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A new method development and validation of Valsartan by RP-HPLC in bulk and pharmaceutical dosage form

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ABSTRACT

Valsartan is an angiotensin-receptor blocker (ARB) that may be used to treat a variety of cardiac conditions. The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 229nm and the peak purity was excellent. Injection volume was selected to be 20µl which gave a good peak area. The column used for study was Inertsil C18. The percent recovery was found to be 98.0-101.50 was linear and precise over the same range. Both system and method precision was found to be accurate and well within range.

KEY WORDS: Valsartan, RP-HPLC, mobile phase.

1. INTRODUCTION

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 (AT1) subtype and prevents the blood pressure increasing effects of angiotensin II. Unlike angiotensin-converting enzyme (ACE) inhibitors, ARBs do not have the adverse effect of dry cough. Valsartan may be used to treat hypertension, isolated systolic hypertension, left ventricular hypertrophy and diabetic nephropathy. It may also be used as an alternative agent for the treatment of heart failure, systolic dysfunction, myocardial infarction and coronary artery disease.

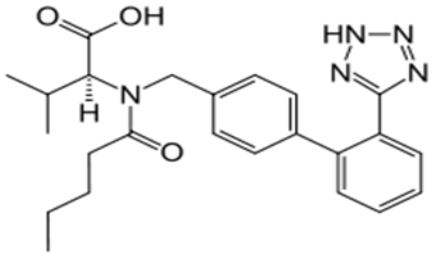

Figure.1: Structure of Valsartan

2. MATERIALS AND METHODS

2.1. Materials: HPLC – WATERS Model NO.486 series Compact System Consisting of Inertsil-C18 ODS column, acetonitrile HPLC Grade, Valsartan Working Standard.

Chromatographic Conditions:

Flow rate	1.0ml/min
Column	Inertsil - C18 ODS column
Detector wavelength	239 nm
Column temp	Ambient
Injection volume	20µl
Run time	10min

Trial: 1

Mobile Phase: 100% pure degaussed methanol.

Preparation of Standard Solution: 10mg of Valsartan drug was weighed and dissolved in 10ml of Mobile phase and taken in 10ml of volumetric flask and sonicated for 20 minutes to get 1000ppm and 1 ml was taken from this and diluted to 10 ml with mobile phase.

Observation: Theoretical plates are less, peak shape is not good and asymmetry is more than limit. Retention time was 6.49 min. The trial 1 chromatogram result was shown in Fig:1

Trail: 2.

Mobile Phase: methanol and Acetonitrile were mixed in the ratio of 90:10V/V and sonicated to degas.

Preparation of Standard Solution:10mg of Valsartan drug was weighed and dissolved in 10ml of Mobile phase and taken in 10ml of volumetric flask and sonicated for 20 minutes to get 1000ppm and 1 ml. was taken from this and diluted to 10ml.with mobile phase.

Observation: Got Base Line Noice, peak tailing occurs. Retention time was 2.193 min. The trial 2 chromatogram result was shown in Fig:2.

Trail: 3.

Mobile Phase: Methanol and Acetonitrile were mixed in the ratio of 80:20 v/v and sonicated to degas.

Preparation of Standard Solution: 10mg of Valsartan drug was weighed and dissolved in 10ml of Mobile phase and taken in 10ml of volumetric flask and sonicated for 20 minutes to get 1000ppm and 1 ml. was taken from this and diluted to 10ml.with mobile phase.

Observation: Got lesss retenton time and peak is spleated. Retention time was 2.199 min. The trial 3chromatogram result was shown in Fig:3

OPTIMIZED METHOD

Mobile Phase: Methanol:Buffer were taken and sonicated to degas in the ratio of 60:40.

Preparation of (KH_2PO_4 0.1M) Buffer: Weight 3.8954g of di-sodium hydrogen phosphate and 3.4023 of potassium dihydrogen phosphate in to a beaker containing 1000ml of distilled water and dissolve completely. Then pH is adjusted with orthophosphoric acid and then filtered through 0.45µm membrane filter.

Preparation of stock solution: 10mg of Valsartan drug was weighed and dissolved in 10ml of Mobile phase and taken in 10ml of volumetric flask and sonicated for 20 minutes to get 1000ppm and 2 ml. was taken from this and diluted to 10ml.with mobile phase.

Preparation of working standard solution: The stock solution equivalent to 20ppm to 80ppm were prepared, sonicated and filtered through 0.45µ membrane.

Preparation of sample drug solution for pharmaceutical formulations: Twenty tablets containing Valsartan of each marketed formulation were taken and powdered. The powder equivalent to 10 mg of the active ingredient was accurately weighed and taken in a 10ml volumetric flask containing 50 ml mobile phase and sonicated for 15 minutes and the solution was made up to volume with mobile phase and filtered through 0.45micron membrane.

Optimized chromatographic conditions:

Parameters	Method
Stationary phase (column)	Inertsil -ODS C_{18} (250 x 4.6 mm, packed with 5 micron)
Mobile Phase	Methanol:Buffer (60:40)
Flow rate (ml/min)	1.0 ml
Run time (minutes)	10
Column temperature (°C)	Ambient
Volume of injection loop (µl)	20
Detection wavelength (nm)	239 nm
Drug RT (min)	3.008

Calculation: The amount of drug present in each pharmaceutical formulation was calculated by using the standard calibration curves (concentration in ppm was taken on x-axis and peak area on y-axis). A typical chromatogram of Valsartan (100ppm) formulation was shown in Fig:5.

Method validation:

System suitability: A Standard solution was prepared by using Valsartan working standard as per test method and was injected Five times into the HPLC system. The system suitability parameters were evaluated from standard chromatograms by calculating the % RSD from five replicate injections for Valsartan**,** retention times and peak areas.

Acceptance criteria:

a. The % RSD for the retention times of principal peak from 5 replicate injections of each Standard solution should be not more than 2.0 %

b. The % RSD for the peak area responses of principal peak from 5 replicate injections of each standard Solution should be not more than 2.0%.

c. The number of theoretical plates (N) for the Valsartan peaks is NLT 3000.

d. The Tailing factor (T) for the Valsartan peaks is NMT 2.0

Observation: The %RSD for retention times and peak areas were found to be within the limit.

Specificity:

Valsartan identification: Solutions of standard and sample were prepared as per the test method are injected into chromatographic system.

Acceptance criteria: Chromatogram of standard and sample should be identical with near Retention time.

Observation: The chromatograms of Standard and Sample were same identical with same retention time.

Precision:

System precision: Standard solution prepared as per test method and injected five times.

Method precision: Prepared six sample preparations individually using single as per test method and injected each solution.
Acceptance criteria: The % relative standard deviation of individual Valsartan, from the six units should be not more than 2.0%. The assay of Valsartan should be not less than 98% and not more than 102.0%.
Observation: Test results are showing that the test method is precise. Refer tables 2 and 3 for system precision and for method precision.
Intermediate precision (analyst to analyst variability): A study was conducted by two analysts as per test method
Acceptance criteria: The individual assays of Valsartan should be not less than 98% and not more than 102% and %RSD of assay should be NMT2.0% by both analysts.
Observation: Individual %assays and %RSD of Assay are within limit and passes the intermediate precision.
Accuracy (recovery): A study of Accuracy was conducted. Drug Assay was performed in triplicate as per test method with equivalent amount of Valsartan into each volumetric flask for each spike level to get the concentration of Valsartan equivalent to 50%, 100%, and 150% of the labeled amount as per the test method. The average % recovery of Valsartan was calculated.
Acceptance criteria: The mean % recovery of the Valsartan at each spike level should be not less than 98.0% and not more than 102.0%.
Observation:

$$\%\text{Recovery} = \frac{\text{Amount found}}{\text{Amount added}} \times 100$$

The recovery results indicating that the test method has an acceptable level of accuracy.
Linearity of test method: A Series of solutions are prepared using Valsartan working standard at concentration levels from 20ppm to 80 ppm of target concentration .Measure the peak area response of solution at Level 1 and Level 6 six times and Level 2 to Level 5 two times.
Acceptance criteria: Correlation Coefficient should be not less than 0.9990.
% of y- Intercept should be ±2.0.
% of RSD for level 1 and Level 6 should be not more than 2.0%.
Observation: The linear fit of the system was illustrated graphically. The results are presented in table6.
Ruggedness of test method:
System to system variability: System to system variability study was conducted on different HPLC systems, under similar conditions at different times. Six samples were prepared and each was analyzed as per test method. Comparison of both the results obtained on two different HPLC systems, shows that the assay test method are rugged for System to system variability.
Acceptance criteria: The % relative standard deviation of Valsartan from the six sample preparations should be not more than 2.0%. The % assay of Valsartan should be between 98.0%-102.0%.
Observation: The % RSD was found within the limit. Ref tables: 3 &7.
Column to column variability: Column to column variability study was conducted by using different columns. Six samples were prepared and each was analysed as per test method
98.0% and 102.0%.
Observation: The results obtained by comparing with both two types were within limit. Refer tables: 3 &9
Acceptance criteria: The% RSD OF Valsartan tablets should be NMT2.0%. The %assay of Valsartan should be between
Robustness:
a) Effect of variation of flow rate: A study was conducted to determine the effect of variation in flow rate. Standard solution prepared as per the test method was injected into the HPLC system using flow rates, 1.0ml/min and 1.2ml/min. The system suitability parameters were evaluated and found to be within the limits for 1.0ml/min and 1.2ml/min flow. Valsartan was resolved from all other peaks and the retention times were comparable with those obtained for mobile phase having flow rates 1.0ml/min.
Acceptance criteria: The Tailing Factor of Valsartan standards should be NMT 2.0 for Variation in Flow.
Observation: The tailing factor for MF was found to be within the limits. As shown in table 10.
b) Effect of variation of temperature: A study was conducted to determine the effect of variation in temperature. Standard solution prepared as per the test method was injected into the HPLC system at 20ºC temperature. The system suitability parameters were evaluated and found to be within the limits for a temperature change of 20ºc. Similarly sample solution was chromatographed at 25ºC temperature. MFH were resolved from all other peaks and the retention times were comparable with those
Acceptance criteria:
The Tailing Factor of Valsartan standard and sample solutions should be NMT 2.0 for Variation in temperature.
Observation: The tailing factor for Valsartan is found to be within the limits.

Limit of detection and quantitation (LOD and LOQ): From the linearity data calculate the limit of detection and quantitation, using the following formula.

$$LOD = 3.3\ \sigma / S, \quad LOQ = 10\ \sigma / S$$

σ = standard deviation of the response, S = slope of the calibration curve of the analyte.

3. RESULTS AND DISCUSSION

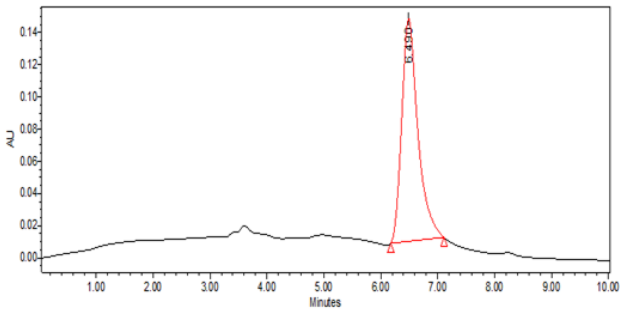

Fig.1.Chromatogram of Trial 1

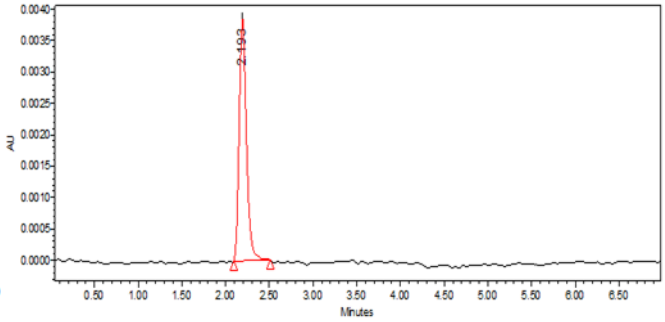

Fig.2.Chromatogram of Trial 2

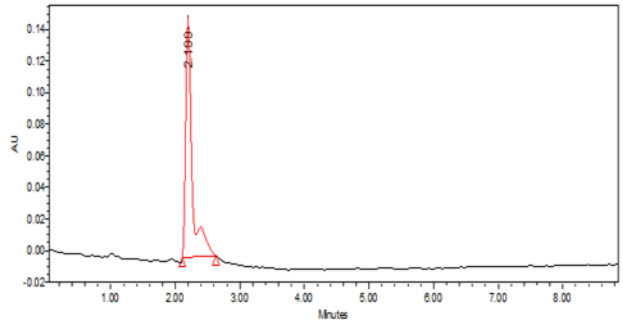

Fig.3.Chromatogram of Trial 3

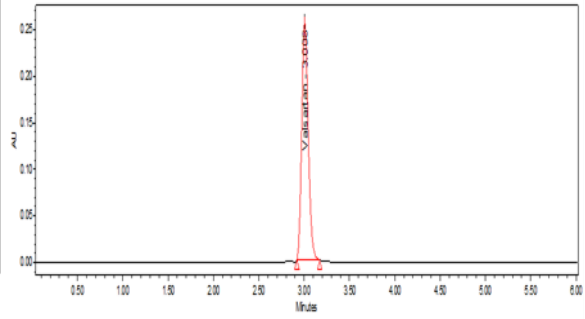

Fig.4.Chromatogram of Standard

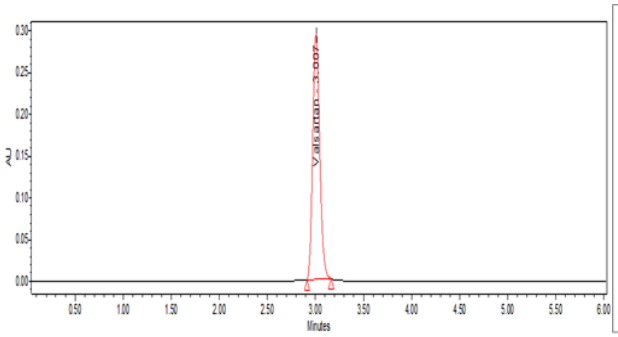

Fig.5.Chromatogram of Sample

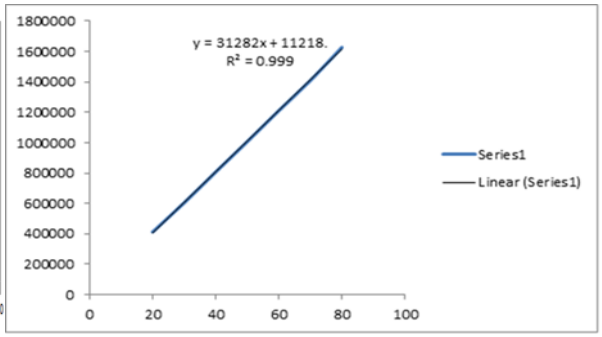

Figure.6.Linearity Plot (Concentration Vs Response)

Table.1.Data of System Suitability

Injection	RT	Peak Area	USP Plate count	USP Tailing
1	3.007	1139272	5890.964069	1.238915
2	3.000	1140892	5915.423628	1.230637
3	3.005	1136301	5934.796986	1.240858
4	3.004	1141067	5976.253744	1.238995
5	3.008	1136024	5953.814152	1.241073
Mean	3.00621	1138711	5934.251	1.236496
SD	0.000837	57540.015	-------	-------
% RSD	0.028363	0.213538	-------	-------

Table.2.Data of Repeatability (System precision)

Conc. 40ppm	Injection	Peak Areas of Valsartan	%Assay
	1	1146923	99.65
	2	1143596	99.08
	3	1158293	99.98
	4	1147283	100.04
	5	1152490	100.16
Statistical **Analysis**	**Mean**	1149717	99.78
	SD	5754.015	0.435569
	% RSD	0.500472	0.43652

Table.3.Data of Accuracy

Concentration % of spiked level	Amount added (ppm)	Amount found (ppm)	% Recovery	Statistical Analysis of % Recovery	
50% Injection 1	20	19.85	99.25	**MEAN**	99.88
50% Injection 2	20	19.96	99.80		
50% Injection 3	20	20.12	100.6	**%RSD**	0.67
100 % Injection 1	40	39.74	99.35	**MEAN**	99.81
100 % Injection 2	40	40.08	100.2		
100% Injection 3	40	40.24	100.6	**%RSD**	0.399
150% Injection 1	60	59.04	98.40	**MEAN**	99.19
150% Injection 2	60	59.62	99.36		
150% Injection 3	60	59.89	99.81	**%RSD**	0.72

4. CONCLUSION

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 229nm and the peak purity was excellent. Injection volume was selected to be 20µl which gave a good peak area. The column used for study was Inertsil C18 chosen good peak shape. Ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time. Different pH and ratios of mobile phase were studied, mobile phase with ratio of Methanol:Buffer (60:40) was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study. Methanol was selected because of maximum extraction sonication time was fixed to be 10min at which all the drug particles were completely soluble and showed good recovery. Run time was selected to be 6min because analyze gave peak around 3.008 and also to reduce the total run time.

The percent recovery was found to be 98.0-101.50 was linear and precise over the same range. Both system and method precision was found to be accurate and well within range. Detection limit was found to be 0.13. Linearity study was, correlation coefficient and curve fitting was found to be. The analytical method was found linearity over the range of 20-80ppm of the target concentration. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

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