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Research Article

DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR THE ESTIMATION OF VALSARTAN IN PURE AND TABLET DOSAGE FORM BY RP-HPLC METHOD

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ABSTRACT

A rapid, precise, accurate, specific and sensitive reverse phase liquid chromatographic method has been developed for the estimation of valsartan in pure and tablet formulation. The chromatographic method was standardized using a Kromasil C18 column (250×4.6 mm I.D., 5 µm particle size) with UV detection at 233 nm and flow rate of 1 ml/min. The mobile phase consisting of a mixture of phosphate buffer and acetonitrile in the ratio of 55:45 v/v was selected. The proposed method was validated for its sensitivity, linearity, accuracy and precision. The retention time for valsartan was 3.943 min. The % recovery was within the range between 99.62 % and 99.88 %. The percentage RSD for precision and accuracy of the method was found to be less than 2 %. This method can be employed for routine quality control analysis of valsartan in tablet dosage forms.

Keywords: Valsartan, Estimation, Validation, Tablets and RP-HPLC.

INTRODUCTION

Valsartan is a nonpeptide, orally active and specific angiotensin II receptor blocker acting on the AT1 receptor subtype. Valsartan is chemically N-(1-oxopentyl)-N-[[2'-(1*H*-tetrazol-5-yl)[1,1'-biphenyl]-4- yl]methyl]-L-valine (Fig. 1) . Angiotensin II is formed from angiotensin I in a reaction catalyzed by angiotensinconverting enzyme (ACE II). Angiotensin II is the principal pressor agent of the renin-angiotensin system, with effects that include vasoconstriction, stimulation of synthesis and release of aldosterone, cardiac stimulation and renal reabsorption of sodium.

Valsartan blocks the vasoconstrictor and aldosterone-secreting effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT1 receptor in many tissues, such as vascular smooth muscle and the adrenal gland. Its action is therefore independent of the pathways for angiotensin II synthesis. A fewer spectrophotometric[2,4] HPLC[5-9] and LC-MS[13] methods were reported earlier for the determination of valsartan in bulk and pharmaceutical dosage forms. In the present study a rapid, sensitive, accurate and precise HPLC method for the estimation of valsartan in bulk samples and in tablet dosage forms is proposed.

MATERIALS AND METHOD

Chromatographic conditions

The analysis of the drug was carried out on a Waters HPLC system equipped with a reverse phase Xterra C18 column (250x4.6 mm., 5 µm), a 515 binary pump, a 20 µl injection loop, a dual absorbance detector and running on Waters Empower software.

Chemicals and solvents

The reference sample of valsartan was supplied by Aurobindo Pharmaceuticals Ltd., Hyderabad. HPLC grade water and acetonitrile were purchased from E. Merck

(India) Ltd., Mumbai. Potassium dihydrogen phosphate and Dipotassium hydrogen ortho phosphate of AR grade were obtained from Qualigens Ltd., Mumbai.

Preparation of Mixed phosphate buffer

Weighed accurately 1.625 gms of KH_2PO_4 and 0.3 gms of K_2HPO4 in a 1000 ml standard flask and dissolved in minimum quantity of HPLC water and made upto the mark with HPLC water.

Preparation of mobile phase and diluents

550 ml of the mixed phosphate buffer was mixed with 450 ml of acetonitrile. The solution was degassed in an ultrasonic water bath for 5 minutes and filtered through 0.45 µ filter under vacuum.

Procedure

A mixture of phosphate buffer and acetonitrile in the ratio of 55:45 v/v was found to be the most suitable mobile phase for ideal separation of valsartan. The solvent mixture was filtered through a 0.45 µ membrane filter and sonicated before use. It was pumped through the column at a flow rate of 1.0 ml/min. The column was maintained at ambient temperature. The pump pressure was set at 800 psi. The column was equilibrated by pumping the mobile phase through the column for atleast 30 min prior to the injection of the drug solution. The detection of the drug was monitored at 233 nm. The run time was set at 7 min. Under these optimized chromatographic conditions the retention time obtained for the drug was 3.943 min. A typical chromatogram showing the separation of the drug is given in Fig.2.

Calibration plot

About 10 mg of valsartan was weighed accurately, transferred into a 100 ml volumetric flask and dissolved in 25 ml of a 55:45 v/v mixture of mixed phosphate buffer and acetonitrile. The solution was sonicated for 10 min and the volume made up to the mark with a further quantity of the diluent to get a 100 µg/ml solution. From this, a working standard solution of the drug (1µg/ml) was prepared by diluting 0.1 ml of the above solution to 10 ml in a volumetric flask. Further dilutions ranging from 1-6 µg/ml were prepared from the solution in 10 ml volumetric flasks using the above diluent. 20 µl of each dilution was injected six times into the column at a flow rate of 1.0 ml/min and the corresponding chromatograms were obtained. From these chromatograms, the average area under the peak of each dilution was computed. The calibration graph constructed by plotting concentration of the drug against peak area was found to be linear in the concentration range of 1-6 µg/ml of the drug. The relevant data are furnished in Table-1. The regression equation of this curve was computed. This regression equation was later used to estimate the amount of valsartan in tablet dosage forms.

Validation of the proposed method

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for linearity, precision, accuracy, specificity, stability and system suitability. Standard plots were constructed with six concentrations in the range of 1-6 µg/ml prepared in triplicates to test linearity. The peak area of valsartan was plotted against the concentration to obtain the calibration graph. The linearity was evaluated by linear regression analysis that was calculated by the least square regression method. The precision of the assay was studied with respect to both repeatability and intermediate precision.

Repeatability was calculated from five replicate injections of freshly prepared valsartan test solution in the same equipment at a concentration value of 5 µg/ml) of the intended test concentration value. The experiment was repeated by assaying freshly prepared solution at the same concentration additionally. Peak area of valsartan was determined and precision was reported as % RSD and the results are furnished in Table-2. The accuracy of the HPLC method was assessed by analyzing solutions of valsartan at 4ppm, 5ppm and 6ppm concentrated levels by the proposed method. The results are furnished in Table-3. The system suitability parameters are given in Table-4.

Estimation of valsartan in tablet dosage forms

One commercial brand of tablets was chosen for testing the suitability of the proposed method to estimate valsartan in tablet formulations. Twenty tablets were weighed and powdered. An accurately weighed portion of this powder equivalent to 10 mg of

valsartan was transferred into a 100 ml volumetric flask and dissolved in 25 ml of a 55:45 v/v mixture of phosphate buffer and acetonitrile. The contents of the flask were sonicated for 10 min and a further 25 ml of the diluent was added, the flask was shaken continuously for 15 min to ensure complete solubility of the drug. The volume was made up with the diluent and the solution was filtered through a 0.45 µ membrane filter. This solution was injected into the column six times.

The average peak area of the drug was computed from the chromatograms and the amount of the drug present in the tablet dosage form was calculated by using the regression equation obtained for the pure drug. The relevant results are furnished in Table-5.

RESULTS AND DISCUSSION

Selection of the detection wavelength

The UV spectra of valsartan in 55:45 v/v mixture of mixed phosphate buffer and acetonitrile was scanned in the region between 200 and 400 nm and shows λmax at 233 nm.

Optimization of the chromatographic conditions

Proper selection of the stationary phase depends upon the nature of the sample, molecular weight and solubility. Mixture of phosphate buffer and acetonitrile was selected as mobile phase and the effect of composition of mobile phase on the retention time of valsartan was thoroughly investigated. The concentration of phosphate buffer and acetonitrile were optimized to give symmetric peak with short run time. A short run time and the stability of peak asymmetry were observed in the ratio of 55:45 % v/v of mixed phosphate buffer and acetonitrile. It was found to be optimum mobile phase concentration. In the proposed method, the retention time of valsartan was found to be 3.94 min. Quantification was linear in the concentration range of 1-6 µg/ml. The regression equation of the linearity plot of concentration of valsartan over its peak area was found to be Y=40.57x+4.261 (r2=0.999), where X is the concentration of valsartan (µg/ml) and Y is the corresponding peak area. The number of theoretical plates calculated was 7958, which indicates efficient performance of the column. The limit of detection and limit of quantification were found to be 0.034 µg/ml and 0.104 µg/ml respectively, which indicate the sensitivity of the method. The use of mixed phosphate buffer and acetonitrile in the ratio of 55:45 v/v resulted in peak with good shape and resolution. The high percentage of recovery indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram of the formulation within the run time indicating that excipients used in tablet formulations did not interfere with the estimation of the drug by the proposed HPLC method.

CONCLUSION

The proposed HPLC method is rapid, sensitive, precise and accurate for the determination of valsartan and can be reliably adopted for routine quality control analysis of valsartan in its tablet dosage form.

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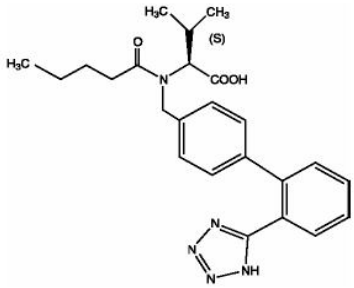

Fig. 1: Chemical structure of Valsartan

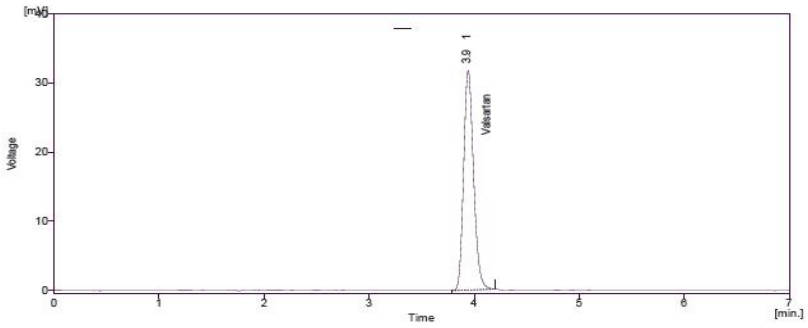

Fig. 2: Typical chromatogram of Valsartan

Table 1: Calibration data of the method

Concentration (µg/ml)	Mean peak area (n=6)
1	50.219
2	85.118
3	125.878
4	166.703
5	206.888
6	247.028

Table 2: Precision of the proposed HPLC method

Injection (5µg/ml)	**Peak Areas of VALSARTAN**
1	205.523
2	205.028
3	205.811
4	205.953
5	206.304
Mean	205.724
SD	0.48
% RSD	0.2085

Table 3: Accuracy studies

Concentration (mcg)	**Amount added (mcg)**	**Amount found (mcg)**	**% Recovery**	**% Mean recovery**
4	0.5	4.48	99.55	99.97
5	0.5	5.52	100.36	
6	0.5	6.50	100.00	

Table 4: System suitability parameters

Parameter	**Result**
Linearity(µg/ml)	1-6
Correlation coefficient	0.999
Theoretical plates(N)	7958
Tailing factor	1.302
LOD	0.034
LOQ	0.104

Table 5: Assay and Recovery Studies

Formulation	Label claim(mg)	Amount found(mg)	% Amount found
VALZAR	40	39.92	99.81

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