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Full Length Original Research Paper

Development and Validation of RP-HPLC method for quantification of Valsartan and its Pharmaceutical Formulations

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Abstract: A simple, rapid, sensitive, reverse phase isocratic RP-HPLC method was developed for determination of Valsartan and its pharmaceutical formulation. The method was carried out using C_{18} column [Agilent ODS UG 5 column, 250mm x 4.5mm] with mobile phase comprised of Acetonitrile: Phosphate buffer (70:30 v/v) with 1.0 ml / min flow rate was quite robust. The optimum wavelength for detection was 273 nm at which better detector response for the drug was obtained. The run time was set at 7 min and the retention time was 3.5 minutes. The method was validated for specificity, accuracy, precision, linearity, limit of detection, limit of quantification, robustness, solubility and stability. LOD and LOQ were found to be 1.83 µg/ml, 5.5 µg/ml respectively. The calibration curve was linear in the concentration range of 10-50 µg/ml with coefficient of correlation 0.9993. The percentage recovery for the valsartan was found to be 99.0-100.2 and the % RSD was found to be less than 2 %. The proposed method was successfully applied for quantitative determination of valsartan in tablet dosage forms.

Keywords: Valsartan, RP-HPLC, Pharmaceutical Formulations, Validation, Mobile Phase, Specific.

Introduction

Valsartan is chemically (*S*)-3-methyl-2-(*N*-{[2'-(2*H*-1, 2, 3, 4-tetrazol-5-yl)biphenyl 4 yl] methyl} pentanamido) butanoic acid [figure 1]. Valsartan is an angiotensin II receptor antagonist (more commonly called an "ARB", or angiotensin receptor blocker), with particularly high affinity for the type I (AT_1) angiotensin receptor. By blocking the action of angiotensin, valsartan dilates blood vessels and reduces blood pressure [1] . In U.S,valsartan is indicated for treatment of high blood pressure, congestive heart failure (CHF), or post-myocardial infarction (MI) in 2005[2]. A study released in 2010, based on 819,491 cases in U.S. Veteran's Administration database from 2002–2006, demonstrated a significant reduction in the incidence and progression of Alzheimer's disease and dementia.[3] An earlier study released by the *Journal of Clinical Investigation* in 2007 found some efficacy in the use of valsartan in the treatment and prevention of Alzheimer's disease.In the present investigation methods such as HPLC [4-6], LC-MS [7-9], Protein precipitation [10], Capillary electrophoresis [11] and Simultaneous UVspectrophotometric methods [12,13] are reported for estimation of Valsartan alone or in

combination with other agents. However, there were few methods reported for determination of valsartan individually.In the present study a rapid, precise, accurate, specific and sensitive HPLC method was developed for quantitative estimation of Valsartan in bulk drug and pharmaceutical formulations using UV detector. The present method was simple, since diluted samples are directly used without any preliminary chemical derivatization or purification steps.

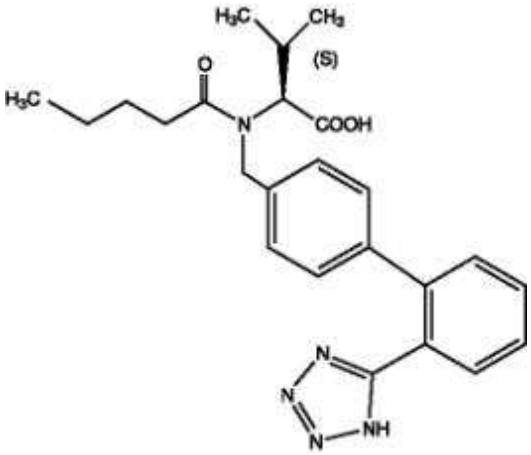

Figure 1: Chemical Structure of Valsartan

Experimental

Instrumentation:

Agilent 1120 compact LC system, with variable wavelength programmable UV detector and Rheodyne injector with 20 µl fixed loop was used for the chromatographic separation. Ezchrome software was used for data analysis. Chromatographic separation was carried out on a C_{18} column [Agilent ODS UG 5 column, 250mm x 4.5mm]. Ultra-sonic bath sonicator, degasser is used.

Preparation of standard stock solution

25mg of pure drug was weighed accurately and transferred in to 25ml volumetric flask and dissolved in 10ml solvent and made up to the mark with solvent to obtain a final concentration of 1000µg/ml (standard stock solution A). From the standard stock solution 2.5ml of aliquot was pippeted in to 25ml volumetric flask and dissolved in 10ml solvent and made up to the mark with the solvent to obtain a final concentration of 100µg/ml (standard stock solution 'B').

Preparation of sample stock solution

Marketed tablet formulation (VALENT) containing 80mg of Valsartan was analyzed by this method. Twenty tablets were accurately weighed and their average weight determined. The tablets were then crushed to fine powder and powder equivalent to 10mg was taken in 100ml volumetric flask and dissolved in 50ml of mobile phase. The solution was kept for sonication for 15min. The solution was made up to the mark with the mobile phase and filtered through 0.45 µ membrane filter to get the concentration of 100µg/ml (sample stock solution A).1.0ml aliquot of the above solution was transferred to a 10ml volumetric flask and diluted to the mark with the mobile phase to obtain a concentration of 10µg/ml (sample stock solution 'B').

Chemicals and reagents

HPLC grade Acetonitrile, Phosphate Buffer, Ortho Phosphoric acid, were used as solvents throughout the experiment.Valsartan was kindly supplied by Dr.Reddys laboratories (Hyderabad, A.P and India). All the solvents used in HPLC method are of HPLC grade. Commercial pharmaceutical preparations of Valsartan from Lupin limited (Aurangabad, India). Which were claimed to contain 80 mg Valsartan was used in analysis. After systematic and detailed study of the various parameters involved, as described under results and Discussion in this chapter, the following procedure was recommended for the determination of valsartan in Bulk samples and pharmaceutical formulations.

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Preparation of Buffer:

A solution of 13.60 gm of potassium bi phosphate was dissolved in 900 ml of HPLC grade water, mix well by using sonicator, and make up the volume to 1000 ml with water. The pH of the resulting solution was adjusted to 3.0 ± 0.5 with 10 % orthophosphoric acid. The above solution was filtered through a 0.45µ membrane filter and degassed by ultra sonicator.

Chromatographic conditions

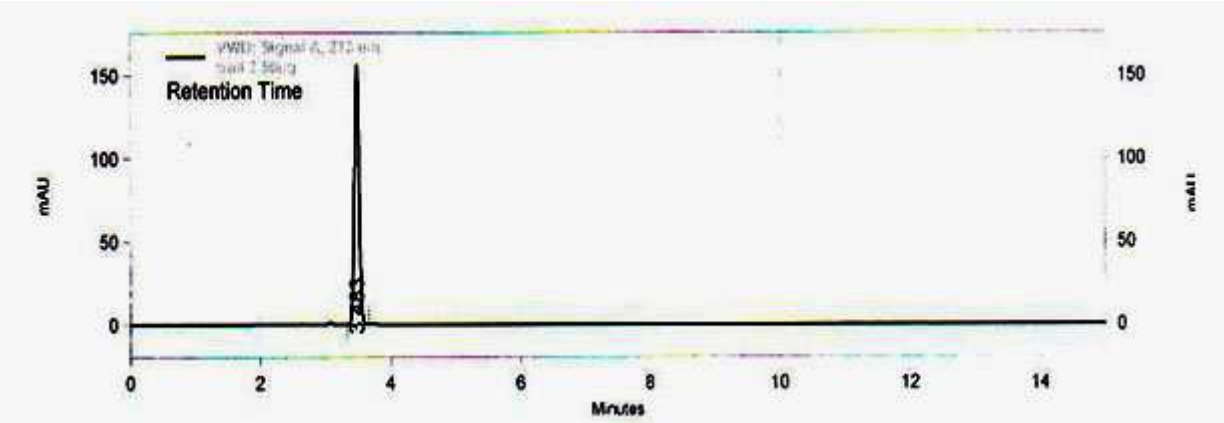
Mode of operation	:	Isocratic
Column temperature	:	Ambient
Detector wavelength	:	273nm
Injection volume	:	20 µL
Flow rate	:	1ml/min
Run time	:	7min

Results and discussion

The objective of this study was to develop a rapid and sensitive HPLC method for the analysis of Valsartan in bulk drug and in its Pharmaceutical dosage forms using the most commonly employed C-18column with UV- detection. Mobile phase and flow rate selection was based on peak parameters (height, capacity, theoretical plates, tailing or symmetry factor), run time, resolution.

The system with Acetonitrile: Phosphate buffer (70:30 v/v) with 1.0 ml / min flow rate was quite robust. The optimum wavelength for detection was 273 nm at which better detector response for the drug was obtained. The run time was set at 7 min and the retention time for was 3.48min.chromatogram was shown in the following figure 2.

Figure 2:



According to USP XXIV (621), system suitability tests are an integral part of chromatographic method. They are used to verify the reproducibility of the chromatographic system. To ascertain its effectiveness, system suitability tests were carried out on freshly prepared stock solutions.

The calibration curve was linear in the concentration range of 10-50µg/ml with correlation coefficient 0.999, that was shown in the following figure.

Figure 3:

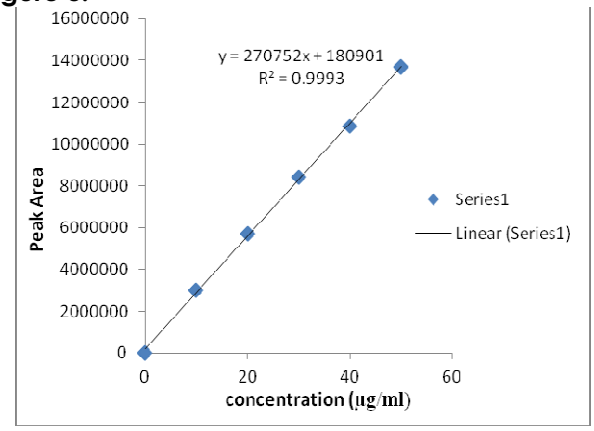


Figure 3: Calibration curve of Valsartan by RP-HPLC

Linearity

To establish the linearity of proposed method, aliquots 1,2,3,4 and 5 of standard stock solution 'B' were transferred in a series of 10ml volumetric flasks and the volume was made up to the mark with mobile phase to obtain final concentration of 10,20,30,40 and 50µg/ml. Three replicates per each concentration were injected and Peak areas of the above solutions were reported. A calibration curve of concentration vs. peak area was established. Regression equations were established and the correlation coefficients were determined in the following table1.

Table 1: Linearity data of Valsartan

S. NO	Concentration (µg/ml)	Retention time (minutes)	Peak area
1	10	3.512	2998701
2	20	3.503	5731686
3	30	3.431	8398017
4	40	3.509	10878087
5	50	3.560	13691768
Correlation coefficient(r^2)	0.9993		
Slope	270752		
Intercept	180901		

Accuracy

Accuracy of the method was examined by performing recovery studies by standard addition method, by adding a known amount of standard solution of pure drug to a pre-analyzed sample solution, at three levels 80%, 100%, 120% of the label claim. Accuracy was evaluated using a minimum of 9 determinations over a minimum of 3 concentration levels covering the specified range. The mean recoveries were found in the range of 99.47-101.1 %. The values were determined in the following table 2.

Table 2: Accuracy data of Valsartan

Peak area	Recovery level		
	80%	100%	120%
Injection 1	6876508	8390615	9856848
Injection 2	6897650	8356948	9887435
Injection 3	6822013	8387345	9890621
Mean	8378303	8378303	9878301
% RSD	0.46	0.18	0.15

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions. The values were reported in the following table 3 and 4.

Table 3: Inter day precision data

S. No	Peak area		
	Day 1	Day 2	Day 3
Mean	8366468	83693028	83742290
Standard Deviation	18387.8	137638.5	38317.57
%RSD	0.21	0.16	0.45

Table 4: Intraday precision data

S. No	Retention time (min)	Peak area
1	3.51	8364912
2	3.50	8390321
3	3.549	8398015
4	3.51	8371612
5	3.512	8351315
6	3.59	8377621
Standard deviation		15508.6
Mean		8375633
% RSD		0.185

Limit of Detection (LOD)

It is the lowest concentration of analyte that can be detected, but not necessarily quantified, by the analytical method. In chromatography the detection limit is the injected amount that results in a peak with a height at least twice or three

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times as high as the baseline noise level (S/N ratio~ 3).

Limit of Quantitation (LOQ)

It is the lowest concentration of analyte that can be determined with acceptable accuracy and precision by the analytical method. LOD was expressed as concentration of analyte generating an instrument response equivalent to ten times the noise (S/N ratio~ 10).

Limit of detection (LOD) and limit of quantitation (LOQ) were manually calculated from the slope of the calibration curve and standard deviation.

The LOD and LOQ values were calculated using the formula given below

LOD = 3.3 x σ/s

LOQ = 10 x σ/s

Where, σ = standard deviation of intercepts of calibration curve

S = slope of the calibration curve.

The LOD and LOQ values were reported in following table 5.

Table: LOD & LOQ data of Valsartan

Parameters	Results
Limit of detection (LOD)	1.83 µg/ml
Limit of quantification (LOQ)	5.5 µg/ml

Robustness

The robustness of a method is its ability to remain unaffected by the deliberate variations in method parameters such as column temperature, analytical wavelength and flow rate.

Detection wave length was initially changed to 271nm and then to 273nm and a series of three injections of 100% concentration were given at each detection wave length and the % RSD was reported separately for each variable.

Flow rate was changed to 0.8ml/min and then to 1.2ml/min and a series of three injections of 100% concentration were given at each detection wave length and the % RSD was reported separately for each variable.

The values were reported in table 6 and 7.

Table 6: Robustness data by change in Detection wave length

Wavelength	Peak area	Mean	SD	%RSD
271nm	13165201	13308085	10402490	0.78
	13349221			
	13409832			
275nm	13567832	13477995	662999	0.49
	13456320			
	13409832			

Table 7: Robustness data by change in Flow rate

Flow rate	Peak area	Mean	SD	%RSD
0.8ml/min	13191723	13324498	119016.7	0.89
	13456320			
	13349221			
1.2ml/min	13567834	13457792	89254.45	0.66
	13456320			
	13349221			

Table 8: Ruggedness data of RP-HPLC

S. No	Peak area	
	Analyst 1	Analyst 2
1	13569870	13308901
2	13459876	13764532
3	13298701	13556720
4	13765490	13618935
5	13553986	13568725
6	13608734	13298654
Mean	13542776	13519411
Standard Deviation	142160	166763.94
%RSD	1.04	1.23

Ruggedness

Ruggedness is a measure of reproducibility of the results under instrument to instrument and analyst

to analyst variations. Six Injections of 100% concentration were given by the co-analyst and the peak areas were recorded and the %RSD was calculated statistically. The values were determined in the following table 8.

Conclusion:

A simple RP-HPLC method has been developed for the determination of Valsartan. The proposed method is rapid, accurate and precise. Its chromatographic retention time of 5 min allows the analysis of a large number of samples in short period of time. Therefore, it is suitable for the routine analysis of Valsartan. The results of the study reveals that the proposed RP-HPLC method for the estimation of Valsartan is simple and accurate in bulk and pharmaceutical dosage forms.

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