(RESEARCH ARTICLE)

RP UFLC method for estimation of valsartan chemometricaly

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Abstract

A simple, sensitive, rapid, precise and economical reverse phase HPLC method has been developed for the estimation of valsartan from pharmaceutical dosage forms. The method was carried out using Chromatographic conditions were established by employing a C18 analytical phenomenal (Kinetex) column (5micron -C18, 250x4.6mm) with a mobile phase of phosphate buffer (PH 3.5) and acetonitrile in a 60:40 ratio. The sample was injected at a volume of 20 microliters, and the mobile phase was degassed using a Sonica ultrasonic sonicator before being pumped into the HPLC system. The flow rate was set at 1 ml per minute, and the wave length 273 nm was chosen for detection. The column temperature was kept at 25°C respectively. The developed method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation and solution stability. The proposed method can be used for the estimation of these drugs in dosage forms.

Key words: Valsartan; UFLC; Chemometricaly; Method development; Method validation

1. Introduction

Analytical chemistry is a division of chemistry that is concerned with the determination of qualitative as well as quantitative make-up of a compound. The qualitative facet of analytical chemistry involves the detecting of chemical composition of the compound under study whereas the quantitative facet of analytical chemistry comprises of the determination of the amount, generally expressed in terms of concentration of a particular chemical component in the compound. Thus, analytical chemistry can be defined as a field of chemistry involving the processes of identifying, separating and quantifying the chemical composition of a complex compound obtained naturally or produced by synthetic means.

The question of why the drug compounds need novel method development and their validation is of significance. The reason behind the requirement of the method development and validation of analytical procedures is that the amount of drugs presented to the pharmaceutical market is up surging year wise. These drugs are either new molecules or formed due to alteration done to the existing form of chemical compound. In majority of cases, the analytical monograph of a drug is introduced into the official books of analysis, namely the pharmacopoeias after a very long period of its introduction to the pharmaceutical market. This interval comes into existence due to various reasons such as adverse events, development of resistance by the patient, introduction of an improved version of drugs of same category, etc. In such circumstances; the analytical techniques might not be available in the pharmacopoeias. Therefore, it becomes essential to develop novel methods and also validate them so as to overcome all the interferences.

Altogether, analytical method development accompanied with its validation prove to certainly take part significantly in the process of discovery, development, manufacture of pharmaceutical products to finally reach the consumer in an effective manner.

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1.1. HPLC - High Performance Liquid Chromatography

High Performance Liquid chromatography (HPLC) has been considered as the chief separation technique employed in most of the chemistry related fields. Since its discovery, the HPLC technique has contributed crucially in industrial as well as in research domains.

The HPLC technique has earned its importance chiefly owing to its consistency (usage of pressure oriented liquid support) as well as adaptability (provision for regulating the stationary phase and mobile phase composition).

1.2. Method development and Validation

Analytical method development and validation contribute a crucial role in the areas of identification, purity and potency estimation of drug formulations. The initial stages of method development include the collection of analyte's information with respect to physico-chemical properties. The important objective of HPLC technique is to separate and quantify the compound of interest from among any reaction impurities, intermediates and degradants. The steps involved in analytical method development and validation includes the following:

- Collection of data regarding the physico-chemical properties of the drug.
- Setting up of HPLC conditions.
- Preparation of sample.
- Optimization of developed method.
- Method validation.

1.3. Method Optimization

After obtaining the suitable separation, the next step is to optimize the experimental settings so as to accomplish adequate sensitivity and resolution. Stability indicating assay procedures will be obtained by means of a strategic and systemized analysis on several parameters such as components of mobile phase, pH, ratio of mobile phase components, mode of elution, Injection volume, temperature, type of solvents and flow rate.

1.4. Method Validation

Method validation in an analytical procedure is a course which establishes that a method meets the proposed requirements by means of experimental studies. The analytical method validation process is initiated with scheduled and organized collection of validation data supported by analytical procedures. The validation of analytical methods usually follows regulatory guidelines.

1.5. Validation parameters

The parameters that are typically performed in order to establish validation are as follows:

- Accuracy
- Precision
- Repeatability
- Intermediate precision
- Linearity
- Detection limit
- Quantitation limit
- Specificity
- Range
- Robustness
- System suitability determination
- Forced degradation studies
- Stability studies

1.6. Advantages of Analytical Method Validation

Analytical method validation gives rise to a degree of confidence, to both developer as well as the end user.

Even though the validation process may seem expensive and time consuming, it demonstrates to be cheap by removing annoying repetitions and provides better time controlling in the end.

The method validation captivates the surprise of variations in analytical settings and recompenses for more than financed on the process.

1.7. Stability Studies

In the course of validation study, the stability of samples and standards are accomplished under normal settings, normal storage conditions, and occasionally in the instrument to assess if different storage conditions are essential, for example, refrigeration or shield from light.
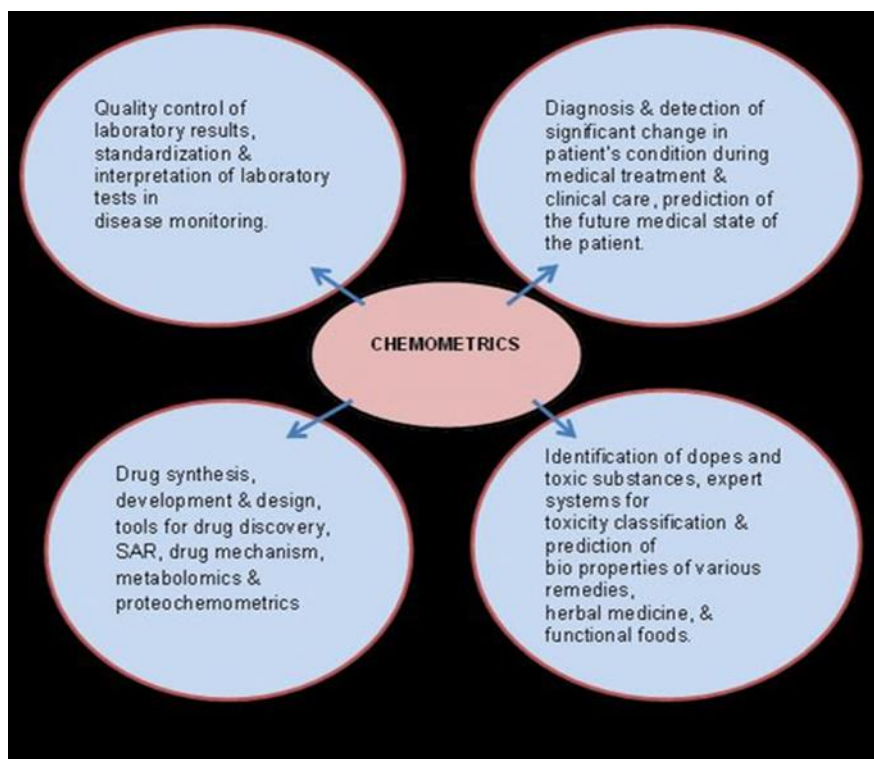

Figure 1 Application of Chemometrics in different fields

Currently available chemometric models for the data analysis are depicted in figure 5.

1.8. Principal Component Analysis

Principal Component Analysis, also known as PCA is a non-parametric process. Here in this method, the appropriate information is extracted from the collection of data. Then the patterns in the datasets are picked up for identifying the similarity as well as the difference in the data. PCA is a multivariate method and is a popular technique due to its application in multivariate data difficulties. In the method, the data in hand in converted to define the equivalent variability. The probable total variance is illustrated by means of first axis. The second axis represents the left over variance. In this case the first axis is not correlated. The third axis represents the total variance which remains later to completion of calculation for former two axes but correlating to neither of the axes. The new axes are also not correlated to one another and are weighted based on the total variance defined by them.

1.9. Partial Least Squares

Partial least squares, also known as PLS is a well-established method utilised for demonstrating the correlation between diverse sets of measured (observed) variables through latent variables. By applying the dimension, regression along with modelling tools, the PLS technique adjusts the relation between sets of the observed variables through a few latent variables.

Though PLS has found wide application in chemometrics, due to the presence of considerable non-linearity it likely provides huge prediction errors.
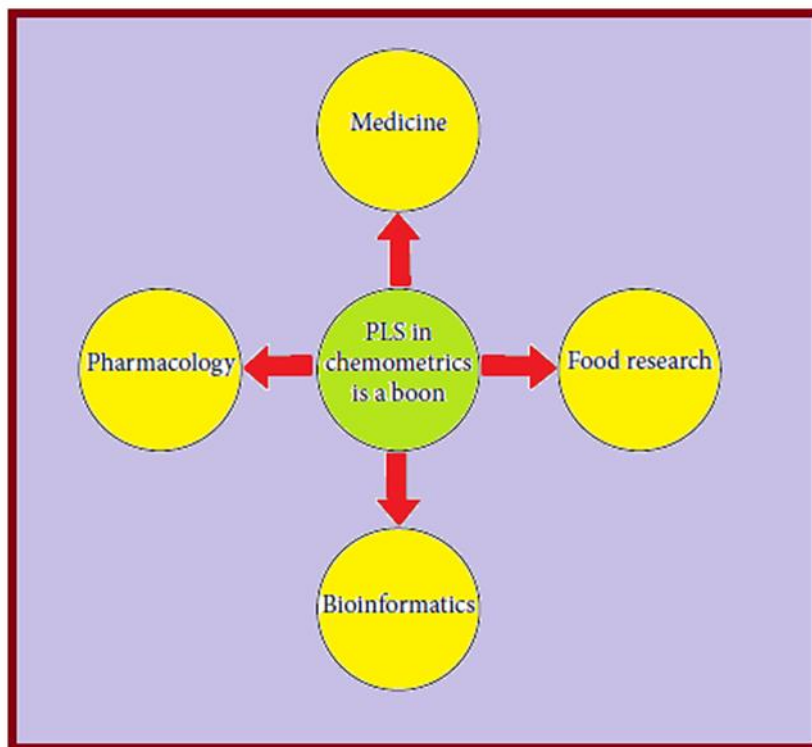

Figure 2 PLS - Application in different fields

1.10. Multiway Models

This type of model is useful for data that is multivariate as well as linear in greater than two dimension arrays. The multiway models are advantageous over bilinear treatment methods, as the latter are incapable of producing adequate results. The multiway models are used extensively for obtaining chemical information from spectral data. This is possible due to its capacity to determine what the chemical compound is comprised of. When the problem of qualitative determination is quiet challenging especially when the spectral data of the chemical constituents overlaps, the multiway modelling has found to be helpful.

Apart from this multiway models are also found applicable in regression analyses as well as process control. The batch data are monitored by tools like multiway partial least squares (MPLS) and multiway principal component analysis (MPCA) methods. This enhances the understanding of the process and condenses their performance in a batch-wise way.

Although the multiway methods are advantageous, when it comes to original data comprising of higher dimensions it becomes cumbersome for interpreting the data that is computed. Hence, in such cases multiway methods accompanied with three-way or higher arrays like parallel factor analysis (PARAFAC and PARAFAC-2, N-partial least squares (N-PLS), and Tucker-3) are preferred.

1.10.1. Parallel Factor Analysis

Parallel factor analysis (PARAFAC) models three-way or higher data mainly proposed for congruent variable profiles within batches. This method actually decomposes multi-dimensional arrays so as to

concentrate on the interest point and attain a complete illustrative result.

1.10.2. Application of Chemometrics in High-Performance Liquid Chromatography (HPLC)

HPLC is a well-known technique for analysing pharmaceutical dosage forms containing multi-components. Various conditions are involved in the technique and unfortunately all the parameters need to be optimized to attain noteworthy results. The components that are necessarily optimized include mobile phase, column, temperature, flow rate of mobile phase and detection wavelength. HPLC is a quiet sensitive analytical technique and any small error such as that found in chromatographic area, linear regression, etc., would affect drastically in the results.

As per the chemometric methodologies, HPLC employ PDA detectors for analysing binary mixtures. The procedures are often pooled with several calibration methods and are popularly known as HPLC-PCR, HPLC-PLS and HPLC-CLS. During the estimation of PSE as well as NAP in tablet formulation or a synthetic mixture, three chemometric methodologies were relevant. In the course of statistical analysis, several tests such as ANOVA test, F test, t-test, etc are done. While analysing the drug, the chromatograms were obtained and saved in the computer to evaluate the response attained from the detector. The detector response depends on the area of the peak obtained in the chromatogram.

The details of HPLC analysis merged with chemometric method approaches are explained as follows:

- HPLC-CLS

This methodology utilises the multilinear regression in order to ratio the peaks obtained from discrete drugs. Matrix equation finds application in this method.

- HPLC-PCR

Here, the ratio re-processing of the concentration of the drug and area of the peak obtained from the individual drug is done through mean centering (Ro and Co).evaluation was performed on the covariance dispersion matrix of the centered matrix Ro. Normalized eigenvalues as well as eigenvectors may be obtained through square covariance matrix. The uppermost value of eigenvalues assists in attaining the number of ideal eigenvector (P). The remaining eigenvectors as well as eigenvalues are overlooked. Coefficient b value is obtained by the following equation:

$$b = P \times q$$

P: eigenvector's matrix. q is the C-loading. It is derived by the following equation:

$$q = D \times TT \times Ro$$

TT signifies the transpose score matrix. T as well as D are diagonal matrices comprising components that are inverse to the chosen values. Drug content was evaluated by the following equation:

$$C \text{ prediction} = b \times R\text{sample}$$

PLS toolbox 3.5 in MATLAB 7.0 software may be employed for data calculation.

- HPLC-PLS

The PLS algorithm includes the dependent variables as well as independent variables in the data operations of decomposition and compression during PLS calibration. The following equations were used to calculate the decomposition of ratio of peaks area matrix as well as concentration into latent variables HPLC- PLS calibration technique:

In order to estimate the drug in the samples, the following equation of linear regression was employed.

Vector b was represented by the following equation:

$$R = T \times PT + E$$

$$C = U \times QT + F$$

$$b = W \times (P^T \times W)^{-1} \times Q$$

$$C\text{prediction} = b \times R\text{sample}$$

In the above equation W stands for weight matrix. PLS toolbox 3.5 in MATLAB 7.0 software is employed in this method.

Several chemometric techniques have been employed for analysing data obtained for a particular quality control test or particular manufacturing process or an instrumental output so as to attain supreme results in precision, accuracy and robustness. Chemometry mainly aims to deliver a speedy quantitative estimation of pharmaceutical products with properties of high sensitivity, simple as well non-destructive. The utilization of chemometric methods with an expectation of guaranteeing whole production course control involves the participation of analytical procedures that are efficient in producing exact outcome in a very simple and quick way.
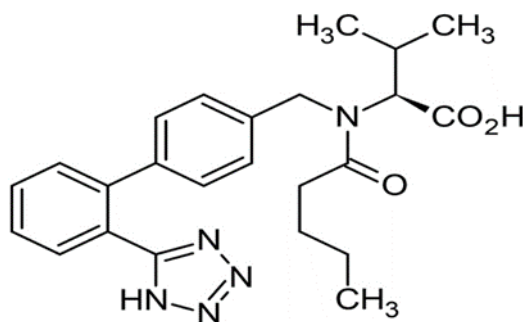
2. Materials And Method

2.1. Drug Profile

2.1.1. Valsartan

Name: Valsartan

Structure

Description: Valsartan belongs to Angiotensin receptor blockers. In order to facilitate easier blood flow, it acts by relaxing blood vessels. Bringing down high blood pressure reduces the risk of heart attacks, renal issues, and strokes.

Chemical formula: $C_{24}H_{29}N_5O_3$

Molecular weight: 435.5g/mol

IUPAC Name: (2S)-3-methyl-2-[pentanoyl-[[4-[2-(2H-tetrazol-5 yl)phenyl]phenyl]methyl]amino]butanoic acid

Categories: Angiotensin II receptor blocker (ARB)

Solubility: soluble in ethanol, DMSO and dimethyl formamide at 30 mg/mL

Pharmacology Class: Anti-hypertensive drug

Mechanism of action: By specifically preventing angiotensin II from binding to the AT1 receptor in several tissues, including vascular smooth muscle and the adrenal gland, valsartan inhibits the actions of angiotensin II that cause the release of aldosterone and vasoconstriction.

Melting point: 116-117 °C

Log P: 1.499

pKa: 3.6

Chemicals and the Reagents

Greensmed lab provided working standards for valsartan. The pill dosage forms were obtained from the local market in malappuram. Valent 40 mg by lupin laboratories were utilised. The weight equivalent of powder to be calculated based on the labelled claim and average weight.

Merck supplied HPLC grade water and acetonitrile, while NICE supplied sodium dihydrogen orthophosphates and orthophosphoric acid of analytical reagent (AR) grade for the preparation of phosphate buffer.

Buffer preparation

In a 1L volumetric flask, accurately weigh 1.0 gm of sodium dihydrogen ortho phosphate, add around 750 ml of HPLC grade water, sonicate for a few minutes to degas, and then make up the volume with HPLC water. The PH was corrected to 3.5 with dilute orthophosphoric acid.

Standard preparation

Accurately weigh 100 mg of valsartan was transfer into a 100 ml clean dry separate standard flask, which was diluted with 75 ml of buffer and sonicated for 45 minutes, and standards flasks were made up to the final volume with buffer, 1 ml of the above stock solutions was pipetted out into a 100 ml standard flask and then makeup to the final volume with buffer (to get 10mcg/ml) solution.

Sample preparation

The weight equivalent of powder (100mg) to be collected from the formulation bought from the local market is estimated from the labelled claim and average weight and the weight equivalent of powder is weighed and was transferred into clean dry separate standard flasks of 100 ml capacity. The powder was dissolved in 75 ml of buffer solution, which was then made up to 100 ml. The contents of the flask was filtered, and 1 ml of filtrate is put into a clean dry 100 ml standard flask and properly diluted to give a composition of 10 mcg/ml solution of medication.

Chromatographic conditions

Chromatographic conditions were established by employing a C18 analytical phenomenal (Kinetex) column (5micron - C18, 250x4.6mm) with a mobile phase of phosphate buffer (PH 3.5) and acetonitrile in a 60:40 ratio. The sample was injected at a volume of 20 microliters, and the mobile phase was degassed using a Sonica ultrasonic sonicator before being pumped into the HPLC system. The flow rate was set at 1 ml per minute, and the wave length 273 nm was chosen for detection. The column temperature was kept at 25°C.

Method development

Several trials were performed for the method development and the best peak with least fronting factor was found in the fifth trials with reaction time of 4.774

Table 1 Chromatographic conditions

SL.No	Chromatographic Conditions	
1	Mode of separation	Isocratic Elution
2	Mobile phase	Phosphate Buffer (pH 3.5) and Acetonitrile (60:40)

3	Column	Phenomenex (250 x 4.6mm, 5micron)
4	Flow rate	1ml/min
5	Detection wave length	273
6	Injection volume	20 micro liter
7	Column over temperature	25°C
8	Run time	10 min
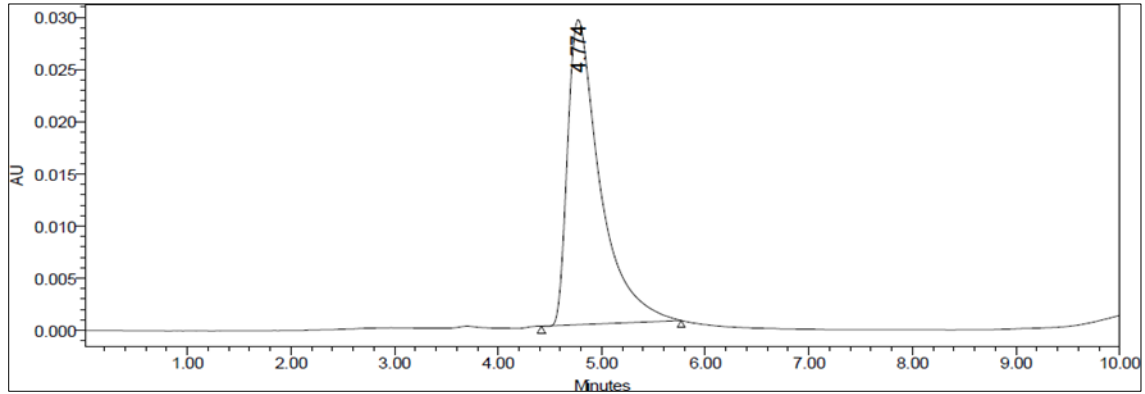
Figure 3 Chromatogram showing the retention time for valsartan
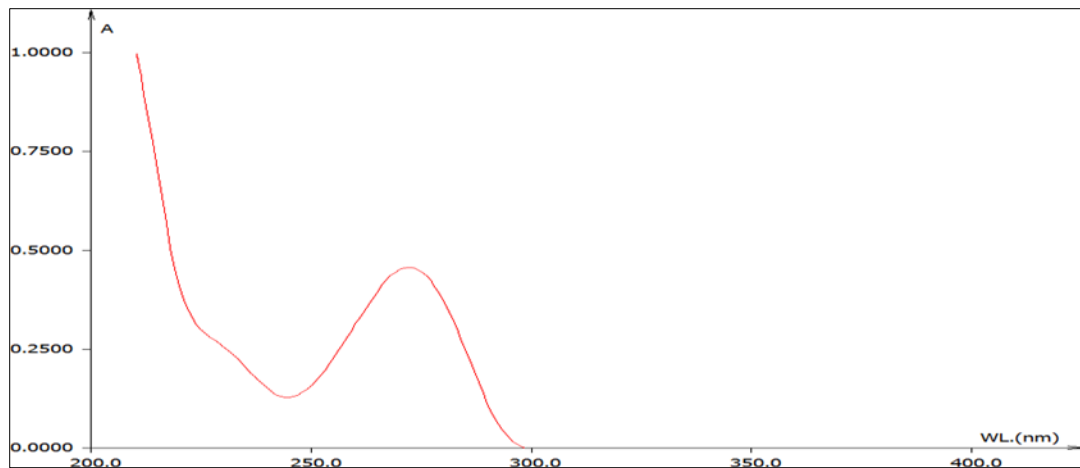

Figure 4 UV spectra
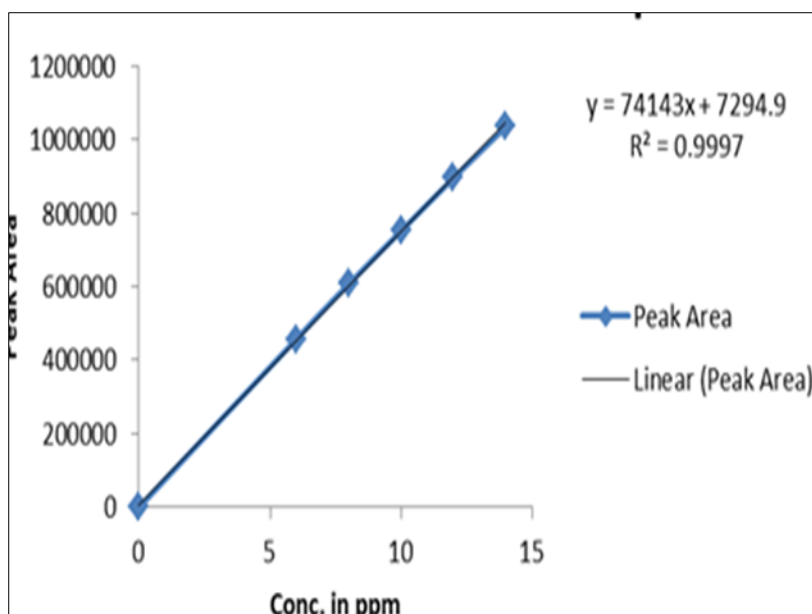
$y = 74143x + 7294.9$
$R^2 = 0.9997$

Figure 5 Linearity study

3. Results and discussion

3.1. Method validation

The final test settings were validated using the validation parameters given in the ICH recommendations. Analytic parameters such as specificity, accuracy, precision, linearity, detection, and quantitation limits were assessed in accordance with the ICH Protocol

3.2. System suitability

As part of the HPLC processes, system suitability tests were done. These tests are used to ensure that the chromatographic system is sufficient for the purpose intended. The system suitability test was carried out for theoretical plates (more than 2000) with a tailing factor less than 2. Table 2 summarizes the results that were within acceptable limits.

Table 2 Evaluation parameters

Sl. No	Parameters	valsartan	Acceptable Criteria
1	Tailing Factor	1.395	Less than 2
2	Theoretical Plates	2841	Not less than 1500
3	Retention time	4.774	Less than 10
4	Area	747557	
5	% RSD	0.35	Less than 2%
6	HETP	92.23	
7	Resolution	1.607	

3.3. Linearity

Linearity studies are the ability of analytical measurements like absorbent diversity, proportion to concentration of the sample linearity experiments were performed for the active ingredient and the response were found to be linear in the study range of 7-15 mcg/ml were confirmed.

3.4. Specificity

Specificity is the ability to detect and assess the analytic in the presence of other components that may be expected to be present in the formulation. In the UFLC study of standard and sample preparations, no interference due to diluents or mobile phase was observed in the analytic retention time, demonstrating that the method was specific.

3.5. LOD and LOQ

The limit of detection is the lowest concentration that can be detected by instruments using a specific analytical procedure, and the limit of quantification is the lowest concentration that can be quantitatively analysed using a specific analytical procedure with acceptable precision, accuracy, and reliability. The signal-to-noise ratio for the analytical processes and instruments utilised for analysis is rigorously monitored and validated.

The LOD values for valsartan the drug for experiment is , 0.92 mcg/ml, while the LOQ values for AR grade, were 1.25 mcg/ml.

3.6. Robustness

Deliberate modifications are made to the approach via flow rate, mobile phase ratio, and temperature, but no discernible variations in the findings were seen, and they are within the range specified by ICH. Robustness conditions such as flow rates of 0.9 ml/min and 1.1 ml/min, mobile phase concentrations of 50:50 and 70:30 for buffer and acetonitrile, and temperature changes at 20°C and 30°C were maintained, and samples were injected in triplicate; system suitability parameters were not significantly affected, and the RSD was within the limit, indicating that the UFLC method development was robust.

3.7. Accuracy

The method's accuracy was studied using recovery analysis, and it was evaluated by completing recovery tests at 75%, 100%, and 125% of the target analyte concentration in the commercial forms chosen. The percentage recovery of analyte at each concentration and the mean percentage recovery for the analyte were investigated; the recovery of each concentration must fall within the permitted range of 2%.

Table 3 Accuracy Data of valsartan

SL.No.	Conc %	Peak Area	Amout Added mg	Amount Found mg	% Recovery	Mean Recovery %	SD	% RSD
1	75%	521341	3.75	3.70	98.66		0.92	0.2
2	100%	738020	5	5.02	100.4	99.58	0.82	0.12
3	125%	1046191	6.25	6.23	99.68		0..1	0.06

3.8. Precision

Precision in the measure of the degree of repeatability of procedures under normal operations and in normally expressed as the relative standard deviation (%RSD). Precision may be performed at different levels: Intraday and Inter day precisions. Precision data presenting the %RSD value for both intraday and inter day studies were less than 2% which indicates that the proposed method in Precise and consistent.

Table 4 Intraday and Inter day data of valsartan

		Intra day	Inter day
1	Retention Time	4.679	4.770
2	Avg. Peak Area	649070	587583
3	SD	13580	5766
4	% RSD	0.2	0.5

4. Conclusion

The stability indicating test method reported is simple, rapid, robust and reliable for estimation of valsartan in bulk and in formulations. No interfering peaks were observed at elution time. system suitability parameters like linearity, precision, accuracy, resolution, theoretical plate, retention time of the proposed method both were were checked and were found to be appropriate, linearity was determined for ingredient and the concentration range of 7-15 mcg/ml. The LOD value of valsartan found to be 0.92 mcg/ml and the LOQ values of AR grade drug was found to be 1.25mcg /ml. Robustness conditions such as flow rates of 0.9 ml/min and 1.1 ml/min, mobile phase concentrations of 50:50 and 70:30 for buffer and acetonitrile, and temperature changes at 20ºC and 30ºC were maintained, and samples were injected in triplicate; system suitability parameters were not significantly affected, and the RSD was within the limit, indicating that the UFLC method development was robust.

Compliance with ethical standards

Disclosure of conflict of interest

There is no conflict of interest in the work presented in this manuscript.

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