



Research Article

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Simple and Validated RP-HPLC Method for the Estimation of Valsartan in Pharmaceutical Tablet Dosage Form

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ABSTRACT

A simple, selective, rapid, precise and economical reverse phase high pressure liquid chromatographic method has been developed for the estimation of valsartan (VALS) from pharmaceutical Tablet dosage form. The method was carried out on a Phenomenex C₁₈ (250 mm x 4.6 mm i.d., 5 μ) column with a mobile phase consisting of acetonitrile: 5mM ammonium acetate (adjusted to pH 4.5 using orthophosphoric acid) (75:25 v/v) at a flow rate of 1.0 ml/min. Detection was carried out at 240 nm. The retention time of valsartan was 3.702 min. The developed method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation and solution stability. The proposed method can be used for the estimation of these drugs in dosage forms.

Keywords: Valsartan; Method development & validation; Tablet Estimation

INTRODUCTION

Valsartan, (S)-N-valeryl-N-[2{[1-(1H-tetrazol-5-yl)biphenyl-4-yl]methyl} valine, is a potent, highly selective, orally active, specific angiotensin II receptor antagonist used as a hypotensive drug [1-3]. Only limited methods have been reported in Spectroscopy, HPLC and LC-MS [4-11]. The aim of this study is development of a simple, precise, rapid and accurate reverse phase HPLC method for the estimation of valsartan in tablet dosage form. The present work describes the development of a validated RP-HPLC method in pharmaceutical dosage form. The present RP-HPLC method was validated following the ICH guidelines (12).

EXPERIMENTAL SECTION

Reagents and chemicals

HPLC grade acetonitrile (ACN), Ammonium acetate and Triethyl amine (A.R. grade) was purchased from Merck, Malaysia. Water HPLC grade was obtained from a Milli-QRO water purification system. Reference standards of Valsartan was Procured from Cipla pharmaceuticals, Mumbai, India.

Apparatus and chromatographic conditions

HPLC chromatographic separation was performed on a Shimadzu liquid chromatographic system equipped with a LC-20AD solvent delivery system (pump), SPD-20A photo diode array detector, and SIL-20ACHT injector with 50 μL loop volume. LC solution version 1.25 was applied for data collecting and processing (Shimadzu, Japan). A C₁₈ column (250 mm x 4.6 mm i.d., 5 μ) was used for the separation, mobile phase of a mixture of acetonitrile and 5mM ammonium acetate (adjusted to pH 4.5 using orthophosphoric acid); (75:25 v/v) was delivered at a flow rate of

1.0 ml/min with detection at 240 nm. The mobile phase was filtered through a 0.2 μ membrane filter and degassed. The injection volume was 50 μ l and the analysis was performed at ambient temperature.

Preparation of standard solutions

Standard stock solutions of 1.0 mg/ml Valsartan was prepared separately using a mixture of water and acetonitrile (1:1 v/v). From the standard stock solution, standard solution was prepared to contain 10.0 μ g/ml of valsartan.

Preparation of sample solutions

Twenty tablets containing 80 mg of valsartan were taken and average weight was determined. Weight equivalent to 80mg of Valsartan was taken in 100 ml volumetric flask and suitable volume (Methanol) was added. Further dilutions were made and used for the analysis.

Assay method

With the optimized chromatographic conditions, a steady baseline was recorded, the mixed standard solution was injected and the chromatogram was recorded. The retention time of Valsartan was found to be 3.702. This procedure was repeated for the sample solution obtained from the formulation. The peak area ratio of the standard solution and sample solution were calculated. The concentration of the drugs was calculated using following formula:

$$\text{Concentration of drugs} = \frac{\text{Sample Peak Area}}{\text{Standard Peak Area}}$$

RESULTS AND DISCUSSION

Estimation of Valsartan in dosage forms

The HPLC procedure was optimized with a view to develop precise and stable assay method. The pure drug Valsartan was run in different mobile phase compositions with different C₁₈. The flow rate was also varied from 0.5 ml to 1.2 ml/min. Finally Phenomenex C₁₈ column (250mm x 4.6mm i.d., 5 μ) with a mobile phase of a mixture of acetonitrile and 5mM ammonium acetate (adjusted to pH 4.5 using orthophosphoric acid) (adjusted to a flow rate of 1.0 ml/min with a detection at 240 nm gave sharp and symmetrical peaks with retention time 3.702 for valsartan respectively. The resolution factor at the above said condition was 1.5. The typical chromatogram of sample solution is shown in Figure 1. Detection was done at 240 nm. The peak area ratio of standard and sample solutions was calculated. The assay procedures were repeated for six times and mean peak area and mean weight of standard drugs was calculated. The percentage of individual drugs found in formulations, mean, standard deviation in formulations were calculated and presented in Table 1. The results of analysis shows that the amounts of drugs were in good agreement with the label claim of the formulation.

Method validation

Accuracy and precision

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out six times and the percentage recovery and standard deviation of the percentage recovery were calculated and presented in Table 1. From the data obtained, added recoveries of standard drugs were found to be accurate.

The precision of the method was demonstrated by inter day and intra day variation studies. In the intra day studies, six repeated injections of standard and sample solutions were made and the response of drug peaks and % CV were calculated and presented in Table 2. In the inter day variation studies, six repeated injections of standard and sample solutions were made for three consecutive days and response factor of drug peaks and percentage % CV were calculated and presented in Table 2. From the data obtained, the developed RP-HPLC method was found to be precise.

Linearity and Range

The linearity of the method was determined at five concentration levels ranging from 2 to 12 μ g/ml for Valsartan. The calibration curve was constructed by plotting response factor against concentration of drugs. The slope and intercept value for calibration curve R²=0.99 for Valsartan. The results show that an excellent correlation exists between response factor and concentration of drugs within the concentration range indicated above.

Limit of Detection and Limit of Quantification

The Limit of Detection (LOD) and Limit of Quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The LOD for Valsartan was found to be 10 ng/ml respectively. The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10). The LOQ was 28 ng/ml Valsartan, respectively (Table 3).

Ruggedness and Robustness

The ruggedness of the method was determined by carrying out the experiment on different instruments like Shimadzu HPLC (LC2010A_{HT}), Agilent HPLC and Water's Breeze HPLC by different operators using different columns of similar type like Hypersil C₁₈, Phenomenex C₁₈ and Hichrom C₁₈. Robustness of the method was determined by making slight changes in the chromatographic conditions. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed, are rugged and robust.

Solution stability

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 5 h at room temperature. The results show that for both solutions, the retention time and peak area of Valsartan remained almost unchanged and no significant degradation within the indicated period, thus indicated that both solutions were stable for at least 5 h, which was sufficient to complete the whole analytical process.

System suitability studies

The column efficiency, resolution and peak asymmetry were calculated for the standard solutions (Table 3). The values obtained demonstrated the suitability of the system for the analysis of this drug combination and the system suitability parameters fall within ± 3 % standard deviation range during routine performance of the method.

Table 1. Results of analysis of formulation and recovery studies

Drug (mg)	Amount mg/ tablet		% Label claim*	% Recovery*
	Labelled	Found *		
Valsartan	80.00	79.85 \pm 1.50	98.9 \pm 1.12	99.85 \pm 1.03

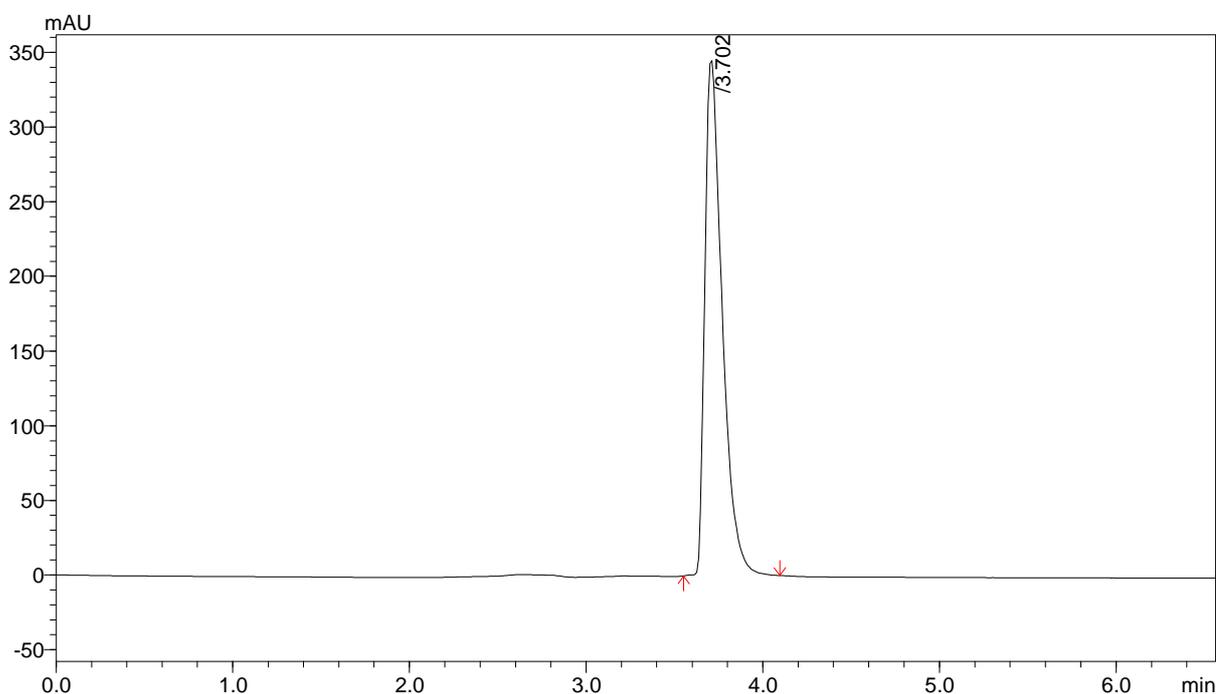
Table 2. Intraday and interday precision studies of Valsartan

	Intraday studies			Interday studies		
	100	400 (ng/ml)	600	100	400 (ng/ml)	600
Mean	99.2253	399.3379	599.7443	99.3253	399.7719	599.5743
SD	0.47	0.62	0.43	0.47	0.43	0.39
%CV	0.47	0.15	0.07	0.47	0.10	0.06
%Accuracy	99.22	99.83	99.95	99.46	99.94	99.92

Table 3. System suitability studies

S. No.	Parameters	Valsartan
1	Theoretical plate/meter	4672
2	Resolution factor	1.5
3	Asymmetric factor	0.93
4	LOD (ng/ml)	10
5	LOQ (ng/ml)	28

Figure 1. Typical Chromatogram of Sample Solution



CONCLUSION

In conclusion, the developed method for the estimation of valsartan is accurate, precise, selective and linear and it can be applicable for the Pharmacokinetics studies. Hence this method is definitely time saving to enable the estimation of valsartan. The proposed method is found to be accurate, precise, linear, robust, and rugged. The proposed method could be useful for the national quality control laboratories in developing countries.

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