

## Simultaneous estimation of ramipril and valsartan by RP-HPLC method in combined dosage form

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### Abstract

For the simultaneous estimation of ramipril and valsartan a chromatographic method has been developed. The method was based on RP- HPLC separation. Drugs were analysed using a mobile phase phosphate buffer (1%): acetonitrile (40:60 v/v, pH 3.2) on reverse phase C<sub>18</sub> (5 µm, 250mm x 4.6 mm) column in isocratic mode followed by UV detection at 225 nm. The retention time of ramipril and valsartan was found to be 3.5 min and 6.6 min, respectively. The method was found to be linear in the range of 6-30 µg/ml for ramipril and 3-15 µg/ml for valsartan respectively. The methods was validated and found to be simple, precise, specific, sensitive and accurate. The method was successfully applied for the determination of both the drugs from combined dosage form.

**Keywords:** Ramipril; Valsartan; Liquid chromatography (LC); HPTLC; Quantitative analysis.

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### Introduction

Ramipril (RAM) [(2S, 3aS, 6aS)-1[(S)-N-[S-1-Carboxy-3-phenylpropyl] alanyl] octahydrocyclopenta pyrrole-2-carboxylicacid, 1-ethylester] is angiotensin-converting enzyme [ACE] inhibitor and prescribed for treatment of congestive heart failure and hypertension. Ramipril is official in British pharmacopoeia [Martindale; 2002, Anonymous; 2004]. Valsartan (VAL) [3-methyl-2-[pentanoyl-[[4-[2-(2H-tetrazol-5-yl) phenyl] phenyl] methyl] amino]-butanoic acid] is angiotensin II receptor antagonist, and prescribed for treatment of hypertension. Combined dosage form of these two drugs is used for management and treatment of hypertension.

Literature survey revealed that various analytical methods like UV, LC, LC-MS [3-6] have been reported for the estimation of RAM alone or in combination with other drugs. Various LC [Francottw, E. *et al*; 1996; González, L. *et al*; 2000, Atana, E. *et al*; 2001, Jing, N. *et al*; 2006] methods have been reported for the estimation of VAL in combination with other drugs in biological fluids and pharmaceutical dosage forms. But no method is reported for simultaneous estimation of ramipril and valsartan in combined dosage form.

HPLC is the most widely used separation technique having different modes of detection. It is non-destructive, sensitive and precise method, which can be applied to thermo labile material. Further analysis time required is very less [Lindsay, S.; 1992, Skoog, D. A., *et al* 2006]. HPTLC is a versatile analytical technique that requires less expensive instrumentation and expertise. The main advantages of HPTLC are the simultaneous separation of several samples on a

single plate and in situ purification and separation of analytes. Moreover, HPTLC analysis needs small amounts of developing solvent and minimal sample preparation. Use of UV/fluorescent detectors coupled with software enables HPTLC on par with HPLC with respect to the sensitivity of analyte detection. The ease of operation and shorter period of analysis is an added advantage of HPTLC [Sethi P.D.; 1996, Fried, J. B. 2003].

The apparent lack of a method for the estimation of RAM and VAL in combined dosage form prompted us to develop accurate, specific and sensitive liquid chromatographic method. In the present study, simultaneous quantification of ramipril and valsartan in capsules by LC and HPTLC methods were developed.

## **Experimental**

### ***Instrumentation***

UV spectral measurements were recorded in Perkin Elmer, U.S.A, Lambda 19, UV-Visible spectrophotometer. The liquid chromatographic system consisted of HPLC 200 Series, Perkin Elmer, U.S.A, with quaternary gradient pump, operating back pressure of 5000 psi, UV-visible diode array detector and rheodyne injector with 20  $\mu$ l loop. The column used was RP- C<sub>18</sub> column (5  $\mu$ m, 250 mm $\times$ 4.6 mm). All the drugs and chemicals were weighed on Shimadzu electronic balance (AX 200, Shimadzu Corp., Japan).

### ***Chemicals and reagents***

Pure drugs RAM and VAL were obtained as gift samples from Lupin pharmaceuticals Ltd., Ankleshwar and Vasudha pharmaceuticals Ltd., Hyderabad respectively. Capsule preparations were procured from local market and designated as RV-1 and RV-2. HPLC grade acetonitrile, methanol and water were obtained from E. Merck Ltd., Mumbai, India while analytical reagent grade O-phosphoric acid and potassium dihydrogen phosphate were obtained from S. D. Fine chemicals, Mumbai, India.

### **RP-HPLC method**

#### ***Preparation of mobile phase***

Mobile phase was prepared by accurately weighing 10 g of potassium dihydrogen phosphate and dissolving in 1000 ml of water, and 400 ml of this solution was mixed with 600 ml of acetonitrile. Mobile phase pH was adjusted to 3.2 with 0.1 M orthophosphoric acid. The solution was filtered using Whatman filter paper (0.45  $\mu$ m) and sonicated for 15 min for degassing prior to use.

#### ***Preparation of standard solutions***

Stock solutions were prepared by accurately weighing 25 mg each of RAM and VAL and transferring to two separate 25 ml volumetric flasks containing a few ml of methanol. The flasks were swirled to dissolve the solids. Volumes were made up to the mark with methanol to yield a solution containing 1000  $\mu$ g/ ml of RAM and VAL. Aliquot from the stock solution of RAM and VAL were appropriately diluted with mobile phase to obtain solutions of 100  $\mu$ g/ ml of each.

### **Method Validation**

The method was validated for accuracy, precision, linearity, detection limit, quantitation limit and solution stability study.

#### **Linearity**

Appropriate aliquots of RAM working standard solution were taken in different 10 ml volumetric flasks. Appropriate aliquots of VAL working standard solution were added to the same flasks. The volumes were made up to the mark with mobile phase to obtain final concentrations of 6, 10, 20, 25, 30  $\mu\text{g}/\text{ml}$  of RAM and 3, 6, 9, 12, 15  $\mu\text{g}/\text{ml}$  of VAL, respectively. The solutions were injected using a 20  $\mu\text{L}$  fixed loop system and chromatograms were recorded. Calibration curves were constructed and regression equations were computed for RAM and VAL.

#### **Precision**

The intra-day and inter-day precision study of RAM and VAL was carried out by estimating the corresponding responses three times on the same day and on three different days for three different solutions containing RAM (6, 10, 30  $\mu\text{g}/\text{ml}$ ) and VAL (3, 9, 15  $\mu\text{g}/\text{ml}$ ) in mixture, and the results are reported in terms of RSD. The instrumental precision was evaluated by injecting mixed solutions containing three different concentrations of RAM (6, 10, 30  $\mu\text{g}/\text{ml}$ ) and VAL (3, 9, 15  $\mu\text{g}/\text{ml}$ ) six times and results are reported in terms of RSD.

#### **Accuracy**

The accuracy of the method was determined by calculating recoveries of RAM and VAL by method of standard additions. Known amount of RAM (0, 6, 10, 20  $\mu\text{g}/\text{ml}$ ) and VAL (0, 3, 6, 9  $\mu\text{g}/\text{ml}$ ) were added to a pre quantified sample solution, and the amount of RAM and VAL were estimated by measuring the peak areas and by fitting these values to the straight-line equation of calibration curve.

#### **Detection limit and Quantitation limit**

Limit of detection and limit of quantification were calculated using following equation as per ICH guidelines.

$\text{LOD} = 3.3 \times \sigma / S$ ;  $\text{LOQ} = 10 \times \sigma / S$ ; Where  $\sigma$  is the standard deviation of y-intercepts of regression lines and S is the slope of the calibration curve.

#### **Solution stability study**

Robustness of the method was studied by observing stability of the sample solution at  $25 \pm 2^\circ\text{C}$  for 24 h.

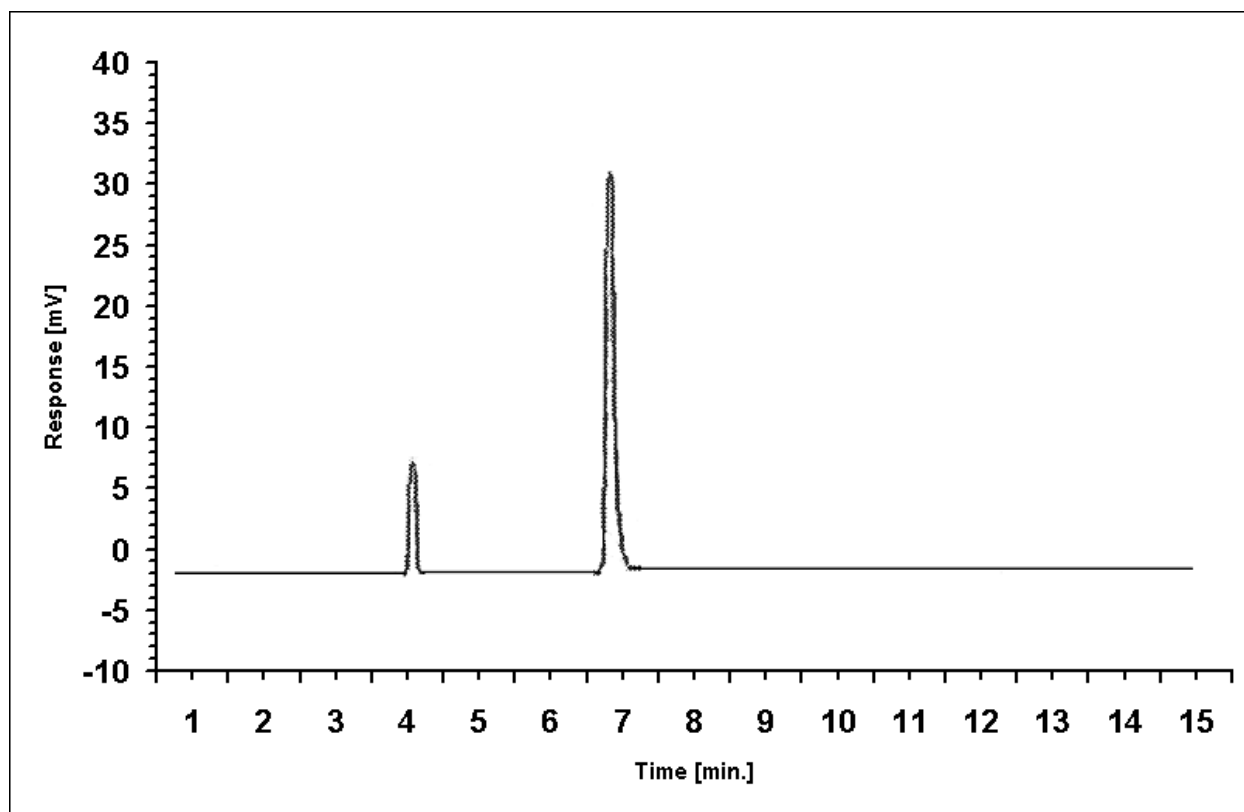
#### **Analysis of marketed formulation**

The contents of 20 capsules (labeled claim: 5 mg of RAM and 80 mg of VAL per capsule), were weighed and finely powdered. The powder equivalent of 2.5 mg of RAM and 40 mg of VAL was weighed accurately and transferred to a 50 ml volumetric flask. The powder was dissolved in the 25 ml methanol and sonicated for 5 min. Volume was made up to mark with methanol and this solution is carefully centrifuged at 400 rpm for 20 min. It was filtered through a Whatman filter paper no 41. Sample solution of 1ml was pipetted out and transferred to 10 ml volumetric flask. 0.75 ml solution of 100  $\mu\text{g}/\text{ml}$  standard stock solution of RAM was added to this sample solution and was suitably diluted to give a solution containing 12  $\mu\text{g}/\text{ml}$  of RAM and VAL. This was used for analysis.

## Results and discussion

The objective of the method development was to resolve chromatographic peaks for active drug ingredients (RAM and VAL) with less asymmetric factor. Various mixtures containing aqueous buffer-methanol, acetonitrile were tried as mobile phases.

The mobile phase consisting of phosphate buffer (1%) : acetonitrile (40:60, pH 3.2) was selected which gave sharp, well resolved peaks for RAM and VAL. The flow rate was maintained at 1.0 ml/ min. The retention times for RAM and VAL were 3.5 min and 6.6 min respectively with the resolution of 4.8 (figure 1).



**Figure 1: Liquid chromatogram showing well resolved peaks of RAM (3.5 min) and VAL (6.6 min).**

UV overlain spectra of both RAM and VAL showed that both the drugs absorbed appreciably at 225 nm, so the same was selected as the detection wavelength during chromatographic studies.

The calibration curves were obtained by plotting the peak area versus concentration over the range of 6 - 30  $\mu\text{g}/\text{ml}$  for RAM and 3 - 15  $\mu\text{g}/\text{ml}$  for VAL, respectively. Instrument precision was determined by performing repeatability test and the RSD values for RAM and VAL were found out. The intra-day and inter-day precision studies were carried out and the results are reported in terms of RSD (table I). The low RSD values indicate that the method is precise.

The accuracy of the method was determined by calculating recoveries of RAM and VAL by method of standard addition. The recoveries were found to be 98.12 – 99.53 % and 97.67 – 100.23 % for RAM and VAL, respectively. The values indicate that the method is accurate. The detection limits for RAM and VAL were 2  $\mu\text{g}/\text{ml}$  and 1  $\mu\text{g}/\text{ml}$ ,

respectively, while quantitation limits were 6 µg/ ml and 3 µg/ ml, respectively. The solution stability study revealed that RAM and VAL stock solutions were stable for 24 h without detectable degradation.

Marketed formulation was analyzed using proposed method which gave percentage recovery of 97.89–100.34 % for RAM and 98.62 – 99.47 % for VAL respectively.

**Table 1 Method validation parameters for estimation of RAM and VAL by the proposed method.**

Parameters	RP -HPLC method	
	RAM	VAL
<b>Instrumental precision</b> (RSD, %, n=7)	0.623	0.764
<b>Precision (CV, n=3)</b>		
<b>Intraday</b>	0.14-0.51	0.31-0.41
<b>Interday</b>	0.17-0.63	0.42-0.48
<b>Repeatability</b> (RSD, %, n=5)	0.322	0.358
<b>Limit of detection</b>	2 µg/ml	1 µg/ml
<b>Limit of quantitation</b>	6 µg/ml	3 µg/ml
<b>Specificity</b>	Specific	Specific
<b>Selectivity</b>	Selectivity	Selectivity
<b>Linearity</b>		
<b>Range</b>	6-30 µg/ml	3-15 µg/ml
<b>Correlation coefficient</b>	0.9932	0.9906

## Conclusion

Proposed study describes RP-HPLC and HPTLC methods for the estimation of RAM and VAL in combined dosage forms. Both the methods were validated and found to be simple, sensitive, accurate, precise and statistical analysis proved that there is no significant difference between both the methods. Both the methods were successfully applied for determination of drugs in their pharmaceutical formulations. Compared to RP-HPLC method HPTLC method is simple, time consuming and can be used for the routine analysis of RAM and VAL from combined dosage forms.

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