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RP—HPLC method development and validation for the estimation of valsartan in tablet dosage form

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Abstract
A rapid, sensitive and simple RP-HPLC method was developed for the estimation of valsartan in tablet dosage form. The separation was carried out by using Agilent technology HPLC. The column used was C_{18} (4.6 ×1000mm) with methanol: 0.05% orthophosphoric acid as mobile phase (75:25%v/v) flow rate was 0.7ml/min. analyte was measured at wavelength of 239nm. The retention time for valsartan was found to be 3.62 min. the validation of selected method was carried out with respect to linearity, precision, accuracy, robustness and LOD & LOQ. Linearity studies were performed in the range of (5-25µg/ml). LOD and LOQ was found to be 0.060 and 0.1837 respectively. Relative standard deviation for accuracy was found to be less than two percent. Force degradation studies were carried out and drug was subjected to stress conditions of hydrolysis, oxidation, thermal degradation and photolysis.

Keywords: RP-HPLC, valsartan, methanol, Method development, validation, Forced degradation

1. Introduction

In drug discovery and development of pharmaceuticals RP-HPLC plays important role in analytical method development and validation. It also has ability to separate, identify and quantify the compounds present in sample which can be dissolved in liquid. There are various factors that are needed to be considered while developing a method [1]. The aim of analytical method development goes into validating the stability indicating HPLC method [2].

Stability testing is crucial part of process of drug product development. Stability testing provides information about how the quality of drug substance is affected with time and shows variation under the change in environmental factors such as light, temperature and recommends about the storage conditions and shelf life. The assay of API valsartan needs to be determined by stability u=indicating method as per ICH guidelines and USP [3, 4]. The separation of normal phase mode and reverse phase mode is adsorption. On introduction of mixture of components into the HPLC column they travel according to their affinity towards the stationary phase [5]. In normal phase chromatography the stationary phase is polar while mobile phase is non-polar hence nonpolar compounds travel through the column faster and gets eluted faster. The reason for it is lower affinity between non-polar compounds and stationary phase. Hence normal phase is generally not preferred in pharmaceutical applications [6, 7]. While in reverse phase the stationary phase is nonpolar and mobile phase as polar. The polar compounds elution is hence faster and non-polar compounds are retained for longer periods. As most of the drugs are found to be polar their elution is faster [8].

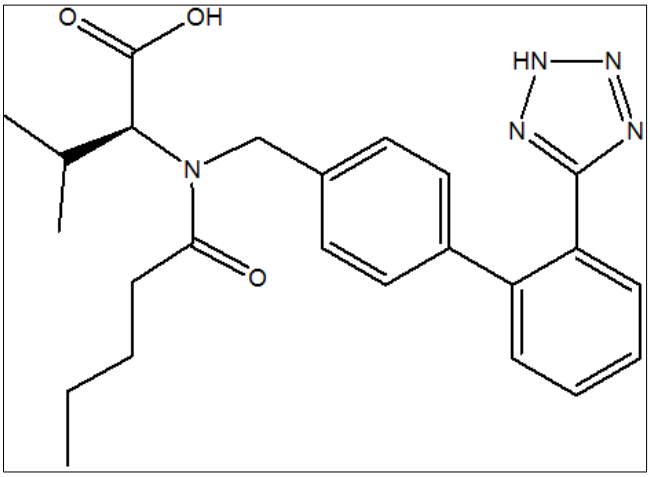

Fig 1: Structure of Valsartan

Valsartan is angiotensin-II receptor blocker, orally active, non-peptide acting on AT1 receptor subtypes. It is chemically N-(1-oxopentyl)-N-[[2'-(1*H*tetrazol- 5-yl) [1, 1'-biphenyl]-4-yl] methyl]-Lvaline (Fig. 1). Angiotensin-II is formed from

angiotensin-I the reaction is catalyzed by (Angiotensin converting enzyme II). As a pressor agent in renin angiotensin system that is responsible for vasoconstriction, release of aldosterone, stimulation of synthesis, renal sodium absorption and cardiac stimulation. Valsartan is found to block the vasoconstriction action of Angiotensin II As well as block the aldosterone secreting effects by selectively blocking binding of Angiotensin-II to the receptors. The literature reveals few spectroscopic HPLC [9-13] and LC-MS [14] methods were reported for determination of valsartan in bulk and pharmaceutical dosage form. Here we have reported rapid, sensitive and accurate method for estimation of valsartan in tablet dosage from.

2. Materials and methods

HPLC grade methanol was obtained from Merck (Darmstadt, Germany) and o-phosphoric acid was obtained from Valsartan was obtained from Sigma-Aldrich (St. Louis, MO). HPLC grade water was used. Solvents used for the study were previously filtered through 0.45µm membranes and were degassed prior to use. The sample was obtained from Swaroop Drugs and Pharmaceuticals, Aurangabad (MS).

HPLC and chromatographic conditions

Chromatographic analysis was performed using HPLC model 1100 Agilent tech gradient system with auto injector and detector UV (DAD). CHEMSTATION 10.1 software was used to control the gradient settings and data acquisition. A C18 reversed-phase column (4.6 mm × 1000 mm, 5 µm particle size) fortis was used. PH meter ultrasonic water bath. Peaks were determined at 239nm having 20µl injection volume.

Standard solution

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Preparation of standard solutions

For stock solution valsartan was taken the solution was sonicated and dissolved in 10ml of methanol in volumetric flask. From stock solution 5, 10, 15, 20 and 25µg/ml solutions were prepared [15-16].

Preparation of Standard Working Solution of valsartan

0.15 ml from standard stock solution was taken sonicated and dissolved in 10ml volumetric flask and volume was made upto mark using mobile phase.

Selection of wavelength

For proper wavelength selection valsartan was scanned between 200-400nm. From the overlay spectra wavelength 239nm was selected (Figure 2).

Fig 2: UV spectra of Valsartan (239 nm)

Optimization of Chromatographic Conditions

The chromatographic conditions were optimized by trials (Using different column, different buffer soultions and different mode of HPLC run)

System suitability parameters

Freshly prepared standard stock solution of valsartan was used under optimized chromatographic conditions and parameters were studied for evaluating suitability of system. (Table 1)

Table 1: System suitability parameter

Parameters	Valsartan
Retention Time	3.626
Theoretical Plates	6911
Asymmetry	0.76
Resolution	-

Assay of marketed formulation

Tablet weight equivalent to 20mg was weighed and transferred into 10ml volumetric flask. 10ml of methanol was added and sonicated for 15min in sonicator. The solution was subsequently filtered through whatman filter pape no 42. From this stock solution 0.15ml solution was taken and volume was made upto 10ml with mobile phase and assay was performed

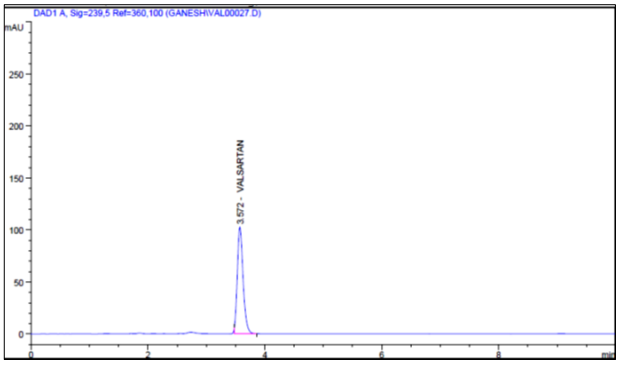

Fig 3: Chromatogram of assay

Table 2: Assay of marketed formulation

Concentration	Area	Label claim	Mean	SD	%RSD
15µg/ml	740.62	100.73	100.97	0.01	0.01

Method Validation

Validation of the method was carried out using ICH guidelines

1. Linearity

It is ability of obtain results which are directly proportional to the concentration of analyte present in given sample. The range of 5-25µg/ml was selected for linearity studies. The chromatogram is shown in figure2. The results of linearity are shown in table 3.

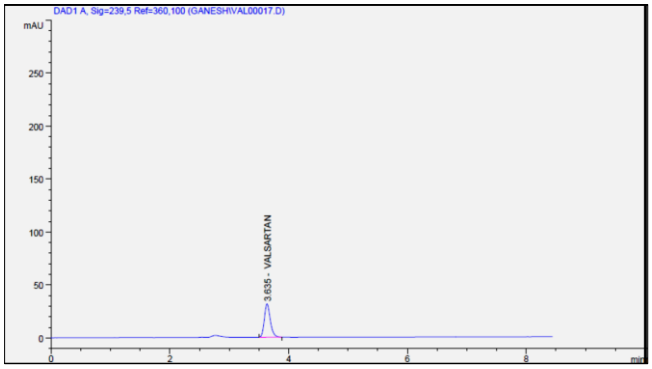

Fig 4: Chromatogram of linearity

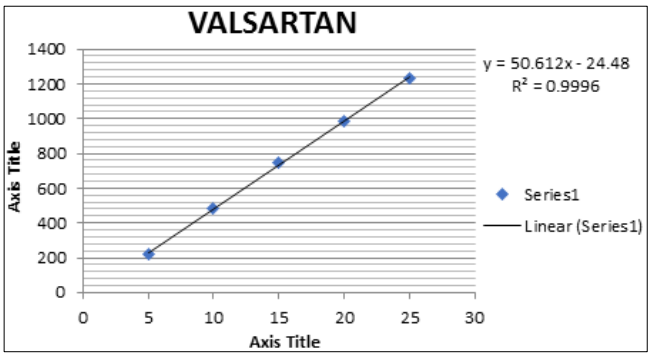

Fig.5: Standard Calibration Curve of Valsartan

Table 3: Results of Linearity

Sr No.	Concentration	Area I	Area II	Mean	SD	%RSD
1	5 (µg/ml)	219.05	217.61	218.33	1.02	0.47
2	10 (µg/ml)	487.12	485.23	486.175	1.34	0.27
3	15 (µg/ml)	746.43	745.26	745.845	0.83	0.11
4	20 (µg/ml)	987.37	988.42	987.895	0.74	0.08
5	25 (µg/ml)	1233.52	1234.56	1234.04	0.74	0.06
				Avg. SD	0.93	

2. Precision

According to ICH guidelines precision is analytical process the closeness of agreement between the series of measurement obtained from homogenous measurement of series of sample under prescribed conditions. The precision was demonstrated using repeatability, intra-day and inter-day studies. The results of precision are given in (Table 4, 5

Table 4: Results of Intraday precision

Sr. No.	Conc.	Area	II	Mean	Amt. Found	% Amt. Found	SD	RSD
1	10 (µg/ml)	485.66	487.35	486.51	10.09	100.90	1.20	0.25
2	15 (µg/ml)	746.2	745.69	745.95	15.22	101.48	0.36	0.05
3	20 (µg/ml)	991.37	990.45	990.91	20.06	100.31	0.65	0.07

Table 5: Results of Interday precision

Sr. No.	Conc.	Area	II	Mean	Amt. Found	% Amt Found	SD	RSD
1	10 (µg/ml)	486.25	487.97	487.11	10.10	101.00	1.22	0.25
2	15 (µg/ml)	744.23	739.56	741.90	15.14	100.93	3.30	0.45
3	20 (µg/ml)	993.37	988.53	990.95	20.06	100.30	3.42	0.35

3. Accuracy

Recovery studies were performed for evaluating whether the developed method was accurate for the analysis of valsartan. The recovery level of 80%, 100% and 120% were selected to perform recovery study. The results of accuracy study are given in (Table 6)

Table 6: Results of Accuracy

Level of % Recovery	Amount of tablet solution taken (µg/ml)	Amount of standard solution add (µg/ml)	Area	Amount found	Amount Recovery	%Recovery	%Mean Recovery ± S.D.
80%	10	8	884.93	17.96	7.96	99.61	99.39
	10	8	883.19	17.93	7.93	99.18	
100%	10	10	988.45	20.01	10.01	100.10	99.69
	10	10	984.1	19.92	9.92	99.28	
120%	10	12	1086.0	21.94	11.94	99.51	100.18
	10	12	1092.8	22.10	12.10	100.85	

Robustness

ICH guidelines defines robustness as analytical process carried out to confirm that analytical process remain unaffected by small variation in optimized process parameters. Change in flow rate, wavelength, composition of mobile phase were recorded. Results were obtained by calculating the %RSD of peak area for each different condition. The results of robustness are given in (Table 7).

Table 7: Result of Robustness

Flow Rate	Sr. no	Conc. (µg/ml)	Area	Mean	SD	%RSD
0.6ml/min	1.	15µl	843.89	844.91	1.44	0.17
	2.	15µl	845.93			
Mobile phase	Sr. no	Conc. (µg/ml)	Area	Mean	SD	%RSD
	1.	15µl	630.98	631.93	1.34	0.21
	2.	15µl	632.87			
Wavelength	Sr. no	Conc. (µg/ml)	Area	Mean	SD	%RSD
238	1.	15µl	748.59	749.4	1.08	0.14
		15µl	750.12			
240	2.	15µl	694.48	695.99	2.14	0.31
		15µl	697.51			

4. LOD and LOQ

The determination of limit of detection (LOD) and limit of quantitation (LOQ) was based on physical evaluation according to the standard derivation of responses and slope. limit of detection was found to be 0.0606 and limit of quantitation was found to be 0.1837.

Forced degradation studies

For carring out forced degradation studies the sample of API was subjected to various stress conditions like

A. Acid degradation
B. Alkaline degradation
C. Peroxide degradation
D. Thermal degradation

Acid degradation

0.4ml of solution was withdrawn from API stock solution to it 5ml of 0.1N HCL was added and volume was made upto 10ml with mobile phase. The sample solution was filtered through 0.45µ membrane syringe filter. To record the chromatogram the sample was injected in system. The degradation of drug sample in acidic condition was found to be 5.70. (Fig 6)

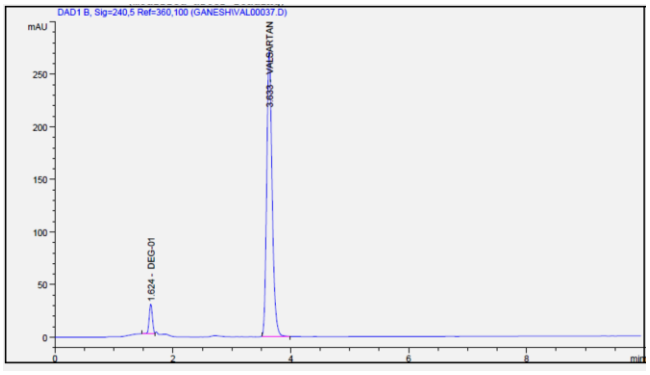

Fig.6: Chromatogram of acid Degradation of Sample

Alkaline degradation

0.4ml of solution was withdrawn from API stock solution to it 5ml of 0.1N NaOH was added and volume was made upto 10ml with mobile phase. The sample was subsequently filtered through 0.45µ membrane syringe filter. To record the chromatogram the sample was injected in system. The degradation of drug sample in acidic condition was found to be 8.9 % (Fig 7).

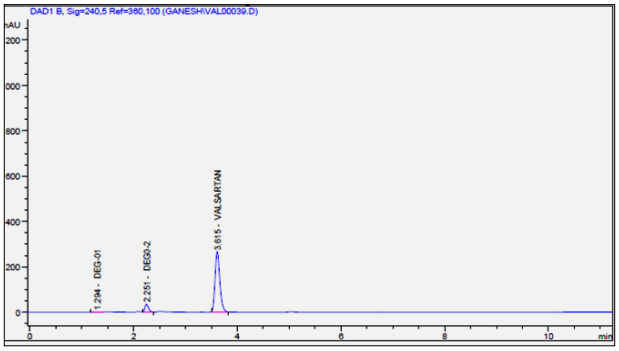

Fig.7: Chromatogram of Alkaline Degradation of Sample

Photo degradation

0.4ml of solution was withdrawn from API stock solution and volume was made upto 10ml with mobile phase. It was kept for 12hrs in sunlight. Sample solution was filtered and then was injected to record the chromatogram. The photo degradation of drug was found to be 2.33%. (Fig.8)

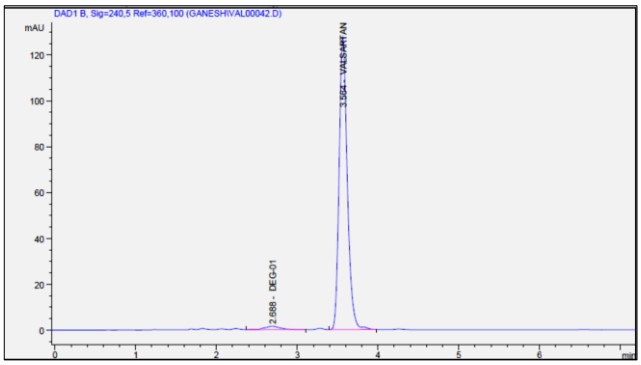

Fig.8: Chromatogram of Photo Degradation of Sample

Peroxide degradation

0.4ml of solution was withdrawn from API stock solution to it 5ml of 3% H_2O_2 was added and volume was made upto 10ml with mobile phase. The resultant solution was filtered injected into system and chromatogram was recorded. Degradation of drug sample under oxidative condition was found to be 7.7% (Fig 9).

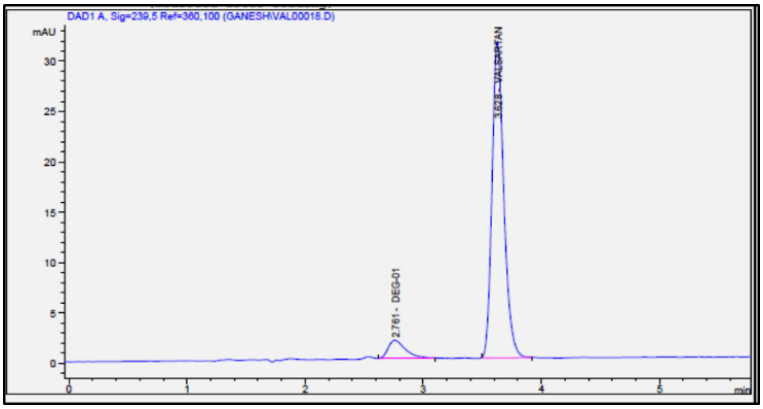

Fig 9: Chromatogram of Peroxide Degradation of Sample

Table 8: Summary of Validation parameter

Sr. No	Parameter	Result
1.	Linearity	5-25µg/ml R^2 =0.996
2.	System suitability test	TP= 6911
3.	Intra dayPrecision	0.12%
4.	Inter-day precision	0.35%
5.	Accuracy	99.75%
6.	Robustness	Change in Flowrate=0.17% Change in composition=0.21 Change in wavelength =
7.	LOD &LOQ	0.060, 0.1837
8.	Assay(%label claim)	100.97% %RSD=0.01

Table 9: Forced degradation studies

Degradation method	% Degradation
Acid	70
Base	8.9
Oxidation	7.7
Thermal	1.74
Photo	2.33

Conclusion

The research work based on stability indicating RP-HPLC method for the estimation of valsartan was successfully carried out. The developed method was found to be simple, accurate, precise, sensitive and rapid. The validation of developed method was done according to ICH guidelines. The method was found to be free from interference of any kind of excipients from formulation. The proposed method can be used for the routine analysis for estimation of valsartan in dosage form.

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