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ISOCRATIC RP-HPLC METHOD VALIDATION OF VALSARTAN IN PHARMACEUTICAL FORMULATION WITH STRESS TEST STABILITY EVALUATION OF DRUG SUBSTANCE

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ABSTRACT

A simple, rapid and accurate and stability indicating RP-HPLC method was developed for the determination of valsartan in pharmaceutical dosage forms. An isocratic RP-HPLC was achieved on Waters 2695 using Xterra C18 (150mm × 4.6mm × 5µm) column with the mobile phase consisting of potassium dihydrogen *ortho*-phosphate, pH adjusted to 3.0 using *ortho*-phosphoric acid (solvent A), and Acetonitrile (solvent B) in the ratio of 55:45 %v/v. The method showed a linear response for concentrations in the range of 10-50 µg/mL using Potassium dihydrogen phosphate (pH 3.0) buffer:Acetonitrile [55:45] as the mobile phase with detection at 286 nm and a flow rate of 0.7 mL min^{-1} and retention time 7.041 min. The method was statistically validated for accuracy, precision, linearity, robustness, forced degradation and selectivity. The proposed method was validated as per ICH guidelines. 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.

KEY WORDS: Valsartan, RP-HPLC, Degradation studies.

1.INTRODUCTION

Stability indicating methods have become an important aspect of any analytical method validation and a part of US FDA requirements (U.S.P.,2002). Valsartan is the second class of drug known as angiotensin receptor blockers (ARBs) which is chemically designated as *N*-(1-oxopentyl)-*N*-{[2'-(1*H*-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl}-*L*-valine (Budavri,1996). Valsartan is a new antihypertensive drug, which is non-peptide potent highly selective, orally active antagonist at the angiotensin II AT1-receptors. It is available as tablets for oral administration containing 40 mg, 80 mg, 160 mg or 320 mg of valsartan. The structure of the drug are shown in Fig.1.

According to current good manufacturing practices, all drugs must be tested with a stability-indicating assay method before release. Stress testing of the drug substance can help identify the likely degradation products, hence it is necessary to validate the stability-indicating power of the analytical procedures used. The nature of the stress testing will depend on the individual drug substance and the type of drug product involved. HPLC method of analysis that separates the drug from the degradation products formed under ICH suggested conditions (hydrolysis, oxidation, photolysis, and thermal stress) (ICH,2003) would be of general interest. A survey on literature reveals that a very few methods were developed for the estimation of valsartan in biological fluids and marketed formulations (Macek,2006; Daneshtalab,2002; Koseki,2007; Gonzalez,2002; Francotte,1996; Senthamil,2007). The literature has demonstrated that a stability-indicating LC method for determination of valsartan in the presence of its impurities and degradation products generated from forced decomposition studies was developed (Agrahari,2009). Nevertheless, in that reported study, the retention time of valsartan was obtained at 27 min. However, in present method the retention time of valsartan was achieved at 7.041 min. Thus, the aim of our study was to develop a simple, selective, economic, specific stability indicating the LC method that can be used to determine the related substances and also the assay of bulk samples of valsartan.

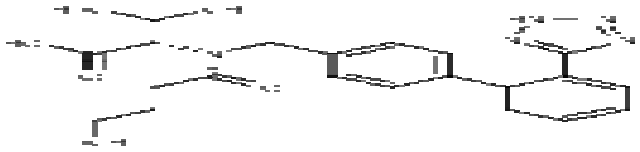

Figure 1.The chemical structure of Valsartan

2.EXPERIMENTAL

Materials and Reagents: Pharmaceutical grade working standards of valsartan (having potency 99.8% on as is basis) obtained as a gift sample from M/s Torrent Pharmaceutical Ltd., (Ahmedabad, India). Acetonitrile (HPLC grade) were obtained from Merck of analytical grade, Mumbai, India. All the other chemicals of analytical grade Potassium dihydrogen phosphate (AR grade), Orthophosphoric acid of HPLC grade from Spectrochemicals. All dilutions were performed in standard volumetric glassware. High pure water was prepared by using Millipore Milli Q plus purification system. Diovan® commercial formulations were purchased from the local market.

Instrumentation: The instrument used was a Waters HPLC, Empower software, Separation module (2695), dual wavelength absorbance detector (2487) as Diode array detector processed by an auto sampler and a rheodyne variable injector fitted with 20 µL volume sample loop. The output signal was monitored and processed using Empower software (designed by Waters Technologies, USA). The samples were injected through a micro liter syringe. Chromatographic separation was performed on XTerra® C-18 column (150 × 4.6mm i.d, 5 µm) column coupled with a guard column of the same material. The mobile phase was composed of potassium dihydrogen *ortho*-phosphate buffer:Acetonitrile (55:45 % V/V) and pH of mobile phase was adjusted to 3.0 ± 0.1 with 10% ortho phosphoric acid and degassed by filtering it under vacuum through a 0.45µm nylon filter. The flow rate of mobile phase through analytical column was 0.7 mL min^{-1}. The column temperature was maintained at 23 ± 1°C. The detection wavelength was set at 286 nm.

Chromatographic conditions: The chromatographic column used was Waters using XTerra® C18 (150mm × 4.6mm × 5µm). The mobile phase consists of a mixture of potassium dihydrogen *ortho*-phosphate, pH adjusted to 3.0 using *ortho*-phosphoric acid (solvent A), and acetonitrile (solvent B) in 55: 45 ratio. The mobile phase was pumped from the solvent reservoir to the column at a flow rate of 0.7 mL/min for 10 min. The column temperature was maintained at 23 ± 1°C. The eluate was monitored at 286 nm using the DAD. The injection volume was 20 µL. Mobile phase was used as diluent during the standard and test samples preparation.

Preparation of standard solutions: Stock solution of valsartan was prepared by dissolving 50 mg of valsartan in 50 mL of volumetric flask containing 20 mL mobile phase. The solution was sonicated for about 20 min and then made up to volume with mobile phase (Stock solution). The mobile phase standards containing valsartan was prepared by appropriately diluting the stocks to give final concentrations of 10-50µg mL^{-1}.

Preparation of sample solution for assay: Twenty tablets were weighed, finely powdered, and an accurately weighed sample of powdered tablets equivalent to 50 mg of valsartan was treated with 20mL of mobile phase in a 50 mL volumetric flask using ultra sonicator. This solution was filtered through 0.45 µm filter paper and made upto the mark with mobile phase. Further dilute the sample from above stock solution into a 10 mL volumetric flask and diluted upto the mark with mobile phase (final concentration of 30 µg mL^{-1}) and 20 µL of this solution was injected for HPLC analysis.

Quality Control Standards: The quality control (QC) standard for valsartan was prepared from stock solutions by dissolving 50 mg of valsartan in 50 mL of mobile phase. The working solutions of valsartan were prepared in the concentration ranges of low (10 µg mL^{-1}), medium (30 µg mL^{-1}), high (50 µg mL^{-1}) as target concentrations using mobile phase as a solvent.

Method Validation: The method was validated in terms of linearity, specificity, accuracy, and precision, limit of detection (LOD) and limit of quantitation (LOQ).

Linearity and Calibration standards: Five different concentrations of a mixture of all three drugs were prepared for linearity studies. The response was measured as peak area. The calibration curve obtained by plotting peak area against concentration showed linearity in the concentration range of 10-30 µg mL^{-1} of valsartan.

Precision: Intra-day precision was found out by carrying out the analysis of sample on five times on the same days. The standard drug solution containing 30 µg mL^{-1} of valsartan was injected into the chromatographic system, the peak area was noted and % RSD was calculated. Inter-day precision was found out by carrying out the analysis of sample on five different days. The standard drug solution containing 30 µg mL^{-1} of valsartan was injected into the chromatographic system, the peak area was noted and % RSD was calculated.

Recovery: The accuracy of the method was checked by spiking the sample with reference compound. 80%, 100% and 120% concentrations of valsartan were prepared with respect to target concentration.

Limit of Detection: The limit of detection is the lowest level of analyte such as valsartan of 0.06% concentration was prepared and that can be detected but not necessarily determined in a quantitative fashion, using a specific method under the required experimental conditions and it was calculated from signal-to-noise ratio method.

Limit of Quantification: The limit of quantification is the lowest concentration of analyte such as valsartan of 0.03% concentration was prepared and that can be determined with acceptable accuracy and precision when the required procedure is applied. It was calculated from signal-to-noise ratio method.

Forced Degradation Behaviour of Valsartan in API: In forced degradation studies all solutions were prepared by dissolving API of 10 mg valsartan in small volume of Acetonitrile in 5 different 10mL volumetric flasks and later add 2 mL of 3% aqueous hydrogen peroxide, 0.1M aqueous hydrochloric acid and 0.1M aqueous sodium hydroxide, it was subjected to forced degradation for about 12 hours. After the degradation these solutions were neutralized and diluted with mobile phase to obtain final concentration of 30 µg mL^{-1}. The no stress treatment sample (as control) has been evaluated relative to the standard concentration. In case of acidic stress the solutions were prepared in Acetonitrile and 0.1M hydrochloric acid (20:80 % V/V), in case of alkaline stress the solutions were prepared in Acetonitrile and 0.1M sodium hydroxide (20:80 % V/V), in case of peroxide stress the solutions were prepared in Acetonitrile and 30%

hydrogen peroxide (20:80 % V/V), in case of the thermal stress bulk powder of valsartan was exposed to 105°C and the resultant samples was kept aside for about 12 hours then the samples that exposed to stress conditions were neutralized, except thermal stress sample and diluted with mobile phase to obtain final concentration of 30 µg mL^{-1}, then the solutions were analyzed after five minutes of the preparation.

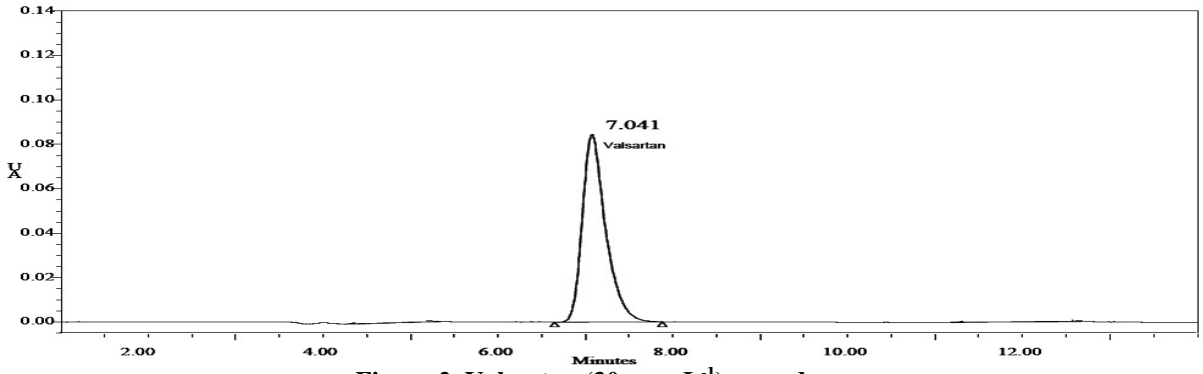

Figure 2. Valsartan (30 µg mL^{-1}) pure drug

3.RESULTS

Optimized Chromatographic Conditions: Spectroscopic analysis of valsartan showed that maximum UV absorbance (λ_{max}) at 286.0 nm. Therefore the chromatographic detection was performed at 286 nm using a UV detector. Chromatographic conditions were optimized by changing the mobile phase composition, by altering the pH of mobile phase a good separation was achieved. The optimized mobile phase was determined as a mixture of pH 3.0 buffer : Acetonitrile (55:45% V/V) at a flow rate of 0.7 mL min^{-1}. Under these conditions valsartan were eluted at a retention time of 7.041 minute respectively with a run time of 10 min. A typical chromatograms for valsartan obtained by using the afore mentioned mobile phase from 20 µL for the assay standard is illustrated in Fig. 2.

Method Validation

System suitability parameters: For system suitability parameters, three replicate injections of valsartan standard solution were injected and parameters such as the Tailing factor, Theoretical plate and Retention time of the peak were calculated. The results are shown in Table 1.

Linearity and Calibration standards: Five different concentrations of valsartan standards were prepared for linearity studies. The response was measured as peak area. The calibration curve obtained by plotting peak area against concentration showed linearity in the concentration range of 10-50 µg mL^{-1}. The best fit for the calibration curve could be achieved by a linear regression equation of valsartan found to be y = 79912.25 x + 29321.7 regression coefficient values (r^2) were found to be 0.9999 indicating a high degree of linearity for all drugs. Calibration results can be shown in Table 2.

Precision: Intra-day precision was ascertained by carrying out the analysis of the sample at a particular concentration five times on the same day. The sample was injected into the chromatographic system, peak areas were noted and the % relative standard deviation was 1.23% was found to be well within the limits indicating the sample repeatability of the method. Inter-day precision was found out by carrying out the analysis of sample on five different days. The sample was injected into the chromatographic system, the peak area was noted and % relative standard deviation was 0.52% was found to be well within the limits indicating the injection repeatability of the method.

Recovery: To check the recovery of the proposed method, recovery studies were carried out at 80%, 100% and 120% of the test concentration as per ICH guidelines. The recovery study was performed 3 times at each level, the method is accurate within the acceptance limit of 2%. The results of recovery study were found to be accurate and are given in Table 3.

Specificity: The specificity of the HPLC method was determined by complete separation of valsartan when it was subjected to forced degradation as per ICH guidelines which were carried out with 0.1M Hydrochloric acid, 0.1M Sodium hydroxide, 3% Hydrogen peroxide and Heat degradation at 105°C. The method does not permit detection of degradation products for valsartan when it was subjected to stress conditions as per ICH guidelines. The drug degrades as observed by the decreased area in the peak of the drug when compared with peak area of the same concentration of the non degraded drug at 0 hr (control), without giving any additional degradation peaks. However it showed stability towards all these stress conditions. Percent degradation was calculated by comparing the areas of the degraded peaks in each degradation condition with the corresponding areas of the peaks of three drugs under non degradation condition. The results of specificity data for degradation study are given in Table 5.

Robustness: The robustness of the method was determined as a measure of the analytical methods capability to be

unaffected by small variations in method parameters. The effect of these variations on the content of valsartan was determined. The different variations are as given below.

a. Variation in flow rates: The flow rate of the mobile phase varied by ± 0.1 mL min^{-1}.

b. Variation in composition: The composition of the organic phase component of Mobile phase was varied by ±5%.

c. Variation in pH: The pH of the mobile phase was varied by ± 0.1.

The robustness study indicated that the selected factors remained unaffected by small variations of flow rate, these were 0.60 mL min^{-1} and 0.80 mL min^{-1} and organic composition of the mobile phase were (50:50 %V/V) and (60:40 %V/V). The results of the analysis of the samples under the conditions of the above variations indicated that the method was robust.

LOD and LOQ: The LOD and LOQ were separately determined based on the S/N Ratio. For LOD the S/N ratio is 3:1 and for LOQ the ratio is 10:1. The limit of detection for valsartan was found to be 0.0180 µg mL^{-1} and the limit of quantitation (LOQ) for valsartan was found to be 0.1016 µg mL^{-1}.

Estimation of valsartan tablet formulation: The value of analysis of tablets obtained by the proposed method was 99.16% for valsartan which can shown in Table 4. This result showed that the estimation of dosage forms was accurate with the acceptance level of 95% to 105%.

Results of forced degradation studies: Intentional degradation was attempted to under different stress conditions to evaluate the ability of the proposed method to separate valsartan from its degradation products. Degradation was not observed in valsartan samples under stress condition like acid hydrolysis. However, mild degradation was observed when the drug was exposed to thermal exposure, oxidative and alkaline hydrolysis. The concentration of valsartan was more slightly decreasing with time in thermal and oxidative hydrolysis. This degradation is mainly observed in terms of loss of assay. Table 5 indicates the extent of degradation of valsartan under various stress conditions. Therefore, it may be concluded that valsartan is susceptible to degrade in oxidative and thermal conditions. Photodiode assay detection was used as an evidence of the specificity of the method and to evaluate the homogeneity of the drug peak.

DISCUSSION

The calibration curves obtained for each drug were linear over a wide range of concentrations. Both precision and accuracy at the LOQ, a low, medium and high concentration of valsartan was within acceptable limits. However the present study we applied our method for stability indicating valsartan in bulk. The peaks due to valsartan was found to be symmetrical and well defined. The total run time is 10 min. The optimum wavelength for detection was found to be 286 nm. The linearity of the calibration curves indicated the suitability of the method over a wide range of concentration of 10-50 µg mL^{-1}. The method was robust and the amounts obtained by the proposed method are between within the acceptance level of 95% to 105%.

Table 1. Data for System suitability of the method

Parameters	Valsartan
Tailing factor	1.2
Theoretical plates	3481.05
Resolution	---
%RSD	1.41
Limit of detection (LOD, µg mL^{-1})	0.0180
Limit of Quantification (LOQ, µg mL^{-1})	0.1016

Table 2. Data for Regression Analysis for Calibration Curves of Valsartan

Parameters	Valsartan
Linearity range(mg mL^{-1})	10-50 µg mL^{-1}
Correlation coefficient (r^2)	0.9999
Slope (m)	79912.25
Intercept (c)	29321.7

Table 3. Data for Recovery of Valsartan

Compound	Conc.(%)	Amount added pure drug(mg)	Amont found (µg mL^{-1})	% Recovery	Mean Recovery
3TC	80	64.0	63.79	99.68	99.56
	100	80.0	79.67	99.59	
	120	96.0	95.42	99.40	

*Average of three determinations

Tables 4. Data for Estimation of Valsartan in tablet formulation

Drug	Qty.claimed(mg/tablet)	Qty.found(mg/tablet)	% Qty.found
Valsartan	80	79.49	99.16

Table No 5. Data for forced degradation Study on Valsartan

Drug Name	Stress behaviour	Time(hrs)	Rt(min)	% Degradation	% of Active drug present after degradation
Valsartan	Control	12	7.041	---	---
	Acid hydrolysis	12	7.007	0.50789	99.49214
	Alkaline hydrolysis	12	6.775	3.735128	96.26487
	Thermal stress	12	6.3387	9.999983	90.00002
	Oxidative stress	12	6.5267	7.328535	92.67147

4.CONCLUSION

It can be concluded that the proposed method were developed and fully validated and it was found to be simple, accurate, precise, reproducible, and robust stability indicating RP - HPLC method to estimate the levels of valsartan considering the fact that the present method involves a shorter running time The results of stress testing undertaken according to the ICH guidelines revealed that the method is selective and stability-indicating. In addition it can be applied to routine quality control analysis for assay of other drugs in this class and its similar formulation studies.

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