Reversed Phase HPLC Analysis of Valsartan in Pharmaceutical Dosage Forms

V. Bhaskara Raju[1] and A. Lakshmana Rao[2*]

[1]Sri Vasavi Institute of Pharmaceutical Sciences, Tadepalligudem- 534 101, A.P., India.
[2]V.V. Institute of Pharmaceutical Sciences, Gudlavalleru- 521 356, A.P., India.
[*] E-mail: dralrao@gmail.com

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 Xterra C_{18} column (100×4.6 mm I.D., 5 µm particle size) with UV detection at 210 nm and flow rate of 1 ml/min. The mobile phase consisting of a mixture of phosphate buffer pH 3 and acetonitrile in the ratio of 50:50 v/v was selected. The proposed method was validated for its sensitivity, linearity, accuracy and precision. The retention time for valsartan was 4.450 min. The % recovery was within the range between 98.6 % and 101.2 %. 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, RP-HPLC.

INTRODUCTION

Valsartan [1] is a nonpeptide, orally active and specific angiotensin II receptor blocker acting on the AT_1 receptor subtype. Valsartan is chemically described as N-(1-oxopentyl)-N-[[2'-(1*H*-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]-L-valine (Fig. 1) [2]. Angiotensin II is formed from angiotensin I in a reaction catalyzed by angiotensin-converting 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 AT_1 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 few spectrophotometric [3-7], HPLC [8-14], UPLC [15] and LC-MS [16-19] methods were reported earlier for the determination of valsartan in bulk and pharmaceutical dosage forms. In the present study the authors report a rapid, sensitive, accurate and precise HPLC method for the estimation of valsartan in bulk samples and in tablet dosage forms.
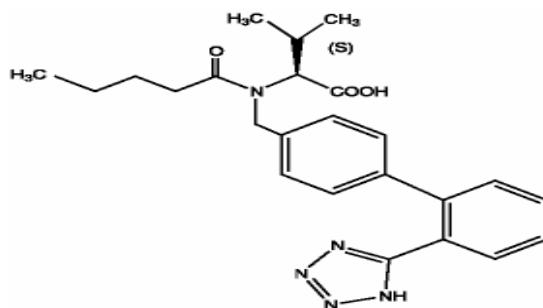

Fig.-1: Chemical structure of valsartan

MATERIALS AND METHOD
Chromatographic conditions

The analysis of the drug was carried out on a Waters HPLC system equipped with a reverse phase Xterra C_{18} column (100x4.6 mm., 5 µm), a 2695 binary pump, a 20 µl injection loop, a 2487 dual absorbance detector and

running on Waters Empower software. The UV spectrum of valsartan was taken using a Elico SL-159 UV-Visible spectrophotometer.

Chemicals and solvents

The reference sample of valsartan was supplied by Lupin Pharmaceutical Industries Ltd., Ahmadabad. HPLC grade water and acetonitrile were purchased from E. Merck (India) Ltd., Mumbai. Potassium dihydrogen phosphate and orthophosphoric acid of AR grade were obtained from S.D. Fine Chemicals Ltd., Mumbai.

Preparation of pH 3.0 phosphate buffer

Seven grams of KH_2PO_4 was weighed into a 1000 ml beaker, dissolved and diluted to 1000 ml with HPLC water. 2 ml of triethyl amine was added and pH adjusted to 3.0 with orthophosporic acid.

Preparation of mobile phase and diluents

500 ml of the phosphate buffer was mixed with 500 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 50:50 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 210 nm. The run time was set at 7 min. Under these optimized chromatographic conditions the retention time obtained for the drug was 4.450 min. A typical chromatogram showing the separation of the drug is given in Fig. 2.
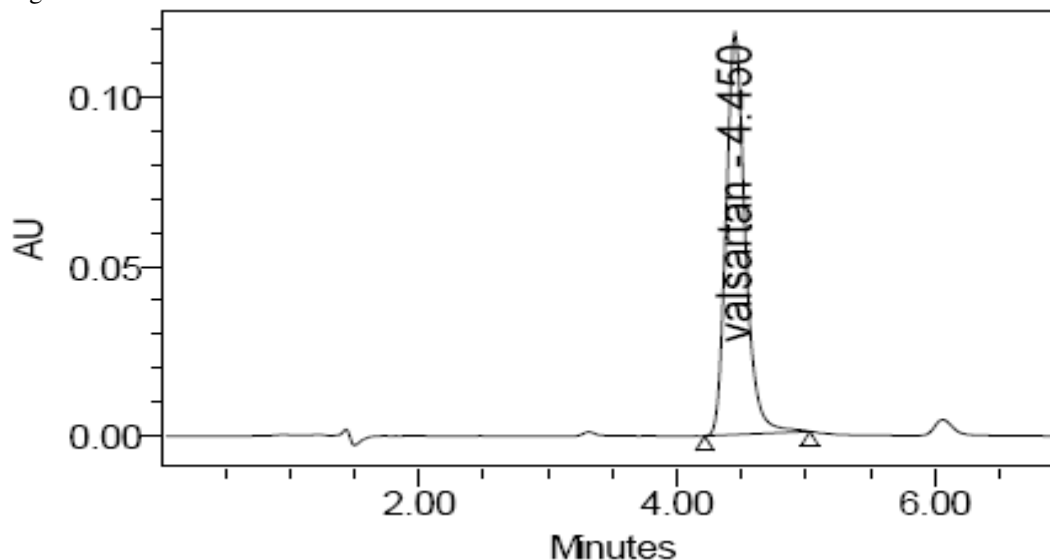

Fig.-2: Typical chromatogram of valsartan

Calibration plot

About 10 mg of valsartan was weighed accurately, transferred into a 100 ml volumetric flask and dissolved in 25 ml of a 50:50 v/v mixture of phosphate buffer and acetonitrile. The solution was sonicated for 15 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 (10µg/ml) was prepared by diluting 1 ml of the above solution to 10 ml in a volumetric flask. Further dilutions ranging from 5-25 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 5-25 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.

Table-1: Calibration data of the method

Concentration (µg/ml)	Mean peak area (n=5)
5	605930
10	1244187
15	1865633
20	2468676
25	3062337

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 [20]. The method was validated for linearity, precision, accuracy, specificity, stability and system suitability. Standard plots were constructed with five concentrations in the range of 5-25 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 100 %   (10 µg/ml) of the intended test concentration value on the same day. The experiment was repeated by assaying freshly prepared solution at the same concentration additionally on two consecutive days to determine intermediate precision. Peak area of valsartan was determined and precision was reported as % RSD and the results are furnished in Table-2.

Table-2: Precision of the proposed HPLC method

Concentration of valsartan (10 µg/ml)	Peak area	
	Intra-day	Inter-day
Injection-1	1237412	1241721
Injection-2	1238580	1241059
Injection-3	1239480	1242984
Injection-4	1241807	1244489
Injection-5	1244696	1247070
Average	1240395	1243465
Standard Deviation	2895.0	2403.4
% RSD	0.23	0.19

The accuracy of the HPLC method was assessed by analyzing solutions of valsartan at 50, 100 and 150 % concentrated levels by the proposed method. The results are furnished in Table-3. The system suitability parameters are given in Table-4.

Table-3: Accuracy studies

Concentration	Amount added (mg)	Amount found (mg)	% Recovery	% Mean recovery
50 %	5.02	4.95	98.6 %	
100 %	10.1	10.17	100.7 %	100.2 %
150 %	15.1	15.28	101.2 %	

Table-4: System suitability parameters

Parameter	Result
Linearity (µg/ml)	5-25
Correlation coefficient	0.9998
Theoretical plates (N)	4547
Tailing factor	1.20

LOD (µg/ml)	0.012
LOQ (µg/ml)	0.040

Estimation of valsartan in tablet dosage forms
Two commercial brands of tablets were 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 25 mg of valsartan was transferred into a 100 ml volumetric flask and dissolved in 25 ml of a 50:50 v/v mixture of phosphate buffer and acetonitrile. The contents of the flask were sonicated for 15 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.

Table-5: Assay and recovery studies

Formulation	Label claim (mg)	Amount found (mg)	% Amount found
Formulation 1	40	40.12	99.70
Formulation 2	40	39.86	100.35

RESULTS AND DISCUSSION
Selection of the detection wavelength
The UV spectra of valsartan in 50:50 v/v mixture of phosphate buffer and acetonitrile was scanned in the region between 200 and 400 nm and shows λmax at 210 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 50:50 % v/v of 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 4.45 min. Quantification was linear in the concentration range of 5-25 µg/ml. The regression equation of the linearity plot of concentration of valsartan over its peak area was found to be $Y=8161.7+122746.06X$ ($r^2=0.9998$), where X is the concentration of valsartan (µg/ml) and Y is the corresponding peak area. The number of theoretical plates calculated was 4547, which indicates efficient performance of the column. The limit of detection and limit of quantification were found to be 0.012 µg/ml and 0.040 µg/ml respectively, which indicate the sensitivity of the method. The use of phosphate buffer and acetonitrile in the ratio of 50:50 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.

REFERENCES
1. Budavari S. The Merck index, Merck and Co. Press: Whitehouse Station, NJ, 12th Edn, 1997.
2. www.rxlist.com
3. Gupta K.R., Wadodkar A.R., Wadodkar S.G., International Journal of Chem Tech Research, **2** (2010) 985.
4. Gupta K.R., Mahapatra A.D., Wadodkar A.R., Wadodkar S.G., International Journal of Chem Tech Research, **2** (2010) 551.

V. Bhaskara Raju and A. Lakshmana Rao

5. Tatar S., Saglik S., Journal of Pharmaceutical and Biomedical Analysis, **30** (2002) 371.
6. Nevin E., Analytical Letters, **35** (2002) 283.
7. Satana E., Altınay S., Goger N.G., Sibel A., Ozkan S.A., Senturk Z., Journal of Pharmaceutical and Biomedical Analysis, **25** (2001) 1009.
8. Kul D., Dogan-Topal B., Kutucu T., Uslu B., Ozkan S.A., Journal of AOAC International, **93** (2010) 882.
9. Patro S.K., Kanungo S.K., Patro V.J., Choudhury N.S.K., E-Journal of Chemistry, **7** (2010) 246.
10. Brunetto M.R., Contreras Y., Clavijo S., Torres D., Delgado Y., Ovalles F., Ayala C., Gallignani M., Estela J.M., Martin V.C., Journal of Pharmaceutical and Biomedical Analysis, **50** (2009) 194.
11. Chitlange S.S., Bagri1 K., Sakarkar D.M., Asian Journal of Research in Chemistry, **1** (2008) 15.
12. Kocyigit-Kaymakcoglu B., Unsalan S., Rollas S., Pharmazie, **61** (2006) 586.
13. Macek J., Klima J., Ptacek P., Journal of Chromatography, B **832** (2006) 169.
14. Daneshtalab N., Lewanczuk R.Z., Jamali F., Journal of Chromatography, B **766** (2002) 345.
15. Krishnaiah CH., Raghupathi Reddy A., Ramesh Kumar., Mukkanti K., Journal of Pharmaceutical and Biomedical Analysis, **53** (2010) 483.
16. Sampath A., Raghupathi Reddy A., Yakambaram B., Thirupathi A., Prabhakar M., Pratap Reddy P., Prabhakar Reddy V., Journal of Pharmaceutical and Biomedical Analysis, **50** (2009) 405.
17. Selvan P.S., Gowda K.V., Mandal U., Solomon W.D.S., Pal T.K., Journal of Chromatography, B **858** (2007) 143.
18. Li H., Wang Y., Jiang Y., Tang Y., Wang J., Zhao L., Gu J., Journal of Chromatography, B **852** (2007) 436.
19. Koseki N., Kawashita H., Hara H., Niina M., Tanaka M., Kawai R., Nagae Y., Masuda N., Journal of Pharmaceutical and Biomedical Analysis, **43** (2007) 1769.
20. ICH, Q2B. Validation of analytical procedures methodology, In Proceedings of The International Conference on Harmonization, Geneva 1993.

[IJCEPR-150/2011]

_____

Highlights of RASĀYAN

- It is a full text open access international journal of Chemical Sciences. Covers all fields related to Chemistry.
- Research papers will be published on the website and also in the printed version simultaneously.
- Manuscript is reviewed and published very quickly.
- Full text of the article is available on the site http://www.rasayanjournal.com all over the world. **Reprints may be downloaded directly from the website.**
- Papers can be submitted through e-mail to rasayanjournal@gmail.com.

Note:

1. *Authors are requested to prepare the manuscript strictly according to RJC guidelines.*
2. *All contacts shall be by e-mail. All the authors are advised to have an email id.*

Adopt **GREEN CHEMISTRY**
Save Our Planet.
We publish papers of Green Chemistry on priority.