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RP-HPLC Method Development and Validation of Valsartan in Bulk and its Tablet Dosage Form

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Abstract

The main objective of this study is to develop a reverse phase HPLC method for the quantitative determination of valsartan and its tablet dosage form with a simple, rapid, specific, validated and sensitive method. An isocratic separation is achieved using Phenomenex C18 (75 x 4.6 mm, 2.6 µ, 100 Å) with mobile phase comprised of water: acetonitrile (30%:70% v/v). Valsartan shows a retention time of 2.71 min at 1 mL/min flow rate and the wavelength was detected at 247 nm. Robustness, specificity, precision, accuracy, linearity, LOD and LOQ was validated using this method. The LOD is 1.24 µg/mL and LOQ is 3.6 µg/mL. The calibration curve in the concentration range of 5-50 µg/mL is linear with coefficient of correlation 0.9999. The % recovery of the valsartan is in the range of 98.9%-102% and the % RSD is <2%. This method is successfully applied for quantitative determination of valsartan in tablet dosage form.

Keywords: High Performance Liquid Chromatography (HPLC); Valsartan; Reverse phase; Mobile phase; Acetonitrile; Wave length

Introduction

The principle of separation in normal phase mode and reverse phase mode is adsorption. When mixtures of components are introduced in to a HPLC column, they travel according to their relative affinities towards the stationary phase. The component which has more affinity towards the adsorbent moves slower and vice versa [1]. In the normal phase mode, the stationary phase is polar and the mobile phase is non-polar. In this technique, non-polar compounds travel faster and are eluted first. This is because of the lower affinity between the non-polar compounds and the stationary phase. Normal phase mode of separation is therefore, not generally used for pharmaceutical applications [2,3]. In this mode, the stationary phase is non-polar hydrophobic packing with octyl or octa decyl functional group bonded to silica gel and the mobile phase is polar solvent. An aqueous mobile phase allows the use of secondary solute chemical equilibrium (such as ionization control, ion suppression, ion pairing and complexation) to control retention and selectivity. The polar compound gets eluted first in this mode and non- polar compounds are retained for longer time. As most of the drugs and pharmaceuticals are polar in nature, they are not retained for longer times and hence elute faster [4].

Most of the drugs in dosage forms can be analyzed by HPLC method because of the several advantages

- Speed (analysis can be accomplished in 20 min or less)
- Greater sensitivity (various detectors can be employed)
- Improved resolution (wide variety of stationary phases)
- Reusable columns (expensive columns but can be used for many analysis)
- Easy sample recovery, handling and maintenance
- Instrumentation tends itself to automation and quantitation (less time and less labor)

Validation of an analytical method is the process by which it is established, by laboratory studies, that the performance characteristics of the method meet the requirements for the intended analytical applications [5,6].

The system suitability test represents an integral part of the method and is used to ensure the adequate performance of the chosen chromatographic system. The simplest form of an HPLC system suitability test involves a comparison of the chromatogram trace with a standard trace. These parameters can be calculated experimentally to provide a quantitative system suitability test report [7-9].

The following methods have been reported for the estimation of Valsartan in bulk and pharmaceutical dosage forms (tablets).

Paladugu et al. [10] proposed a method in which quantitative estimation of valsartan in tablets determined by a stability-indicating RP-HPLC method for the quantitative analysis of valsartan in pharmaceutical tablets. The calibration curve was linear in the concentration range of 10-50 µg/mL with coefficient of correlation 0.9993. LOD and LOQ were found to be 1.83 µg/mL, 5.5 µg/mL respectively. The percentage recovery for the valsartan was found to be 99.0-100.2 and the % RSD was found to be less than 2%.

Kendre and Banerjee [11] proposed a method in which quantitative estimation of valsartan in tablets determined by RP-HPLC method development and validation of valsartan in its pure form and tablet dosage form. The flow rate of 1.0 mL/min was used and Perkin Elmer series 200 UV/VIS detector wavelength was set at 250 nm. The retention time of Valsartan was found to be 5.19 min. The percentage recovery was found to be up to 99% to nearly 100% and percentage RSD was found to be less than 2.0%.

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Vinzuda DU, et al. [12] proposed a method in which quantitative estimation of valsartan related substances determined by a stability indicating RP-UPLC method for estimation of valsartan related impurities in bulk drugs and its pharmaceutical dosage forms. 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%.

Haque et al. (2012) [13] proposed a method in which quantitative estimation of valsartan in tablets determined by RP-HPLC method development and validation of valsartan in its pure form and tablet dosage form. Linear regression analysis data for the calibration plot showed that there was good linear relationship between response and concentration in the range of 10- 100 µg/mL respectively. The LOD and LOQ values for HPLC method were found to be 0.02 and 0.06 µg/mL respectively. No chromatographic interference from tablet excipients was found.

The main objective of method development is to obtain a good separation with minimum time and effort. Based on the goal of separation, the method development is preceded [13]. The steps involved are:

- Information on sample, define separation goals
- Need for special HPLC procedure, sample pretreatment, etc.
- Choose detector and detector settings
- Choose LC method, preliminary run, Estimate best separation conditions
- Optimize separation conditions
- Check for problems or requirement for special procedure
- Validation for release to routine laboratory

Materials and Methods

Chemicals and reagents

The following table lists the materials that were used in this study (Table 1).

Procurement of drug

The valsartan working standard and the commercial formulation of valsartan were procured from the Formulation division of SIGMA Aldrich Limited, Mumbai and the test drug was procured from Al-Karrem Medical Stores, Hyderabad.

Drug: Valsartan

Label claim: 40 mg

Method development

Solubility: The solvents are selected on the basis of drug solubility. The solubility was performed using common solvents like methanol, water, acetonitrile [14] (Table 2).

Diluent: Acetonitrile:Water (70:30)

Determination of adsorption maxima by UV-visible spectrophotometer

Instrument specification: Spectrophotometer: Double beam UV-visible spectrophotometer with 1 cm; matched quartz cells

Chemicals/Reagents	Grade
Acetonitrile	HPLC
Water	HPLC
Valsartan	Working standard and test sample

Table 1: Chemicals and their grades.

Solvent	Solubility
Water	Slightly soluble
Methanol	Freely soluble
Acetonitrile	Freely soluble

Table 2: Solvents and their solubility.

Model: TG60

Software: UV WIN

Preparation of standard solution: 20 mg of Valsartan and 60 mL of diluent was taken in a 100 mL volumetric flask. The solution was sonicated and volume was made up to using diluent. 10 mL of this solution was taken and made up to using the diluent. The concentration of Valsartan is 20 µg/mL. From the spectrum, the λ_{max} of the solution was found to be 247 nm under 200-400 nm [15].

Preparation of test solution: 20 mg of sample and 60 mL of diluent was transferred to 100 mL of volumetric flask. The solution was sonicated for 15 min and then diluted with the diluent. Filter the solution through 0.45 µm Nylon filter. 1 mL of this solution is transferred to 100 mL volumetric flask and diluted with the diluent to get the concentration about 20 µg/mL [16].

Optimized method

Chromatographic conditions: Column: C_{18} (75 x 4.6 mm, 2.6 µ, 100 Å)

Mobile phase: Acetonitrile:Water (70:30% v/v)

Diluent: Acetonitrile

Flow rate: 1.0 mL/min

Column temperature: 40°C ± 2°C

Injection volume: 20 µL

Run time: 5 min

Detector: UV -detector

Detection wavelength: 247 nm

Elution: Isocratic

Assay

Mobile phase: De-gassed and filtered mixture of 70% of acetonitrile and 30% water is used to make the mobile phase.

Test solution preparation: The average weight of 20 tablets was calculated. 20 mg of valsartan tablet powder and 60 mL of diluent was taken in a 100 mL of volumetric flask and sonicated for 15 min and the remaining diluent was added to make the volume, filter the solution through 0.45 µm Nylon filter. 10 mL of this solution was diluted to get the concentration of 20 µg/mL [17].

Procedure: The column was equilibrated using the mobile phase for sufficient time until stabile baseline is obtained. The chromatograms were recorded by injecting the 20 µL of blank solution, standard solution and test solution into the chromatographic system [18]. The peak due to blank was disregarded. Tailing factor was <2.0.

Blank preparation: About 5 mL of dilute was taken in a 50 mL volumetric flask and then, the volume was made up with mobile phase.

Formula for calculating the assay of the given sample

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

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.

Method validation

System suitability test: System suitability test was performed by injecting the blank solution once and 100% test concentration standard solution for six times into HPLC system. The system suitability parameters were evaluated from the chromatograms obtained [19].

Linearity

Preparation of working standard solutions: 10, 20, 30, 40 and 50 µg/mL working standard solutions were prepared.

Procedure: Linearity was demonstrated over the range of 5-50 µg/mL of test concentration. The solution at six levels of concentration was prepared and 10 µL of each of working standard solutions were injected into the HPLC system to obtain the chromatograms.

By plotting average peak areas against concentration, the linearity curve was constructed and regression equation was calculated by the method of least squares. The correlation coefficient, y- intercept and slope of the regression line were reported.

Accuracy: The closeness of agreement between a test result and the true value (combination of random and systematic errors).

Accuracy (%) =100 × Test value/Reference value

Determination of accuracy: a) Comparison to a reference standard

b) Recovery of the analyte spiked in to blank matrix

c) Standard addition of analyte

Procedure: Accuracy of the method was established by performing recovery studies. Recovery studies was performed by spiking sample solution with pure authenticated standard drug at 3 different concentration levels i.e. 50, 100 and 150% each in triplicate. Mean recovery of the five different concentrations of the drug was calculated.

Precision: The standard stock solution and working standard solution were prepared as per the procedure which has been followed in case of determining the linearity of the method [20].

Intra-day and inter-day precision of the method were demonstrated by taking one of the test concentration. The concentration injected in triplicate into HPLC system to obtain the chromatograms and the peak areas were recorded from the obtained peaks. Then average and the standard deviation of three peak areas of at each concentration level were calculated.

Finally %RSD can be calculated by applying the formula,

% RSD=100 SD/X

Where, SD=Standard deviation of 'n' responses, X=mean of 'n' responses

Specificity: 10 mL of each prepared solution of blank, standard and sample were injected individually and chromatograms were developed. The retention time of individual injection of standard and sample in the chromatogram was observed to check whether there is any interference in the blank chromatogram at that retention time [21].

Limit of detection: The Limit of Detection (LOD) can be defined as the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value, using a specific method under the required experimental conditions.

LOD=3σ/S

Where,

σ=Standard deviation of the response, and

S=Slope of the calibration curve

Limit of quantitation: The Limit of Quantitation (LOQ) can be defined as the smallest concentration of analyte which gives a response that can be accurately quantified. The LOQ can be determined at S/N ratio of 10:1

LOQ=10 σ/S

Where,

σ=Standard deviation of the response, and

S=Slope of the calibration curve

Robustness: For the determination of method's robustness, deliberate change in flow rate, mobile phase composition, pH, temperature was made to evaluate the impact of this variation on the method.

Effect of variation of mobile phase composition: The effect of variation of mobile phase ratio was determined by changing the ratio of buffer: acetonitrile. Standard solution of 100% test concentration was injected into HPLC system and the chromatogram as recorded. The retention time, tailing factor and %RSD values were calculated.

Effect of variation of flow rate: The effect of variation of flow rate was determined by keeping flow rates at 1 mL/ min, 1.1 mL/min, and 0.9 mL/min .Standard solution of 20 µg/mL test concentration was prepared and injected into HPLC system and the chromatogram as recorded. The retention time, tailing factor and % RSD values were calculated.

Results and Discussions

Initially, the solubility of valsartan was checked in various solvents. The drug is soluble in acetonitrile, methanol and water. From the overlain spectrum, it was observed that maximum absorbance of valsartan was shown at 290 nm. Hence, this value has been selected as detection wave length for the analysis.

The chromatographic conditions was optimized by running several trials to obtain retention time, peak symmetry, plate count and relative standard deviation within the limits and possible optimal.

After several trails, a method using mobile phase consisting of acetonitrile and buffer in the ratio of 70:30 at a flow rate of 1 mL/min, on Phenomenex C_{18} (75 x 4.6 mm, 2.6 µ, 100 Å) column at 247 nm, was found to be the most appropriate and acceptable. Optimized method resulted in chromatogram with valsartan eluting at 2.71 min with a tailing factor of 1.09 and USP plate count of 5955.8.

System suitability

Tailing factor, % RSD, number of theoretical plates found, were calculated by performing the system suitability test. The retention time of 2.71 min was exhibited by the chromatograms. From the system suitability studies, it was observed that % RSD of peak areas was to be 1.93 for standard preparation of valsartan. Theoretical plate count is > 2000 and the tailing factor is <2 (Tables 3, 4 and Figure 1).

Linearity

The calibration curve was constructed with concentration on x-axis and peak area on y-axis to establish the linearity of the drug. From the calibration curve, it was observed that the method was linear over concentration range of 5 µg/mL-50 µg/mL for valsartan and correlation coefficient (r^2) is 0.999 (Figures 2-7 and Table 5).

Acceptance criteria: The value of correlation coefficient (r^2) should be NLT (Not Less Than) 0.99 and NMT (Not More Than) 1. The straight line equation for the calibration curve was found to be y=650.8x. Correlation coefficient (r^2) was found to be 0.999. Linearity was observed with in the range of 5-50 µg/mL for valsartan and r^2 was notless than 0.999. Hence, the method was found to be linear.

Injection	Retention time (min)	Peak area µV x sec	USP Plate count	USP Tailing factor
1	2.71	13235	6532	1.12
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 Standard Deviation	252.82695	13089.333	5955.6667	1.09
%RSD	1.9315495			

Table 3: Results of system suitability test for valsartan.

System suitability Parameter	Acceptance criteria
% RSD	An RSD of ≤ 2% for n ≥ 5 is desirable
Tailing factor (T)	T should be ≤ 2
Theoretical plates (N)	In general N should be > 2000

%RSD for six replicate injections of peak area response for valsartan standard preparation is <1%, tailing factor is <2 and number of theoretical plates is >2000.

Table 4: System suitability acceptance criteria.

Observation: Valsartan retention time is 2.71 min. Slight fronting of the peak was observed.

Accuracy: The recovery studies were performed to check the accuracy of the method at 25%, 50%, 100%, 120% and 150%. The mean recovery of the drug from the analyzed solution of formulation is in the range of 98.6-100.5%. Hence, the method is said to be accurate (Figure 8 and Table 6).

Acceptance criteria: The mean % recovery of valsartan at each spiked level should within the range 98.3%-102%. As per the recovery results, the test method has an acceptable level of accuracy shown in Table. The mean recovery of valsartan is in the range of 98.3%-102%.

Precision: Precision determined by preparing the working standard solutions and injecting thrice a day for three days. The % RSD of peak area of chromatograms of valsartan is <2% for intraday and interday precision respectively (Tables 7 and 8).

Acceptance criteria: % RSD of peak area of precision solution should not be more than 2.0%**.** Percentage RSD of peak areas of five injections of valsartan working standard solutions is in the range of 0.50%-1.57% and 1.88%, for inter-day and intra-day precision respectively. The % RSD value is <2%.

Specificity

The specificity is determined by injecting blank, sample and standard preparation. No interference was seen due to mobile phase solvent (blank) and excipients at the retention times of valsartan which confirms that this method is specific (Figures 9 and 10).

Acceptance criteria: No interference is observed in the blank chromatogram at the retention time of standard and sample preparation of valsartan.

Sensitivity

Formula to calculate LOD:

LOD=3σ/S

Where, σ=Standard deviation of the response, and

S=Slope of the calibration curve

From the results,

3×246.9/650.8=1.24

The LOD for valsartan is 1.24 µg/mL.

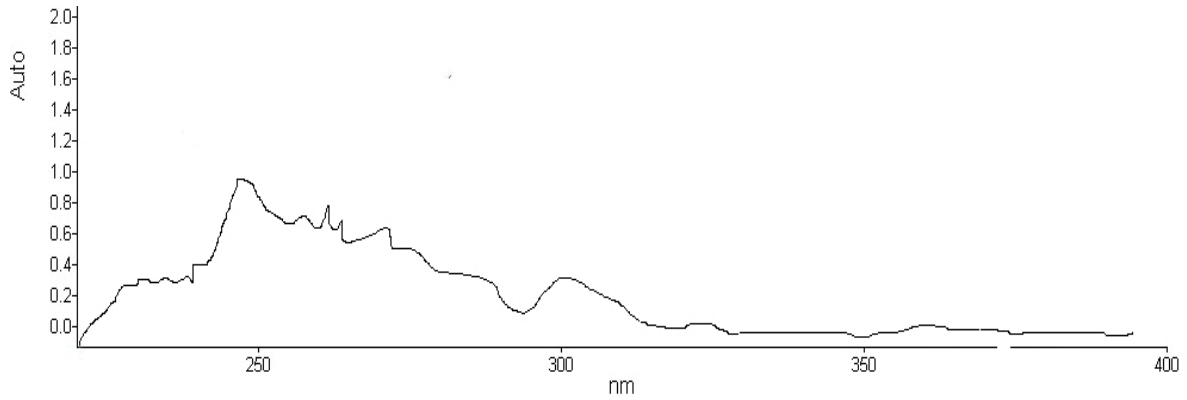


Figure 1: UV spectrum of valsartan showing absorption maxima of 247 nm.

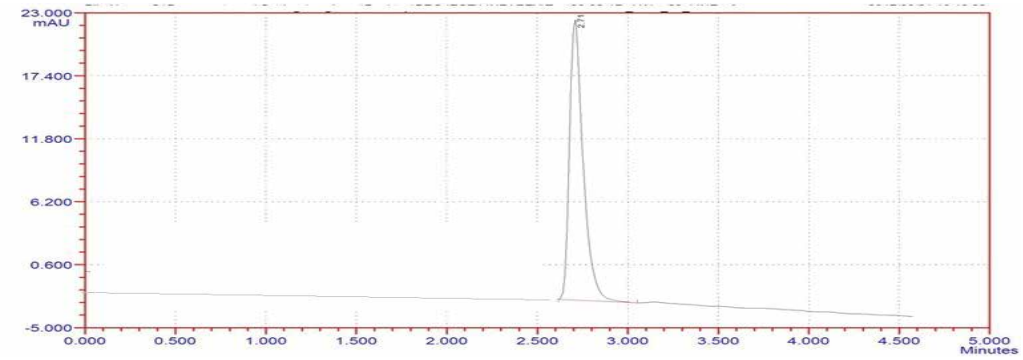
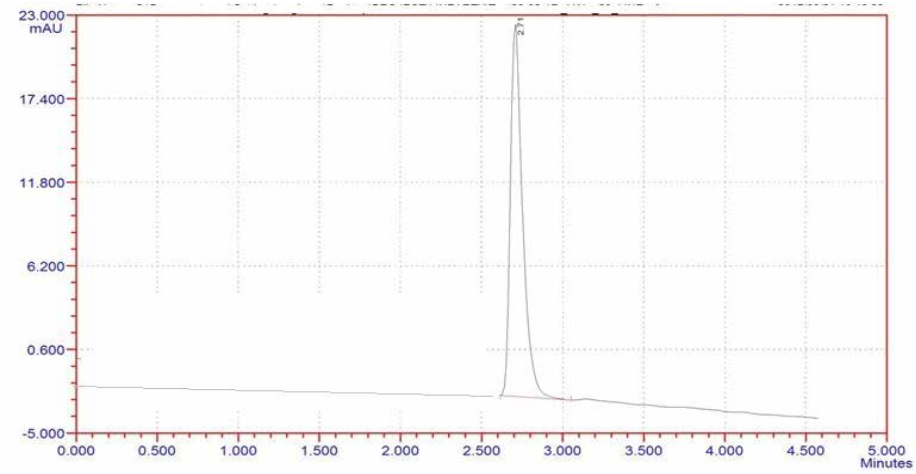


Figure 2: Chromatogram of optimized.



% RSD for six replicate injections of peak area response for valsartan standard preparation is <1%, tailing factor is <2 and number of theoretical plates is >2000.

Figure 3: System suitability test for chromatogram.

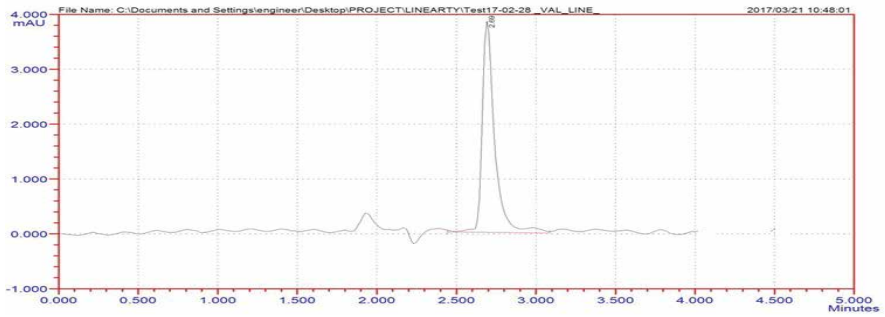


Figure 4: Chromatogram of 5 µg/mL solution of valsartan (Linearity).

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

Table 5: Peak areas of linearity standard solution of valsartan.

% Level	Amount added (µg/mL)	Standard solution peak area	Spiked Average peak area	% Recovery
50%	10	6772	6654	98.3
100%	20	13762	14103	102
150%	30	19570	19756	99

Table 6: Percentage recovery data for valsartan.

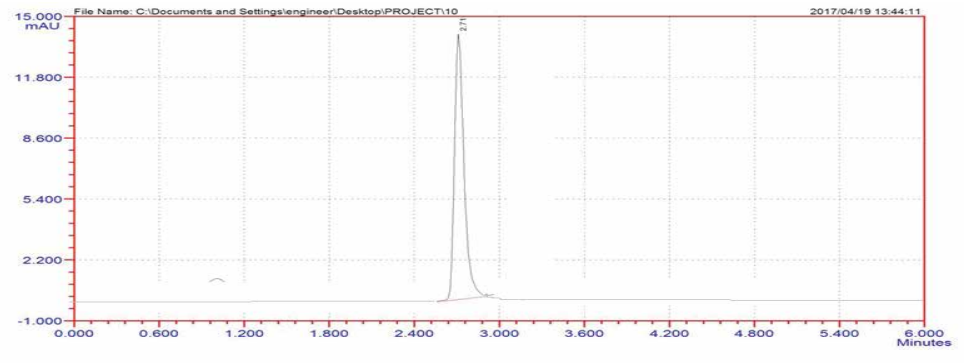


Figure 5: Chromatogram of 10 µg/mL solution of valsartan (Linearity).

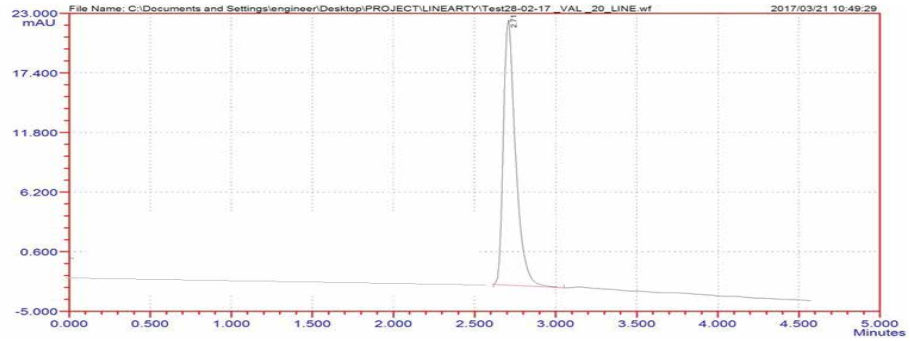


Figure 6: Chromatogram of 20 µg/mL solution of valsartan (Linearity).

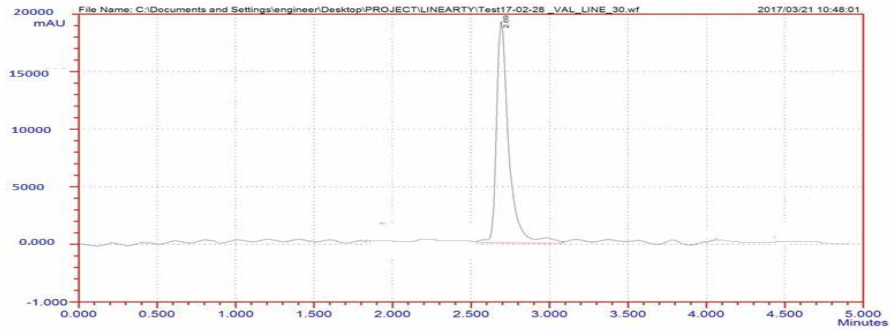


Figure 7: Chromatogram of 30 µg/mL solution of valsartan (Linearity).

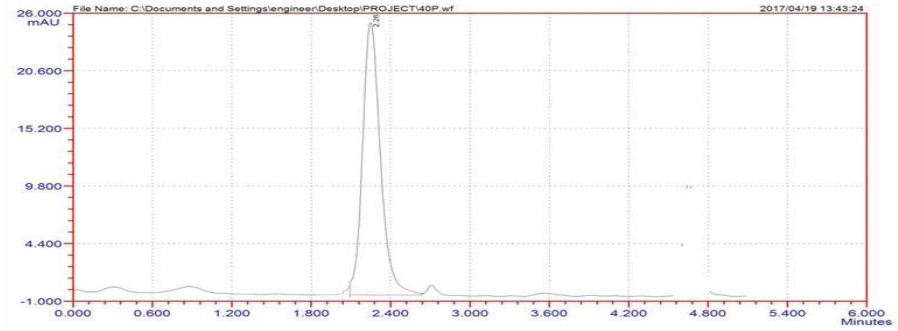


Figure 8: Chromatogram of 40 µg/mL solution of valsartan (Linearity).

No. 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
STDEV	246.8366
%RSD	1.888068

Table 7: Results of Intra-day Precision.

Formula to calculate LOQ:

LOQ=10 σ/S

Where, σ=Standard deviation of the response; S = slope of the calibration curve

LOQ=10×246.9/650.8=3.6

The LOD and LOQ are 1.24 µg/mL and 3.65 µg/mL respectively.

Robustness: The standard chromatograms of valsartan were within limits for variation in flow rate (± 0.1 mL), the flow rate within the range of 1.4 to 1.6 mL was allowable and variation in mobile phase

Concentration (20 µg/mL)	DAY-1	DAY-2	DAY-3	Mean	STD DEV	% 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

Table 8: Results of inter-day precision.

Variation of Parameter		System suitability parameters			
		Retention time(min)	% RSD	USP Tailing factor	USP Plate count
Mobile phase ratio(±1)Water: Acetonitrile	29:71	2.7	0.65	1.12	4589
	30:70	2.715	0.54	1.04	6823
	31:69	2.69	0.63	1.1	3561
Flow rate (±0.1 mL)	1.1 mL	2.7	0.59	1.04	5423
	1 mL	2.7	0.91	1.04	2963
	0.9 mL	2.71	0.72	1.06	4327

Table 9: Results of robustness.

Peak name	Retention time	Peak Area	% Area	USP Tailing	USP Plate count
Valsartan (Standard)	2.710 min	12997	100	1.23	4564
Valsartan (Test)	2.70 min	13540	100	1.13	5661

Table 10: System suitability parameters of valsartan (standard & test).

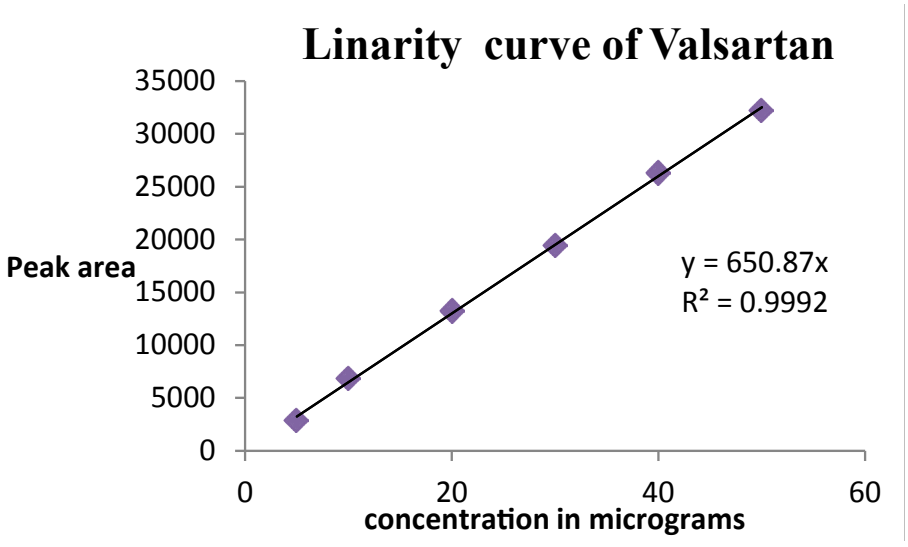


Figure 9: Calibration curve of valsartan.

composition (± 1%), pH (± 0.2) and temperature (± 5 ^{0}C) (Figures 11-14 and Tables 9 and 10).

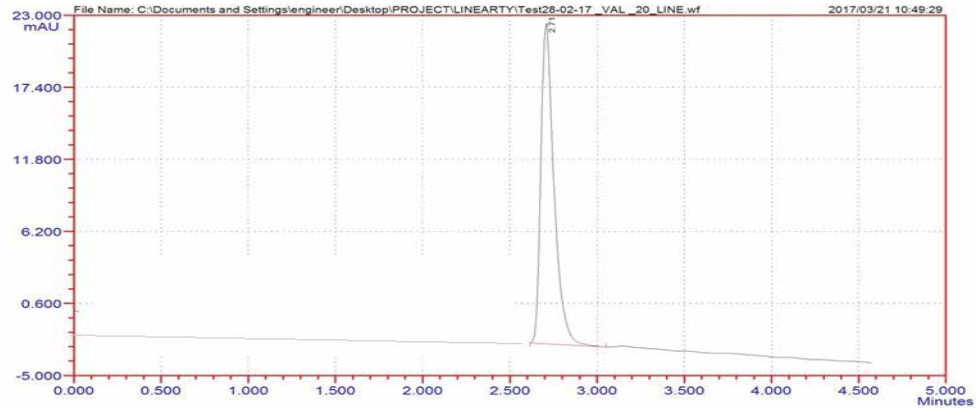
The % RSD values is <2.0%, hence the method is proved to be robust.

Assay: Assay of the given sample preparation is calculated by using following formula Figures 15 and 16.

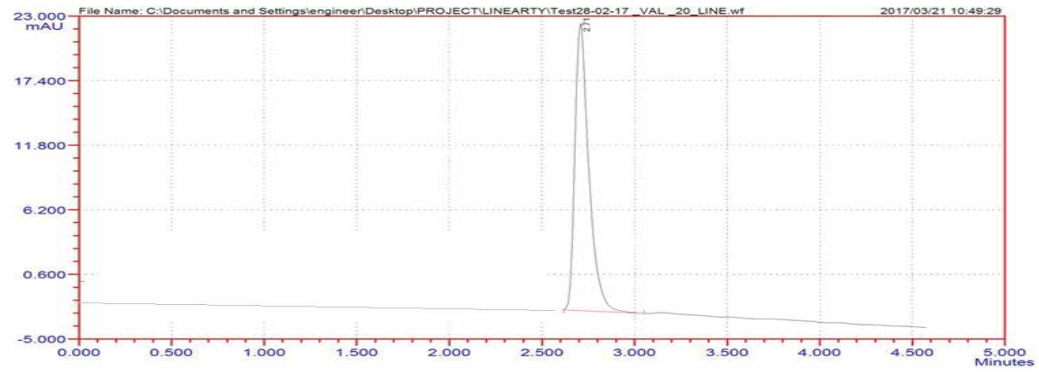
$$\text{Assay\%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{\text{AVG Wt}}{\text{Label Claim}} \times 100$$

Average weight of 20 tablets: 81 mg

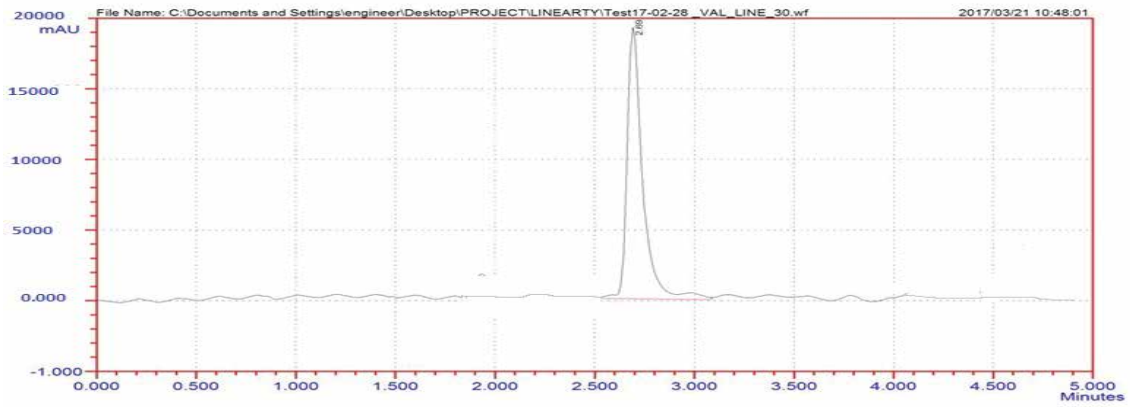
Label claim: 40 mg



(a)



(b)



(c)

Figure10: Chromatograms of Accuracy at (a) 50%, (b) 100%, (c) 150%.

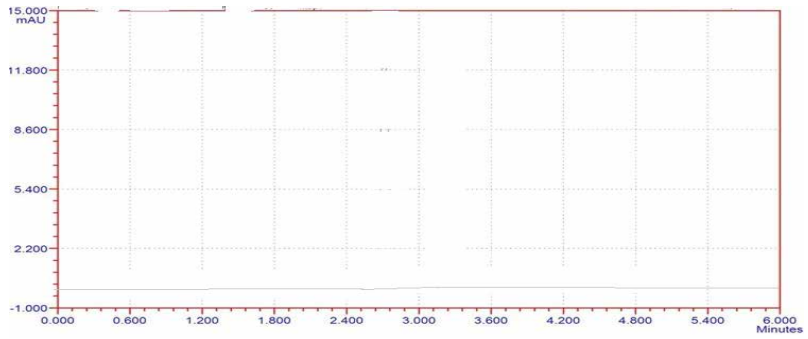


Figure 11: Chromatogram of blank.

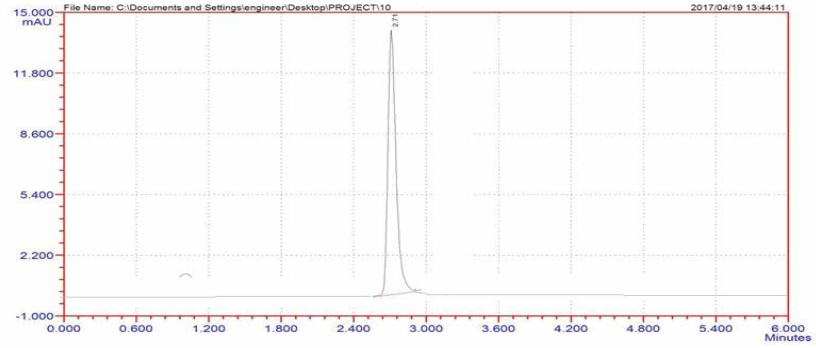


Figure 12: Standard solution chromatogram.

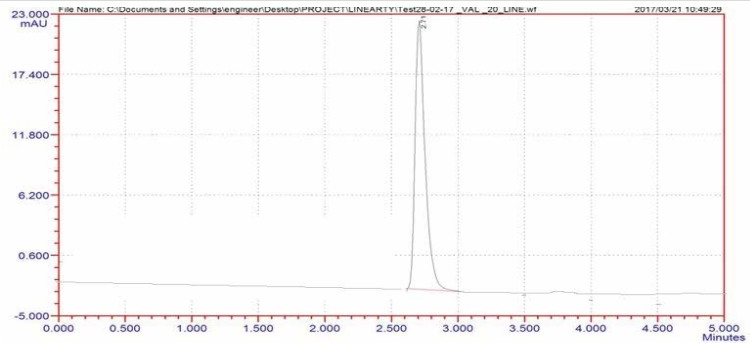


Figure 13: Chromatogram of standard solution with mobile phase ratio 29:71 (Water: ACN).

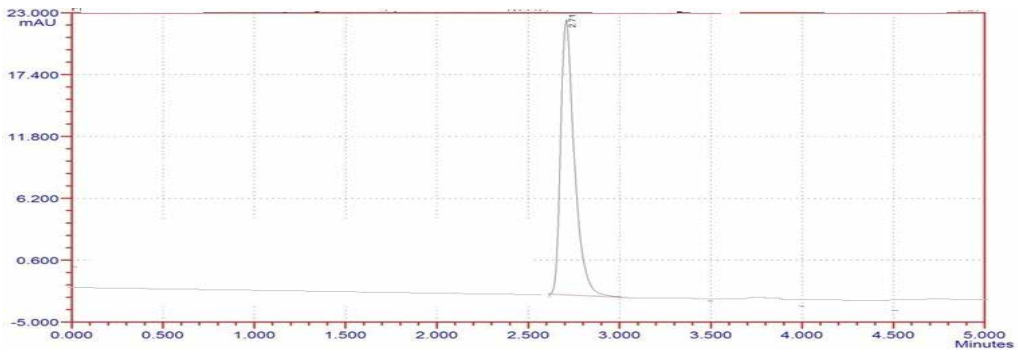


Figure 14: Chromatogram of standard solution with flow rate 1.1 mL/min.

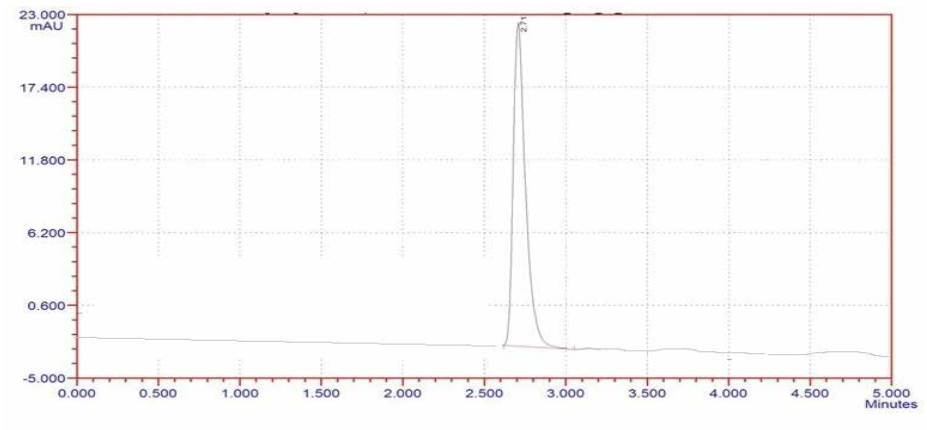


Figure 15: Chromatograms of standard solution.

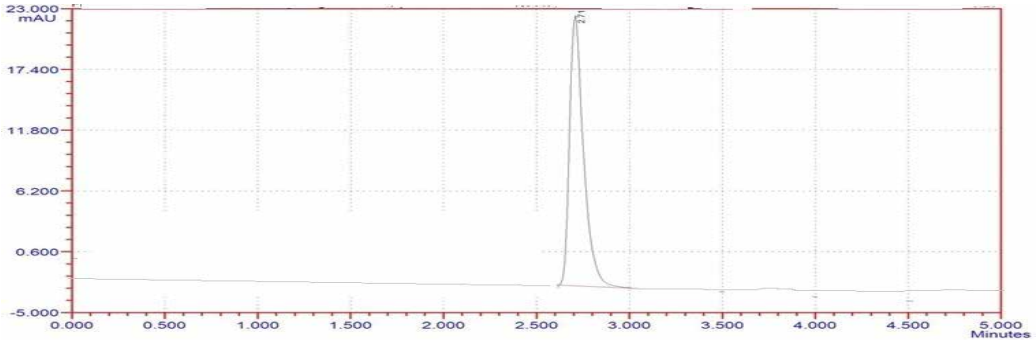


Figure 16: Chromatograms of test (tablet) solution.

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

The percentage purity of taken sample is 99.7%.

Conclusion

As there is no official method available in Indian Pharmacopoeia, British Pharmacopoeia and United States Pharmacopoeia for the estimation of valsartan in bulk and in commercially available tablets with less retention time, accuracy and sensitivity, so attempts were made to develop a method by drug amount present in the given sample can be quantified.

In this method of Reverse Phase HPLC, the parameters were optimized to obtain suitable conditions for the valsartan analysis. The optimum flow rate was found to be 1 mL/min using water and acetonitrile (30:70) as mobile phase.

The optimum wavelength for detection was 247 nm at which better detector response for valsartan was obtained. The retention time is 2.71 min. To ascertain the effectiveness of the system used, system suitability tests were done on new stock solutions and results met with acceptance criteria. The calibration was linear in concentration range was 5-50 µg/mL with correlation coefficient 0.999.

No interference was seen due to mobile phase solvents (blank) and excipients at the retention times of valsartan which confirms that the method was specific. The LOD and LOQ for valsartan were found to be 1.24 µg/mL and 3.6 µg/mL respectively which specify the method's sensitivity.

The method of precise is indicated when the value of % RSD is below 2%. The mean recoveries are in the range of 92%-102% indicating that this is an accurate method. The method was found robust as the % RSD was below 2.0%.

The proposed method was validated in accordance with ICH parameters and the applied for analysis of the same in marketed formulations.

Finally, it can be concluded that this method is accurate, precise, robust, specific sensitive and less retention time than previous methods and can be successfully applied for the routine analysis of valsartan in bulk and in commercially available tablet.

References

1. Thammana M (2016) A Review on High Performance Liquid Chromatography (HPLC). Research & Reviews: Journal of Pharmaceutical Analysis 5: 22-28.
2. Marie-Isabel Aguilar (2004) Reversed-Phase High-Performance Liquid Chromatography. Methods in Molecular Biology 251: 9-21.
3. Hemilton RJ, Swell (1982) Introduction to HPLC. Edition (2nd Edtn), ISBN 978-94-009-5938-5: 2-94
4. Kumar RB, Anjum A, Rani, Mounika P, Hima Bindu S, et al. (2016) Method

development and validation of tradozone by RP-HPLC. Der Pharmacia Lettre 8: 241-250.

5. Green JM (2011) Peer Reviewed: A practical guide to analytical method validation. Anal Chem 68: 305A-309A.
6. ICH: Q2B (1996) Analytical validation-methodology.
7. ICH: Q2A (1994) Text on validation of analytical procedure.
8. ICH: Q2 (R1) (2005) Validation of analytical procedures: text and methodology.
9. https://www.chromacademy.com/lms/sco5/01-Reverse-phase-chromatography-Aims-and-Objectives.html
10. Paladugu ND, Rao GD, Bonthu S, Poloju D (2013) Development and Validation of RP-HPLC method for quantification of Valsartan and its Pharmaceutical Formulations. Int J Drug Dev Res 5: 199-205.
11. Kendre MD, Banerjee SK (2012) Precise and accurate RP-HPLC method development for quantification of valsartan in tablet dosage form. International Journal of Pharmaceutical Sciences and Drug Research 4: 137-139
12. Vinzuda DU, Sailor GU, Sheth NR (2010) RP-HPLC Method for Determination of Valsartan in Tablet Dosage Form. Int J Chem Tech Res 2: 1461-1467.
13. Haque MA, Amrohi SH, Kumar PK, Nivedita G, Kumar PT, et al. (2012) Stability indicating RP-HPLC method for the estimation of Valsartan in pharmaceutical dosage form. IOSR Journal of Pharmacy 2: 12-18.
14. Ravali R, Phaneendra M, Bhanu Jyothi K, Ramya Santhoshi L, Sushma K (2011) Recent Trends in Analytical Techniques for the Development of Pharmaceutical Drugs. Journal of Bioanalysis & Biomedicine S11: 1-7.
15. Saeed Arayne M, Sultana N, Bahadur SS (2007) Multivariate calibration in UV Spectrophotometric analysis. Pak J Pharm Sci 20: 163-74.
16. Malviya R, Bansal V, Pal OP, Sharma PK (2010) High performance liquid chromatography: A short review. Journal of Global Pharma Technology 2: 22-26.
17. Mohammed NS, Mohammed AJ (2016) Development and Validation of RP-HPLC Method for the Determination of Hydrochlorothiazide in Bulk Drug and Pharmaceutical Dosage Form. Chromatography Research International 1693024.
18. Vinzuda DU, Sailor GU, Sheth NR (2013) Development of RP-HPLC Method for Estimation of Valsartan and Hydrochlorthiazide in Tablets. International Journal of Pharmaceutical Science and Nanotechnology 6: 2240-2244.
19. ICH, Specifications: Test procedures and acceptance criteria for new drug substances and new drug products: Chemical substances. International Conference on Harmonisation, IFPMA, Geneva, 1999.
20. Hokanson GC (1994) A life cycle approach to the Validation of analytical methods During Pharmaceutical product development. Part I: The initial validation process, Pharm Tech 118-30.
21. Hokanson GC (1994) A life cycle approach to the validation of analytical methods during Pharmaceutical product development. Part II: Changes and the need for additional validation. Pharm Tech pp: 92-100.