ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 10, Issue 12, 2017

Online - 2455-3891
Print - 0974-2441

Research Article

A SIMPLE REVERSE PHASE-HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY METHOD DEVELOPMENT AND VALIDATION OF VALSARTAN IN BULK AND IT'S TABLET DOSAGE FORM

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Received: 15 July 2017, Revised and Accepted: 15 September 2017

ABSTRACT

Objective: The most important objective of the present research work is to develop simple, specific, rapid, accurate, and sensitive reverse-phase high-performance liquid chromatography method and validated for the qualitative and quantitative determination of valsartan in its active pharmaceutical ingredient and tablet dosage form according to ICH guidelines.

Proposed Method: An isocratic separation was done using Phenomenex C_{18} column possess 75×4.6 mm, 2.6 µ,100 A^0 dimensions with mobile phase composition of water:acetonitrile (30:70% v/v) by maintaining 1 ml/minute flow rate and response detected at a wavelength of 247 nm.

Results: The retention time of valsartan was found to be 2.71 minutes, limit of detection and limit of quantification were observed at 1.24 µg/ml and 3.6 µg/ml concentration, respectively, and a calibration curve was linear in the concentration range of 5-50 µg/ml with coefficient of correlation 0.99. The percentage recovery (accuracy) was in the range of 98.9-102%, and the % relative standard deviation was observed to be <2%.

Conclusion: The proposed method was validated for accuracy, precision, sensitivity, linearity, and robustness and successfully employed for the quantitative determination of valsartan in tablet dosage form in quality control department of pharmaceutical industry.

Keywords: Reverse-phase-high-performance liquid chromatography, Retention time, Limit of detection, Limit of quantification, Robustness.

 DOI: http://dx.doi.org/10.22159/ajpcr.2017.v10i12.21312

INTRODUCTION

Valsartan (2S)-3-methyl-2-[pentanoyl-[[4-[2-(2H-tetrazol-5-yl) phenyl] phenyl] methyl] amino] butanoicacid (Fig. 1) is a angiotensin II receptor antagonist used in the treatment of hypertension. It works by relaxing the blood vessels. The blood vessels to lower blood pressure.

Literature survey revealed that various analytical methods have been used for the qualitative and quantitative estimation of valsartan in bulk and pharmaceutical dosage forms using ultraviolet (UV)-visible spectroscopy [1,2], high-performance thin-layer chromatography [3], and reverse phase-high-performance liquid chromatography (RP-HPLC) [4-7]. Extensive literature survey reveals that there is no RP-HPLC method with less retention time with simple mobile phase system was not reported for quantitative estimation of valsartan in bulk drugs and pharmaceutical dosage forms.

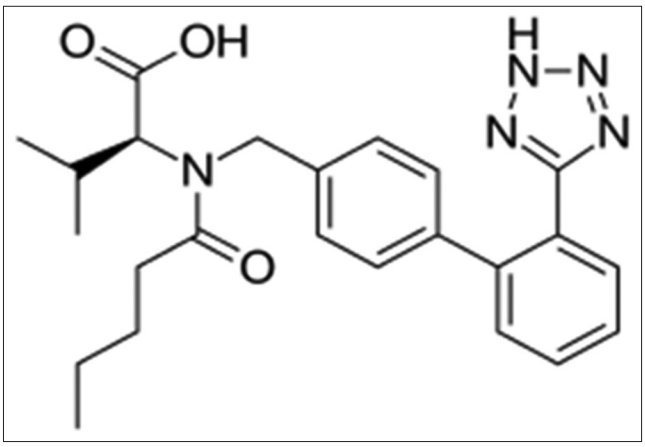

Fig. 1: Structure of valsartan

The objective of the present research work was to develop and validate simple, precise, and accurate analytical method with less retention time and simple solvent system for the estimation of valsartan in bulk and commercially available tablets in routine analysis. Chromatographic analysis is the most popular method for the analysis of most of the formulations; hence, a new RP- HPLC method was developed and validated for the estimation of valsartan.

METHODS

The valsartan reference standard (claim 99.18%) was provided by Aizant pharma. Tablets of valsartan (VALZAAR-40 mg) were purchased from a local pharmacy. HPLC grade acetonitrile was obtained from Merck India Limited, Mumbai, India. All the glass wares used were made of Borosilicate glass, and the solvents and prepared solutions were filtered through Nylon (0.45 µm) filters.

Chromatography

RP-HPLC method [8] was performed with Cyberlab HPLC equipment with UV detector and manual injector with a 20 µL loop. The equipment was connected to a multi-instrument data acquisition and data processing system (LC-solution software). The chromatographic system was performed using C_{18} (250 × 4.6 mm, 2.6 µ, 100 A^0) column. Separation was achieved using a mobile phase consisting of acetonitrile:water (70:30 v/v) at a flow rate of 1 ml/minute. The eluent was monitored using UV detection at a wavelength of 247 nm. The column temperature was maintained at 28°C, and the injection volume 10 µL was used. The mobile phase was filtered through a 0.45 µm nylon filter before use.

Determination of absorption maxima by UV-visible spectrophotometer

Weigh accurately 20 mg of valsartan, transfer into 100 ml of volumetric flasks, add 60ml of diluent, sonicate to dissolve, and make up to the volume with diluent. Pipette out 10 ml of this solution into 100 ml of volumetric flask dilute to the volume with diluents, concentration of the valsartan was found to be 20 µg/ml. The solution was scanned in the range of 200-400 nm, and from the spectrum, the λ_{max} was found to be 247 nm, resulted spectrum was shown in Fig. 2.

Preparation of standard solution

Weigh accurately 20 mg of valsartan and transfer into 100 ml of volumetric flasks, add 60 ml of diluent, sonicate to dissolve, and make up to the volume with diluent. Pipette out 10 ml of this solution into 100 ml of volumetric flask dilute to the volume with diluents, the concentration of the valsartan was found to be 20 µg/ml.

Preparation of sample solution

Weigh and transfer powder equivalent to 20 mg of valsartan into 100 ml volumetric flask, add 60 ml of diluent and sonicate for 15 minutes and diluted to the volume with diluent, filter the solution through 0.45 µm nylon filter. Pipette out 1 ml of this solution into 100 ml volumetric flask and diluted to the volume with diluent to get the concentration about 20 µg/ml.

Assay of the given sample preparation is calculated using following formula.

$$\text{Assay}\,\% = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{AVG\,Wt}{\text{Lable claim}} \times 100$$

Where:
AT = Peak area of obtained with test preparation
AS = Peak area of obtained with standard preparation
WS = Weight of working standard taken in mg
WT = Weight of sample taken in mg
DS = Dilution of standard solution
DT = Dilution of sample solution
P = Percentage purity of working standard.

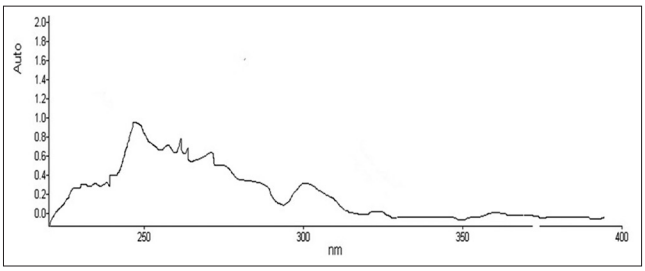

Fig. 2: Ultraviolet spectrum of valsartan showing absorption maxima of 247 nm

Method validation

Validation is establishing documented evidence, which provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality characteristics.

System suitability test

Test for system suitability was performed by injecting blank solution once and standard solution of 100% test concentration (prepared as per the assay method) for 6 times into HPLC system. The system suitability was established by evaluating the system suitability parameters from the chromatograms thus obtained. Typical system suitability parameters include % relative standard deviation (RSD), tailing factor (T), and theoretical plates (N).

Linearity

The linearity of an analytical method aims to elicit whether the test result is directly proportional to concentration. This is well understood by plotting a graph with peak area versus concentration. The linearity of the drug was established by constructing the calibration curve with a concentration on X-axis and absorbance on Y-axis over a concentration range of 5 µg/ml-50 µg/ml.

ACCURACY

The accuracy of the method was determined by performing recovery studies which were carried out by standard addition method at three different levels (80%, 100%, and 120%). A known amount of valasartan added separately to pre-analyzed samples, and percent recoveries were calculated at each level.

Precision

Intraday precision was determined by analyzing valsartan for 3 times in the same day (intraday). Interday precision was determined by analyzing valsartan daily for 3 days, and % RSD was calculated.

Sensitivity (limit of detection [LOD] and limit of quantification [LOQ])

LOD is defined as the lowest amount of an analyte that can reliably be differentiated from background levels. LOQ of an analytical procedure is the lowest amount of analyte that can be quantitatively determined with suitable precision and accuracy. LOD and LOQ were calculated using following equation as per the ICH guidelines.

$LOD = 3.3 \times \sigma / S$

$LOQ = 10 \times \sigma / S$

Where,

σ is the standard deviation of y-intercepts of regression lines

S is the slope of the calibration curve.

Table 1: Results of system suitability test for valsartan 20 µg/ml

Injection	Retention time (minute)	Peak area µV x seconds	USP plate count (N)	Acceptance limit (N)	USP tailing factor (T)	Acceptance limit (T)
1	2.71	13235	6532	N should be >2000	1.12	T should be ≤2
2	2.7	12960	5532		1.08	
3	2.71	12801	6209		1.12	
4	2.69	13506	6461		1.1	
5	2.701	13102	5087		1.12	
6	2.72	12932	5913		1.04	
Mean*		13089.3	5955		1.09	
SD	252.8					
% RSD	1.9					

*Average of six determinations. RSD: Relative standard deviation, SD: Standard deviation

Table 2: Peak areas of linearity standard solution of valsartan

Concentration (µg/ml)	Peak area
5	2903
10	6872
20	13235
30	19471
40	26312
50	32230

Robustness

Robustness of an analytical procedure was performed by slightly changing the mobile phase composition and flow rate.

RESULTS

Initially, the solubility of valsartan was checked in various solvents. The drug was found to be soluble in methanol, acetonitrile, and water. From the UV spectrum, it was observed that maximum absorbance of valsartan was shown at 247 nm.

Method optimization

Optimization of the chromatographic conditions was carried out by running several trials to obtain retention time, peak symmetry, plate count, and relative standard deviation within the limits and possible optimal. After several trials, a method using mobile phase composition of acetonitrile and water in the ratio of 70:30 at a flow rate of 1 ml/minute, on Phenomenex C_{18} (250×4.6 mm, 2.6 µ,100 A^0) column at 247 nm, was found to be the most suitable and acceptable. The optimized method resulted in chromatogram with valsartan eluting at 2.71 minutes (Fig. 3) with a tailing factor of 1.09 and USP plate count of 5955.

Method validation

The proposed method was validated according to Q2 specifications of the ICH guidelines [9].

System suitability

The system suitability was established by assessing the system suitability parameters from the chromatograms thus obtained. Typical system suitability parameters include % RSD, tailing factor (T), and theoretical plates (N). All the parameters measured values (Table 1) were satisfying the acceptance criteria.

Linearity

The linearity of detector response was determined by preparing a series of solution of the working standards over the range of 5-50 µg/ml of concentration. These solutions were injected onto the chromatographic system, and response area was recorded. A calibration curve was constructed by plotting area against concentration, and regression equation was computed. R^2 value of linearity curve was 0.999, plots with values were shown in Fig. 4 and Table 2.

Accuracy

The percentage recovery of drug from the spiked sample solutions was found to be in the range of 98.3-102% (Table 3) which indicates that the accuracy of the proposed analytical method was within the acceptance criteria of the ICH guidelines.

Precision

Variation of results of the concentration (20 µg/ml) within the same day (intraday) and variation of results concentrations (5-50 µg/ml) between days (inter-day) were analyzed. % RSD of peak area values of valsartan working standard solutions was found to be in the range of 1.8% and 0.50-1.57%, for intra- and inter-day precision, respectively. The low values of (≤2) these statistical parameters represent the method with good precision (Tables 4 and 5).

Sensitivity and robustness

LOD and LOQ were found to be 1.24 µg/ml and 3.6 µg/ml, which indicate the method has good sensitivity. The % RSD values were below 2.0% by changing the parameters such as flow rate (±0.1 ml) and mobile phase composition ratio (±1), and hence, the method was said to be robust (Table 6).

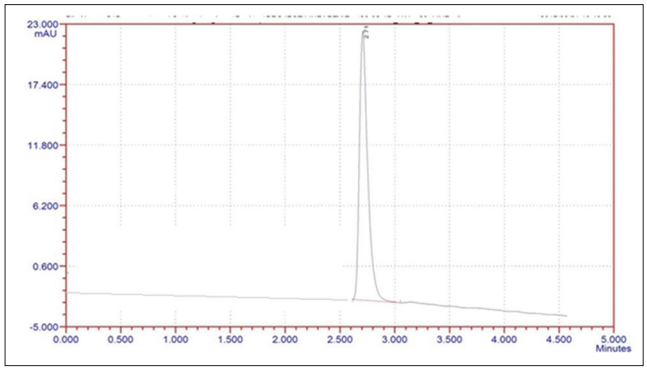

Fig. 3. Optimized chromatogram of valsartan

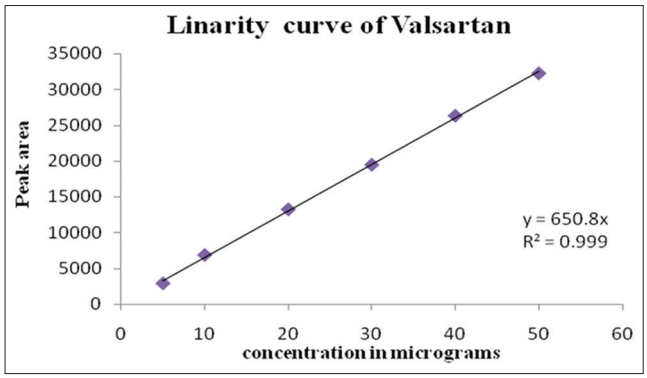

Fig. 4: Calibration curve of valsartan

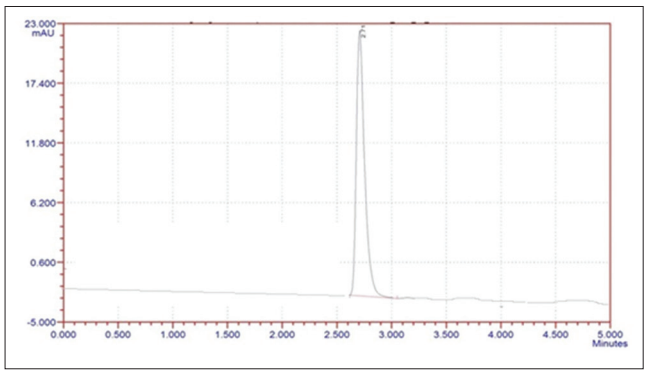

Fig. 5: Chromatograms of standard solution of valsartan

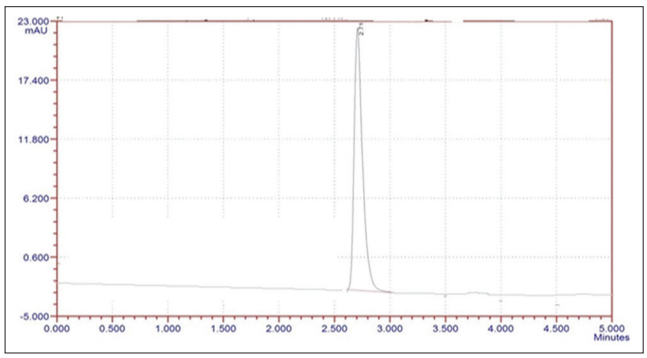

Fig. 6: Chromatograms of test (tablet) solution of valsartan

Table 3: % Recovery data for valsartan

% Level	Amount added (µg/ml)	Standard solution peak area	Spiked average peak area	% Recovery	Acceptance limit
50	10	6772	6654	98.3	±2%
100	20	13762	14103	102	
150	30	19570	19756	99	

Table 4: Results of intraday precision

Number of injections (20 µg/ml)	Peak area
Injection-1	13205
Injection-2	12960
Injection-3	12901
Injection-4	13506
Injection-5	13032
Injection-6	12837
Mean*	13073.5
SD	246.8366
% RSD	1.888068

*Average of three determinations. RSD: Relative standard deviation, SD: Standard deviation

Table 5: Results of intraday precision

Concentration (µg/ml)	Day-1	Day-2	Day-3	Mean*	SD	% RSD
5	2903	2953	2863	2906.333	45.0925	1.551525
10	6872	6772	6812	6818.667	50.33223	0.738154
20	13235	13362	13207	13268	82.60145	0.622561
30	19270	19445	19281	19332	98.0153	0.507011
40	26312	26230	26991	26511	417.7092	1.575607
50	32230	33230	32730	32730	500	1.52765

*Average of three determinations. RSD: Relative standard deviation, SD: Standard deviation

Table 6: Results of robustness

Variation of parameter	System suitability parameters			
	Retention time (minutes)	% RSD	USP tailing factor	USP plate count
Mobile phase ratio (±1) water:acetonitrile				
29:71	2.70	0.65	1.12	4589
30:70	2.71	0.54	1.04	6823
31:69	2.69	0.63	1.10	3561
Flow rate (±0.1 ml)				
1.1	2.70	0.59	1.04	5423
1.0	2.70	0.91	1.04	2963
0.9	2.71	0.72	1.06	4327

Table 7: System suitability parameters of valsartan (standard and test) in assay method

Peak name	Retention time	Peak area	% Area	USP tailing	USP plate count
Valsartan (standard)	2.710 minutes	12997	100.00	1.23	4564
Valsartan (test)	2.70 minutes	13540	100.00	1.13	5661

Assay

Percentage purity of the tablet dosage form was done by injecting the 20 µg/ml standard solution and a sample solution equivalent to 20 µg/ml valsartan. The percentage purity of valsartan in tablet dosage form was found to be 99.7% based on the data showed in (Figs. 4 and 5, Table 7).

$$\frac{13241}{12997}\times\frac{20}{100}\times\frac{10}{100}\times\frac{100}{41}\times\frac{100}{10}\times\frac{99.1}{100}\times\frac{81}{40}\times100=99.7\%$$

DISCUSSION

The RP-HPLC assay method plays a vital role in both qualitative and quantitative analysis of drug in its pure and tablet dosage. Till date, many RP-HPLC methods have been developed for qualitative and quantitative analysis of valsartan. However, the retention times in the reported studies were in a range 7-4 minutes [5-7], and the method with high retention time could not be treated as economical as it requires more amount of solvents and needs to be run for long time. If the retention time could be reduced solvent consumption and runtime for sample analysis can be lowered, hence rapid analysis of more number samples can be done. In previously reported methods, very expensive solvents such as glacial acetic acid and buffers were used and more effective linearity range could not be accomplished. In the present study, RP-HPLC method with retention time of 2.7 minutes was observed by a simple mobile phase composition of acetonitrile:water (70:30), better accuracy and huge linearity range of 5-50 µg/ml was attained with this simple mobile phase composition disclose the

cost-effectiveness of the method as compared to previously reported methods. The statistical results of the validated parameters within the limits stated by international conference on harmonization of technical requirements for registration of pharmaceuticals for human use. Compared to previously reported methods, the proposed method has a great advantage in terms of retention time, linearity, and sensitivity, and hence, this method could be used for better qualitative and quantitative analysis of valsartan.

CONCLUSION

The isocratic HPLC method was developed for the study of valsartan in pharmaceutical dosage form. The validated method is very rapid, accurate, and precise. Moreover, it has advantages of short runtime and the possibility of analysis of a large number of samples, both of which significantly reduce the analysis time per sample. Hence, this method can be conveniently used for routine quality control analysis of valsartan in its pure and tablet dosage forms.

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