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RP-HPLC Method for Determination of Valsartan in Tablet Dosage Form

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Abstract: A simple, rapid, sensitive, reverse phase isocratic RP-HPLC method was developed for determination of Valsartan in tablet dosage form. The method was carried out using Thermo-hypersil ODS column (150 mm × 4.6 mm i.d., 5 µm particle size) with mobile phase comprised of water: acetonitrile: glacial acetic acid (500:500:01). The flow rate was set at 1.0 ml/min and effluent was detected at 273nm. The retention time of valsartan was found to be 4.6 minute. The method was validated for specificity, accuracy, precision, linearity, limit of detection, limit of quantification, robustness and solubility stability. LOD and LOQ were found to be 2.72 µg/ml and 8.25 µg/ml respectively. The calibration curve was linear in the concentration range of 40-140 µg/ml with coefficient of correlation 0.9990. 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 form.
Key words: Valsartan, HPLC, Validation.

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 AT_1 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[1,2]. Methods such as HPLC [3-5], LC-MS [6-8], Protein precipitation [9], Capillary electrophoresis [10] and Simultaneous UV-spectrophotometric methods [11,12] 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 tablet dosage form.

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, Ahmedabad, India. The HPLC grade solvent used were of E-Merck (India) Ltd, Mumbai. HPLC grade water prepared using Millipore System (Millipore, Molesheim France, Model Elix-10). All other reagents were of AR grade.

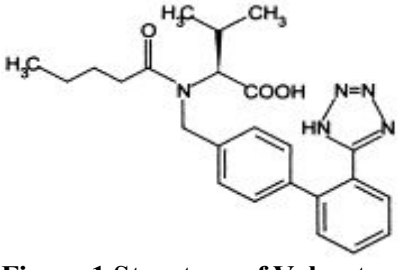


Figure 1 Structure of Valsartan

Instruments
The instrument was used Shimadzu LC 2010C integrated system equipped with quaternary gradient pump, 2010C UV-VIS detector in isocratic mode. The system was equipped with Millennium 32 Software. The analytical column used was Thermo-hypersil ODS(150 mm × 4.6 mm i.d., particle size 5 µm).

Chromatographic Conditions
For HPLC, mobile phase, water: acetonitrile: glacial acetic acid (500:500:01) was filtered and degassed. The injection volume was injected 20µl with a flow rate of 1.0ml/min. Detection was carried out at 273 nm at column temperature 25^0c and run time set at 10 minutes.

Assay Procedure:
Standard preparation
A stock solution of valsartan was prepared by accurately weighing 100 mg of drug, transferring to 100 ml volumetric flask, dissolving in 50 ml of diluent water:acetonitrile (50:50) and was sonnicated to dissolve. Make volume upto mark with diluent. Dilute 5.0 ml of this solution to 50ml with diluent to obtained final standard solution of 100 µg/ml of valsartan.

Sample Preparation
Weigh 20 tablets and calculate the average weight. Crush the tablets into a fine powder. Transfer an accurately weighed quantity of tablet powder equivalent to about 100 mg of valsartan into a 100 ml volumetric flask. Add about 50ml of diluent and sonnicate for 30 minutes with occasional shaking to dissolve. Make volume upto the mark with diluent and mix. Filter the solution through 0.45µm filter, collect the filtrate by discarding first few ml of the filtrate. Dilute 5.0 ml of this solution to 50 ml with diluent to obtained standard solution of 100 µg/ml.

Assay of valsartan
Standard and sample solution of valsartan was injected. Assay was performed as per given chromatographic conditions. The amount of valsartan present in the sample was computed from the linearity curve.

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

System suitability
System suitability of the method was evaluated by analyzing the repeatability, peaks symmetry (symmetry factor), theoretical plates of the column, peak area and retention time.

Range and Lineariry
To evaluate the linearity, serial dilution of analyte were prepared from the stock solution by taking suitable volume and diluted up to 50 ml to get the desired concentrations (40, 80, 100, 120 and 140 µg/ml) for linearity in the range of 40-140 µg/ml. The prepared solutions were filtered through 0.45 µm membrane filter and each of the dilutions was injected five times into the column. Absorbance at 273nm was measured and calibration curve for valsartan was constructed by plotting the mean peak area (Y-axis) against the concentration (X-axis).

Precision
The precision of method was investigated with respect to repeatability and ruggedness.
Method Precision (Repeatability):
Method precision for assay was established by determining the assay of six sample preparations under same conditions. Six replicates of valsartan sample solution was prepared at sample concentration by one analyst and analyzed on same day.
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.

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.

Specificity
Specificity is a procedure to detect quantitatively the analyte in the presence of component that may be expected to be present in the sample matrix. Commonly used excipients in tablet preparation were spiked in a pre weighed quantity of drugs and then absorbance was measured and calculations done to determine the quantity of the drugs.

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. Change in flow rate of mobile phase to 0.9 ml/min and 1.1ml/min (± 10%) or column oven temperature (± 5°C absolute) to 25°C and 35°C or organic phase ratio

of mobile phase by (±2% absolute) as Water: Acetonitrile (49:51), Water: Acetonitrile (51:49) and to observe their effect on system suitability.

Standard and sample solution stability

Standard and sample preparation was prepared as per test procedure and assay of standard and sample was determined as per method. Standard and sample solution was stored for 24 hours at room temperature. Assay of standard and sample solution was determined after different hours till 24 hours using freshly prepared standard. The assay obtained was compared with the initial assay value and recorded.

Detection and Quantification limit

Limit of detection and limit of quantification was calculated by the proposed method which was based on the standard deviation (σ) of the response and the slope (S) of the calibration curve at levels approximating the LOD and LOQ, LOD= 3.3 (σ/S) and LOQ= 10 (σ/S).

Table 1 Data derived from System suitability experiment for Valsartan

Sr. No.	Parameters	Valsartan
1.	Peak area	1425053
2.	Theoretical plates	4882.90
3.	Retention time (min)	4.63
4.	Asymmetry	1.11
5.	% RSD	0.05

Table 2 Data derived from linearity experiment of Valsartan.

Levels	ml Added	Diluted to	ppm	Absorbance
25%	2	200	5	0.159
50%	2	100	10	0.313
100%	2	50	20	0.620
125%	1	20	25	0.803
150%	3	50	30	0.921

Table 3 Data derived from Method Precision experiment of Valsartan.

Set	Test Wt.(mg)	Test Reading	mg/Tab	% Assay	Mean % Assay	% RSD
1	360.30	1446042	320.47	100.1	101.6	1.5
2	360.00	1442130	319.87	100.0		
3	360.00	1465730	325.11	101.6		
4	360.00	1500679	332.86	104.0		
5	360.10	1476739	327.46	102.3		
6	360.30	1466463	325.00	101.6		

Table 4 Data derived from Intermediate precision (Ruggedness) experiment of Valsartan.

Set	Test Wt.(mg)	Test Reading	mg/Tab	% Assay	Mean % Assay	% RSD
1	360.30	1431913	320.89	100.3	100.5	1.2
2	360.30	1469703	329.35	102.9		
3	360.20	1427392	319.96	100.0		
4	360.10	1426762	319.91	100.0		
5	360.10	1426638	319.88	100.0		
6	360.00	1426750	319.99	100.0		

Table 5 Data derived from Accuracy experiment of Valsartan

Levels	Sets	Area	mg Added	Mg Added (Actual)	Mg Recovered	% Recovery	Mean % Recovery	% RSD
50%	1	716155	50.20	50.13	50.29	100.3	100.2	0.3
50%	2	715299	50.10	50.03	50.23	100.4		
50%	3	712169	50.20	50.13	50.01	99.8		
100%	1	1414634	100.40	100.26	99.34	99.1	99.2	0.1
100%	2	1415360	100.30	100.16	99.39	99.2		
100%	3	1417169	100.40	100.26	99.52	99.3		
150%	1	2110922	150.10	149.89	148.24	98.9	99.0	0.1
150%	2	2116916	150.20	149.99	148.66	99.1		
150%	3	2114882	150.10	149.89	148.52	99.1		

Table 6 Data derived from Robustness experiment for Valsartan

Sets	System suitability	Temp. +°5c	Temp. -5°c	Flow Rate +10%	Flow Rate - 10%	Organic Solvent ratio - 2%	Organic Solvent ratio +2%
1	1426885	1424865	1423492	1588196	1293693	1413993	1416310
2	1426049	1425047	1423435	1589272	1293934	1414270	1416215
3	1426644	1425401	1423424	1588967	1294665	1414549	1416655
4	1426469	1424931	1423649	1588620	1295069	1414255	1416546
5	1427763	1425196	1424120	1589590	1294528	1414597	1416457
Mean	1426762	1425088	1423624	1588929	1294377	1414332	1416436
SD	637.43	215.46	291.46	545.16	558.73	245.97	176.93
%RSD	0.04	0.02	0.02	0.03	0.04	0.02	0.01

Table 7 Characteristics of the Analytical Method Derived from the Standard Calibration Curve.

Compound	LOD (µg/ml)	LOQ (µg/ml) n= (5)	Linearity (µg/ml)	Correlation co-efficient (r^2)	Residual std regression (σ)	Slop of Regression (S)
Valsartan	2.72	8.25	40-140	0.9999	11951.55	14490.14

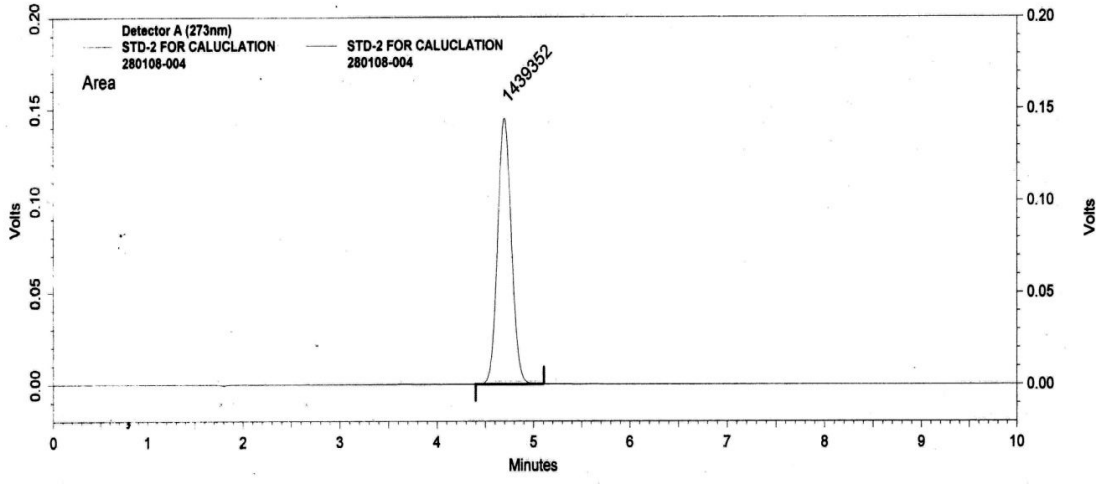


Figure 2 Chromatogram of Standard solution of Valsaran

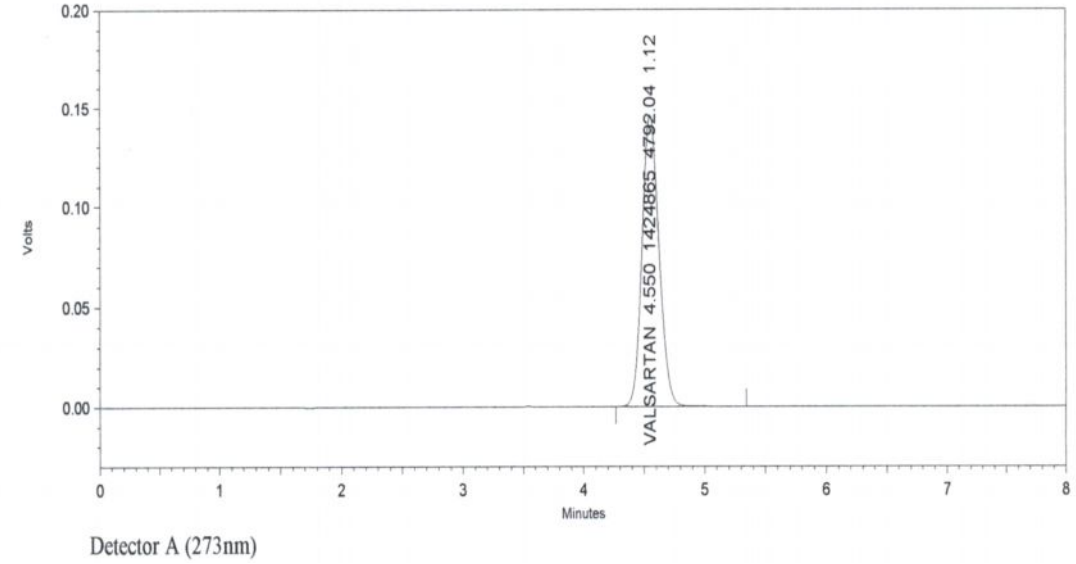
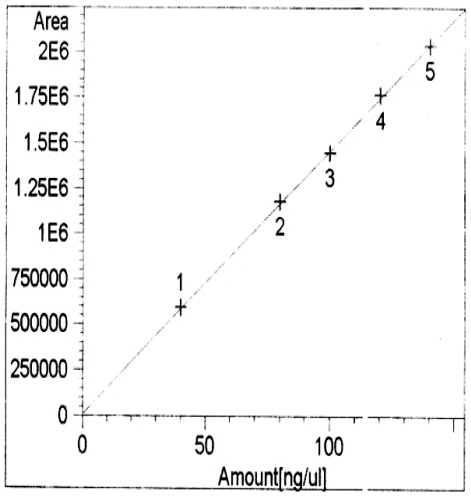


Figure 3 Chromatogram of Test solution of Valsaran



VALSARTAN at exp. RT: 8.928
VWD1 A, Wavelength=280 nm
Correlation: 0.99990
Residual Std. Dev.: 11951.55056
Formula: y = mx + b
m: 14490.13987
b: 6499.34314
x: Amount[ng/ul]
y: Area

Figure 4 Linear calibration curve for Valsartan

Result and Discussion:

Method Development

The aim of this study was to develop a simple, accurate and precise HPLC method for the analysis of valsartan in bulk and tablet dosage forms using mobile phase and commonly employed Thermo-hypersil ODS column with UV detector at 273 nm. The typical chromatogram of valsartan was shown in fig.2 and fig.3. The optimal retention time found to be 4.6 minute.

Method Validation

The described method has been validated for the assay of drugs in bulk drug and its dosage. The method was validated for the parameters like system suitability, specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision, ruggedness and robustness.[13,14].

System suitability testing

System suitability shall be performed and calculated at the start in study of each validation parameters. Results of system suitability obtained during the entire study were summarized in table-1. System suitability test are an integral part of chromatographic methods and reproducibility of the system are adequate for the analysis to be performed.

Linearity

The range of an analytical method is the interval between the upper and lower analytical concentration of a sample where the method has shown to demonstrate acceptable accuracy, precision, and linearity[15]. Linearity was studied by preparing standard solutions at different concentration levels. The linearity of an analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in samples within a given range. The linearity of the method was observed in the expected concentration range demonstrating its suitability for analysis. The calibration curve was carried out and found to be linear in the concentration range of 40-140 µg/ml (Fig.4). The result of linearity was given in Table 2.

Precision

Precision is a measure of the ability of the method to generate reproducible results. The precision of method was evaluated using repeatability and ruggedness. Repeatability refers to the use of the analytical procedure within a laboratory over a short period of time using the same analyst with the same equipment and is expressed as the % RSD. The results of method precision are shown in Table 3. The % RSD for method precision was found to be 1.5. The ruggedness of an analytical method is the degree of reproducibility of the test results obtained by the same samples under a variety of conditions, such as different laboratories, different analysts, different instruments, different lots of reagents and different days. The assay result indicated that the method was capable with high precision (Table.4). The result of % RSD (1.2 %) prove the ruggedness of developed method.

Accuracy

Accuracy of an analytical method is the closeness in agreement between the accepted true value or a reference value and the actual result obtained. Accuracy studies are usually evaluated by determining the recovery of a spiked sample of the analyte into the matrix of the sample to be analyzed[16]. The results of accuracy studies are shown in table 5. Results of recoveries and coefficient of variation (%RSD) indicate that the method is accurate within the desired range.

Specificity

Specificity is the ability of a method to discriminate between the analyte of interest and other components that are present in the sample[17,18]. The method demonstrated good separation between the peaks and was found to be free of interference. For demonstrating the specificity of the method for drug formulation, the drug was spiked, wherein the excipients used in different formulation products did not interfere with the drug peak and thus the method was specific for valsartan.

Robustness

This was done by small deliberate changes in the chromatographic conditions. The results of robustness study are summarized in table 6 indicate that the method was robust enough that the selected factors remained unaffected by small variations of these parameters.

Stability Studies

Stability of reagents, mobile phase, standard and sample solutions were studied for 48 hours and compared with the freshly prepared solutions and was found to be stable.

Detection & Quantification limit

The detection limit or LOD is the lowest amount of analyte in a sample that can be detected, but not necessarily quantitated. LOD was expressed as a concentration that gives a signal to noise ratio of 2:1 or 3:1. Quantitation limit or LOQ, on the other hand is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. LOQ is measured in terms of signal to noise ratio of 10:1.

LOD and LOQ were calculated by the equation given in ICH guidelines. This may be expressed as LOD = 3.3 σ /S and LOQ = 10 σ / S, where σ is the standard deviation of the response, S is the slope of the calibration curve which may be estimated from the calibration curve of the analyte[17,18]. The limit of detection and limit of quantification of the proposed method at 273 nm were found to be 2.72 µg/ml and 8.25 µg/ml respectively shown in table 7.

The proposed high-performance liquid chromatographic method has been evaluated as per ICH guidelines, Parameters such as linearity, precision, accuracy, LOD, LOQ, specificity and robustness are proved to be convenient for the quality control of valsartan in tablet dosage form. The proposed RP-HPLC method enables the determination of valsartan because of good separation of chromatographic peaks. The method can be used successfully for the analysis of valsartan in tablet dosage form.

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