

## Stability indicating RP-HPLC method for the estimation of Valsartan in pharmaceutical dosage form

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**Abstract**—A simple, specific, accurate, precise and stability-indicating reverse phase high performance liquid chromatography (RP-HPLC) method is developed for estimation of Valsartan in tablet dosage form. The method employed, Phenomenex C<sub>18</sub>, 5µm, 25cm x 4.6 mm i.d. column in isocratic mode, with mobile phase of methanol & phosphate buffer pH 3.0 in ratio of 65:35(v/v). The flow rate was 1mL min<sup>-1</sup> and effluent was monitored at 210 nm. Retention time was found to be 6.22 min. The method was validated in terms of Linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ) etc. in accordance with ICH guidelines. Linear regression analysis data for the calibration plot showed that there was good linear relationship between response and concentration in the range of 10- 100 µg/ml respectively. The LOD and LOQ values for HPLC method were found to be 0.02 and 0.06 µg/ml respectively. No chromatographic interference from tablet excipients was found. The proposed method was successfully used for estimation of Valsartan in tablet dosage form.

**Keywords**—Valsartan, Reverse phase- High performance liquid chromatography (RP-HPLC).

### I. INTRODUCTION

Valsartan is chemically 3-methyl-2- (pentanoyl-(4-(2-(2H-tetrazol-5-yl) phenyl) phenyl) methyl)amino)- butanoic acid (Fig. 1), angiotensin II receptor antagonist, acting on the AT1 subtype & used for treatment of high blood pressure, of congestive heart failure, and post-myocardial infarction(MI). By blocking the action of angiotensin, valsartan dilates blood pressure. Methods such as HPLC, LC-MS, Protein precipitation, Capillary electrophoresis and Simultaneous UV-spectrophotometric methods are reported for estimation of Valsartan alone or on combination with other agents. However, there were few methods reported for determination of valsartan individually. The focus of present study was to develop & validated a rapid, stable & economic HPLC method for the estimation in tablet dosage form.

### II. MATERIALS AND METHODS

#### 2.1. Chemicals and reagents:

Valsartan Active Pharmaceutical Ingredient (API) was provided by cipla laboratories, Goa. Methanol & water of HPLC grade were from Standard reagents, Hyderabad. Commercial formulations of VALZAR-40 [Torrent pharmaceuticals] procured from Ahmadabad (GUJARAT).

#### 2.2. HPLC and Chromatographic conditions:

The HPLC system employed was Shimadzu LC 2010c with PDA & UV absorbance detector. The monitor & processor was equipped with Winchrom softwar. Stationary phase used was Phenomenex C<sub>18</sub>, 5µm, 25cm x 4.6 mm i.d. column and the mobile phase was methanol and phosphate buffer in the ratio of 65:35 v/v was used. The mobile phase was filtered using 0.45µm membrane filter. The mobile phase flow rate was 1ml/min and injection volume was 20µl.

#### 2.3. HPLC METHOD

##### 2.3.1. STANDARD & SAMPLE PREPARATION:

The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent mixture of methanol & diluting with the same solvent.(After optimization of all conditions). HPLC spectrum of valsartan (200 ppm) in optimized conditions is shown in Fig.2.

## 2.4. METHOD VALIDATION:

### 2.4.1. Accuracy: *Recovery study:*

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of pure drug of VALSARTAN were taken and added to the pre-analyzed formulation of concentration 10 µg/ml. From that percentage recovery values were calculated.

### 2.4.2. Precision:

#### *Repeatability*

The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of five replicates of a fixed amount of drug. Valsartan (API) The percent relative standard deviation were calculated for valsartan are presented in the table.

#### *Intra-assay & inter-assay:*

The intra & inter day variation of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for valsartan revealed that the proposed method is precise

### 2.4.3. Linearity & Range:

The calibration curve showed good linearity in the range of 10 – 100 µg/ml, for valsartan (API) with correlation coefficient ( $r^2$ ) of 0.999 (Fig. 4). A typical calibration curve has the regression equation of  $y = 83907x - 78449$  for valsartan.

### 2.4.4. Method Robustness:

Influence of small changes in chromatographic conditions such as change in flow rate ( $\pm 0.1$  ml/min), Temperature ( $\pm 2^\circ\text{C}$ ), Wavelength of detection ( $\pm 2$  nm) & methanol content in mobile phase ( $\pm 2\%$ ) studied to determine the robustness of the method are also in favour of (Table-4, % RSD < 2%) the developed RP-HPLC method for the analysis of valsartan (API).

### 2.4.5. LOD & LOQ:

The Minimum concentration level at which the analyte can be reliably detected (LOD) & quantified (LOQ) were found to be 0.02 & 0.06 µg/ml respectively.

### 2.4.6. Specificity & stability in analytical solution:

The results of specificity indicated that the peak was pure in presence of degraded sample. It is important to mention here that the valsartan (API) was stable in solution form up to 24 hrs at  $25^\circ\text{C}$ .

The results of linearity, precision, inter & intraday assays, method robustness, LOD, LOQ, specificity and stability in analytical solution established the validation of the developed RP-HPLC method for analysis of valsartan.

## 1.5. ASSAY OF VALSARTAN IN DOSAGE FORM

The assay of valzar and valtans tablets containing valsartan was found to be 100.88 % and 98.18 % as per the method.

## 2.6. OPTIMIZATION OF CHROMATOGRAPHIC CONDITIONS:

The chromatographic conditions were optimized by different means. (Using different column, different mobile phase, different flow rate, different detection wavelength & different diluents for sample preparation etc.

## III. STABILITY INDICATING STUDIES

### PROCEDURE

The API (Valsartan) was subjected to stress conditions in various ways to observe the rate and extent of degradation that is likely to occur in the course of storage and/or after administration to body. This is one type of accelerated stability studies that helps us determining the fate of the drug that is likely to happen after along time storage, within a very short time as compare to the real time or long term stability testing. The various degradation pathways studied are acid hydrolysis, basic hydrolysis, dry heat degradation, moist heat degradation, Photolytic degradation and oxidative degradation.

### 3.1. ACID HYDROLYSIS:

An accurately weighed 10 mg. of pure drug was transferred to a clean & dry 10 ml volumetric flask. To which 0.1 M Hydrochloric acid was added & make up to the mark & kept for 24 hrs. from that 1 ml was taken in to a 10 ml volumetric flask & make up to the mark with methanol, than injected into the HPLC system against a blank of HCl & methanol ( after all optimized conditions )

### 3.2. BASIC HYDROLYSIS

An accurately weighed 10 mg. of pure drug was transferred to a clean & dry 10 ml volumetric flask. To which 0.1 M Sodium hydroxide was added & make up to the mark & kept for 24 hrs. from that 1 ml was taken in to a 10 ml volumetric flask & make up to the mark with methanol, than injected into the HPLC system against a blank of . NaOH and methanol ( after all optimized conditions )

### 3.3. OXIDATION WITH (3%) H<sub>2</sub>O<sub>2</sub>:

An accurately weighed 10 mg. of pure drug was transferred to a clean & dry 10 ml volumetric flask. To which 3% Hydrogen Peroxide was added, make up to the mark & kept for 24 hrs. from that 1 ml was taken in to a 10 ml volumetric flask & make up to the mark with methanol, than injected into the HPLC system against a blank of . H<sub>2</sub>O<sub>2</sub> and methanol ( after all optimized conditions )

### 3.4. THERMAL DEGRADATION:

An accurately weighed 10 mg. of pure drug was transferred to a clean & dry 100 ml volumetric flask, make up to the mark with methanol & was maintained at 50 °C. for 24 hrs. than injected into the HPLC system against a blank of methanol ( after all optimized conditions )

### 3.5. PHOTOLYTIC DEGRADATION:

Approximately 10 mg. of pure drug was taken in a clean & dry Petridis. It was kept in a UV cabinet at 254 nm wavelength for 24 hours without interruption. Accurately weighed 1 mg. of the UV exposed drug was transferred to a clean & dry 10 ml. volumetric flask. First the UV exposed drug was dissolved in methanol & make up to the mark. than injected into the HPLC system against a blank of methanol ( after all optimized conditions ).

### Results of degradation studies:

The results of the stress studies indicated the **specificity** of the method that has been developed. Valsartan was degraded only in 3% H<sub>2</sub>O<sub>2</sub> & temperature stress conditions.

## IV. RESULTS & DISCUSSION

To develop a precise, linear, specific & suitable stability indicating RP-HPLC method for analysis of valsartan , different chromatographic conditions were applied & the results observed are presented.

Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution.

In case of RP-HPLC various columns are available, but here Phenomenex , C<sub>18</sub>, 5µm, 25cmx4.6mm i.d.. column was preferred because using this column peak shape, resolution and absorbance were good.

Mobile phase & diluent for preparation of various samples were finalized after studying the solubility of API in different solvents of our disposal (methanol, acetonitrile, dichloromethane, water, 0.1M NaOH, 0.1M HCl).

The drug was found to be highly soluble in acetonitrile & dichloromethane and methanol. Drug was insoluble in water. Using these solvents with appropriate composition newer methods can be developed and validated .

Detection wavelength was selected after scanning the standard solution of drug over 200 to 800nm. (Fig. 29). From the U.V spectrum of racecadotril it is evident that most of the HPLC work can be accomplished in the wavelength range of 215-240 nm conveniently. Further, a flow rate of 0.5ml/min & an injection volume of 20 µl were found to be the best analysis.

The result shows the developed method is yet another suitable method for assay and stability studies which can help in the analysis of valsartan in different formulations.

## V. CONCLUSION

A sensitive & selective RP-HPLC method has been developed & validated for the analysis of Valsartan API. Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility.

The result shows the developed method is yet another suitable method for assay, impurity studies which can help in the analysis of Valasartan in different formulations

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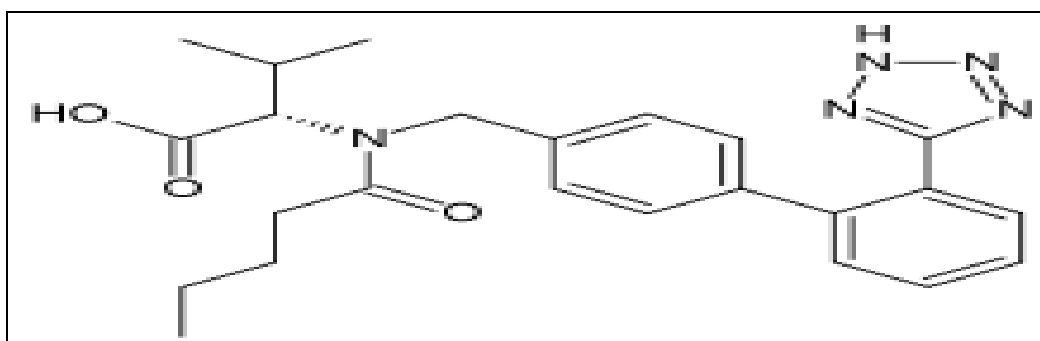


Fig.1: Structure of Valsartan

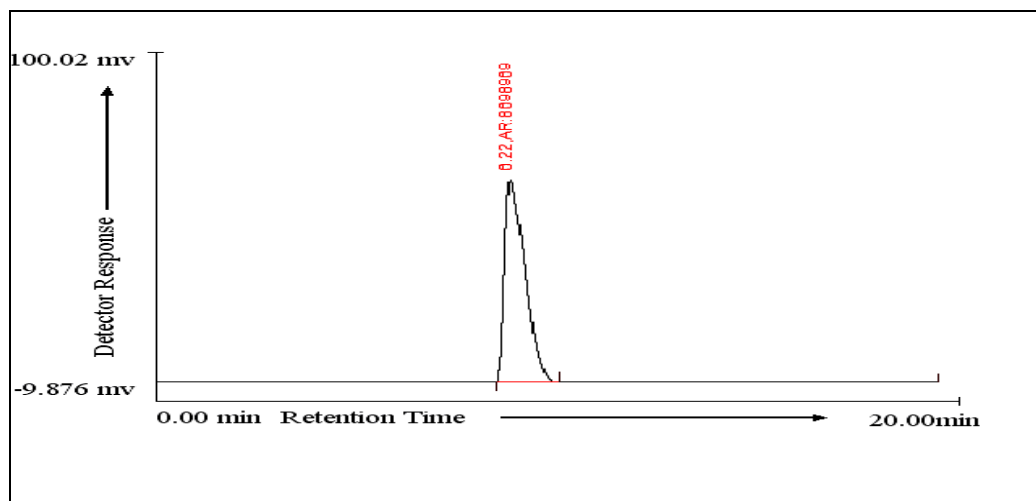


Fig.2: HPLC spectrum of valsartan (200 ppm) in optimized conditions

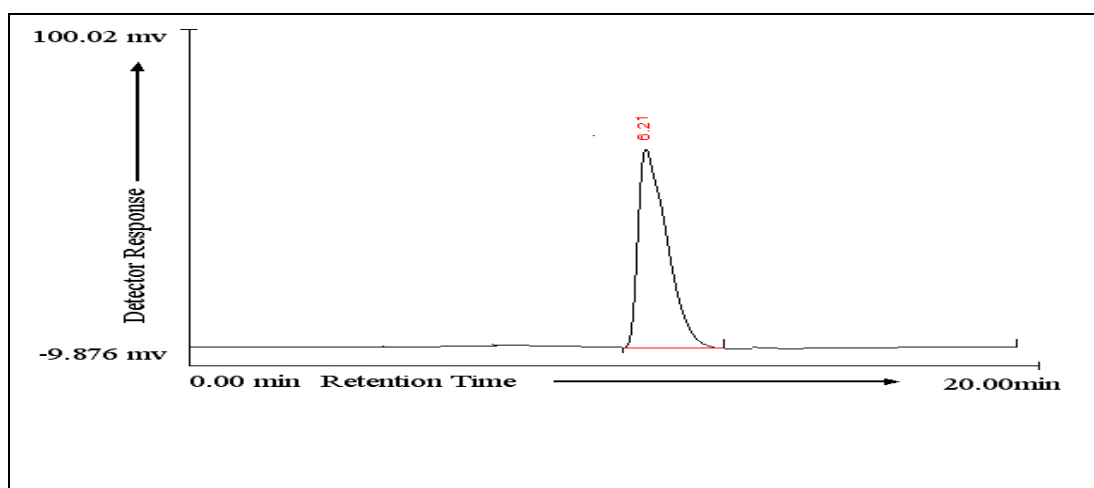


Fig.3: Chromatogram showing the degraded products in 0.1N HCl

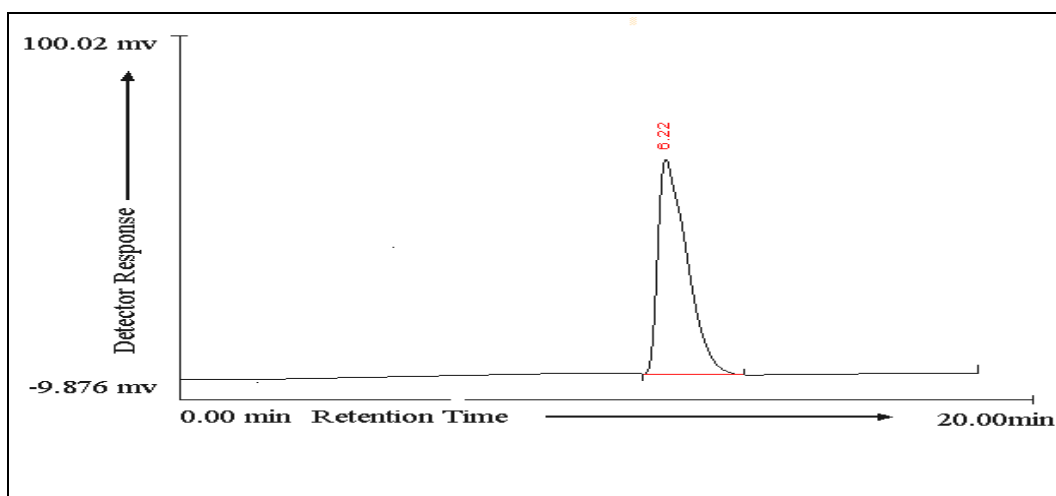


Fig.4: Chromatogram showing the degraded products in 0.1N NaOH

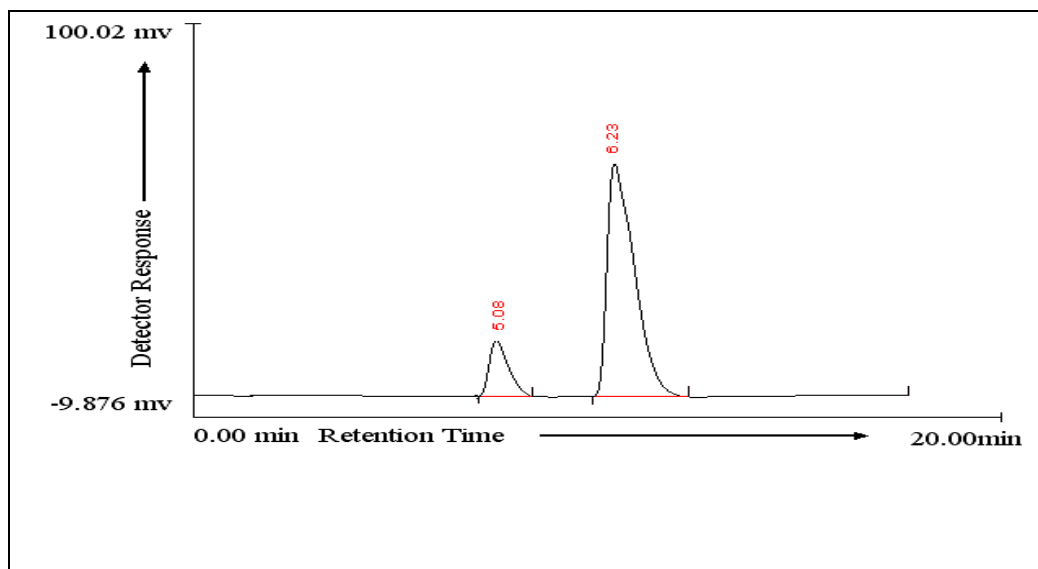


Fig.5: Chromatogram showing the degraded products after thermal degradation

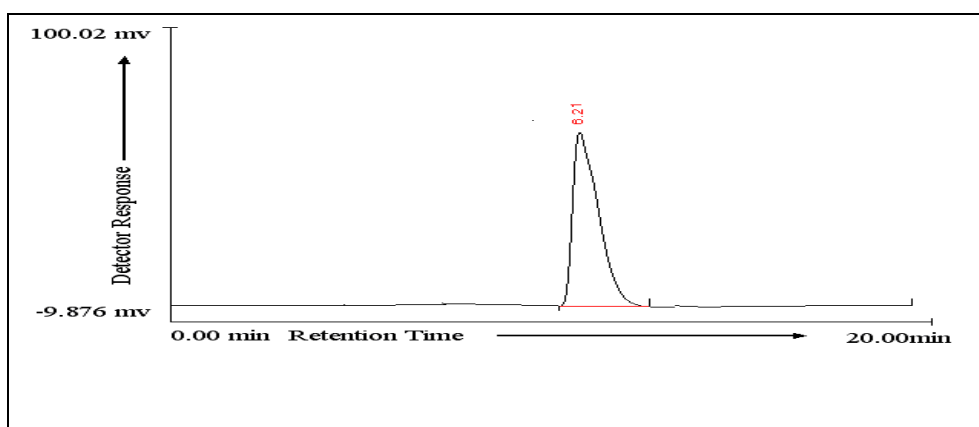


Fig.6: Chromatogram showing valsartan only (no degradation products) in photolytic studies.

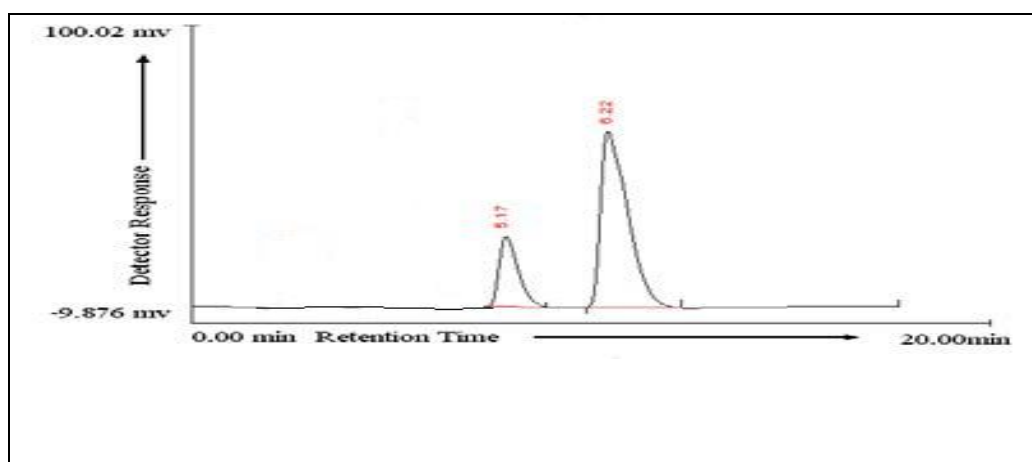


Fig.7: Chromatogram showing valsartan, degraded product in  $H_2O_2$

Sample ID	Concentration ( $\mu\text{g/ml}$ )		%Recovery of Pure drug	Statistical Analysis
	Pure drug	Formulation		
S <sub>1</sub> : 80 %	8	10	99.13	Mean= 98.94667% S.D. = 0.171561 % R.S.D.= 0.1733
S <sub>2</sub> : 80 %	8	10	98.79	
S <sub>3</sub> : 80 %	8	10	98.92	
S <sub>4</sub> : 100 %	10	10	99.72	Mean= 99.76% S.D. = 0.045826 % R.S.D.= 0.0459
S <sub>5</sub> : 100 %	10	10	99.81	
S <sub>6</sub> : 100 %	10	10	99.75	
S <sub>7</sub> : 120 %	12	10	99.36	Mean= 99.37667% S.D. = 0.105987 % R.S.D. = 0.1066
S <sub>8</sub> : 120 %	12	10	99.28	
S <sub>9</sub> : 120 %	12	10	99.49	

**Table1: Results of recovery study**

Conc. Of valsartan ( $\mu\text{g/ml}$ )	Observed Conc. Of valsartan ( $\mu\text{g/ml}$ ) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
10	10.01	0.86	10.03	0.87
30	30.02	0.30	30.03	0.32
100	99.97	0.13	99.95	0.11

**Table2: Results of intra-assay & inter-assay**

Change in parameter	% RSD
Flow (1.1 ml/min)	0.05
Flow (0.9 ml/min)	0.06
Temperature (27°C)	0.04
Temperature (21°C)	0.04
Wavelength of Detection (212nm)	0.02
Wavelength of detection (209nm)	0.02

**Table3: Result of method robustness test**

Brand name of tablets	Labeled amount of Drug (mg)	Mean ( $\pm$ SD) amount (mg) found by the proposed method (n=6)	Mean ( $\pm$ SD) Assay (n = 6)
Valzar	40	40.35 ( $\pm$ 0.06)	100.88 ( $\pm$ 0.48)
Valtan	80	79.34 ( $\pm$ 0.04)	99.18 ( $\pm$ 0.04)

**Table4: Assay of valsartan capsules**