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METHOD DEVELOPMENT AND VALIDATION OF VALSARTAN IN BULK AND PHARMACEUTICAL DOSAGE FORMS BY ISOCRATIC RP- HPLC TECHNIQUE

Kishanta Kumar Pradhan ^{*1},

Uma Shankar Mishra¹, Subasini Pattanaik², Gourishyam Pasa¹, Kanhu charana Sahu¹, Debananda Mishra¹

¹Department of pharmaceutical analysis and quality assurance
Royal college of pharmacy and health sciences, Andhapasara Road, Berhampur

²Berhampur university, Bhanja bihar, Berhampur

ABSTRACT

A reverse phase high performance liquid chromatographic method (HPLC) has been developed for the method development validation of valsartan in bulk and pharmaceutical formulation using RP-C8 column. The mobile phase was Methanol: Acetonitrile: Distilled water (70:15:15) and P^H was adjusted to 3 by glacial acetic acid pumped at a flow rate of 1 ml/min and the eluents were monitored at 249nm. Linearity was obtained in the concentration range of 10-200 µg/ml. The retention time of valsartan was found to be 3.9 minute. The method was validated for specificity, accuracy, precision, linearity, and limit of detection, limit of quantification, robustness and solubility stability. LOD and LOQ were found to be 0.261 µg/ml and 0.791µg/ml respectively. The method was statistically validated and RSD was found to be less than 2% indicating high degree of accuracy and precision of the proposed HPLC method. Due to its simplicity, rapidness, high precision and accuracy, the proposed HPLC method may be used for determining Valsartan in bulk drug samples or in pharmaceutical dosage forms.

Correspondence to Author



Kishanta Kumar Pradhan

Royal College of Pharmacy and
Health Sciences Andhapasara
road, Berhampur Dist: Ganjam ,
Pin: 760002 Orissa, India

Email

kishantakumar@gmail.com

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INTRODUCTION

Valsartan is chemically 3-methyl-2-[pentanoyl-[4-[2-(2H-tetrazol-5-yl) phenyl] phenyl] methyl]amino] - butanoic acid (Fig. 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. Methods such as HPLC, LC-MS, Protein precipitation Capillary electrophoresis and Simultaneous UV spectrophotometric methods are reported for estimation of Valsartan alone or in combination with other agents. However, there were few methods reported for determination of valsartan individually. The focus of present study was to develop & validated a rapid, stable, & economic HPLC method for the estimation of valsartan in bulk and tablet dosage forms.

MATERIALS AND METHODS

Chemicals & Reagents

Valsartan API was purchased from Cadila Healthcare Ltd, Ahmedabad, India. Valsartan tablets, claimed to contain 320mg of Valsartan procured from Zydus Cadila Ltd, Ahmadabad, India. The HPLC grade solvent used were of Merck (India) Ltd, Mumbai. HPLC grade water prepared using Millipore System (Millipore, Molesheim France, Model Elix-10). All other reagents were of AR grade.

Instrumentation

Quantitative HPLC was performed on Shimadzu HPLC with LC-20AT pumps besides SPD-20A UV-Visible detector. Shimadzu spinchrom-CFR software is used along with C-18 (250 x 4.6 mm, packed with 5 μ) column for the separation.

Chromatographic Conditions:

For HPLC, mobile phase, Methanol:water:acetonitrile (70:15:15) and P^H 3 adjusted with glacial acetic acid was filtered and degassed. A reverse phase C-18 column was equilibrated with the mobile phase. Mobile phase flow rate was maintained at 1ml/min and eluents were monitored at 249nm. The samples were injected using a 20 μ l fixed loop. All determinations were performed at ambient temperature for a run time of 6 min.

Optimization

Selection of Mobile Phase

Selected Drugs were 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. The different mobile phases were containing one or the combinations of three of the following: Methanol (HPLC grade), Acetonitrile (HPLC grade), Dist. Water (HPLC grade) in the ratio 70:15:15 and P^H 3 adjusted by Glacial Acetic acid (HPLC Grade) solutions.

Preparation of Mobile Phase

Mobile phase was prepared by mixing 700 ml of HPLC grade methanol, with 150 ml of water of HPLC grade and 150ml of Acetonitrile to get the proportion of 70:15:15 v/v and finally the pH was adjusted to 3 with Acetic acid (0.2%v/v). The mobile phase was sonicated for 10 minutes and filtered through 0.45 μ membrane filter.

Preparation of Standard Drug Stock Solutions

The standard stock solutions of 100 μ g/ml of drugs were prepared by dissolving 50mg of pure drugs in mobile phase, in 50ml volumetric flask and the volume was made up to the mark. Resulting solutions were further diluted with mobile phase to obtain final concentration of 100 μ g/ml and sonicated for 10 minutes.

Calibration Curve

Aliquots of standard stock solutions of the drugs were taken in 10 ml volumetric flask and diluted up to the mark with mobile phase in such a way that final concentration of drugs were in the range of 10-100 μ g/ml. Triplicate injections of 20 μ l were made and chromatographed under the conditions as described above. Evaluation of drugs was performed and peak areas were recorded. Calibration curves were plotted by taking peak area on y-axis and respective concentration of drugs on x-axis.

Estimation of Drugs in Dosage Forms

Twenty tablets were taken, weighed and powdered. Powder equivalent to 10 mg of drugs were accurately weighed and transferred to 100 ml volumetric flask. The drug was extracted four times by adding solvent in portions, 20 ml each and then the volume was made up to mark with the same mobile

phase. The above solutions were filtered using Whatman filter paper No. 41. Appropriate volumes of the aliquots were transferred and diluted with mobile phase to get 30 µg/ml of drug concentration. The solutions were injected at above chromatographic conditions and peak areas were measured. The quantification was carried out by keeping these values to the straight line equation of calibration curve.

Validation

Accuracy:

The accuracy of the method was determined by calculating recoveries of drug by method of standard addition. 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.

Precision:

The intraday and Interday 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.

Specificity:

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:

Robustness of the method was studied by deliberate variations of the analytical parameters such as flow rate (0.5 ± 0.1 ml/min), concentration of methanol ($90 \pm 2\%$).

Ruggedness:

Ruggedness is the degree of reproducibility of the results obtained under a verity of conditions, expressed

Table 1: Optimized chromatographic conditions of Valsartan

Parameters	Conditions
Stationary phase(column)	Phenomenex ODS C-18(250×4.6 mm, packed with 5 micron)
Mobile Phase	Methanol: Acetonitrile: Water (75:15:15) p ^H Adjusted to 3 with Glacial acetic acid

as %RSD. These conditions include different laboratory conditions and different analysts.

Detection Limit and Quantification Limit:

Calibration curves were plotted by using concentration in the expected detection limit range of the drug. The standard deviation of y-intercept of regression line was determined and substituted in the following equation for the determination of detection limit and quantification limit. Detection limit = $3.3 \sigma/s$; quantification limit = $10 \sigma/s$; where σ is the standard deviation of y-intercept of regression line and s is the slope of the calibration curve.

RESULTS AND DISCUSSION

In the proposed method, the retention time of Valsartan was found to be 3.9 min. Quantification was linear in the concentration range of 10-200 µg/ml. The regression equation of the linearity plot of concentration of Valsartan over its peak area was found to be $y = 18.61x + 16.52$ ($r^2=0.999$), where X is the concentration of Valsartan (µg/ml) and Y is the corresponding peak area. The number of theoretical plates calculated was 4484, which indicates efficient performance of the column. The limit of detection and limit of quantification were found to be 0.261 µg/ml and 0.791 µg/ml respectively, which indicate the sensitivity of the method. The use of Methanol: Acetonitrile:Distilled water (70:15:15) and P^H was adjusted to 3 by glycial acetic acid in the ratio of 70:15:15 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.

Flow rate (ml/min)	01
Run time (minutes)	06
Column temperature(^o C)	Ambient
Volume of injection loop (μl)	20
Detection wave length (nm)	249
Drug R _t (min)	3.9
Assymetric factor	1.592
Theoretical plates	4484
Peak width	0.141
LOD (μg/ml)	0.261
LOQ (μg/ml)	0.791
Linearity (μg/ml)	10-200

Table 2: Calibration of Valsartan for the RP-HPLC Method

Conc. (μg/ml)	Peak Area	Statistical Analysis
10	204.335	Slope= 18.61 Intercept= 16.52 S.D.= 1.473 % R.S.D= 0.084
20	387.462	
30	584.547	
40	764.304	
50	950.573	
60	1137.311	
70	1316.981	
80	1501.931	
90	1684.35	
100	1867.397	
150	2798.415	
200	3754.691	

Table 3: Accuracy data of the RP-HPLC Method for Valsartan

Samples	Concentration (μg/ml)		% Recovery	Statistical Analysis
	Amount present in Formulation	Amount of drug added		
S1: 80%	30	24	99.8	Mean=99.9 SD=0.521 % RSD=0.522
S2: 80%	30	24	99.6	
S3: 80%	30	24	100.3	
S4: 100%	30	30	99.5	Mean=99.86 SD=0.566
S5: 100%	30	30	98.9	

S6: 100%	30	30	100.4	% RSD=0.652
S7: 120%	30	36	100.1	Mean=100.03 SD=0.487 % RSD=0.487
S8: 120%	30	36	99.7	
S9: 120%	30	36	100.3	

Table 4: Precision data Showing Repeatability of the RP-HPLC Method for Valsartan

Sl. No.	Concentration (µg/ml)	Peak Area	Calc. Amt. (µg/ml)	Statistical Analysis
1	30	586.116	30.607	Mean= 30.16 SD=0.195 % RSD=0.646
2	30	569.404	29.709	
3	30	579.491	30.251	
4	30	573.963	29.954	
5	30	573.219	29.914	
6	30	585.278	30.562	

Table 5: Intraday Precision data of the RP-HPLC Method for Valsartan

Sl. No.	Concentration (µg/ml)	Peak Area	Calc. Amt. (µg/ml)	Statistical Analysis
1	30	576.308	30.08	Mean=29.98 SD=0.169 % RSD=0.563
2	30	576.867	30.11	
3	30	570.911	29.79	
4	30	568.306	29.65	
5	30	576.494	30.09	
6	30	578.728	30.21	

Table 6: Interday Precision data of the RP-HPLC Method for Valsartan

Sl. No.	Concentration (µg/ml)	Day 1	Day 2	Day 3	Statistical Analysis
1	30	584.418	568.465	586.574	Mean= 30.171 SD=0.17 % RSD=0.563
2	30	582.367	573.254	576.821	
3	30	580.351	579.652	577.639	
4	30	571.516	570.325	577.482	
5	30	575.684	572.658	585.658	
6	30	581.628	580.567	579.281	
	Mean Peak Area	579.327	574.153	580.575	
	Calc.Amt. (µg/ml)	30.242	29.964	30.309	

Table 7: Ruggedness Data of the RP-HPLC Method by Different Analysts for Valsartan

Analyst-1				Analyst-2			
Conc. (µg/ml)	Peak Area	Calc. Amt. (µg/ml)	Statistical Analysis	Conc. (µg/ml)	Peak Area	Calc. Amt. (µg/ml)	Statistical Analysis
30	565.244	29.4	Mean=29.8 SD=0.183 % RSD=0.615	30	570.154	29.7	Mean=30.1 SD=0.163 % RSD=0.543
30	569.726	29.7		30	577.451	30.1	
30	578.351	30.1		30	583.135	30.4	
30	579.562	30.2		30	581.625	30.3	
30	564.486	29.4		30	567.435	29.6	
30	581.653	30.3		30	584.621	30.5	

Table 8: Robustness Data of the RP-HPLC Method at Different p^H for Valsartan

p ^H -2.8				p ^H -3.2			
Conc. (µg/ml)	Peak Area	Calc. Amt. (µg/ml)	Statistical Analysis	Conc. (µg/ml)	Peak Area	Calc. Amt. (µg/ml)	Statistical Analysis
30	571.324	29.8	Mean=29.9 SD=0.119 % RSD=0.398	30	580.403	30.3	Mean=30 SD=0.191 % RSD=0.641
30	568.457	29.6		30	578.513	30.2	
30	580.741	30.3		30	567.376	29.6	
30	584.625	30.5		30	582.415	30.4	
30	574.582	29.9		30	577.826	30.1	
30	569.965	29.7		30	563.654	29.4	

Table 9: Robustness Data of the RP-HPLC Method at Different Flow Rate for Valsartan

Flow Rate-0.8				Flow Rate-1.2			
Conc. (µg/ml)	Peak Area	Calc. Amt. (µg/ml)	Statistical Analysis	Conc. (µg/ml)	Peak Area	Calc. Amt. (µg/ml)	Statistical Analysis
30	587.445	30.6	Mean=30.3 SD=0.125 % RSD=0.451	30	590.116	30.8	Mean=30.5 SD=0.103 % RSD=0.337
30	589.627	30.8		30	586.419	30.6	
30	574.135	29.9		30	593.216	30.9	
30	569.895	29.7		30	580.356	30.2	
30	584.761	30.5		30	580.403	30.3	
30	580.325	30.3		30	587.847	30.7	

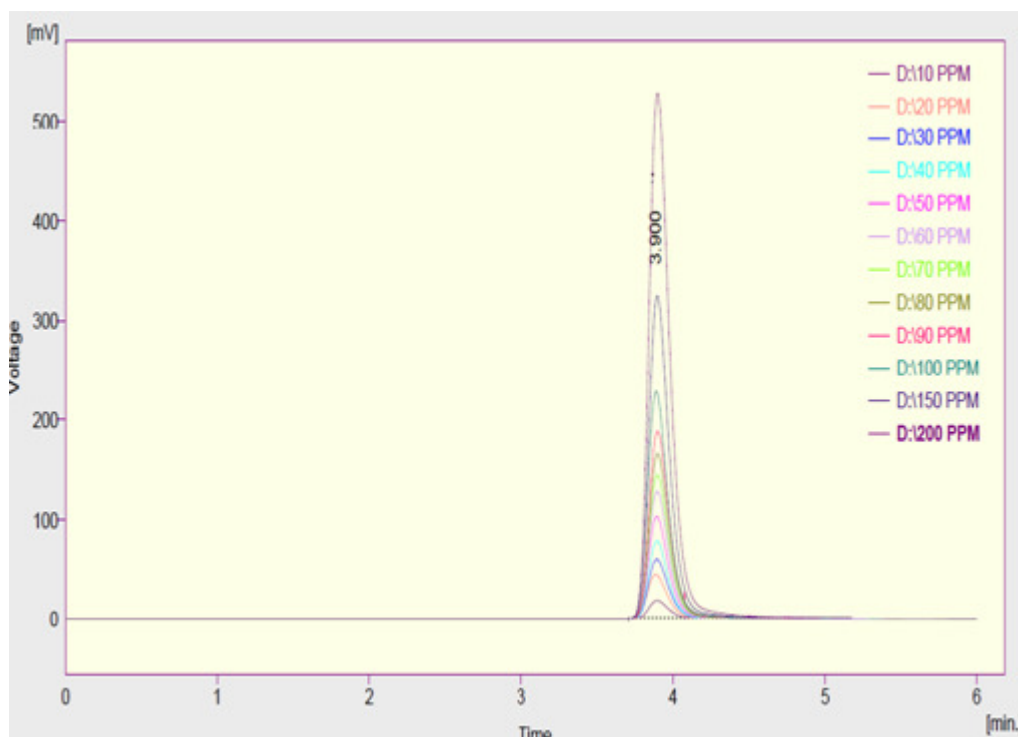


Fig.1: Typical HPLC Overlay chromatogram of Valsartan

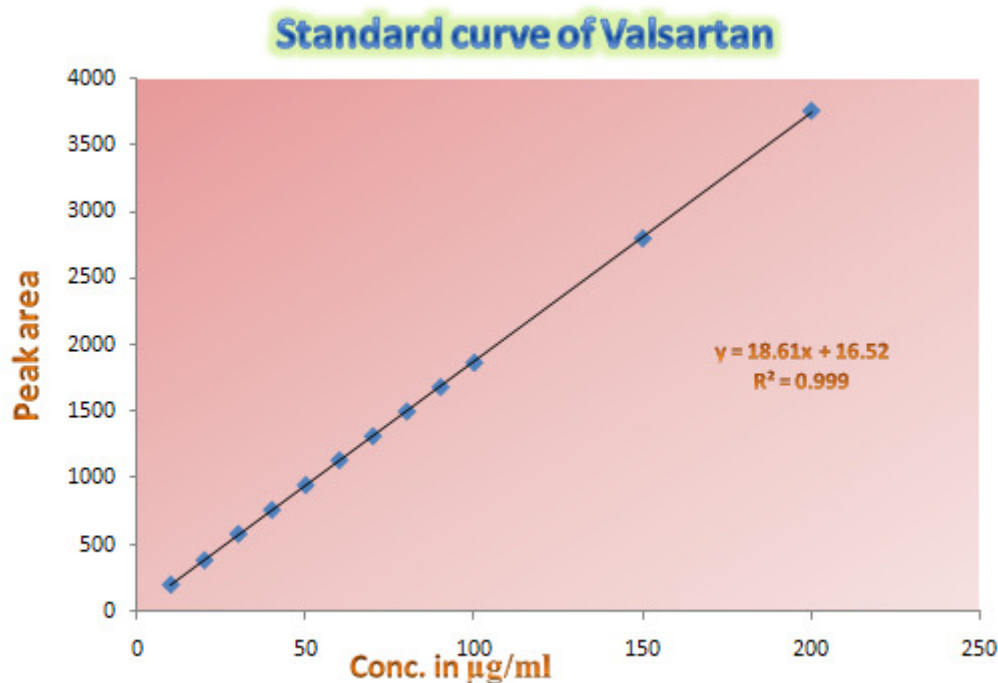


Fig.2: Calibration curve of Valsartan

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