Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Issue 2, 2012

Research Article

DEVELOPMENT AND VALIDATION OF A HPLC METHOD FOR THE DETERMINATION OF VALSARTAN AND ITS DEGRADATION PRODUCTS IN PHARMACEUTICAL FORMULATION

TRIPTI SHARMA*[1], SWAPAN K. MOITRA[1], SUDAM C. SI[1], DANNANA G. SANKAR[2]

[1]Department of Pharmaceutical Analysis, School of Pharmaceutical Sciences, Siksha'O' Anusandhan University, Bhubaneswar-751003, India, [2]Department of Pharmaceutical Sciences, Andhra University, Visakhapatnam-530003, India. Email: tripti_neema@ yahoo.co.in

Received: 16 Nov 2011, Revised and Accepted: 23 Jan 2012

ABSTRACT

A simple and rapid reverse phase high performance liquid chromatography (RP-HPLC) method was developed and validated for quantitative determination of Valsartan in bulk drug samples and formulations. Valsartan was analyzed by using reverse phase column Hypersil ODS, (150 mm X 4.6 mm), 5µm with mobile phase consisting of Sodium dihydrogen orthophosphate buffer pH 4.2 and Methanol (40:60 v/v) . The flow rate was set 1.0 mL/min and analysis was performed at wavelength 254 nm using Photo Diode Array (PDA) detector at ambient temperature. The method was validated and stability studies were conducted under different conditions. The retention time for valsartan was around 4.63 minutes. The calibration curves were linear (*r*_0.9990) over a concentration range from 40.0 to120.0 µg/mL. Limit of detection (LOD) and Limit of quantitation (LOQ) were 0.065 and 0.197 µg/mL respectively. The developed method was successfully applied to estimate the amount of Valsartan in tablet formulations.

Keywords: RP-HPLC, Valsartan, Degradation products, Pharmaceutical dosage forms

INTRODUCTION

Valsartan is chemically N-(1-Oxopentyl)-N-[2'-(1H-tetrazol- 5-yl) [1, 1'-biphenyl]-4-yl] methyl]-L-valine, is a potent angiotensin receptor blocker[1]. Literature survey revealed that HPLC [2-4], LC-MS[5-8], Protein precipitation[9], Capillary electrophoresis[10] and simultaneous UV spectrophotometric methods [11-12]are reported for estimation of valsartan alone or in combination with other drugs. Two stability-indicating high performance liquid chromatography methods were reported for the determination of impurities and assay of valsartan [13-14]. But the methods are very complicated in terms of complex mobile phase composition and longer run time; hence the method is not feasible and economical for pharmaceutical industry. At present study attempts were made to develop simple, rapid, robust and economic method for the estimation of valsartan in the presence of degradants. It separates drugs from the degradation products under ICH suggested stress conditions (hydrolysis, oxidation, photolysis and thermal stress) [17]. Due to short run time and simple mobile phase composition the proposed method will be of immense help to the pharmaceutical industry for routine analysis of valsartan in quality control as well as stability studies of bulk drug and pharmaceutical formulations.

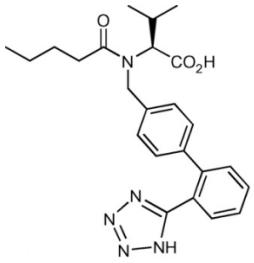

Fig. 1: Chemical structure of Valsartan

MATERIALS AND METHODS

A reference standard sample of Valsartan was obtained from Macleod's Pharmaceuticals Ltd and

Commercial dosage form containing the studied drug were purchased Cipla Ltd. HPLC-grade methanol, Orthophosphoric acid, triethylamine and water were HPLC grade purchased from E. Merck, Mumbai, India. All the other chemicals and reagents used were of AR grade and Purchased from S.D. Fine Chemicals, Mumbai, India.

Instrumentation

Chromatography was performed with Jasco, Japan equipment comprising a PU-2089 plus quaternary pump, degasser and a photo diode array detector (Jasco MD-2010 Plus). A Rheodyne injector fitted with a 20 µL loop was also used and data were recorded and evaluated by use of Chrompass software. The detector wavelength was set at 254nm. The chromatographic separations were performed at ambient temperature on a Hypersil ODS, (150 mm X 4.6 mm), 5µm. The mobile phase was a mixture of Sodium dihydrogen orthophosphate buffer (the pH of the solution was adjusted to 4.2±0.05 with triethylamine) and Methanol (40:60 v/v) filtered and degassed for 30 mins prior to use and flowing at the rate of 1.0mL/min and run time of 10 min. Analytical Balance (Sartorius) and pH meter (Lab India) were used.

Preparation of stock solution:

Stock solutions were prepared by accurately weighing 10 mg of valsartan and transferring to 10 mL volumetric flasks containing 6 mL of methanol. The flasks were sonicated for 10 minutes to dissolve the solids. Volumes were made up to the mark with methanol, which gave 1000 µg/mL the drugs. Aliquots from the stock solutions were appropriately diluted with mobile phase to obtain working standards of 100 µg/mL of each drug.

Calibration standards and quality control sample:

Different calibration standards ranging from 40, 60, 80, 100 and 120 µg/mL were prepared by appropriate dilution of standard solution (1000 µg/mL) with mobile phase. Three quality control samples at concentrations 40, 80 and 120 µg/mL representing 50,100 and 150% respectively of assay concentration (80µg/mL) were prepared from the standard solution. An aliquot of 20 µL of solution was injected into HPLC system.

Preparation of assay solution:

To determine the Valsartan content of tablet formulations, twenty tablets were weighed, to determine the average weight of the tablets, and then crushed and mixed using a mortar and pestle. A portion of powder equivalent to 1000 µg mL^{-1} was accurately weighed into each of three 10 mL volumetric flasks and 5 mL methanol was added. Each solution was sonicated for 20 min to achieve complete dissolution of the valsartan and the solutions were then diluted to volume with mobile phase, to yield concentrations of 1000 µg mL^{-1}, and filtered through a 0.22-µm Nylon membrane filter. The solution obtained was analyzed by HPLC.

Forced degradation study

50 mg of Valsartan was accurately weighed and dissolved in 10 mL of methanol, then 5 mL of 0.1N HCl were added and kept at 80^{0}C about 2 h in a water bath, the solution was allowed to attend ambient temperature then the solution was neutralized by 0.1N NaOH to pH 7 and the volume made up to 50mLwith methanol.

50 mg of Valsartan was accurately weighed and dissolved in 10 mL of methanol, then 5 mL of 0.1N NaOH was added and kept at 80^{0}C about 2 h in a water bath. Then the solution was neutralized by 0.1 N HCl to pH 7 and the volume made up to 50mL with methanol.

50 mg of valsartan was accurately weighed and dissolved in 10mL of methanol, then 5 mL of 10% H_2O_2 solution were added and kept at 80^{0}C about 2 h in a water bath then volume was made up to 50 mL with methanol.

50mg of valsartan was spread in a borosilicate glass Petri dish and placed in a hot –air oven maintained at 80^{0}C for 24 hours, then the solution was prepared to achieve a final concentration of 80µg mL^{-1}with methanol.

50mg of valsartan was (covered with aluminum foil) and exposed in the UV chamber for 24 hours, then the solution was prepared to achieve a final concentration of 80µg mL^{-1}with methanol

Method validation

The method was validated according to International Conference on Harmonization[17] guidelines for validation of analytical procedures.

RESULTS AND DISCUSSION

System suitability

System-suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (t_R), number of theoretical plates (N), tailing factor (T), and RSD of peak area were evaluated for six replicate injections of the drug at a concentration of 80 µg / ml. The results given in Table 1 were within acceptable limits.

Table 1: Results from system suitability studies

Sr. no.	Parameters	Results	Acceptance limit
1	Theoretical plates (N)	4633	$N > 2000$
2	Peak area	1504141	RSD ≤ 1%
3	Retention Time (t_R)	4.63 min	4633
4	Tailing factor (T)	1.08	$T \leq 2$

* Mean ± S.D. from six determinations

Linearity and range

Appropriate aliquots of standard Valsartan stock solutions (1000 µg / ml) were taken in different 10 ml volumetric flask and resultant solution was diluted up to the mark with diluents to obtain final concentration of 40-120 µg / ml. The solutions were prepared in triplicate. Calibration curve were constructed by plotting the concentration of valsartan versus corresponding mean peak area. The results show that an excellent correlation exists between peak area and concentration of drugs within the concentration range and the results given in Table 2.

Table 2: Calibration data of valsartan by RP-HPLC method

Sr. No.	Concentration (µg / ml)	Retention time (min)	Peak area (mv)
1	40	4.629	758727
2	60	4.635	1146217
3	80	4.630	1504141
4	100	4.636	1878575
5	120	4.633	2250255

Precision

The intra-day precision was determined by analyzing standard solution of concentration 80 µg / ml for 6 times on the same day while inter-day precision was determined by analyzing corresponding standards daily for 6 day over a period of one week. The values of percentage relative standard deviation (% RSD) for intra-and inter-day variation are given in Table 3.

Table 3: Precision results for Valsartan

Sr. No	Concentration (µg / ml)	Intraday precision (Area)	Interday precision (Area)
1	80	1504842	1505802
2	80	1504543	1503693
3	80	1505248	1502560
4	80	1506765	1506662
5	80	1504102	1503482
6	80	1506131	1506131
Mean		1505272	1504722
Std.Dev		1005.261	1684.397
%RSD		0.066	0.111

Accuracy

Accuracy of the method was checked by recovery study using standard addition method known amount of standard valsartan was added into pre analyzed sample and subjected it to the proposed high performance liquid chromatographic method. These studies were carried out at three levels i.e., (50, 100 and 150%). The recovery studies were carried out and the % recovery and standard deviation of the % recovery were calculated and presented in Table 4.

Table 4: Results from recovery studies (n=3)

Formulation	Label claim (mg/ml)	Initial amount (µg/ml)	Amount added (µg/ml)	Amount recovered (µg/ml)	Recovery ± SD* (%)	% RSD
VALZAAR-80	80	80	40	120.09	100.07 ± 0.13	0.108
		80	80	159.78	99.86 ± 0.16	0.100
		80	120	199.93	99.96 ± 0.08	0.040

Sensitivity

The sensitivity of measurement of Valsartan by use of the proposed method was estimated in terms of the limit of quantitation (LOQ) and the limit of detection (LOD). The limits of detection and quantitation were calculated by the method based on the standard deviation (σ) of responses for triplicate blank injections and the slope (S) of the calibration plot, using the formulae LOD = $3.3\sigma/S$ and LOQ = $10\sigma/S$. The LOD and LOQ of the method were 0.065 and 0.197 µg mL^{-1}, respectively, which indicates the method is suitable for detection and quantification of valsartan over a very wide range of concentrations.

Ruggedness and robustness

Ruggedness is a measure of the reproducibility of a test result under normal, expected operating condition from instrument to instrument and from analyst to analyst. The results of ruggedness testing are reported in the Table 5.

Robustness is a measure of capacity of a method to remain unaffected by small but deliberate variations in the method conditions, and is indications of the reliability of the method. A method is robust, if it is unaffected by small changes in operating conditions. The small changes made included: the mobile phase

ratio, the mobile phase pH, the flow rate, the detection wavelength. **Table 6** shows that the percent recoveries of valsartan were good under most conditions and did not show a significant change when the critical parameters were modified. The tailing factor for Valsartan and the related compounds was always less than 2.0 and the components were well separated under all the changes carried out. Considering the modifications in the system suitability parameters and the specificity of the method would conclude that the method conditions are robust.

Table 5: Ruggedness studies of Valsartan

Formulation	Label claim(mg/ml)	Analyst I Amount found(mg/ml)	Recovery± SD*(%)	Analyst II Amount found(mg/ml)	Recovery ± SD*(%)
VALZAAR-80	80	79.87	99.84 ± 0.097	79.96	99.95± 0.25

Table 6: Effect of experimental parameters on the percent recoveries of Valsartan

Conditions	Modification	(%) Recovery of valsartan
Mobile phase composition (Methanol: Buffer) (v / v)	58:42	100.10
	60:40	99.95
	62:38	100.30
Mobile phase pH	4.0	99.98
	4.2	99.88
	4.4	100.5
Flow Rate Of Mobile Phase	0.8	99.42
	1	99.94
	1.2	100.02
Wavelengths (nm)	252	100.32
	254	100.15
	256	100.2

Forced degradation study

When establishing the stability-indicating properties of analytical methods, the intermediate degradation products should not interfere with any stage of drug analysis**.** Valsartan was found to be stable at light and oxidation experiments. In acidic condition valsartan degraded up to 13.1 %, in basic condition up to 5.8 % and in thermal condition 14.6 % degradation was observed for valsartan. The results from forced degradation studies are given Table 8.Chromatograms obtained from after degradation under different stress conditions are shown in Fig: 2, respectively. No peaks co eluted with the drug peak, suggesting the method enabled specific analysis of valsartan in the presence of its degradation products.

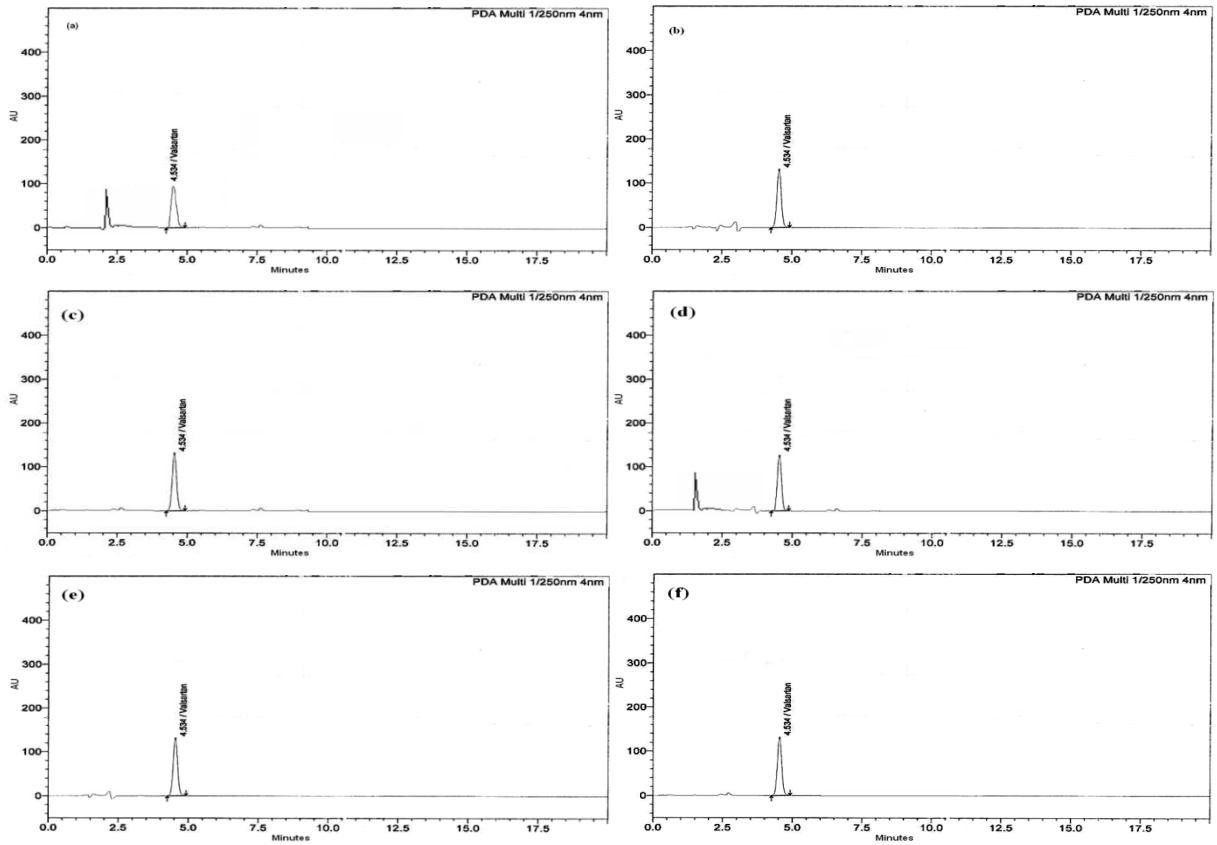

Fig. 2: LC chromatograms of Valsartan (a) After acidic degradation (b) After basic degradation (c) After oxidative degradation (d) After thermal degradation (e) After photo degradation (f) Standard solution (80µg/mL)

Table 7: Characteristic parameters of Valsartan

Parameters	RP-HPLC
Calibration range (µg / ml)	40-120
Detection wavelength	250nm
Mobile phase (Methanol: Buffer)	60:40
Retention time	4.63 min
Regression equation (Y*)	Y=18577x+21417
Slope (b)	18577
Intercept (a)	21417
Correlation coefficient(r2)	0.9990
Limit of detection (µg / ml)	0.065
Limit of quantitation (µg / ml)	0.197

Table 8: Results from analysis of samples by the forced degradation study, showing percentage Degradation of valsartan

Condition	% Assay	% Degradation	Peak purity
Treated with 5 mL of 0.1 N HCl solution and kept at water bath 80°C for 2 hours	86.5	13.1	0.999999
Treated with 5 mL of 0.1 N NaOH solution and kept at 80°C for 2 hours on water bath	93.8	5.8	0.999999
Treated with 5 mL of 10.0 % H_2O_2 solution and kept at 80°C for 2 hours	97.6	2.0	0.999999
Heated at 80°C for 24 hours in oven	85.0	14.6	0.999998
Exposed in the UV chamber for 24 hr	97.3	2.3	0.999999

CONCLUSION

The results of the various validation studies showed that the LC method was very simple, sensitive, accurate, reproducible and stability indicating quantitative analysis of Valsartan in tablets, which separates all degradants. The method was validated as per ICH guidelines and it is one of the rare studies where forced degradation studies were done under all different suggested conditions and all the products were resolved in a single isocratic run. Literature survey showed that two stability indicating method has been published but the methods are very complicated in terms of complex mobile phase composition and longer run time. That's why the method is not feasible and economical for pharmaceutical industry. The developed method is very sensitive enough with LOD of 0.065 and 0.197 µg mL^{-1}. Due to simpler mobile phase and short run time, it ultimately increases the productivity of this method, thus reducing the cost of sample analysis. So the developed analytical method is more economical to pharmaceutical industry and can be used for stability testing as well as routine quality control analysis of valsartan in bulk drug and pharmaceutical formulations.

ACKNOWLEDGMENT

The authors express their sincere thanks to Macleod's Pharmaceuticals Ltd., India for providing gift sample of Valsartan. Thanks are also due to the Professor and Chairman Siksha 'O' Anusandhan University, Bhubaneswar for providing laboratory facility to carry out the present research work.

REFERENCES

1. Budavari S, The Merck index, Merck and Co. Press. Edn 12, Whitehouse Station, New Jersey, 1997.
2. Koçyiğit KB, Unsalan S, Rollas S Determination and validation of Ketoprofen, Pantoprazole and Valsartan together in human plasma by high performance liquid chromatography. Pharmazie 2006; 61: 586-589.
3. Daneshtalab N, Lewanczuk RZ, Jamali F High performance liquid chromatographic analysis of angiotensin II receptor antagonist Valsartan using a liquid extraction method. Journal of Chromatography B, Analytical Technology and Biomedical Life Science 2002; 766: 345-349.
4. González L, López JA, Alonso RM, Jiménez RM Fast screening method for the determination of angiotensin II receptor antagonists in human plasma by high-performance liquid chromatography with fluorimetric detection. Journal of Chromatography *A* 2002; 949: 49-60.
5. Koseki N, Kawashita H, Haraa H, Niina M, Tanaka M, Kawai R, Nagae Y, Masuda N Development and validation of a method for quantitative determination of Valsartan in human plasma by liquid chromatography-tandem mass spectrometry. Journal of Pharmaceutical and Biomedical Analysis 2007; 43: 1769-1774.
6. Hao L, Yingwu W, Yao J, Yunbiao T, Jiang W, Limei Z, Jingkai G A liquid chromatography/tandem mass spectrometry method for the simultaneous quantification of Valsartan and Hydrochlorothiazide in human plasma. Journal of Chromatography B Analytical Technologies in the Biomedical and Life Sciences 2007; 852: 436-442.
7. Selvan PS, Gowda KV, Mandal U, Solomon WDS, Pal TK Simultaneous determination of fixed dose combination of Nebivolol and Valsartan in human plasma by liquid chromatographic-tandem mass spectrometry and its application to pharmacokinetic study. Journal of Chromatography B, Analytical Technologies in the Biomedical and Life Sciences 2007; 858: 143-150.
8. Jing N, Bingren X, Yuqi F, Danhua W Isolation and Identification of Process Impurities in Crude Valsartan by HPLC, Mass Spectrometry, and Nuclear Magnetic Resonance Spectroscopy. Journal of Liquid Chromatography & Related Technologies 2006; 29(4): 553 -568.
9. Macek J, Klíma J, Ptácek P Rapid determination of Valsartan in human plasma by protein precipitation and high performance liquid chromatography. Journal of Chromatography B, Analytical Technologies in the Biomedical and Life Sciences 2006; 832: 169-172.
10. Hillaert S, Bossche VW Simultaneous determination of Hydrochlorothiazide and several angiotensin-II-receptor antagonists by capillary electrophoresis. Journal of Pharmaceutical and Biomedical Analysis 2003; 31: 329-339.
11. Satana E, Altmay S, Göger NG, Özkan SA, Sentürk Z Simultaneous determination of Valsartan and Hydrochlorothiazide in tablets by first-derivative ultraviolet spectrophotometry and LC. Journal of Pharmaceutical and Biomedical Analysis 2001; 25: 1009-1013.
12. Tatar S, Sağlík S Comparison of UV- and second derivative-spectrophotometric and LC methods for the determination of Valsartan in pharmaceutical formulation. Journal of Pharmaceutical and Biomedical Analysis 2002; 30: 371-375.
13. Agrahari V, Kabra V, Gupta S, Nema RK, Nagar M, Karthikeya C, Trivedi P Determination of Inherent Stability of Valsartan by Stress Degradation and Its Validation by HPLC. International Journal of Pharmaceutical and Clinical Research 2009; 1: 77-81

14. Bhatia M. Sudesh and Kokil S. Uttamrao Determination and validation of valsartan and its degradation products by isocratic HPLC. J. Chem. Metrl. 2009; 3: 1-12.
15. Sharma T, Moitra SK, Si SC, Sankar DG Stability indicating method for the determination of Ranolazine hydrochloride in the bulk drug and in pharmaceutical dosageform. Int J Pharm Pharm Sci 2011; 3 Suppl 4, *327-332*
16. Channabasavaraj KP, Modiya J S, Sathrath H M Development and validation of RP-HPLC Method for estimation of Varenicline tartate in bulk and tablet dosage form. Journal of Pharmacy and pharmaceutical sciences 2011; 3(2):59-61.
17. ICH. Stability Testing of New Drug Substances and Products. International Conference on Harmonization, IFPMA, Geneva, 2003.