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"DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHODS FOR ESTMATION OF VALSARTAN AND ITS DEGRADATION PRODUCTS"

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ABSTRACT

A simple, rapid, economic, sensitive and precise RP-HPLC method has been developed and validated for estimation of valsartan and its degradation product. The method was carried out using the gradient programmer and mobile phase was used Methanol and Water (85:15v/v), at a flow rate of 0.7ml/min, on HPLC system containing UV- visible detector with was carried out at 249 nm. The method gave the good resolution and suitable retention time. The method was validated in terms of specificity, linearity, precision, accuracy, specificity, limit of detection, limit of quantitation and robustness. Linearity of Valsartan was found to be 0.9996 in the range of 10 to 50µg/ml. The LOD and LOQ was found to be 0.6442µg/ml and 1.9522µg/ml. The method was also applied for the determination of Valsartan in the presence of their degradation products formed under variety of stress conditions, like acidic hydrolysis, basic hydrolysis, oxidation, photo and thermal degradation. The method was found to be suitable for the quality control of valsartan in bulk and pharmaceutical dosage forms as well as the stability-indicating studies.

KEYWORDS: Valsartan, RP-HPLC, Method Development, Stress Testing, Validation.

INTRODUCTION

Pharmaceutical analysis deals with qualitative and quantitative analysis of drug, dosage form and in biological samples. Analytical chemistry play an important role in resolution of chemical compound, determination of its elements or of the foreign substances. Analytical chemistry may be defined as the science and art of determining the composition of material in

terms of elements or compounds contents in it. Analytical chemistry divided in to two branches quantitative ans qualitative. A qualitative method is the information about the identity of atomic or molecular species or function group in sample. A quantitative method provides numerical information as to the relative amount of one or more of those components.

For the analysis of this drugs different analytical method are routinely being used in that one chromatographic technique

Chromatographic technique: Seperation process is used to decrease the complexity of material mixture. The most utilised seperation method is chromatography.

Mainly two types

1) High-performance liquid chromatographic technique.
2) High performance TLC chromatography.

Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice.

These terms are referred to as "analytical performance parameters", or sometimes as "analytical figures of merit". The ICH divided the "validation characteristic" somewhat differently.

Stability indicating assay method can be defined as the "validated quantitative analytical methods that can detect the changes with time in the chemical, physical or microbiological properties of the drug substance and drug product, and that are specific so that the contents of active ingredient, degradation products, and other components of interest can be accurately measured without interference.

This is employed for the analysis of stability samples in pharmaceutical industry. With the advent of International Conference on Harmonization (ICH) guidelines, the requirement of establishment of explicitly require conduct of forced decomposition studies under a variety of Conditions like pH, oxidation, dry heat, etc. and separation of drug from degradation products. The method is expected to allow analysis of individual degradation product.

VALSARTAN (figure 1) is Antihypertensive agent chemically (S)-3methyl-2(N-{[2-(2H-1,2,3,4-tetrazol-5-yl)biphenyl 4yl]methyl}pentanamido)butanoic acid.

valsartan blocks the vasoconstrictor and aldosterone-secreting effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT1 receptor in many tissues, such as vascular smooth muscle and the adrenal gland. Its action is therefore independent of the pathways for angiotensin II synthesis. Valsartan inhibits the pressor effect of angiotensin II infusions. An oral dose of 80 mg inhibits the pressor effect by about 80% at peak with approximately 30% inhibition persisting for 24 hours.

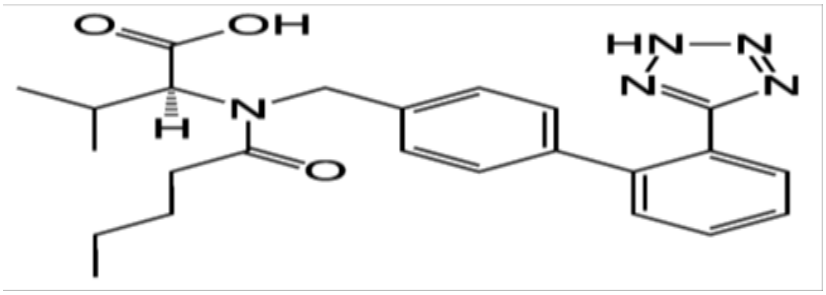

Fig.1 Structure of Valsartan

Literature reveals that there is no report on HPLC method of analysis for this new drug. Therefore, it was considered to develop a Robust method while subjecting the drug to various stress conditions.

MATERIAL AND METHODS

Pharmaceutical grade Valsartan was supplied as gift sample by Wockhardt research center, Aurangabad, India. and used without purification. The solvents used for extraction were of analytical grade. The HPLC Grade Chemical used were Methanol, Hydrochloric acid, Hydrogen peroxide, sodium hydroxide. All the reagent prepared by carbon dioxide free water and whereas the sample solution prepared in carbon dioxide free water double Distilled water for HPLC Purpose.

Apparatus: UV- Visible Double beam spectrophotometer *Shimadzu* (2450), along with two matched cuvettes was used. Stock solution of the samples were prepared in AR grade methanol and used for analysis. The HPLC system consisted of a pump (P-3000-M Reciprocating (40MPa) with manual Rheodyne injector facility operate at 20µl capacity per injection. The column used was C18 (Primsil)(4.6ID × 250mm; 5µm) for validation studies.

The detector consisted of UV/VIS (UV-3000-M) operated at 249nm. The data were acquired and processed by the use of Autochro -3000 software.

Optimized Chromatographic Conditions: Different mobile phase were tested in order to find the suitability for seperation of the drug and its degradation products. In view of solubility of the drug in methanol and also in phosphate buffer 6.8, initially the mobile phase containin atleast one of these was tried. The experimentation was started with 1:1 composition of methanol and water and tried at different levels of composition containing these solvents. The optimal condition of mobile phase was determined as methanol: water (80:15 v/v). The mobile phase was filtered through 0.45µm nylon filter, then sonicated for atleast 20 min. The injection volume was 20µl and mobile phase flow rate was mainatined at 0.7ml/min and Pressure:10-11Mpa.

Standard Stock Solution: Accurately weighted about 100 mg of Valsartan was taken in 100 ml clean dry volumetric flask and dissolved in methanol (HPLC grade) and volume was made upto 100 ml(1000µg/ml solution A). 0.2 ml of solution A was pipetted out in 10 ml volumetric flask and volume was made upto 10 ml (20µg/ml solution B) with mobile phase containing methanol: water (80:15 v/v). 20 µl of solution B was injeced in HPLC system. Corresponding peak was injected in HPLC system. Corresponding peak area was measured and respecive concentration was calculated from the calibration curve. A system suitability test was performed for six replicate standard injections.

Validation of RP-HPLC method for Valsartan in Bulk Drug

Calibration curve (Linearity and Range): Linearity was performed by diluting standard stock solution to give final concentration in the range of 10 to 50µg/ml for Valsartan 20µl of concentration injected and calibration curve was constructed by plotting the peak area versus the drug concentration to obtain the calibration graph.

Precision: Precision method was determined by analyzing standard drug in duplicate at different levels/day for consecutive six days and results were expressed as % RSD (25 µg/ml was taken as 100%).

Accuracy: The accuracy of the method was determined through the recovery test of the samples, using known amounts of valsartan reference standard. For the LC method, aliquots of 0.8, 1.0, and 1.2 ml of a valsartan standard solution (10µg/ml) were added to three sample

solutions containing a fixed amount of valsartan (10µg) in diluent, respectively. Therefore, this recovery study was performed at a final concentration solution of 8, 10 and 12µg/ml of valsartan. All solutions were prepared in triplicate and analyzed and results obtained were compared with expected results.

SPECIFICITY

Forced Degradation Study: Forced degradation studies were performed under different stress conditions on drug.

Acid Hydrolysis: An accurately weighed 10 mg. of pure drug was transferred to a clean & dry 10 ml volumetric flask. To which 0.1 M Hydrochloric acid was added & make up to the mark & kept for 24hrs from that 1 ml was taken in to a 10 ml volumetric flask & make up to the mark with methanol, then injected into the HPLC system against a blank of HCl & methanol.

Alkali Hydroysis: An accurately weighed 10 mg. of pure drug was transferred to a clean &dry 10 ml volumetric flask. To which 0.1 M Sodium hydroxide was added & make up to the mark & kept for 24 hrs. From that 1 ml was taken in to a 10 ml volumetric flask &make up to the mark with methanol and then injected into the HPLC system against a blank of NaOH and methanol.

Oxidation with (3%) H2O2: An accurately weighed 10 mg. of pure drug was transferred to a clean& dry 10 ml volumetric flask. To which 3% Hydrogen Peroxide was added, make up to the mark & kept for 24hrs from that 1 ml was taken in to a 10 ml volumetric flask &make up to the mark with methanol, then injected into the HPLC system against a blank of H2O2and methanol.

Photodegradation: Approximately 10 mg. of pure drug was taken in a clean & dry Petridis. It was kept in a UV cabinet at 254 nm wavelength for 24 hours without interruption. Accurately weighed 1 mg. of the UV exposed drug was transferred to a clean & dry 10 ml. volumetric flask. First the UV exposed drug was dissolved in methanol & make up to the mark then injected into the HPLC system against a blank of methanol.

Thermal degradation: An accurately weighed 10 mg. of pure drug was transferred to a clean & dry 100 ml volumetric flask, make up to the mark with methanol & was maintained at 500C for 24 hrs then injected into the HPLC system against a blank of methanol.

RESULT AND DISCUSSIONS

Optimization of chromatographic conditions: Several columns were used for optimizing the chromatographic condition. The parameters being focused were improvisation of retention time, seperation of degradation producs and column life. The symmetry C_{18}, Thermo C_{18} and Hypersil BDS C_{18} columns provides good peak shapes and no peak splitting was observed with any impurity.

Linearity and range: For linearity, all solutions were prepared in mobile phase. All solutions showed linear responses with concentrations level ranging from 10µg/ml-50µg/ml. The correlation coefficient value was found to be 0.999. The relative response factor was determined by slope method.(figure 2)

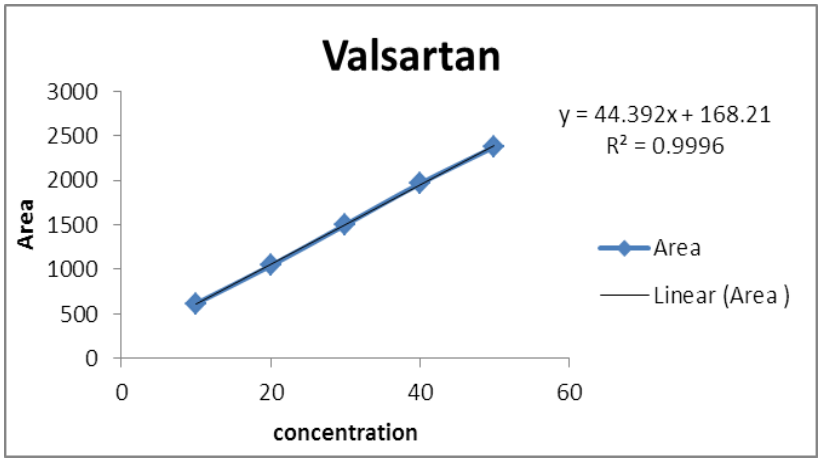

Fig.2 Calibration Curve indicating linearity of RP-HPLC response for drug concentrations.

Precision: The precision method was found to be precise after six replications for the quantification of Valsartan and %RSD was found to be less than 2.0%.

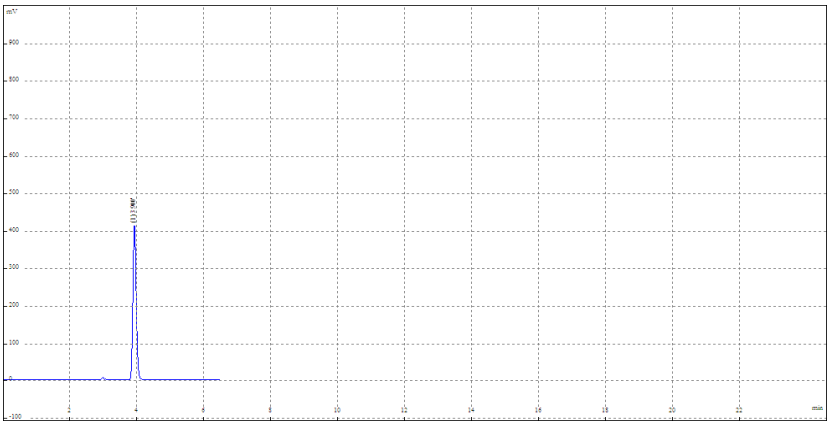

Fig.3 HPLC chromatogram of Valsartan (Standard drug)

Rank	Time (minute)	Area	Resolution	T. Plate	Asymmetry
1	3.900	2880418	0.00	8118	1.17

Table No.1 Repeatability of Valsartan

Sr. No.	Conc	Peak Area	Amt Found	% Amt Found
1.	40	1928.96	39.66	99.16
2.	40	1926.76	39.61	99.03
		Mean	39.64	99.09
		SD	0.04	0.10
		%RSD	0.09	0.10

Accuracy: The recovery of drug was determined by spiking drug at three different levels ranging from 80-120% of the label claim. The recovery range was found to be between 99-102% with RSD between 0.40% and 0.73%(Table no.3).

Table no.2 Result of Accuracy

Sr. No.	Level	Replicate	Recovery		
			% Recovery	Mean	%RSD
1.	Recovery-80%	1	100.57	100.29	0.40
		2	100.00		
2.	Recovery -100%	1	101.89	101.32	0.80
		2	100.74		
3.	Recovery-120%	1	100.91	100.40	0.73
		2	99.88		

Specificity: Forced degradation of drug was carried out as per the ICH guidelines (ICH Q2B) by subjecting to acidic, alkaline. with hydrogen peroxide, Thermal and Photolytic conditions. The acidic, alkaline, neutral and oxidative stress studies was carried out by refluxin drug for 24 hours with 0.1 N HCL(Figure 4.),0.1N NaOH(Figure.5), Hydrogen peroxide(figure 6) respectively. Under photo and thermal stability (fig.7 & 8), drug was found to be stable.

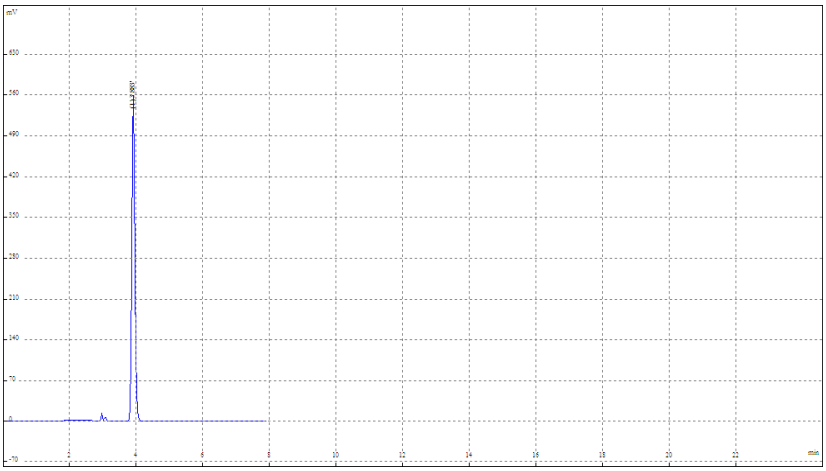

Fig 4. HPLC Chromatogram representing acid degradation of drug

Rank	Time (minute)	Area	Resolution	T. Plate	Asymmetry
1	3.883	2483731	0.00	9318	1.17

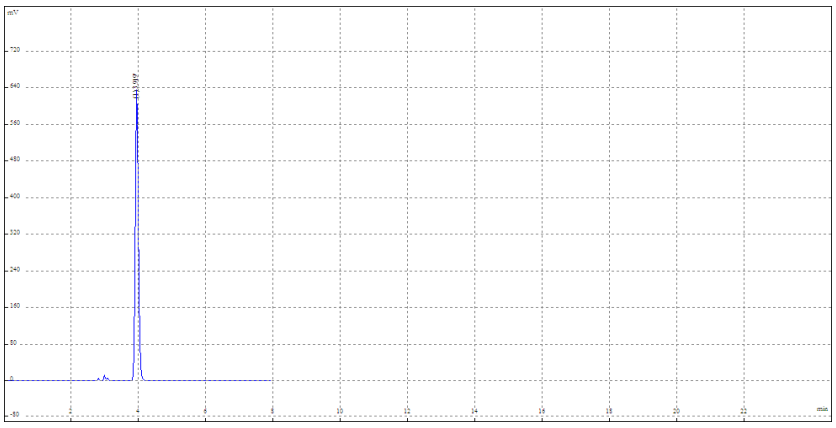

Fig 5. HPLC Chromatogram representing alkaline degradation of drug

Rank	Time (minute)	Area	Resolution	T. Plate	Asymmetry
1	3.919	4213231	0.00	9303	1.17

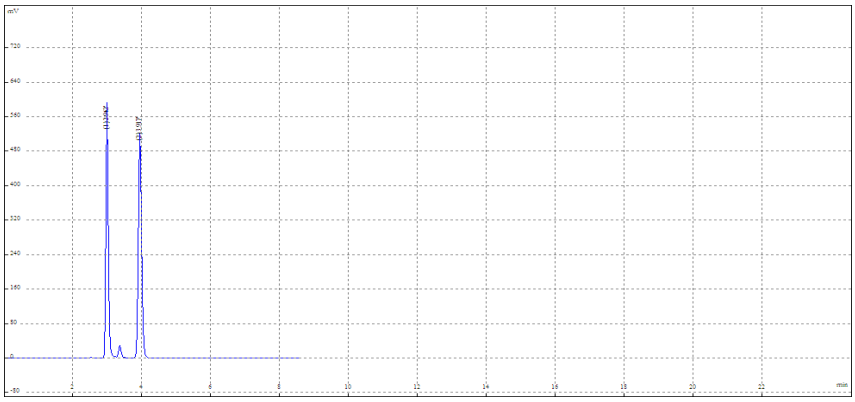

Fig 6. HPLC Chromatogram representing oxidation (H_20_2) degradation of drug

Rank	Time (minute)	Area	Resolution	T. Plate	Asymmetry
1	2.962	2523132	6.43	9395	1.28
2	3.917	2532963	0.00	8448	1.17

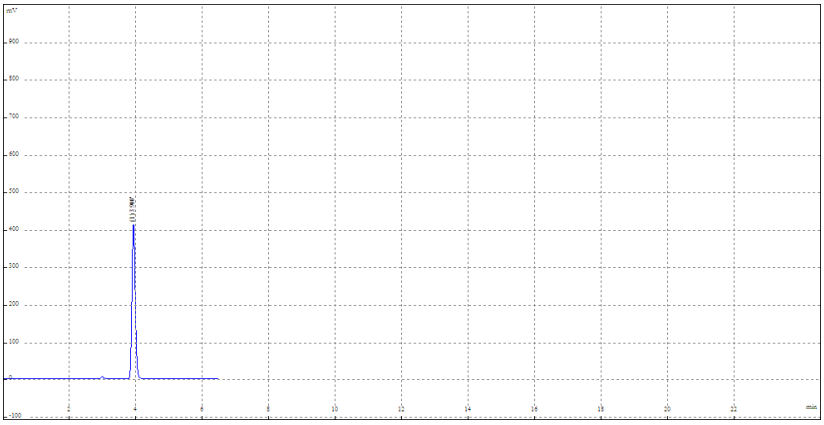

Fig 7. HPLC Chromatogram representing photolytic degradation of drug

Rank	Time (minute)	Area	Resolution	T. Plate	Asymmetry
1	3.900	2850213	0.00	8231	1.19

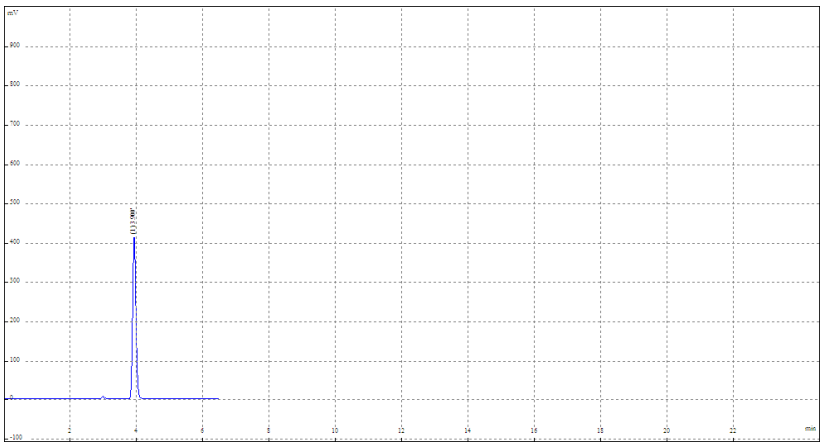

Fig. 8 HPLC Chromatogram representing Thermal degradation of drug

Rank	Time (minute)	Area	Resolution	T. Plate	Asymmetry
1	3.900	2840993	0.00	9132	1.16

Robustness: The robustness was investigated by varying the conditions with respect to change in flow rate(± 0.1 ml/min), mobile phase composition (± 2 ml) and using two allied columns other than regularly used column. The method was found to be robust with respect to flow rate, compositions and column with out any changes in system suitability parameters such as tailing,resolution and theoretical plate.

Valsartan was exposed to different humidity conditions as per ICH guidelines and found to be stable for 6 months.

CONCLUSION

On the basis of the above work it was concluded that the developed method of assay for candidate drug was validated as per the ICH guideline with following objectives

a) A simple, precise, accurate, robust, and economical analytical for assay of candidate drug had been developed by using RP-HPLC method.

b) The developed method has been validated as per ICH guidelines, and it meets all the accepted criteria given in ICH guideline.

c) Here on the basis of the results of forced degradation study it was proved that no any kind of degradation peak was merged that of the drug.

A simple, rapid, accurate, and precise stability-indicating HPLC analytical method has been developed and validated for the routine analysis of valsartan in API and tablet dosage forms.

The results of stress testing reveal that the method is selective and stability-indicating. The proposed method has the ability to separate the drug from their degradation products, related substances and excipients found in tablet dosage forms and can be applied to the analysis of samples obtained during accelerated stability experiments.

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