WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 16, 1258-1268. Research Article ISSN 2277– 7105

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING REVERSE PHASE HPLC METHOD FOR THE QUANTIFICATION OF VALSARTAN IN BULK AND PHARMACEUTICAL DOSAGE FORM

Himani Tiwari* and Goyal Pradeep[1]

*Research Scholar, Pacific University, Udaipur (Raj.).

[1]Associate Professor, B.N. University, Udaipur (Raj.).

Article Received on
12 July 2018,

Revised on 01 August 2018,
Accepted on 22 August 2018

DOI: 10.20959/wjpr201816-13228

***Corresponding Author**
Himani Tiwari
Research Scholar, Pacific University, Udaipur (Raj.).

ABSTRACT

Valsartan is an angiotensin-receptor blocker (ARB) that may be used to treat a variety of cardiac conditions including hypertension, diabetic nephropathy and heart failure. A simple, accurate and précised reverse phase high performance liquid chromatographic method was developed for the estimation of Valsartan in bulk and tablet dosage form. An Inertsil ODS-4, 50 mm×4.6mm, 3µm column was used as a stationary phase with a mobile phase A having 1000 ml Water: 1.0 ml TFA and Mobile Phase B having 400 ml water: 600 ml Acetonitrile: 1.5 ml TFA. The flow rate was 1.0 mL/min. The effluent was monitored at 270 nm and eluted at 11.890 min. Calibration curve was plotted with a range from 0.5-3 µg/ml for Valsartan and the correlation was found to be 0.9958. The accuracy range was found between 98-102%. The % RSD values for precision were less than 2.0%. The developed method was validated for the parameters like system suitability, precision, accuracy, and robustness parameters as per ICH guidelines. The proposed method can be useful for the routine analysis of Valsartan in Bulk and pharmaceutical dosage form.

KEYWORDS: Anti-hypertensives, Valsartan, RP-HPLC, Columns, ICH, Validation, Mobile Phase etc.

INTRODUCTION

Elevated arterial pressure brings pathological changes in the vasculature and hypertrophy of the left ventricle. As a result, hypertension is the principal cause of stroke and also leads to disease of the coronary arteries with myocardial infarction and sudden cardiac death. It is a major contributor to cardiac failure, renal insufficiency, and dissecting aneurysm of the

aorta.[1] The antihypertensive drugs are categorised into different classes based on their mechanism of action.[2-3] Valsartan is an angiotensin-receptor blocker (ARB) that may be used to treat a variety of cardiac conditions including hypertension, diabetic nephropathy and heart failure. Valsartan lowers blood pressure by antagonizing the renin-angiotensin-aldosterone system (RAAS); it competes with angiotensin II for binding to the type-1 angiotensin II receptor (AT_1) subtype and prevents the blood pressure increasing effects of angiotensin II.[4-5] In recent years development of the analytical methods for estimation, purity evaluation and qualification of drugs has received a great deal of attention in the field of pharmaceutical sciences. The rational of present work is to develop and validate HPLC method for estimation of Valsartan in bulk and pharmaceutical dosage form and also conduct forced degradation studies for stability testing in different stress conditions.[6] This method was successfully applied to the estimation studies of Valsartan in marketed products. An attempt has been made to develop new simple, reliable, and reproducible, RP-HPLC methods to estimate the Valsartan in bulk and pharmaceutical formulation with good precision, accuracy, linearity and reproducibility respectively.[7] Hence in present work, we developed a simple, rapid and inexpensive liquid chromatographic method for the analysis of Valsartan in pure and pharmaceutical dosage form. The proposed HPLC method is validated using standard ICH guidelines.[8]

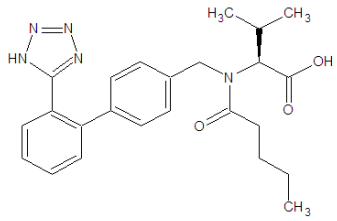

Figure I: Structure of Valsartan.

MATERIALS AND METHODS

The pure drug of Valsartan was procured as gift sample from Lupin Pharmaceuticals, Ankleshwar, Gujrat. All the chemicals used were of analytical grade and solvents used were of HPLC grade. Diovan (80mg) of Novartis and Valent (80mg) of Lupin were procured from local shops. Membrane filters 0.22 µm were procured from Millipore Pvt. Ltd. Bangalore, India.

INSTRUMENTATION

Agilent Technologies 1200 series RRLC equipped with UV-Visible detector & Diode array detector, with Chem-station software was used in the analysis.

CHROMATOGRAPHIC CONDITIONS

Chromatographic separation was performed at 35°C temperature on a reverse phase Inertsil ODS-4, 50 mm×4.6mm, 3µm column with use of a filtered and degassed mobile phase consisting of Mobile Phase A: Buffer 0.2ml TFA in 1000ml Water) and Mobile Phase B: 400 ml water: 600 ml Acetonitrile: 1.5 ml TFA. The flow rate of mobile phase was adjusted to 1.0 ml/min. The UV detector wavelength was set at 270 nm.

PREPARATION OF THE MOBILE PHASE

Mobile phase-A

1 ml of Trifluoro acetic acid was added to 1000 ml of water, mixed well and filtered through 0.22µ filtered and degassed.

Mobile Phase - B

Mixture of water and Acetonitrile (400:600), add 1.5 ml of Trifluoro acetic acid was prepared, mixed well, filtered through 0.22µ filtered and degassed.

Water: Acetonitrile (50:50) was used as diluent.

PREPARATION OF SOLUTIONS

Standard Stock Solution and Working Standard Solution

A stock solution of valsartan was prepared by accurately weighing 32 mg of drug, transferring to 100 ml volumetric flask, dissolving in 70ml of diluent water: acetonitrile (50:50) and was sonicated to dissolve. Volume was made upto the mark with diluent. Diluted 5.0 ml of this solution to 100ml with diluent and again 5ml of this resulting solution was diluted to 50ml to obtain final standard solution of 1.6 µg/ml of valsartan.

Sample Solution

Weighed accurately and transferred 10 intact tablets into a specified volume (A) of volumetric flask, add specified volume (B) of water and sonicated for 10 minutes with intermittent shaking to disperse the tablets. Then added specified volume (C) of diluent and sonicated for 30 minutes with intermittent shaking, allowed to cool to room temperature and made the volume up to mark with diluent, mixed and allowed to settle-down. Transferred 5.0

ml of this solution to 25 ml volumetric flask and diluted to volume with diluent and mixed. It was filtered through 0.22µ PVDF filter, discarding first 3 ml of the filtrate.

Sample Preparation

Strength	First dilution		
Valsartan	Volumetric flask (A)	Volume of Water (B)	Volume of Diluent (C)
80 mg	250 ml	12.5 ml	175 ml

METHOD VALIDATION

The method was validated for the parameters like specificity, range and linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision and robustness.

a. Specificity

Specificity is a procedure to detect quantitatively the analyte in the presence of component that are expected to be present in the sample matrix. The peak purity index for the main peak in standard preparation and sample preparation was determined and record in table- 1 suggested that there was no interference from blank and placebo.

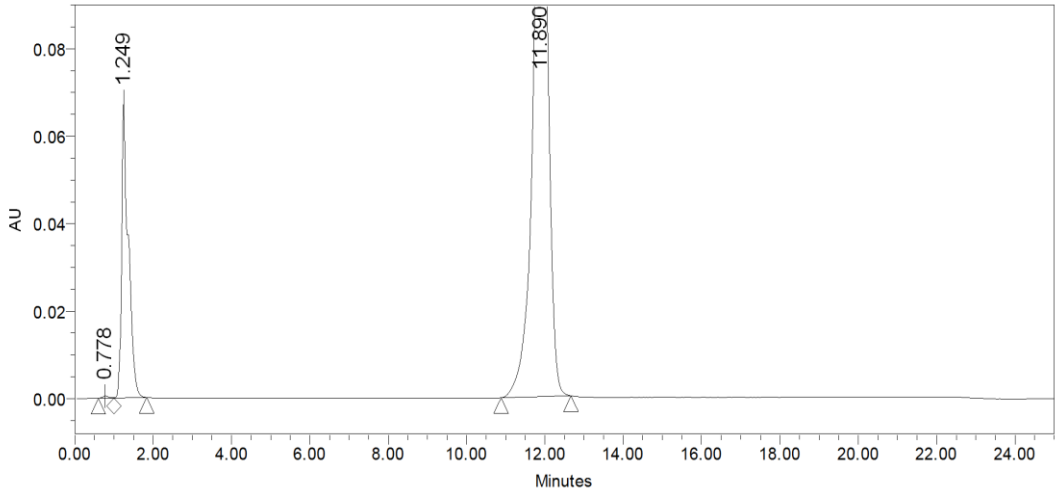

Figure II: Chromatogram of standard solution of Valsartan.

Table I: Interference from blank and placebo.

Sample	Peak Purity Index
Standard preparation	0.9996
Sample preparation	0.9998

b. Linearity and Range

To evaluate the linearity, serial dilution of analyte were prepared from the stock solution in the concentration range of 0.5µg/ml to 3µg/ml. The calibration curve was made between peak area and concentrations at in the range. The results of Linearity study are shown in table 2.

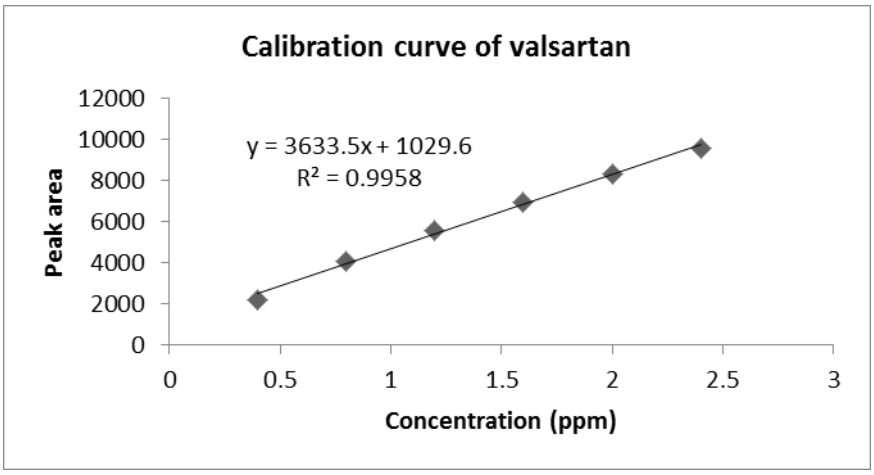

Figure 3: Calibration curve Valsartan.

Table II: Linearity data of Valsartan.

Sr. No.	Linearity Level	Actual Conc. in % of Valsartan	Peak Area
1	0.05	0.3998	2218
2	0.1	0.7996	4089
3	0.15	1.1994	5563
4	0.20	1.5992	6928
5	0.25	1.9990	8297
6	0.3	2.3988	9589
Correlation coefficient (r)		0.9958	
Slope of regression line		3633.5	
y-intercept		1029.6	

c. LOD and LOQ

Limit of detection and limit of quantification was calculated by the proposed method which was based on the standard deviation (s) of the response and the slope (S) of the calibration curve at levels approximating the LOD and LOQ, LOD= 3.3 (s/S) and LOQ= 10 (s/S). The results are given in table 3.

Table III: Results for LOD and LOQ determination

Sr. No.	Samples	LOD	LOQ
1	Valsartan	0.02	0.06

d. Accuracy

Accuracy was determined over the range 50% to 150% of the sample concentration. Calculated amount of Valsartan from standard stock solution was added in placebo to attain 50%, 100% and 150% of sample concentration. Each sample was prepared in triplicate at each level. Blank and standard preparations were injected and the chromatograms were recorded. The reported value from recovery studies is shown in table 4.

Table IV: Results of Recovery Studies of Valsartan (n=3).

Accuracy Level (%)	Amount Added (mcg/ml)	Amount Found (mcg/ml)	% Recovery	% Mean Recovery	% RSD
50%	0.8088	0.8043	99.4	100.5	3.3
	0.8088	0.7922	97.9		
	0.8088	0.8426	104.2		
100%	1.6715	1.5729	97.2	100.3	4.8
	1.6715	1.5819	97.8		
	1.6715	1.7113	105.8		
150%	2.4263	2.3566	97.1	98.2	1.0
	2.4263	2.3918	98.6		
	2.4263	2.4026	99.0		

e. Precision

The precision of method was investigated with respect to repeatability and ruggedness.

a. Method Precision (Repeatability): Method precision was established by determining the area response of six replicate injections prepared under same conditions and the results are shown in table 5.

Table V: Results for Area response of standard for Repeatability.

Sr. No.	Valsartan (standard)	
	Retention time	Area
	11.89 min	11278.28
2	11.87 min	11286.30
3	11.85 min	11290.26
4	11.89 min	11294.24
5	11.86 min	11288.25
6	11.87 min	11268.32
Mean	**11.872** min	**11284.28**
SD		**9.44**
% RSD		**0.08**

b. Intermediate Precision (Ruggedness): Different analyst, using a different system, repeated the procedure followed for method precision on a different day using same lot of sample. The results obtained from Intermediate Precision study are recorded in table 6.

Table VI: Result for intermediate precision.

Sr. No.	Valsartan (standard)	
	Area	% Assay
	11274.45	98.7
2	11282.34	99.6
3	11288.28	99.4
4	11290.26	100.0
5	11289.44	100.6
6	11287.48	99.8
Mean	99.68	
St. Dev.	0.63	
% RSD	0.64	

f. Robustness

The method was found to be robust, as small but deliberate changes in the method parameters have no detrimental effect on the method performance. There was a change in flow rate of mobile phase to 0.9 ml/min and 1.1ml/min (± 10%) and column oven temperature (± 5°C absolute) to 35°C and 40°C. The results of Robustness study of the method are shown in table 7(a-d).

Table VIIa: Result for robustness (flow 0.9 ml/min).

Sr. No.	Valsartan (standard)	
	Conc. (ppm)	Area
1	100	1127428
2		1123248
3		1126843
4		1129021
5		1123945
Mean	1126097	
St. Dev.	2430.32	
%RSD	0.22	

Table VIIb: Result for robustness (flow 1.1 ml/min)

Sr. No.	Valsartan (standard)	
	Conc. (ppm)	Area
1	100	1123684
2		1127485
3		1125486
4		1124785
5		1127889
Mean	1125866	
St. Dev.	1788	
%RSD	0.15	

Table VIIc: Result for robustness (temp. 30°C)

Sr. No.	Valsartan (standard)	
	Conc. (ppm)	Area
1	100	1133684
2		1129485
3		1128486
4		1129785
5		1125889
Mean	1129466	
St. Dev.	2812.87	
%RSD	0.25	

Table VIId: Result for robustness (temp. 40°C).

Sr. No.	Valsartan (standard)	
	Conc. (ppm)	Area
1	100	1129684
2		1134485
3		1131486
4		1129785
5		1133889
Mean	1131866	
St. Dev.	2246.54	
%RSD	0.2	

g. Forced Degradation study

The forced degradation of tablets and API was done by subjecting Valsartan to different Stress conditions like Acidic, Alkaline, Thermal and Oxidative degradation. This study is done for stability check of the drug in different conditions. The results were tabulated and are shown in table 8.

Table VIII: Results for Peak Purity index and % Degradation of Valsartan.

Solution	% Degradation	Peak Purity Index
Acid degradation	20.55%	0.8996
Base degradation	1.14%	0.9996
Oxidation degradation	15.34%	0.8994
Thermal degradation	0.28%	0.9998

RECOVERY STUDIES OF COMMERCIAL FORMULATIONS OF VALSARTAN

Recovery studies were carried out for developed method by addition of known amount of standard drug solution of Valsartan to pre-analyzed tablet sample solution at three different concentration levels. Determined the concentration of drug in final dilution after addition of known concentration of pure drug and determined the percentage recovery after deduction of concentration of drug. Results of recovery studies were found to be satisfactory and are reported in table 9.

Table IX: Results of analysis of commercial formulation.

Brand Name	Label claim (mg/tablet)	% Label claim estimated*	Standard deviation	Relative standard deviation	Coefficient of variance
Diovan	80	99.56	0.676	0.0067	0.678
Valent	80	99.61	0.550	0.0055	0.552

**** Each value is an average of five determinations.***

RESULTS AND DISCUSSION

Valsartan is an angiotensin-receptor blocker (ARB) that may be used to treat a variety of cardiac conditions including hypertension. The optimization of the mobile phase and selection of column for analysis was done with different trials and the one showing optimum resolution and fulfilling the specificity conditions was chosen for the drug. The separation was achieved on Inertsil ODS-4, 50 mm×4.6mm, 3µm by mobile phase A: having 1000 ml Water: 1ml TFA and mobile phase B: having 400 ml water: 600 ml Acetonitrile: 1.5 ml TFA. The linear regression analysis data for the calibration plots showed good linear relationship in concentration range of 0.5 to 3µg/ml having linearity equation y = 3633.5x + 1029.6 and correlation co-efficient of 0.9958. The methods were validated for specificity, accuracy, precision, linearity, repeatability and robustness as per ICH guideline Q2 (R1). Forced degradation studies of drug was performed for bulk and dosage form of Valsartan in acidic, basic, peroxide and heat stress conditions where Valsartan showed instability in acidic and basic conditions. Thus the specificity studies shows that developed method can be applied in

the QC laboratory for routine quality check as well as for the stability studies for the Valsartan API and tablet dosage form.

CONCLUSION

A linear, precise, accurate, robust, stability indicating RP-HPLC method has been developed for the estimation of valsartan in active pharmaceutical ingredient and Tablet dosage form. Simplicity of the method, economical nature and low limit of detection and quantitation makes the method superior to the other reported HPLC methods. The developed method was applied for the stability studies of Valsartan in bulk and Pharmaceutical dosage form. The proposed method has the capability to separate the analyte from their degradation products obtained during forced degradation studies. The method can be employed for the routine analysis of Valsartan in Bulk and tablet dosage form.

ACKNOWLEDGEMENTS

The authors express their sincere gratitude to Lupin Pharmaceuticals Ankleshwar, Gujrat, for providing the pure drug sample of Valsartan.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

FINANCIAL SUPPORT AND SPONSORSHIP: Nil.

REFERENCES

1. Barar FSK. “Essentials of pharmacotherapeutics”. S. chand and company ltd; reprint, 2004; 298-301, 239-249.
2. Rang HP, Dale MM, Ritter JN and Moore PK. Pharmacology. 5th edition. Churchill livingstone; Elsevier science ltd, 2003; 307-317.
3. Haque MA, Amrohi SH, Kumar P, Nivedita G, Kumar PT, Mohanty D, Diwan P. Stability indicating RP-HPLC method for the estimation of Valsartan in pharmaceutical dosage form. IOSR Journal of Pharmacy, 2012; 2(4): 12-18.
4. Vinzuda DU, Sailor GU, Sheth NR. RP-HPLC Method for Determination of Valsartan in Tablet Dosage Form. Int. J. Chem Tech Res, 2010; 2(3): 1461-1467.
5. Nissankara rao S, Kumar AA, Sravanthi S L, Naga S J. Method development and validation for the estimation of valsartan in bulk and tablet dosage forms by RP-HPLC. Der Pharma Chemica, 2013; 5(2): 206-211.

6. Bakshi M. Singh S. Development of Validated stability-indicating assay methods-Critical review. Journal of Pharmaceutical and Biomedical Analysis, 2002; 1011–1040.
7. ICH, Q1A (R2), Stability Testing of New Drug Substances and Products, International Conference on Harmonization, Geneva, 6th February 2003; 1-18.
8. ICH, Q2B, Validation of Analytical procedures, Methodology, International Conference on Harmonization, Geneva, November 1994; 1-8.