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Original Article

DEVELOPMENT AND VALIDATION OF A STABILITY INDICATING RP-HPLC METHOD FOR THE DETERMINATION OF VALSARTAN

K. K. PRADHAN[a]*, U. S. MISHRA[b]

[a]Department of Pharmaceutical Sciences & Technology, Birla Institute of Technology, MESRA, Ranchi 835215, [b]Royal College of Pharmacy and Health Sciences, Berhampur, Odisha 76002
Email: kkpradhan@bitmesra.ac.in

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ABSTRACT

Objective: A stability indicating RP-HPLC method was developed and validated for the determination of Valsartan using Telmisartan (10 µg/ml) as the internal standard.

Methods: In this procedure Phenomenex ODS C-18(250×4.6 mm, packed with 5 micron) column was used with a new mobile phase consisting of methanol: acetonitrile: water (70:15:15 v/v) and the pH was adjusted to 3 by 0.1% glacial acetic acid with a flow rate of 1 ml/min. The eluents were monitored at 249 nm. Valsartan was subjected to stress conditions including hydrolytic degradation in acidic, basic and neutral conditions, oxidation, photolytic, UV degradation and thermal degradation.

Results: Linearity was obtained in the concentration range of 10-90 µg/ml (R^2 =0.999) and with a regression equation y=0.074x+0.005. The LOD and LOQ values were 0.261 and 0.791 µg/ml respectively. The drug had shown promising degradation in the acidic, basic, neutral, thermal and oxidative stress conditions.

Conclusion: The method was validated for accuracy, precision, linearity, specificity and robustness and revealed that it is specific, accurate, rapid, precise, reliable and reproducible enough to analyze commercial dosage forms as per ICH guidelines.

Keywords: Valsartan, Stability Studies, Stability Indicating RP-HPLC, Stress Degradation, ICH Guidelines.

INTRODUCTION

Valsartan is chemically 3-methyl-2-[pentanoyl-[[4-[2-(2H-tetrazol-5-yl) phenyl] phenyl]methyl]amino]-butanoic acid (fig. 1)[1], Angiotensin II receptor antagonist, acting on the AT1 subtype &used for treatment of high blood pressure, of congestive heart failure (CHF), and post-myocardial infarction (MI). By blocking the action of Angiotensin, Valsartan dilates blood vessels and reduces blood pressure [2]. The focus of the present study was to develop & validated a rapid, stable, & economic stability indicating HPLC method for the estimation of Valsartan in bulk and tablet dosage forms. As per ICH Q1A (R2) guidelines the purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors, such as temperature, humidity, and light, and to establish a retest period for the drug substance or a shelf life for the drug product and recommended storage conditions.

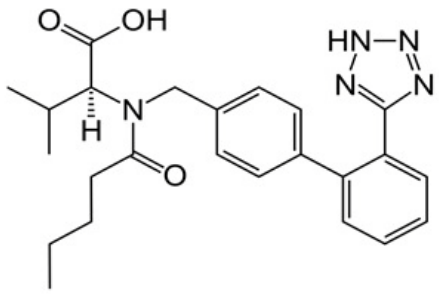

Fig. 1: Chemical structure of valsartan

From the literature survey [3-10] it has been found that many HPLC methods are available for the determination of valsartanin bulk as well as in formulations along with stability indications. But with the use of this new mobile phase i.e. methanol: acetonitrile: water (70:15:15 v/v) pH adjusted to 3 by glacial acetic acid, the detection of degradant peaks of UV, Oxidative and Thermal stresses was found to be better and precise. It has also been found that using the new mobile phase, the developed RP HPLC method became more robust, accurate and highly précised. As the separation of the degradants using this mobile phase is quite good, isolation of the degradants with preparative techniques can also be achieved using this mobile phase. Further LC MS-MS analysis will help to deduce the structures of the degradants which can help to establish the possible degradation pathway of this drug. So this method can be economically very useful in both research and industrial aspect.

MATERIALS AND METHODS

Chemicals and reagents

Valsartan API was purchased from Cadila Healthcare Ltd, Ahmedabad, India. The HPLC grade solvents used were obtained from Merck (India) Ltd, Mumbai. HPLC grade water was prepared using Millipore System (Millipore, Molesheim France, and Model Elix-10). All other reagents were of HPLC grade.

Equipments

HPLC was performed on Shimadzu HPLC with LC-20AT pumps besides SPD-20A UV-Visible detector. Shimadzu spin crom-CFR software was used along with Phenomenex ODS C-18(250×4.6 mm, packed with 5 microns) for the separation.

Chromatographic conditions

Selected drug was injected to the column with different mobile phases of different ratios with different flow rates till sharp peaks without any interference peaks containing spectra were obtained. Final mobile phase was chosen as methanol: water: acetonitrile (70:15:15) and pH was adjusted to 3 with 0.1 N glacial acetic acid. The mobile phase was then sonicated for 10 minutes and filtered through 0.45µ membrane filter. C-18 column was equilibrated with the mobile phase. Mobile phase flow rate was maintained at 1 ml/min and eluent were monitored at 249 nm wavelength. The samples were injected using a 20 µl fixed loop. All determinations were performed at ambient temperature for a run time of 6 min.

Preparation of stock and working solutions

About 50 mg of valsartan was weighed accurately and was taken in 50 ml volumetric flasks. It was dissolved in the mobile phase and the volume was made up to the mark to prepare the 1000 µg/ml stock solution. From the above prepared stock solution of valsartan 5 ml was pipette out in to a 50 ml volumetric flask and the volume was made to up to the mark with mobile phase to yield the 100 µg/ml working solution of valsartan.

About 50 mg of telmisartan was weighed accurately and was taken in a 50 ml volumetric flask. It was dissolved in the mobile phase and the volume was made up to the mark to make a stock solution of 1000 µg/ml. From this stock, 5 ml was pipette out and diluted to 50 ml with the mobile phase to yield the working solution of Telmisartan (100 µg/ml).

Method validation

Validation of the established HPLC method was done as per the ICH guidelines to assess the accuracy, precision, specificity, ruggedness, robustness, LOD and LOQ values. The accuracy of the method was determined by calculating recoveries of drug by the method of standard drug. Known amounts of standard drug corresponding to 80%, 100% and 120% of the label claim was added to pre quantified sample solution and the amounts of drug were estimated by measuring the peak areas and by fitting these values to the straight line equation of calibration curve. The intraday and inter-day precision studies of the drugs were carried out by estimating the corresponding responses on the same day and consecutive three days respectively.

The results were reported in terms of standard deviation and % RSD. The specificity of the proposed RP-HPLC method was determined by complete separation of two peaks with parameters like retention time (R_t), resolution (R_S) and tailing factor (T). Robustness of the method was studied by deliberate variations of the analytical parameters such as flow rate (1±0.2 ml/min), concentration of methanol (90±2%). Ruggedness is the degree of reproducibility of the results obtained under a variety of conditions, expressed as %RSD. These conditions include different laboratory conditions and different analysts.

Forced degradation studies

The force degradation studies were conducted on the sample using acid, alkaline, oxidative, thermal, and photolytic and UV degradations. Neutral stress was given by dissolving 10 mg of drug into 10 ml of methanol followed by volume made up with water and refluxing for 6 h. Acidic and basic stresses were given by using 0.1 N. 0.5 N and 1 N of hydrochloric acid and sodium hydroxide followed by 6 h reflux. Oxidative stress was put on by using 1 % w/v, 2 % and 3 % w/v hydrogen peroxide. Photo degradation was done upon exposure to sunlight for 12,24,72 h. UV illumination of 1.2×10^6lux h for 12,24,72 h was used as the stress factor for UV degradation. Thermal degradation was carried out at 70 °C dry heat for 10, 20 and 30 days.

RESULTS

Percentage recovery was calculated from differences between the peak areas obtained for fortified and unfortified solutions.

As shown from the data in table 1, good recoveries were made at each added concentration, confirming that the method was accurate. Data obtained from analysis of the samples on the same day (n = 6) and on consecutive days (n = 6) are given in table 1. As the % RSD values of the data obtained were well below 2%, thereby the values indicate that the method was sufficiently precise.

Linear calibration plots of each drug for the previously mentioned method were obtained over the calibration ranges 10–90 µg/ml; the correlation coefficient obtained was 0.999 (fig. 3). The chromatogram is shown in fig. 2. The results show that the good correlation existed between the peak area and concentration of the analyte. The LOD and LOQ values found for the analysis were 0.261 and 0.791 µg/ml respectively.

Robustness study was being performed by changing two parameter pH and flow rate. pH had been altered to 2.8 and 3.2 whereas flow rate was changed to 0.8 an 1.2. Ruggedness of the established method was assessed by performing 6 different injections of a particular concentration of the drug by two different analysts. Results obtained (shown in table 1-5) and % RSD values calculated clearly illustrate the ruggedness of the method.

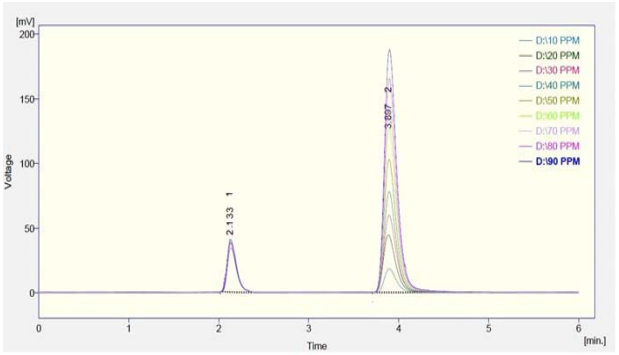

Fig. 2: Linearity Chromatogram of valsartan along with I. S. telmisartan

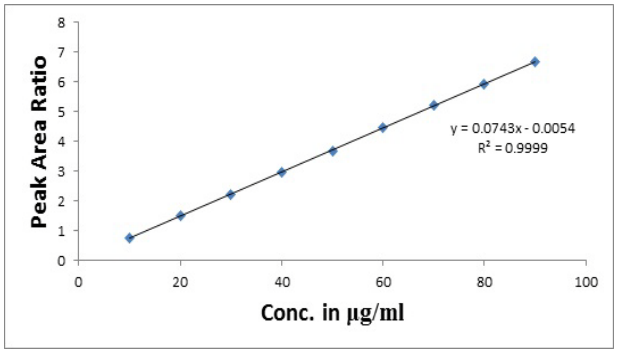

Fig. 3: Calibration curve of valsartan

Table 1: Accuracy data of the method

Samples	Concentration (µg/ml)		% Recovery	Statistical Analysis
	Amount present in Formulation	Amount of drug added		
S1: 80%	30	24	100.02	Mean=99.88
S2: 80%	30	24	99.89	SD=0.169
S3: 80%	30	24	99.75	% RSD=0.168
S4: 100%	30	30	100.07	Mean=99.94
S5: 100%	30	30	99.79	SD=0.132
S6: 100%	30	30	99.98	% RSD=0.132
S7: 120%	30	36	99.59	Mean=99.78
S8: 120%	30	36	99.73	SD=0.194
S9: 120%	30	36	100.04	% RSD=0.193

Table 2: Intraday precision data

S. No.	Concentration (µg/ml)	Peak Area Ratio	Calc. Amt. (µg/ml)	Statistical Analysis
1	30	2.216	30.01	
2	30	2.209	29.91	Mean=29.99
3	30	2.228	30.17	SD=0.117
4	30	2.207	29.89	% RSD=0.390
5	30	2.219	30.05	
6	30	2.212	29.95	

Table 3: Inter-day precision data

S. No.	Concentration (µg/ml)	Day 1	Day 2	Day 3	Statistical analysis
1	30	2.231	2.236	2.232	
2	30	2.237	2.229	2.241	Mean=30.09
3	30	2.205	2.208	2.201	SD=0.076
4	30	2.217	2.227	2.205	% RSD=0.252
5	30	2.211	2.206	2.209	
6	30	2.225	2.239	2.245	
Mean Peak Area Ratio		2.221	2.224	2.222	
Calc. Amt. (µg/ml)		30.08	30.12	30.09	

Table 4: Robustness data

pH 2.8				pH 3.2			
Conc. (µg/ml)	Peak Area Ratio	Calc. Amt. (µg/ml)	Statistical Analysis	Conc. (µg/ml)	Peak Area Ratio	Calc. Amt. (µg/ml)	Statistical Analysis
30	2.207	29.89		30	2.235	30.27	
30	2.218	30.04	Mean=29.99	30	2.229	30.18	Mean=30.13
30	2.211	29.94	SD=0.124	30	2.233	30.24	SD=0.143
30	2.226	30.14	% RSD=	30	2.203	29.83	% RSD=0.475
30	2.207	29.89	0.415	30	2.214	29.98	
30	2.219	30.05		30	2.236	30.28	

Table 5: Ruggedness data

Analyst-1				Analyst-2			
Conc. (µg/ml)	Peak Area Ratio	Calc. Amt. (µg/ml)	Statistical Analysis	Conc. (µg/ml)	Peak Area Ratio	Calc. Amt. (µg/ml)	Statistical Analysis
30	2.241	30.35	Mean=	30	2.221	30.08	
30	2.232	30.22	30.13	30	2.237	30.29	Mean=30.17
30	2.230	30.20	SD=0.132	30	2.238	30.31	SD=0.139
30	2.209	29.91	% RSD=	30	2.227	30.16	% RSD=0.462
30	2.204	29.85	0.439	30	2.201	29.81	
30	2.235	30.27		30	2.243	30.37	

In case of acidic, neutral, basic, photo, UV and thermal stress 100 µg/ml solutions were made and injected into the system. For oxidative stress, 60 µg/ml solutions was made and injected. The chromatograms of the drug and degradants under different stress conditions are given in the fig. 4. Individual degradation study results are elaborated from table 6-12 and overall summary of the results of the degradation study is given in table 13.

Table 6: Hydrolytic degradation in neutral condition of valsartan

S. No.	Reten. Time (min)	Area (Mv. s)	Height (Mv)	Area (%)	Height (%)	W05 (min)	Results
1	2.120	280.393	25.156	10.0	13.0	0.11	Drug Remaining=
2	2.173	68.824	4.843	2.5	2.5	0.16	85.85 µg/ml
3	2.863	83.632	3.474	3.7	1.8	0.42	%Degradation=
4	3.637	32.965	1.598	1.1	0.8	0.27	14.15%
5	3.937	1614.188	154.788	79.4	80.3	0.16	
6	4.870	95.254	3.020	3.3	1.6	0.29	
Total		2175.256	192.879	100	100	-	

Table 7: Degradation in acidic condition

S. No.	Reten. Time (min)	Area (Mv. s)	Height (Mv)	Area (%)	Height (%)	W05 (min)	Results
1	3.883	1544.773	140.349	81.6	84.9	0.16	Drug Remaining=
2	4.393	281.473	17.391	18.4	14.1	0.17	82.12 µg/ml
Total		1826.246	157.74	100	100	-	%Degradation= 17.88%

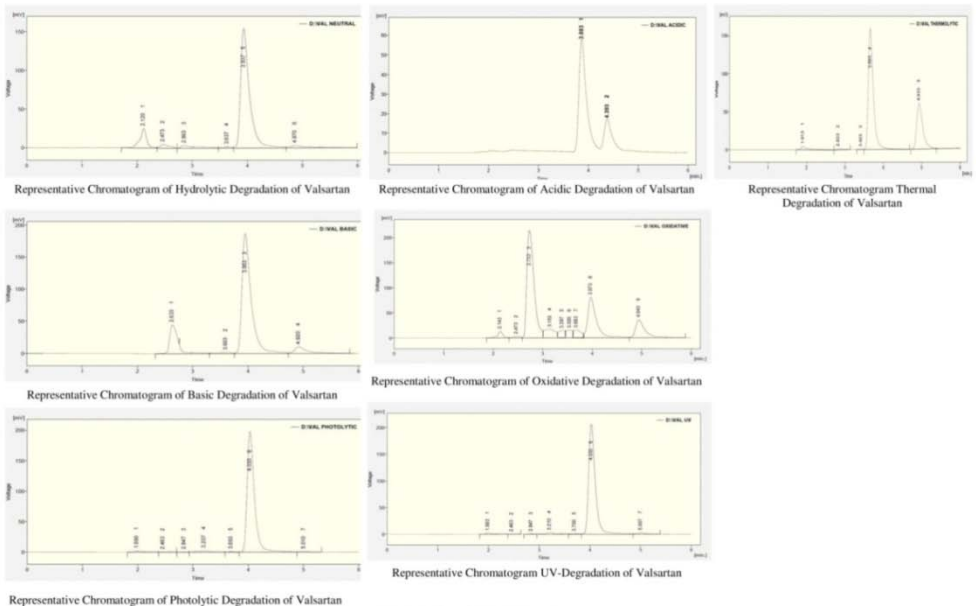

Fig. 4: Degradation chromatograms under different stress conditions

Table 8: Degradation in basic condition

S. No.	Reten. Time (min)	Area (Mv. s)	Height (Mv)	Area (%)	Height (%)	W05 (min)	Results
1	2.633	368.736	41.639	16.5	18.3	0.15	Drug Remaining=
2	3.603	35.599	1.663	1.3	0.7	0.45	74.16 µg/ml
3	3.953	1396.637	127.092	74.2	74.7	0.16	%Degradation=25.84
4	4.920	169.072	10.427	8.0	6.3	0.20	
Total		1907.044	180.821	100	100	-	

Table 9: Thermal degradation

S. No.	Reten. Time (min)	Area (Mv. s)	Height (Mv)	Area (%)	Height (%)	W05 (min)	Results
1	1.913	61.636	4.333	1.6	1.8	0.13	
2	2.833	11.999	1.120	0.5	0.5	0.24	Drug Remaining=
3	3.423	1.760	0.279	0.1	0.1	0.11	72.14µg/ml
4	3.663	1359.045	159.154	72.14	71.6	0.13	%Degradation=
5	4.933	567.501	49.941	25.66	26.0	0.16	27.86%
Total		2001.941	214.827	100	100	-	

Table 10: Oxidative degradation

S. No.	Reten. Time (min)	Area (Mv. s)	Height (Mv)	Area (%)	Height (%)	W05 (min)	Results
1	2.143	96.239	13.276	2.2	3.2	0.10	Drug Remaining=
2	2.473	34.131	3.271	0.8	0.8	0.19	49.88µg/ml
3	2.733	2175.281	214.507	53.8	50.1	0.16	%Degradation=
4	3.153	261.389	16.908	5.8	4.1	0.29	16.86%
5	3.397	132.315	15.605	3.0	3.8	0.16	
6	3.550	130.210	14.824	2.9	3.6	0.15	
7	3.683	170.121	15.579	3.8	3.8	0.22	
8	3.973	944.861	80.041	22.0	21.8	0.16	
9	4.943	380.859	31.115	5.7	8.8	0.18	
Total		4325.406	405.126	100	100	-	

Table 11: Photolytic degradation

S. No.	Reten. Time (min)	Area (Mv. s)	Height (Mv)	Area (%)	Height (%)	W05 (min)	Results
1	1.990	21.850	1.748	1.1	0.9	0.15	
2	2.463	7.106	0.629	0.4	0.3	0.18	Drug Remaining=
3	2.847	5.013	0.604	0.3	0.3	0.15	94.16µg/ml
4	3.207	38.440	2.696	2.0	1.3	0.15	%Degradation=
5	3.693	7.383	0.728	0.4	0.4	0.17	5.84%
6	4.033	1768.837	191.886	95.3	96.5	0.14	
7	5.010	10.525	0.846	0.6	0.4	0.20	
Total		1859.154	199.136	100	100	-	

Table 12: UV degradation

S. No.	Reten. Time (min)	Area (Mv. s)	Height (Mv)	Area (%)	Height (%)	W05 (min)	Results
1	1.993	24.852	2.215	1.2	1.0	0.15	
2	2.463	3.414	0.468	0.2	0.2	0.12	Drug Remaining=
3	2.847	8.294	0.868	0.4	0.4	0.18	98.44µg/ml
4	3.210	46.247	3.349	2.3	1.5	0.14	%Degradation=
5	3.700	11.733	1.162	0.6	0.5	0.17	1.56%
6	4.030	1848.488	206.241	93.7	95.0	0.13	
7	5.007	30.627	2.713	1.5	1.3	0.17	
Total		1973.657	217.016	100	100	-	

Table 13: Overall summary of degradation study

Stress condition	Stressing agents	Time (h)	Degradation
Neutral	WATER	6	14.15%
Acidic	0.1 N	6	Stable
	0.5 N	6	Stable
	1 N	6	17.88%
Basic	0.1 N	6	Stable
	0.5 N	6	Stable
	1 N	6	25.84%
Oxidation	1% H_2O_2	6	Stable
	2% H_2O_2	6	Stable
	3% H_2O_2	6	16.86%
Light	SUN LIGHT	12	Stable
		24	Stable
		72	5.84%
Thermal	70 °C HEAT	240	Stable
		480	Stable
		720	27.86%
UV Radiation	1.2×10^6 lux h (UV illumination at 256 nm)	12	Stable
		24	Stable
		72	1.56%

DISCUSSION

The developed RP-HPLC stability indicating assay method was found suitable for the analysis of drug in their pure form in presence of their respective degradants since the resolution between the drugs with its corresponding degradants was better. ADVANTAGE: Previously reported methods for analysis of valsartan in bulk are having retention times more than 4 minutes whereas in this method it is less than 4 minutes. More to that 99.94% accuracy and huge linearity range of 10-90 µg/ml with such a low LOD and LOQ values i.e. 0.261 and 0.791 µg/ml was not reported in earlier methods. Previously linearity was assessed in between 16-320 µg/ml range and LOD and LOQ values were also more than 10 µg/ml. The sensitivity and accuracy of the method were also ascertained by using internal standard telmisartan which is not reported earlier. With the use of the newly developed mobile phase and internal standard, further bio-analytical method can be developed. CONCLUSION: The results of stability studies of valsartan suggest that the drug is more prone to thermolytic, hydrolytic and oxidative degradations. So measures should be focused to reduce the exposure of valsartan to atmospheric conditions.

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CONFLICT OF INTERESTS

Declared None.

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