93.79) and a_{x2} (30.52) are absorptivities values for VAL at 210.0 nm and 249.0 nm respectively, a_{y1} (46.05) and a_{y2} (4.312) are absorptivities values for RAMI at 210.0 nm and 249.0 nm respectively.

Method 2

Absorbance correction method[16]

This method is used to calculate the concentration of drug even in the presence of interferents. If the identity, concentration and absorbtivity of the absorbing interferents are known, it is possible to calculate their contribution to the total absorbance of the mixture. The concentration of absorbing component of interest is then calculated from the corrected absorbance. The wavelength selected for this method is at 274.5 nm because at this wavelength the absorbance for Ramipril is practically nil. Refer spectrum in figure 1.

The concentration of both drugs was calculated in 4 steps:

(a) Calculate the concentration of Valsartan in g/100ml by using the formula:

$$A_1 = a_{x3}\, bc$$

Where, A_1 = Absorbance of mixture at 274.5nm; a_{x3} = Absorbtivity value of Valsartan at 274.5nm (10.96); c = Con

TABLE 1: Absorbtivity values for method I and method II

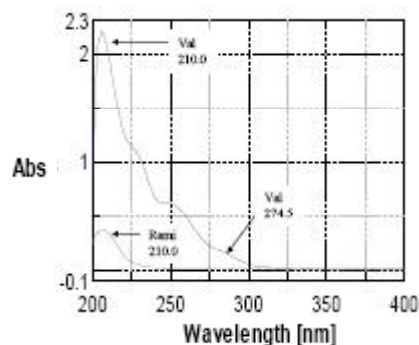| Drug	Absorbtivity value at 210.0nm	Absorbtivity value at 249.0nm	Absorbtivity value at 274.5nm
Ramipril (y)	46.05(a_{y1})	4.312(a_{y2})	-
Valsartan(x)	93.79(a_{x1})	30.52(a_{x2})	10.96(a_{x3})

Figure 1: Overlain spectra of Ramipril and Valsartan in 0.1N HCl

centration in g/100ml; b = path length (1cm)

(b) Calculate the absorbance value of Valsartan at 210 nm using the formula:

$$A_2 = a_{x1} \, bc$$

Where, A_2 = Absorbance value of Valsartan at 210nm; a_{x1} = Absorbtivity value for Valsartan at 210nm (93.79); c = Concentration of Valsartan in g/100ml; b = path length (1 cm)

(c) Corrected absorbance at 210.0nm = Absorbance of mixture at 210nm - A_2

(d) Concentration of Ramipril = Corrected absorbance at 210.0nm / $a_{y1} \times b$

Where, a_{y1} = Absorbtivity of Ramipril at 210nm (46.05); b = path length (1cm)

Method validation

Linearity and range

The relationship between absorbance and concentration was found linear for Ramipril in the range of 1.25 - 10μg/ml and 5 - 40μg/ml for Valsartan. The dilutions were made in 0.1N HCl by taking appropriate amount of solution from working standard of both the drugs. The solutions were scanned over the range 200-400nm. The Zero-order overlain spectrum is shown in figure1. The linear regression equations are:

For Ramipril at 210.0nm　　y = 0.0480x - 0.0122 (r = 0.995)
For Valsartan at 249.0nm　　y = 0.0288x + 0.0237 (r = 0.997)
For Valsartan at 274.5nm　　y = 0.0101x + 0.0137 (r = 0.999)

Precision

The precision of the method were checked by interday and intraday variation studies. In the Interday studies the absorbance of all the solutions was measured on three consecutive days.

In the Intraday studies the absorbance of all the solutions was measured thrice a day. The Percentage RSD values were calculated and the developed UV method was found to be precise. Results are shown in TABLE 2.

Accuracy

The accuracy of the method were determined by recovery experiments. The recovery studies were carried out by standard addition method at 80%, 100%, 120% level. The percentage recovery was calculated and found to be within range of 98.0 – 102%. A known amount of working standard of both the drugs was added to the tablet solution. The percentage recovered

TABLE 2: Validation parameters for method I and method II

Parameters	Method I		Method II	
	RAMI	VAL	RAMI	VAL
Beer's law range(mcg/ml)	1.25 - 10	5-40	1.25-10	5-40
Wavelength (nm)	210.0	249.0	210.0	274.5
Regression equation y = mx+c				
Slope (m)	0.0480	0.0288	0.0480	0.0101
Standard deviation of slope	1.56×10^{-3}	2.8×10^{-4}	1.52×10^{-4}	3.5×10^{-4}
Intercept (c)	0.0122	0.0237	0.0122	0.0137
Standard deviation of intercept	1.0×10^{-3}	1.6×10^{-3}	1.3×10^{-3}	4.04×10^{-3}
% RSD (n=3)				
Interday precision	0.20-0.58	0.39-1.05	0.25-0.46	1.54-0.93
Intraday precision	0.31-1.15	0.55-0.65	0.39-1.75	0.18-0.47
LOD (mcg/ml)	0.089	0.184	0.088	1.33
LOQ (mcg/ml)	0.271	0.589	0.272	4.04

was calculated by comparing the absorbance before and after addition of working standard solution.

Limit of detection and limit of quantitation

The Limit of Detection (LOD) is smallest concentration that can be detected but not necessarily quantified as an exact value. LOD was calculated using the following formula:

LOD = 3.3 σ /S

σ = Standard deviation of the response, S = slope of the calibration curve

The Limit of Quantitation (LOQ) is the lowest amount of analyte in the sample that can be quantitatively determined with suitable precision and accuracy. LOQ was calculated using the following formula:

LOQ = 10 σ/S

σ = Standard deviation of the response, S = slope of the calibration curve

Robustness

In this study, effect on the final result was noted by changing the wavelength of measurement by ±1nm.

RESULTS AND DISCUSSION

The two proposed methods for simultaneous estimation of Ramipril and Valsartan were found to be accurate and precise. A straight-line calibration graph was obtained for Ramipril and Valsartan. Robustness studies showed that by varying the wavelength the % ab

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TABLE 3: Assay for tablet formulations

Methods	Label claim (mg/tablet)		% % of label claim estimated ± S.D	
	RAMI	VAL	RAMI	VAL
Method-I	5	80	99.59±0.181	99.08 ± 0.170
Method-II	5	80	99.74 ±1.510	98.53 ± 0.455

TABLE 4: Recovery studies

Recovery level	Amount present(mg/tab)		Amount added (mg)		Amount found(mg)		% Recovery ± S.D	
	RAMI	VAL	RAMI	VAL	RAMI	VAL	RAMI	VAL
Method-I								
80%	5.0	80	4.0	64.0	9.01	144.80	100.11 ±1.679	99.44 ±0.450
100%	5.0	80	5.0	80.0	9.92	159.21	99.20 ±0.976	99.50 ±0.649
120%	5.0	80	6.0	96.0	10.99	176.00	99.99 ±1.504	100.00 ±0.616
Method-II								
80%	5.0	80	4.0	64.0	8.98	144.34	99.70 ±1.145	100.23 ±1.242
100%	5.0	80	5.0	80.0	9.96	159.51	99.60 ±1.273	99.69 ±1.485
120%	5.0	80	6.0	96.0	11.04	176.05	100.36 ±1.155	100.02 ±1.190

sorbance value was deviated by not more than ± 0.069% for Ramipril and ± 0.0019 for Valsartan, which shows that the methods are robust. Percentage RSD value is not more than 1.5 and percentage recovery was found within the range of 99.20-100.36% indicating reproducibility and accuracy of the methods. The summary of Validation parameters was shown in TABLE 2. The methods were also evaluated by carrying out assay. The details were shown in TABLE 3. The results for recovery studies were shown in TABLE 4.

CONCLUSION

The two Validated Spectrophotometric methods developed were found to be simple, precise and accurate and hence can be used for routine analysis of these drugs in combined dosage form.

ACKNOWLEDGMENT

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