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# Method development and validation for the estimation of valsartan in bulk and tablet dosage forms by RP-HPLC

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**ABSTRACT**

*A simple, precise and accurate RP - High performance liquid chromatography (Reverse Phase - HPLC) method has been developed for the estimation of Valsartan in tablet formulations. The separation was achieved on a X terra ,RP-18(100mm X 4.6mm 5µm) using a mobile phase consisting of a degassed mixture of water, Acetonitrile & Glacial acetic acid in the ratio of 550:450:1v/v with a flow rate of 2.0 mL/min. The mobile phase showed the most favorable chromatographic parameter for analysis. The detection of the constituent was done using UV detector at 248 nm. The retention time of valsartan was found to be 2.530 minutes. The method was validated for system suitability, precision, accuracy, linearity, robustness. The linearity range for Valsartan was found to be 4 – 12 µg / ml. The method is validated for accuracy, precision, linearity, specificity and robustness in accordance with ICH guidelines and revealed that the method established is specific, accurate, rapid, precise, reliable and reproducible for the method has been successfully used to analyze commercial dosage forms and its percentage recovery was found to be 99.65%.*

**Key words:** Valsartan, High Performance Liquid Chromatography (HPLC), Mobile phase, Validation.

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## INTRODUCTION

Valsartan (VL) is N-(1-oxopentyl)-N-[[2′-(1H-tetrazol-5-yl)[1,1′-biphenyl]-4- yl]methyl]-L-valine, is an Anti-hypertensive and used in the treatment of congestive heart failure, post-myocardial infarction[1-3].Valsartan blocks the vasoconstrictor[4] and aldosterone-secreting effects of angiotensin II by selective binding of angiotensin II to the AT1 receptor in many tissues, such as vascular smooth muscle and the adrenal gland[2]. A few methods were reported earlier for the determination of valsartan in bulk and pharmaceutical dosage forms [5, 6]. In The present study is designed to develop a simple, precise and accurate reverse phase HPLC method with good recoveries and shorter retention time for the estimation of Valsartan in the tablet formulations.

## MATERIALS AND METHODS

### 2.1 Materials

Valsartan, Acetonitrile HPLC Grade- (E. Merck Ltd. Mumbai, INDIA), Glacial acetic acid, Water HPLC Grade- (E. Merck Ltd. Mumbai, INDIA), Purified water-mille Q grade.

### 2.2 Instrument: Highperformanceliquid chromatography (HPLC)

Model - shimadzu-LC-10ADVP, Pump -LC-10ATVP, operates back pressure 5000 psi.

Detector - UV-Visible, **Analytical Balance –** Afcoset, **Vacuum filter -** BV– 40**,** Smart Labtech Pvt. Ltd., **Sonicator -** Fast clea.

**2.3 Chromatographic Conditions**
Chromatographic separation was performed at ambient temperature on a reverse phase X terra, RP-18(100mm X 4.6mm 5µm) column with use of a filtered and degassed mobile phase consisting of water & Acetonitrile & glacial acetic acid in the ratio of 550:450:1. The flow rate of mobile phase was adjusted to 2.0 mL/min. The UV detector wavelength was set at 248 nm. The injection volume of the standard and sample solutions was 20 mcL. Run time was 15 min.

**2.4 PROCEDURE**
**2.4.1 Preparation of 0.05 M Glacial Acetic Acid**
Accurately measured volume of 2.85 mL of glacial acetic acid (0.05 M) was transferred to a 1000 mL volumetric flask containing 500 mL of distilled water and sonicate for 5 min and final volume was made with distilled water. The solution was filtered through 0.45 µm membrane filter.

**2.4.2 Preparation of Mobile Phase**
A mixture of water, Acetonitrile and 0.05M glacial acetic acid in the ratio of 550:450:1 previously filtered through 0.45µm membrane filter was used as a mobile phase.

**2.4.3 Preparation of Standard Stock Solution**
Accurately weigh and transfer about 100 mg of valsartan working standard into 100 ml volumetric flask.ad about 60 ml of methanol and sonicated to dissolve. Cool the solution to room temperature and dilute to volume with methanol. Transfer 5 ml of above solution into 50 ml volumetric flask and dilute to volume with mobile phase.

**2.4.4 Preparation of Working Standard Solution**
From the prepared stock solution further dilution were made in order to get concentrations of 4, 6,8,10, 12 µg / ml for the construction of calibration curve

**2.4.5 Preparation of Sample Solution**
Weigh a finely power not fewer than 20 tablets. Transfer an accurately weigh portion of the powder tablet, equivalent to 100 mg of valsartan into 100 ml volumetric flask .add about 50 ml of methanol an sonicate for 30 min with occasional shakings. Cool the solution to room temperature and dilute to volume with methanol and mix filter the solution through 0.45µ nylon filter. Transfer 5 ml of the above solution into a 100 ml volumetric flask and dilute to volume with mobile phase.

**2.5 Method Development**
It was done by using different made of $C_{18}$ stationary phase like Thermo column, X terra, RP-18(100mm X 4.6mm 5µm) column with use of a filtered and degassed mobile phase consisting of water & Acetonitrile & glacial acetic acid in the ratio of 550:450:1. The flow rate of mobile phase was adjusted to 2.0 mL/min. The UV detector wavelength was set at 248 nm. Individual drug solution of 20 mcL was injected into the column at ambient temperature at a concentration of 10 ppm and it was chromatographed for 15 min using mobile phase at a flow rate of 2.0 mL/min and the UV spectra of valsartan was recorded at a wavelength of 248 nm. The retention time of Valsartan was found to be 2.530 minutes.

**3.0 METHOD VALIDATION**
To confirm its suitability for its intended purpose, the method was validated in accordance with ICH guidelines, for system suitability, linearity, specificity, precision, accuracy, and limit of detection, limit of quantification, robustness, and solution stability. All the validation studies were performed by replicate injections of sample and standard solutions.

**3.1 Linearity and Range:**
Linearity was established for the analyte peaks from 40 -120% of the test concentrations and were subjected to linear least square regression analysis. The calibration and linearity equation relating Y (drug: peak height ratio) to X (concentration µg $ml^{-1}$) was fitted and correlation coefficient ($r^2$), slope, intercept were calculated. The intra and inter day linearity values were established. The values were tabulated in table no 1.

**3.2 System Suitability**

To know reproducibility of the developed RP - HPLC method, system suitability performance parameters were determined by injecting standard solutions. Parameters such as retention time RT), peak area, number of theoretical plates (N), tailing factor (As) were determined. The results were shown in Table I, indicating good performance of the system.

System repeatability was determined by ten replicate injections of a standard solution, the relative standard deviation (RSD) of retention time and peak area of Valsartan was less than 2.0 %.

**3.3 Detection and Quantitation limit:**

Detection and Quantitation limit were calculated by the method based on the standard deviation (σ) and slope (S) of the calibration plot, using the formulae LOD = 3.3 σ/ S and LOQ = 10 σ/ S. and were mentioned in table no.1

**3.4 Specificity of Valsartan**

Specificity was assessed by comparison of chromatograms obtained from tablets and blank solution and from the drug standards. Because the retention times of Valsartan from standard solution and from dosage forms were identical & no-co-eluting peaks from diluents were observed [fig.2 (a); fig.2 (b); fig.2(c)], the method was specific for quantitative estimation of drug in commercial formulations (table no.2).

**3.5 Accuracy**

Accuracy was determined by recovery study. It was carried out by taking the known amount of drug corresponding to 80%, 100% and 120% of the label claim of Valsartan.

$\%Recovery = [(c_t - c_u)/ c_a] \times 100.$

Where $c_t$ is the total conc. of the analyte found; $c_u$ is the conc. of the analyte present in formulation; and $c_a$ is the conc. of the pure analyte added to the formulation. The values were tabulated in the table no.3, indicating good accuracy of the method.

**3.6 Precision**

Precision was determined by analyzing variation of results within the same day (intra day) and variation of results between days (inter day). Intra-day and inter-day precision and accuracy were evaluated by analyzing quality-control samples containing low, medium, and high concentrations of Valsartan 4, 8, 12 ppm. For intra-day variation, sets of five replicates of the three concentrations were analyzed on the same day; for inter-day variation, five replicates were analyzed on three different days. The values were listed in the table. No.4

## RESULTS AND DISCUSSION

The method was carried out using X terra, RP-18(100mm X 4.6mm 5µm) column with use of a filtered and degassed mobile phase consisting of water & Acetonitrile & glacial acetic acid in the ratio of 550:450:1. The flow rate of mobile phase was adjusted to 2.0 mL/min. The UV detector wavelength was set at 248 nm. The retention time of Valsartan was found to be 2.536 min and % Recovery for Valsartan was 99.82.

**Table no 1. Linearity and System Suitability**

| Method characteristic | Valsartan |
|---|---|
| Linearity Range ( µg / ml) | 4-12 |
| Regression equation | Y= 4.4675X+5.4148 |
| Retention time | 2.534 |
| Correlation coefficient($r^2$) | 0.9992 |
| SD of intercept | 0.146 |
| %RSD of peak areas | 0.513 |
| Theoretical plates | 2744.549 |
| Tailing factor | 1.069 |
| LOD (ng / ml) | 0.1078 |
| LOQ (ng / ml) | 0.3268 |

**Mean of ten observations*

It shows that the method is accurate and free from interference of the excipients. The low value of standard deviation obtained confirms the precision of the method. A linear relationship was obtained in the range of 4-12 µg / ml for Valsartan. When pharmaceutical preparations containing Valsartan was analysed, the results obtained by the proposed method has good agreement with the labeled amount.

**Table no 2. Specificity of the method**

| S No. | Sample name | Average Retention time (minutes)[a] |
|---|---|---|
| 1 | blank | 1.251 |
| 2 | Standard | 2.530 |
| 3 | Sample | 2.536 |

*a average of four replicate injections of four samples*

**Table 3: Results of Accuracy of Valsartan**

| Drug | % of spiked Level | *Amount of drug added(mg) | *Amount of drug found(mg) | %*Recovery | Mean % Recovery |
|---|---|---|---|---|---|
| Valsartan | 80% Sample | 6.4 | 6.33 | 98.43 | 99.65 |
| | 100% Sample | 8 | 8.12 | 101.5 | |
| | 120%Sample | 9.6 | 9.52 | 99.16 | |

**Mean of five observations*

**Table no. 4 Precision of Valsartan**

| Drug amount Con (µg / ml) | Intra-day [a] | | Inter-day[b] | |
|---|---|---|---|---|
| | Mean ± SD | %RSD | Mean ± SD | %RSD |
| 4 | 3.95 ± 0.022 | 0.5569 | 4.032 ± 0.064 | 1.5873 |
| 8 | 7.96 ± 0.014 | 0.1758 | 8.034 ± 0.036 | 0.4480 |
| 12 | 11.942 ± 0.028 | 0.2344 | 12.048 ± 0.049 | 0.4067 |

*a. Intra-day accuracy and precision were determined by five replicate analyses for each concentration*
*b Inter-day accuracy and precision were determined by fifteen replicate analyses (day 1, n = 5; day 2, n = 5; day 3, n = 5) for each concentration*

**Table no: 5 Results for the assay of drugs.**

| Formulation | Active ingredient (Label claim mg) | Amount found * ± SD | %purity* ±SD |
|---|---|---|---|
| VALZAR | 40 | 39.45± 0.003 | 98.62 ± 1.42 |
| DIOVAN | 40 | 40.92 ± 0.0026 | 102.34 ± 1.06 |

- *-- a mean of 5 injections of each formulation*

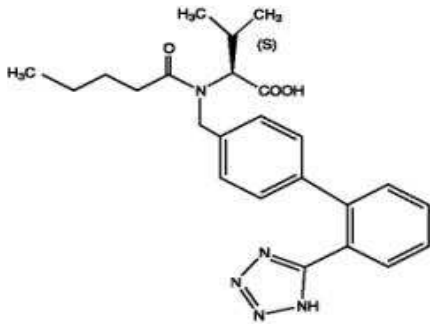


**Fig. 1. Structure of Valsartan**

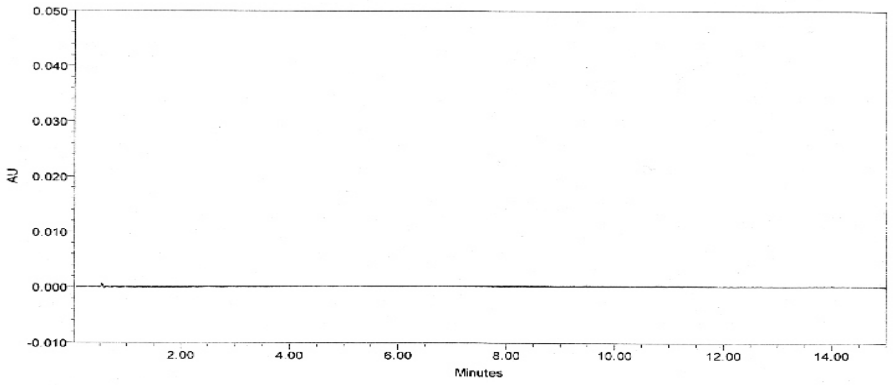


**Fig.2 (a). Chromatogram of blank**

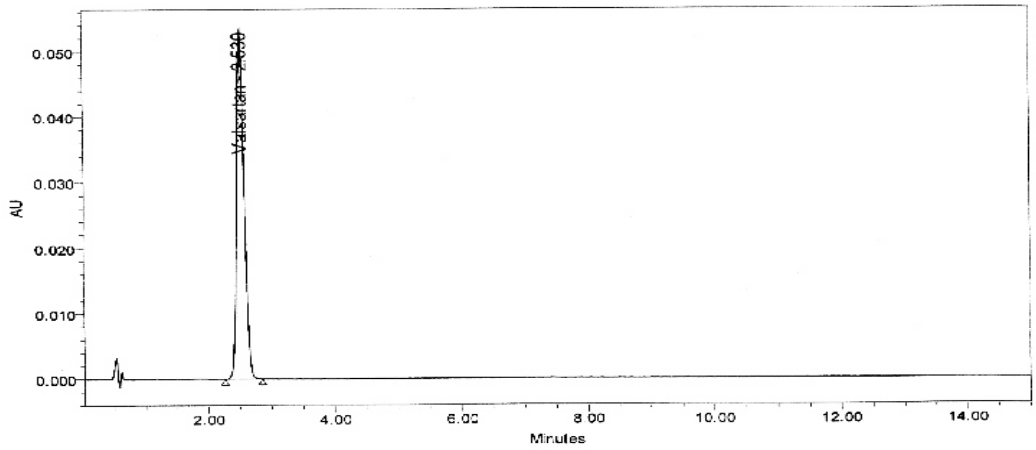


**Fig.2 (b). Chromatogram of standard Valsartan**

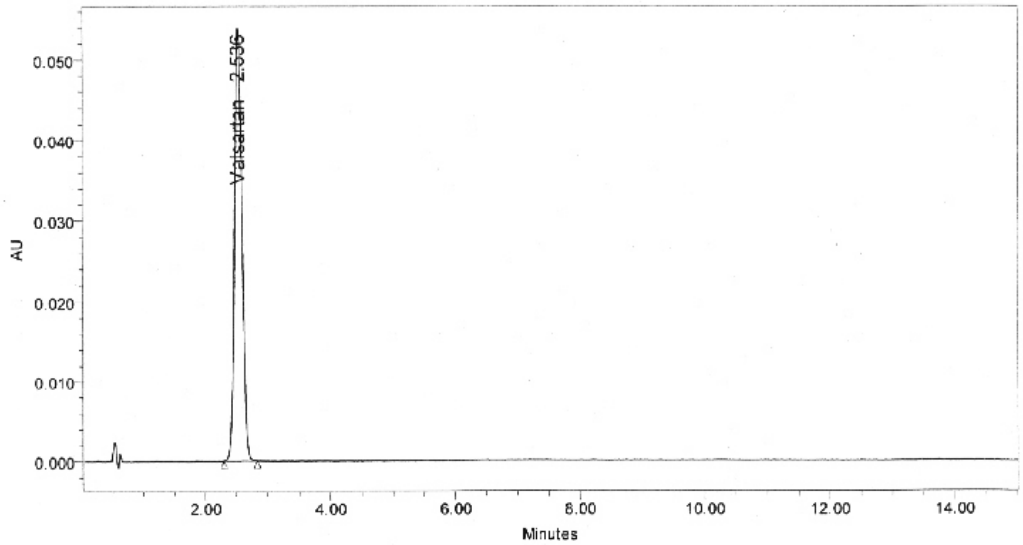


**Fig.2 (c). Chromatogram of sample Valsartan**

## CONCLUSION

A new, reversed-phase HPLC method has been developed for the analysis of VALSARTAN in marketed tablets. It was shown that the method is accurate, repeatable, linear, precise, specific, and selective, and therefore reliable. The run time is relatively short, i.e. 2.534 min, which enables rapid quantitation of many samples in routine and quality-control analysis of the tablet formulation. The same solvent was used through out the experimental work and no interference from any excipient/ placebo was observed. The method could therefore find practical application as a quality-control tool for estimation of drug in tablet dosage forms.

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