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# DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR QUANTITATION OF VALSARTAN TABLET DOSAGE FORM

**Gogula Bhargava*, Anusha K., G. Kulayappa***

*Assistant Professor Department of Pharmaceutical Chemistry, K. R. College of Pharmacy, Bengaluru, Karnataka, India.

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***Corresponding Author**

**Gogula Bhargava**

Assistant Professor, Department of Pharmaceutical Chemistry,
K. R. College of Pharmacy,
Bengaluru.

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## ABSTRACT

The UV spectroscopic method used to measure Tadalafil involves dissolving the drug in dichloromethane and then diluting it with methanol. The absorbance of Tadalafil is measured at a wavelength of 284 nm, with methanol serving as the reference. This method adheres to Beer's law within the concentration range of 10 to 40 mcg/ml. The resulting linear equation is y = 0.01013x + 0.10100, and the correlation coefficient is 0.99940. The UV spectroscopic method for measuring Valsartan relies on determining the ultraviolet absorbance maximum of the drug at 230 nm, using acetonitrile as the diluent. The method follows Beer's law in the concentration range of 2.5 to 25 mcg/ml, with the linear equation y = 0.05702x - 0.02740 and a correlation coefficient of 0.997. Additionally, the drug obeys Beer's law within the concentration range of 2.5 to 15 mcg/ml. Both methods are simple, fast, and do not involve complicated sample preparation steps, making them economical and suitable for routine quality control applications. In conclusion, the developed methods are effective for the analysis of the selected drugs in both bulk and tablet forms.

**KEYWORDS:** Tadalafil, UV-Spectroscopic method, Valsartan, Absorbance, Beer 'law, Analytical- techniques, Quality Control, Analyte, Separation Techniques, Immovable phase.

## INTRODUCTION

Pharmaceutical analysis involves using different analytical methods and processes to evaluat the purity, safety, and overall quality of medicines and chemical substances. In simple term, it covers both the identification and measurement of pharmaceuticals products and materials, including raw drug substances. Pharmaceutical analysis plays a key role in areas like "quality control" and "quality assurance." The concept of "quality" in relation to drugs and drug products includes all factors that directly or indirectly affect their purity, safety, effectiveness and dependability. The main purpose of quality control is to test a drug for its quality and amount. To accomplish both these tasks—identifying what a substance is and determining how much of it is present—pharmaceutical analysis is divided into two main categories:

¬ Qualitative analysis (identification, i.e., figuring out what the substance is)

¬ Quantitative analysis (estimation, i.e., measuring how much of the substance is present)

## QUALITATIVE ANALYSIS

The composition of natural or synthetic substance is established by Qualitative analysis and it is also used for the identification of the chemical's compounds. A variety of qualitative tests include detection of evolved gas, formation of precipitates, limits tests, colour change reactions, melting point and boiling point tests, molecular weight, solubility, half-life etc.

## QUANTITATIVE ANALYSIS

This method is performed for the determination of exact amount of individual component or mixture of components of interest, which are present in the sample. This method is mainly used for the estimation of the any compound or substance present in the sample. Quantitative analysis should require the identification (qualitative) data of the analyte for the estimation of that particular analyte to be determined.

## TYPES OF ANALYTICAL METHODS[2]

There are various analytical techniques used to analyze drugs. Different methods are available for the quantitative estimation of substances. These methods can be classified based on the type of analysis performed and whether they use instruments or not.

**Non-Instrumental methods (Classical methods):** These are the chemical reactions quantitatively is the basis of the traditional or classical methods of analysis.

- **Gravimetric analysis**: In this method, the weight of the sample can be determined for the

quantitative determination after the precipitation.

- **Titrimetric analysis:** In this method the volume of the solution can be determined after the reaction such as Neutralization, Complex formation, Precipitate formation and Oxidation - reduction.
- **Volumetry (Gasometry):** In this method, the volume of the gas evolved by the reaction can be determined.

- **Instrumental methods**

The main basis for these methods is measurement of an electrical property, and some are based on the measurement of intensity of absorption or emission of radiation, all require the use of suitable instrument.

- **Electrochemical methods**: This method is used for the measurement of the current, voltage or resistance. A few examples are as follows: Examples
  - **Conductometry:** measures the conductivity
  - **Potentiometry:** measures the potential or emf
  - **Coulometry:** current at specified voltage
- **Optical methods**: This method is based upon the measurement of absorbed or emitted radiation measurement. Some examples are as follows:
  - *Absorption methods*: visible, Ultraviolet (UV), Infrared (IR).
  - *Emission methods*: example like Plasma spectroscopy, flame spectroscopy, and fluorimetry.

- **Chromatographic methods**

Separation techniques and analysis of mixture of compounds based on the differential affinity of component towards either movable phase or an immovable phase.

- Thin layer Chromatography (TLC), Column Chromatography (CC), Paper Chromatography (PC), High Performance Chromatography (HPLC), Ion exchange Chromatography (IEC), Gas Chromatography (GC).

- **Thermal methods**

  A group of techniques in which a property of the sample is monitored against time or temperature while the temperature of the sample, in a specified atmosphere, is programmed.

- Differential Scanning Calorimetry (DSC): Monitor differences in some sample

property as the temperature increases, or differences in temperature between a sample and a standard as a function of added heat.

- Thermo Gravimetric Analysis (TGA): Measurement of the mass of a sample as the temperature increases.
- Differential Thermal Analysis (DTA): Measurement of the difference in temperature between a sample and a reference as heat is applied to the system.

- **Other methods**

X-ray diffraction method, radioactive methods, Mass spectrometry, Refractometry and Polarimetry.

### 1.1. SPECTROSCOPY

Spectroscopy is the qualitative & quantitative study of interaction between matter and electromagnetic radiation. It involves the measurement of absorption or emission radiation by the molecules/atoms/ ions. The instrument that can measure the intensity as a function of colour, or more specifically, the light wavelength is called spectrophotometer. The difference in incident radiation intensity of light and emitted/transmitted radiation intensity is measured by spectrophotometer. Spectroscopy consists of numerous dissimilar applications like atomic absorption spectroscopy, atomic emission spectroscopy, ultraviolet-visible spectroscopy, x-ray fluorescence spectroscopy, infrared spectroscopy, Raman spectroscopy, dual polarization interferometer, nuclear magnetic resonance spectroscopy, photo emission spectroscopy, Mossbauer spectroscopy and so on.[3]

#### 1.1.1. Ultraviolet–Visible spectroscopy

Ultraviolet–visible spectroscopy is the absorption spectroscopy within the UV- Visible spectral region. It means UV- Visible spectroscopy use light source in the visible as well as adjacent (near-UV) and near-infrared (NIR) ranges. The visible light which is absorbed in the UV- visible region affects directly the apparent colour of drugs concerned. The molecules undergo electronic transitions in the region of electromagnetic spectrum.[4]

In Pharmaceutical Analysis, the most frequently employed technique is Ultraviolet- visible spectrophotometry. In which the amount of absorbed UV (400- 200nm) radiation by a substance in solution was measured. While a beam of light is passed through a transparent cell, which is having a solution of an absorbing substance, reduction of the incident light intensity occurs. This is due to:

- Reflections occur at the inner and outer surfaces of the cell
- Scatter by particles in the solution
- Absorption of light by molecules in the solution.

If a comparison cell is used, the value of Ir, which is very small, can be eliminated for air-glass interfaces, under this condition's equation write as follows.

The absorbed light intensity is then given by

$$I_{absorbed} = I_O - I_T$$

IO is the original incident light intensity on the cell

IT is the reduced intensity of transmitted from the cell.

**Principles of Ultraviolet Spectroscopy**

**A. Types of Excitations**

In UV-Visible region molecules undergo electronic transitions[5] involving σ, π and n electrons. Four types of electronic transitions are possible as represented in:

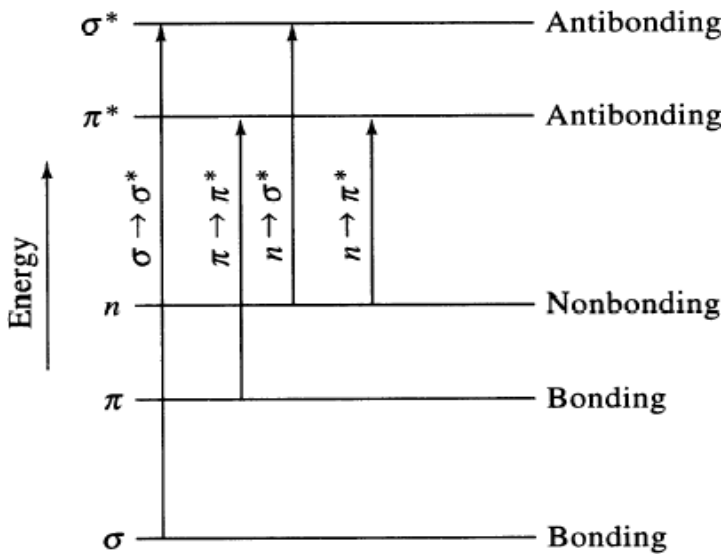


**Fig. No. 1.4.1.1: Electronic Transitions in Ultraviolet Region.**

**σ → σ* Transitions**

An electron in a bonding σ orbital is exited to the corresponding antibonding orbital. The energy required is large. For example, methane which has only C-H bonds can only undergo σ → σ* transitions and shows an absorbance maximum at 125 nm.

**n → σ* Transitions**

Saturated compounds containing atoms with lone pairs (non-bonding electrons) are capable of n → σ* transitions. These transitions usually need less energy than σ → σ* transitions. They can be initiated by light whose wavelength is in the range 150-250 nm. The number of organic functional groups with n → σ* peaks in the UV region is small.

**i. n → π* and π → π* Transitions**

Most absorption spectroscopy of organic compounds is based on transitions of n or π electrons to the π* excited state. This is because the absorption peaks for these transitions fall in an experimentally convenient region of the spectrum (200 -700 nm). These transitions need an unsaturated group in the molecule to provide the π electrons. Molar absorptivity's n → π* from transitions are relatively low and range from 10 to 100 $Lmol^{-1}cm^{-1}$. π → π* transitions normally give molar absorptivity's between 1000 and 10,000 L $mol^{-1}$ $cm^{-1}$.

**Lambert-Beer's Law**

**I. Lamberts Law**

The relationship between absorption of radiation and length of the path through the absorbing medium was formulated by Lambert (1768). "When beam of monochromatic light passes through a transparent medium, the rate of decrease in intensity with the thickness of the medium is proportional to the intensity of the light".

**Beer's Law**

It is analogous to Lamberts law. "The intensity of a beam of monochromatic radiation decreases exponentially as the concentration of absorbing species increases arithmetically.

These two laws are readily combined to give a convenient expression as

$$\text{Log } I_O/I_t = K_{bc}$$

Where, Io = Intensity of incident radiation, It = Intensity of transmitted radiation,

K= constant,

b = thickness of the absorbing medium and

c = concentration of the solution.

This is the fundamental equation for Calorimetry or Spectrophotometry. The term log Io/It is called as absorbance (A) or optical density (OD).

Therefore, $A= \log I_O/I_t = Kbc$

K is replaced with ε when concentration is taken in moles/lit.

Hence, A = εbc, ε is known as molar absorptivity and expressed in $Lmol^{-1}cm^{-1}$.

This equation indicates that the absorbance is directly proportional to the concentration of the solution.

The term It/Io is called as transmittance (T). Hence, T = $I_t/I_o$

Now, %T = $I_t/I_o$ x 100 Rearranging,

$I_o/I_t$ = 100 / %T

Applying logs log $I_o/I_t$ = log 100 – log (%T)

i.e., A = 2- log (%T)

Hence, if the transmittance of a solution is known, then its absorbance is calculated. These two laws are readily combined to give a convenient expression as

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## I. Instrumentation[6]

Instruments for measuring the absorption of U.V or Visible radiation have the following components (Fig. No. 1.4.1.2).

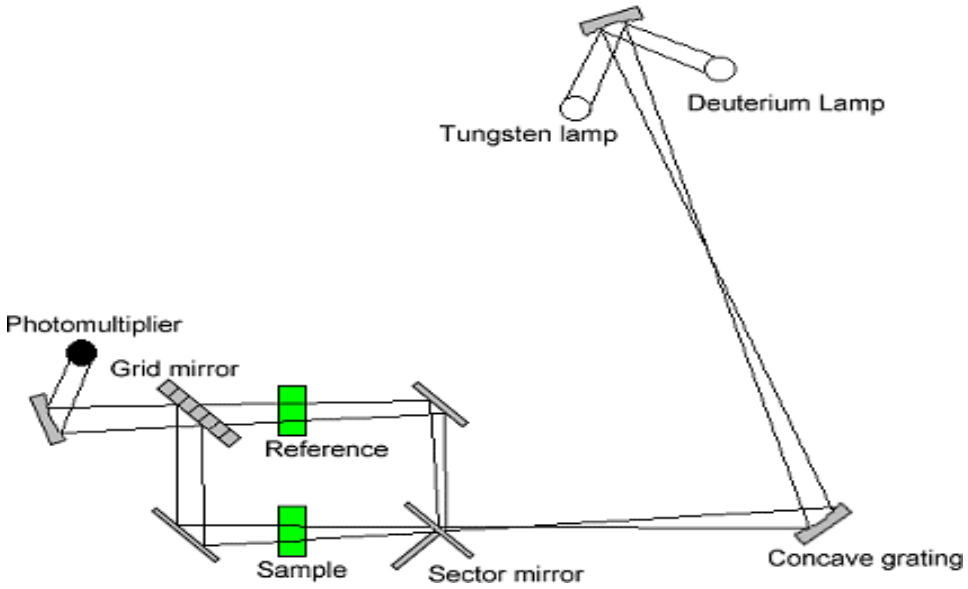



**Fig. 1.4.1.2: Block Diagram of a Double Beam UV-Visible Spectrophotometer.**

### i. SOURCES OF RADIATION

Ultraviolet light is generally derived from a deuterium arc that provides emission of high intensity and adequate continuity in the 190 - 380 nm range. A quartz or silica envelope is necessary not only because of the heat generated but also to transmit the shorter wavelengths of the ultraviolet radiation. The limiting factor is normally the lower limit of atmospheric transmission at about 190 nm.

Visible light is normally supplied by a tungsten lamp or, in modern systems, by a tungsten-halogen (also described as quartz-iodine) lamp which has higher relative output in the cross-over region (320 - 380 nm). The long wavelength limit is usually the cut-off of the glass or quartz envelope, normally well beyond the useful visible limit at 900 nm.

**Fig. 1.4.1.3: Deuterium lamp & Tungsten lamp.**

**ii. WAVELENGTH SELECTOR (MONOCHROMATOR)**

The function of a monochromator is to produce a beam of monochromatic (single wavelength) radiation that can be selected from a wide range of wavelengths. The essential components are (1) entrance slit, (2) collimating device (to produce parallel light), (3) a wavelength selection or dispersing system, (4) a focusing lens or mirror and (5) an exit slit. Two basic methods of wavelength selection may be noted, filters and a dispersing system (e.g. a prism or diffraction grating).

Polychromatic radiation (radiation of more than one wavelength) enters the monochromator through the entrance slit. The beam is collimated, and then strikes the dispersing element at

an angle. The beam is split into its component wavelengths by the grating or prism. By moving the dispersing element or the exit slit, radiation of only a particular wavelength leaves the monochromator through the exit slit.

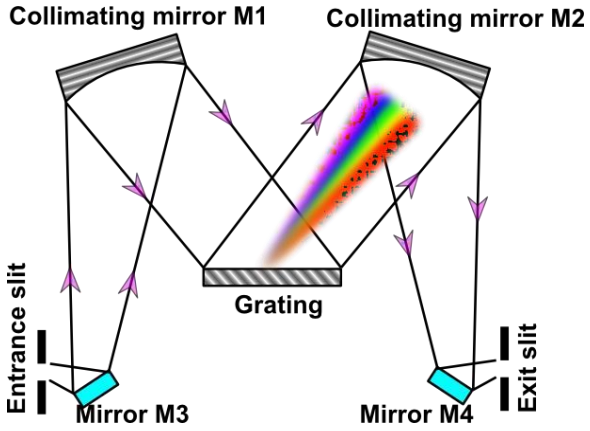



**Fig. 1.4.1.4: Monochromator.**

### iii. CUVETTES

The containers for the sample and reference solution must be transparent to the radiation, which will pass through them. Quartz or fused silica cuvettes are required for spectroscopy in the UV region. These cells are also transparent in the visible region. Silicate glasses can be used for the manufacture of cuvettes for use between 350 and 2000 nm.

 ]

**Fig. 1.4.1.5: Cuvettes.**

### iv. DETECTORS

The photomultiplier tube is the commonly used detector.

**Fig. 1.4.1.6: Photo Multiplier Tube.**

It consists of a photo emissive cathode. (a cathode which emits electrons when struck by photons of radiation), several dynodes (which emit several electrons for each electron striking them) and an *anode.* A photon of radiation entering the tube strikes the cathode, causing the emission of several electrons. These electrons are accelerated towards the first dynode, causing the emission of several electrons for each incident electron. These electrons are then accelerated towards the second dynode, to produce more electrons, which are accelerated towards dynode three and so on. Eventually, the electrons are collected at the anode. By this time, each original photon has produced $10^6$-$10^7$ electrons. The resulting current is amplified and measured. Photomultipliers are very sensitive to UV-Visible radiation and have fast response times.

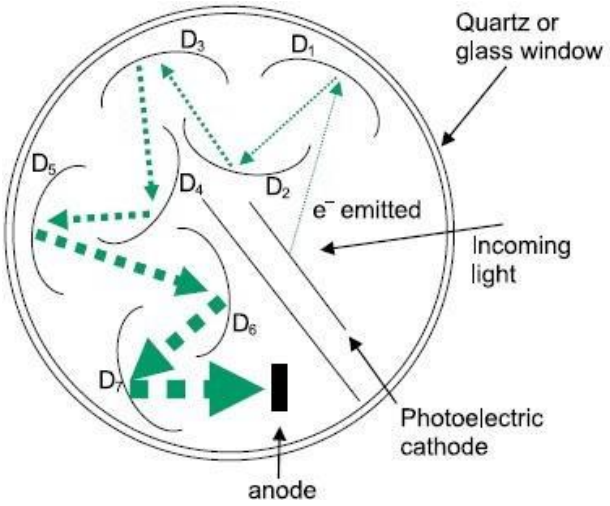



**Fig. 1.4.1.7: Schematic representation of working of PMT.**

**Figure 1.4.1.8: UV-Visible spectrophotometer.**[7]

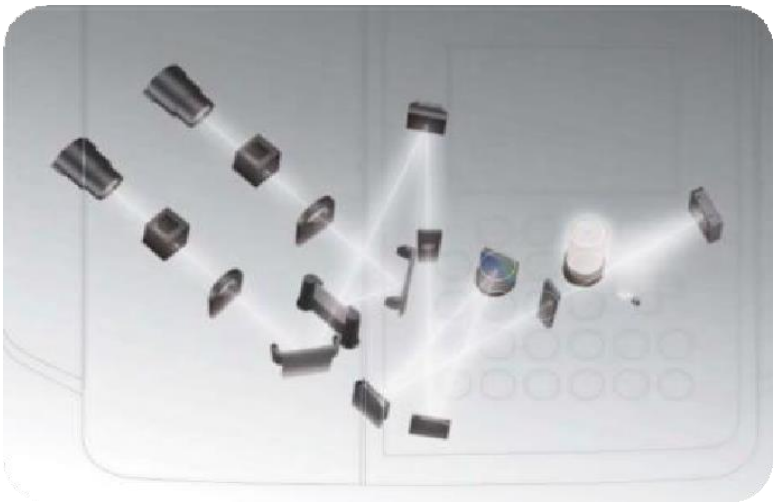


**Figure 1.4.1.9: UV-1800 Optics.**[7]

### 1.1.2. Quantitative estimation by UV- spectroscopy Assay of single component samples

The quantitative analysis of absorbing substance can be carried out by preparing a solution in a suitable transparent solvent for its absorbance measurement at its absorption maximum (where small errors in setting wavelength has little effect on measured absorbance).[7]

The absorbing substance concentration is then calculated from measured absorbance by following methods.

### A. Use of Standard Absorptivity Value

Absorptivity value A (1%, 1cm) or  avoids the need of standard solution of reference substance. It is advantageous where it is difficult or expensive to get pure sample of reference substance.

**B. Use of calibration graph**

In this method, absorbance of reference standard solutions at concentrations encompassing test concentrations are measured, through these measurements a calibration graph is plotted by taking concentration on x- axis and absorbance on y- axis. Analyte concentration in the sample solution read from the graph as concentration corresponding to solution absorbance. Calibration data is essential if absorbance has nonlinear relationship with concentration.

$y= \alpha + \beta x$ may be calculated by least squares method.

$$Intercept = \frac{(\Sigma y)\,(\Sigma x^2) - (\Sigma x) - (\Sigma xy)}{N(\Sigma x^2) - (\Sigma x)^2}$$

$$Slope\ (\beta) = \frac{N\Sigma xy - (\Sigma x)(\Sigma y)}{N\Sigma x^2 - (\Sigma x)}$$

Where y = absorbance value at concentration x.

N = number of measurements.

The data may be further evaluated statistically to confirm a linear relationship between x and y and provides confidence limits for the slope intercept and estimated concentration of sample.

**A. Single- or Double-Point Standardization**

In this procedure absorbance of both sample and a reference standard solution of substance is measured. Standard and sample solutions are prepared in same manner. The standard solution concentration of must be close to sample solution. The concentration of substance in sample is calculated with the following formula

$$C_{test} = \frac{A_{test}}{A_{std}} \times C_{std}$$

$C_{test}$ & $C_{std}$ = Concentration of test & standard

$A_{test}$ & $A_{std}$ = absorbance of Test and Standard solutions.

### 1.1.3. Development of UV- Spectrophotometric method[8]

Analytical method is detailed description of different steps essential to perform analytical test. The very first step in the development of new analytical method is to decide what has to

be measured, as well as how accurately it should be measured. The next step is the literature search. Based literature search and knowledge of the chemistry involved, a method is chosen. After the nucleus of the method has been decided, development process can be undertaken. The experiments should be repeated several times for its repeatability and ruggedness. If there is a need for reference standard, it should be developed at this stage.

Various steps in the development of UV spectroscopic method are described below:

- Suitable solvent selection.
- Analytical wavelength Selection.
- Beer-Lambert's Law Study
- To perform analysis of standard laboratory mixture as well as formulations by developed method.
- Validate the developed methods as per regulatory guidelines.

**Selection of the solvent**

A suitable solvent for Ultra-Violet spectroscopy should meet the following requirements:

- It should not itself absorb radiations in the region under investigation.
- It should be less polar so that it has minimum interaction with the solute molecules
- It should be transparent to UV radiation.

**Analytical wavelength selection**

The wavelength range of UV spectrum is 200- 400 nm, and visible spectrum wavelength range is 400- 800 nm.

The basis for selection of wavelength is solvent choice and nature of analyte i.e., whether that molecule UV active or inactive. The selected wavelength is such that the absorbance units should be in the range of 0.2 -1.2 readings are taken for no of wavelengths and the wavelength where it gives maximum absorption peak is selected.

**Study of Beers-lamberts law**

Beer- Lambert law indicates the analyte response is increase upon increase concentration. The validity of the Beer – Lambert law is affected by number of factors. If absorbing species undergoes association, dissociation, photo degradation, salvation, complexation or adsorption or emits fluorescence, the positive or negative deviation from beer- Lambert law may be observed. Stray light effects and the type of solvent used can also lead to non- compliance

with the Beers law. Concentration range is selected from the Beer – Lamberts law by plotting the graph.

## 1.2. CALIBRATION OF UV-VIS SPECTROPHOTOMETER[9]

In order to use a standard value, the instrument used to make the measurement must be properly calibrated with respect to its wavelength and absorption scales. In addition, checks for stray light and spectral resolution are run.

### Calibration of Absorbance Scale

Potassium dichromate solution is used to calibrate the absorbance scale of a UV spectrophotometer. The absorbance scale calibration wavelengths with corresponding A (1%, 1cm) values for 0.0065% w/v Potassium dichromate solution are 235nm (122.9 -126.2), 257nm (142.4 – 145.7), 313nm (47.0 – 50.3), 350nm (104.9 – 108.2).

### Calibration of Wavelength Scale

The wavelength scale of a UV-Visible spectrophotometer is checked by determining the specified wave length maxima of a 5% w/v solution of holmium per chlorate.

The tolerances for calibration wavelengths are 241.15 ± 1nm, 287.15 ± 1nm, and 361.5 ±1nm.

### Determination of Instrumental Resolution

The resolving power of an instrument is controlled by its slit width settings. The resolving power of an instrument can be assessed by using a 0.02% w/v solution of toluene in hexane. The ratio of the absorbance for this solution at 269nm to that at 266nm should be at least 1.5.

### Determination of Stray Light

stray light is checked by measuring the absorbance of a 1.2% solution of KCl in water against a water blank at a wavelength of 200nm. If the absorbance of the sample is less than 2 then stray light is present and the instrument needs to be serviced.

## 1.5. METHOD VALIDATION

Method validation is documented evidence of developed method by which it is established through laboratory studies, that the performance characteristics of the developed method meet the wishes for the intended analytical applications.

Commonly various analytical procedures to be validated are as follows:

- Identification tests
- Quantitative tests for content of impurities
- Limit tests for the control of impurities
- Quantitative tests of the active moiety in samples of drug substance or drug product or other selected component(s) in the drug product.

Identity of an analyte in a sample performed by means of identification tests, by comparing properties of the sample like spectrum, chromatographic behavior, chemical reactivity etc., with reference standard. A quantitative test or a limit test for control of impurities in a sample. Moreover, the above tests are proposed to accurately indicate the purity characteristics of the sample.

**ICH method validation[10-11]**

Analytical performance characteristics:

- Precision
- Accuracy
- Detection limit
- Quantitation limit
- Specificity
- Linearity
- Range
- Robustness
- System suitability

**Table 1.5.1: Data elements required for Validation (ICH).**

| Type of analytical procedure characteristics | Identification | Testing for impurities | | Assay -Dissolution (measurement only) -Content/potency |
|---|---|---|---|---|
| | | Quantitative | Limit test | |
| Accuracy | - | + | - | + |
| Precision | | | | |
| -Repeatability | - | + | - | + |
| -Intermediate precision | - | +**(1)** | - | + **(1)** |
| Specificity **(2)** | + | + | + | + |
| tection limit | - | - | + **(3)** | - |
| ntitative limit | - | + | - | - |

| | | | | |
|---|---|---|---|---|
| Linearity | - | + | - | + |
| Range | - | + | - | + |

-Signifies that this character is not normally evaluated

+ Signifies that this character is normally evaluated

1. If reproducibility has been performed, then no need of intermediate precision
2. Be deficient in specificity of one analytical procedure could be rewarded by other following analytical procedures.
3. Might be required in some cases.

**1. Accuracy**

The accuracy of an analytical method expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. Sometimes this is termed as reality of method.

**a) Assay of Drug Substance**

Numerous methods are offered the accuracy of the analytical procedure:

1. By comparing the results of developed analytical procedure with an analyte of known purity i.e., reference material.
2. By means of comparing the results of the developed analytical procedure with those of a second well-characterized procedure, the accuracy of which is stated and/or defined.

**b) Assay of Drug Product**

A number of methods for determining method accuracy are as follows:

a. Use of the analytical procedure to synthetic mixtures present in drug product components to which known quantities of the drug substance to be analyzed have been added.
b. If it is not possible to attain all sample components of drug product, it may be acceptable moreover to add well-known quantities of the analyte to the drug product or by comparing the results obtain from a second, well considered procedure, the accuracy of which is stated and/or defined.
c. Once method precision, linearity and specificity have been established, then the accuracy is performed.

**Suggested Data**

Accurateness of proposed analytical procedure must be assessing by using a minimum of 9 determinations at three different concentration levels cover the specified range (e.g., 3concentrations / 3 replicates at each level concentration analytical procedure). Accuracy must be reported as percent recovery by taking assay of well-known added amount of analyte in the sample.

**2. Precision**

Precision of an analytical method indicates the ability of method to produce repeatable and reproducible results when a sequence of measurements attained from multiple sampling of the similar homogeneous sample by using the prescribed conditions. In other words, it is the extent of individual test results from a series of standards agrees. The measured standard deviations can be categorized into three levels: Repeatability, Intermediate precision and Reproducibility. Precision have to be investigated using uniform, valid samples. On the other hand, if it is not possible to find an uniform samples it may be investigated using artificially prepared samples or a sample solution. The meticulousness of an analytical method is usually uttered as the variance, standard deviation or coefficient of variation of a sequence of measurements.

**Repeatability**

Repeatability obtained by carried out analysis under the same operational conditions over a relatively short time span. Repeatability is in addition termed intra- assay precision. Repeatability must be assessed by using minimum of 9 determinations covering the specified range for the procedure (e.g., 3concentrations/3 replicates each) or With minimum of 6 determinations at 100% of the test concentration.

**Intermediate precision**

It indicates the long- term variability of the measurement by analytical procedure within-laboratories variations: different days, different analysts, different equipment, etc. The main objective of intermediate precision is to prove that in the same laboratory the method will produce the same results. The level to which intermediate precision have to be established depends very much on the type of analysis and circumstances under which the procedure is planned to be used. Distinctive variations to be studied include different days, analysts, equipment, etc.

**Reproducibility**

Reproducibility of analytical method represents the precision obtained between laboratories (collaborative studies, usually applied to standardization of methodology). Reproducibility is carried out by means of an inter-laboratory trial. Reproducibility must be considered in case of the standardization of an analytical method, for case in point, for enclosure of procedures in pharmacopoeias.

**Suggested Data**

The standard deviation, relative standard deviation (coefficient of variation) and confidence interval should be reported for each type of precision investigated.

**Specificity**

Method specificity is the capability to measure unambiguously the analyte in the being there of components which might be expected to be present. Characteristically these may include impurities, degradants, matrix, etc. be deficient in specificity of an individual analytical method may be remunerated with other supporting analytical procedure(s).

**Assay (content or potency)**

To afford exact results which allow an accurate declaration on the content or potency of the analyte present in a sample, an investigation of specificity must be conducted for the period of the validation of identification tests, the resolve of impurities and assay. The procedures used to express specificity will depend on the Proposed purpose of the analytical procedure. It is not possible for all time to show that an analytical procedure is specific for a particular analyte (whole discrimination). In that case combination of two/ more analytical procedures is optional to attain the essential level of discrimination.

**3. Detection limit and Quantification limit**

Detection limit is lowest concentration of analyte in a sample that can be identify, but not inevitably quantified, under the stated experimental conditions. With UV detectors, it is not easy to assure the recognition accuracy of small level compounds due to probable regular loss of sensitivity of detector lamp with age, or noise level variation by detector manufacturer.

Quantitation limit is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions.

Several approaches for determining the detection limit are possible, depending on whether

the procedure is a non –instrumental or instrumental.

- Based on visual assessment
- Based on signal-to-noise
- Base on standard deviation (SD) of the response and the slope The Detection limit (DL) may be expressed as

$$\mathbf{DL = 3.3\ \sigma/S}$$

$$\mathbf{QL = 10\ \sigma/S}$$

Where, σ= standard deviation of the response S=Slope of calibration

The slope S may be predictable from the calibration curve of the analyte.

### 4. Robustness

The concept of robustness of an analytical procedure has been defined by the ICH as "A measure of its ability to stay unchanged by minute, but purposeful variations in method parameters". A good practice is to vary, important parameters in the method, systematically and measure their effect on separation.

### 5. Ruggedness

Degree of reproducibility of test results attain by the analysis of identical samples in a variety of condition such as special laboratories, different analysts, different instruments etc., normally expressed as the lack of influence on test results of operational and environmental variable of the analytical method. Ruggedness is a measurement of reproducibility of the test results under the variable condition usually expected from one laboratory to another laboratory and from one analyst to another analyst. Degree of representation of test results is then determined as the function of the assay variable.

### 6. Linearity and Range

The linearity of an analytical method is its capability (within a specified range) to elicit test results that are directly proportional to the concentration (amount) of analytes in the sample. A linear association must be evaluated across the range of the analytical procedure. It may be established directly on the drug substance (by dilution of a standard stock solution) and/or separate weighing of synthetic mixtures of the drug product components, using the proposed procedure. The later aspect can be studied during investigation of the range.

Linearity must be evaluated by visual inspection of a plot of signals as a function of analyte concentration or content. In case there is a linear association, test results must be evaluated by

suitable statistical methods, for example, by calculation of a regression line by the method of least squares. In a few cases, to find linearity between assays and sample concentrations, the test data necessitate subjected to a mathematical transformation proceeding to the regression analysis. Data from the regression line itself may be helpful to provide mathematical estimates of the degree of linearity. The correlation coefficient, y-intercept, slope of the regression line and residual sum of squares should be submitted. A plot of the data should be included. In addition, an analysis of the deviation of the actual data points from the regression line may also be helpful for evaluating linearity.

The range of an analytical method is the intermission between the higher and inferior concentration (amounts) of analyte in the sample (together with these concentrations) for which it has been confirmed that the analytical procedure has a suitable level of precision, accuracy and linearity.

The following minimum specific ranges have to be considered:

- For drug substance or a finished product assay: normally from 80-120% of the test concentration.
- For content uniformity, covering a minimum of 70-130% of the test concentration,
- For dissolution testing: +/-20 % over the specified range e.g., if the specifications for a controlled release product cover a region from 20%, after 1 hour, up to 90%, after 24 hours, the validated range would be 0- 110% of the label claim.
- For the estimation of an impurity: from the reporting level of an impurity to 120% of the specification.
- For impurities which are known to be abnormally potent or to produce toxic or unexpected pharmacological effects, the detection/quantitation limit should be commensurate with the level at which the impurities must be controlled.

**System suitability testing**

System suitability testing is an essential part of many analytical procedures. These are to be established for a particular procedure depending on the type of procedure being validated.

**Note**

**I.** Establishment of Detection Limit and Quantitation limit are not required for assay validation as per International Conference on Harmonization Guidelines.

**II.** Robustness and system suitability testing are not applicable for assay by UV

spectrophotometry

## 2. LITERATURE REVIEW[13-39]

### 2.1. Analytical Profile of Tadalafil

Extensive literature survey has done on the Tadalafil and found few spectroscopic methods, HPLC, and UPLC methods have been reported for the estimation of Tadalafil in bulk, other dosage forms.

1. **Mohammad yunoos *et al.*,**[13] has developed UV Spectrophotometric method for determining the tadalafil in bulk and tablet dosage forms which is simple, sensitive, precise and highly accurate. Tadalafil in methanol shows the max. Absorbance at 284 nm. It was observed to obey Beer's law in the concentration range of 2 to 20 µg/ml with $1.65x10^4$/mol/cm, correlation coefficient, intercept, the slope, quantitation and detection limits have also been calculated. The proposed method was successfully applied for the drug analysis in pure form and also in its tablet's dosage forms. Percentage recovery and the placebo interference results show that this method was unaffected even if common excipients are present. Then validation of method by determining the accuracy, sensitivity and precision which proves the suitability of the method, that has been developed, for estimating Tadalafil in both bulk and solid dosage form.

2. ***M.M. Mathpati et al.,***[14] developed and validated a new stability-indicating LC assay method for quantitatively in presence of forced degradation products exterminating Tadalafil in both pharmaceutical dosage form and Bulk drug. An isocratic reverse phased LC method was implemented to separate the drug from its degradation products using mobile phase as water – Acetonitrile mixture and column Zorbax SB-C18 at a wavelength of 235 nm. Stress conditions were performed on Tadalafil in a view to perform its acid, base, photolytic, oxidative and thermal degradation. Tadalafil was observed to degrade in the presence of acid, base, and 30% H2O2. It was found that the drug would stabilize itself under other stress conditions. Percentage recovery ranges between 98.89% to 101.25% of the Tadalafil in the pharmaceutical dosage form. By Forced degradation studies the method was proved as stability indicating.

3. **Bojanapu a *et al.*,**[15] reported a RP-HPLC method for rapid assay of Tadalafil in pharmaceutical dosage form which is simple, precise and accurate method. Isocratic elution at a flow rate of 1.0ml/minute was employed and column of a symmetry C18 at

ambient temperature. Acetonitrile: buffer 50:50(v/v) as mobile phase and the detection wavelength was at 284nm.Linearity was observed in concentration range of 20-70µg/ml. The retention time for Tadalafil was 2.98 minutes. The method was validated as per the ICH guidelines.

4. **B. Prsannareddy *et al*.,**[16] developed a RP-HPLC method for the analysis of Tadalafil which is stability indicating. The samples separated by using an inertsil C18 (150mmx4.6mm,5µm) as column by isocratic run using mobile phase acetonitrile and phosphate buffer, with a flow rate of 0.8ml/minute, and 260 nm was the determined wavelength for analysis of tadalafil. The method was linear within the range of 70-130µg/ml. The precision, ruggedness and robustness values were also within the prescribed limits. The sample was meant for force degradation studies and the stressed samples were analysed by the proposed method. Chromatographic peak purity results indicated the absence of co-eluting peaks within the same peak of Tadalafil, which demonstrated the specificity of assay method for estimation of Tadalafil in presence of degradation products.

5. **Gudipatiy Yedukondalu *et al*.,**[17] developed a RP-HPLC method for rapid assay of Tadalafil tablet dosage form which is simple, precise and accurate. Isocratic elution at a flow rate of 1.3ml/minute by using column as symmetry chromosil C18 (250x4.6mm, 5µm in particle size) at ambient temperature. Methanol: acetonitrile 65:35 v/v used as mobile phase. The wavelength was 222nm and injection volume as 20µl sample injected. The retention time for tadalafil was 7.8minutes. As per the ICH guidelines the method was validated.

6. **M. Sankar*et al*.,**[18] has developed a stability indicating, RP-HPLC method for determining of tadalafil and validated in oral dosage form which is simple, selective but precise. The chromatographic conditions of a mixture of buffer: acetonitrile in a ratio 55: 45 (v/v) as mobile phase and the column Zorbax C18 measuring 250X4.6 mm, 5µ. The detection was observed at 225nm with a flow rate of 1.5ml/minute. It has been validated according to USP and ICH guidelines... A forced degradation analysis under varied conditions of light, thermal, basic, acidic, peroxide and UV radiation was performed on the drug. The results have proved the selectiveness and reproducibility for the determination of tadalafil, of the method. The so-proposed stability indicating RP-HPLC method can be employed for the effective determination of tadalafil in routine drug

analysis of the oral jelly dosage form.

7. **P.H. Sonawane *et al.*[19]** has developed and validated reverse-phase high- performance liquid chromatography method for tadalafil in bulk and tablet dosage form which is the simple, selective, precise and accurate method. The Inertsil C18 (150×4.6 mm; 5 µm) as column in isocratic mode. The mobile phase is a mixture of acetonitrile (50:50% v/v) and phosphate buffer (10 mM, pH 3.2) at the flow rate of 1.0 ml/min with UV detection at 295 nm. The tadalafil was observed a retention time around 4.01 min. Linearity was observed in the concentration range from 60 to 140 µg/ml for tadalafil with a correlation coefficient of ($\mathbf{r^2}$) 0.9998. The method was validated in accordance to International Conference on Harmonization guidelines in terms of accuracy, linearity, specificity and precision. Hence, the method proposed can be employed for routine quality control of tadalafil in both the bulk and tablet dosage forms.

8. **Alivelu Samala, *et al.,*[20]** has developed a simple and accurate method for the estimation of Tadalafil in tablet dosage form by HPLC. A sharp peak has been Observed at 3.068min, on a column Agilent Eclipse XBD C18 (150 X 4.6mm I'd., 5µm particle size) with mobile phase as acetonitrile, and buffer solution (50:50v/v), delivered at a flow rate of 1.2ml/min. at 282nmin isocratic mode, and the diluents as methanol. The linear dynamic response was observed to be in the concentration range of 10-150µg/ml and shows a correlation coefficient ($r^2$) of 0.999. Recovery studies have determined accuracy and it ranges from 100.3- 100.8% in tablet dosage form.

9. **Sutar**, ***et al.,*[21]** A reverse phase high performance liquid chromatography has been developed for the estimation of tadalafil in form of tablet dosage which is simple, accurate, precise and specific method, For HPLC method, column is Hi-Qsil C18- 10 in isocratic mode has Acetonitrile; acetate buffer pH (2.8) in a ratio of 45: 55 v/v as a mobile phase. The detection wavelength at 283 nm. Retention time was found to be 4.46 ± 0.03 minutes and flow rate was 1 mL/min. Method in terms of accuracy, linearity and precision was validated. Results of the analysis prove that the method can be effectively used for the estimation of tadalafil in form of tablet dosage.

10. **Dhanesh war SR, *et al.,*[22]** developed and validated for simultaneous quantitation of Tadalafil and Dapoxetine HCL in bulk drug and formulation by HPLC method. This method is based on separation of the two drugs by HPLC on the column thermo hypersil

BDS-C18 (250mm×4.6mm, 5.0µ) from Germany with isocratic conditions and acetonitrile: 0.1% triethyl amine in water pH adjusted to 4.0 with ortho phosphoric acid (80:20) as a simple mobile phase at flow rate of 1mL / min using UV detection at 229nm. The linear regression analysis data for the calibration plots showed a good linear relationship over the concentration range of 0.25-4 µg / mL for Tadalafil and 0.75-12µg/mL for Dapoxetine HCL respectively. The limit of detection (LOD) and limit of quantitation (TOQ) was 0.010 µg/mL and 0.025µg/mL for Tadalafil and 0.005µg/mL and 0.020µg/mL for Dapoxetine HCL respectively.

11. **Divya Yada *et al.*[23]** developed a HPLC method which is of the simple yet reliable and also reproducible have been developed for the analysis of the Tadalafil and Sildenafil citrate (API). YMC-Pack ODS AQ of dimensions 150 mm x 4.6 mm, I'd as column. Phosphate buffer (10mM, pH 3.0) and acetonitrile as a mobile phase gradient run at the flow rate of 1mL/min with UV (PDA) detector at 220nm at ambient temperature. Tadalafil and Sildenafil citrate from tablet was extracted by carried out using methanol. Linearity was observed in the range from 50 to 150µg/ml for Tadalafil with a correlation coefficient ($r^2$) 0.99 and 10ng/ml as the limit of detection. The values of linearity range, correlation coefficient ($r^2$) and limit of detection were 50 to 150µg/ml, 0.99 and 20ng/ml respectively for Sildenafil. Validation parameters prove the precision and stability of the method and it's applicability for the Assay of Tadalafil and Sildenafil citrate. The method is suitable for routine analysis of the drug.

12. **Sree Ganesh K *et al.*,**[24] developed a UPLC method was developed and validated for simultaneous determination of tadalafil and their impurities in tablets which is stability indicating. The chromatographic separation was performed on Acquity HSS T3 column (1.8 µm, 2.1 mm × 150 mm) using gradient conditions with methanol and ammonium acetate buffer (0.02 M; pH 4.0 adjusted with acetic acid) at flow rate of 0.35 mL/min. UV detection was performed at 262 nm. Total run time was 10 min for performing the analysis in which main compound; five known and other unknown impurities were separated. The method was suitably validated with respect to linearity, limit of detection, limit of quantification, accuracy, precision and selectivity. The calibration curves obtained for the five impurities were linear over the range 0.112 to 1.96 µg/ml. The relative standard deviations of intra and inter day experiments were less than 1.5%. The detection limits ranged from 0.039 to 0.040 µg/mL depending on the impurity.

13. **C. Rambabu *et al.,***[25] developed a two simple and sensitive UV-Visible Spectrophotometric methods A and B for the determination of tadalafil have been described. These methods are based on the formation of bluish green colour and blue colored chromogens obtained when the drug was condensed with Isatin and Xanthydrol exhibiting λ max at 665 and 640nm respectively. These methods obey Beer's Law in the concentration ranges of 0.4-2.0 and 4-20 µg/mL. The optimum conditions for all colour development are described and the proposed methods have been successfully applied for the determination of tadalafil in bulk drug and pharmaceutical formulations. The common excipients and additives did not interfere in this method.

14. **Nief Rahman Ahmed**[26] developed a simple, accurate, precise, rapid, economical and a highly sensitive Spectrophotometric method has been developed for the determination of tadalafil in pharmaceutical preparations and industrial waste water samples, which shows a maximum absorbance at 204 nm in 1:1 ethanol- water. Beer's law was obeyed in the range of 1-7µg/ mL, with molar absorptivity and Sandel's sensitivity of $0.783x10^5$ l/mol.cm and 4.97 ng/cm$^2$ respectively, relative standard deviation of the method was less than 1.7%, and accuracy (average recovery %) was 100 ± 0. 13. The limits of detection and quantitation are 0.18 and 0.54 µg.ml$^{-1}$, respectively. The method was successfully applied to the determination of tadalafil in some pharmaceutical formulations (tablets) and industrial wastewater samples. The proposed method was validated by sensitivity and precision which proves suitability for the routine analysis of tadalafil in true samples.

15. **Safwan Fraihat**[27] developed two Spectrophotometric methods for the determination of Tadalafil (TDF) in pure and pharmaceutical form. Method A is based on oxidation of (TDF) with a known excess amount of Ce (IV) then estimation of the unreacted amount of Methyl orange (MO) dye at 507 nm, Method B is based on the oxidation of (TDF) with excess N-bromosuccinamide then estimation the amount of Indigo carmine (IC) at 610 nm. The methods were linear in the concentration ranges 18–60 and 10–55 µg/ml with correlation coefficients of 0.993 and 0.992 and limits of detection LOD of 10.5 and 5.3 µg/ml for the two methods respectively. The proposed methods were applied for the determination of the drug in pharmaceutical formulations with recovery and relative standard deviations of 97% ± 1.4 and 98% ± 1.1 for the two methods. The developed methods are equally accurate, precise and reproducible compared to the official methods.

16. ***Ayman A. Gouda et al.***[28] developed two simple, rapid, and extractive Spectrophotometric methods were developed for the determination of tadalafil (TDF) in both pure and tablet dosage form. These methods are based on the formation of ion-pair complexes between the basic nitrogen of the drug with bromocresol purple (BCP) and methyl orange (MO) in acidic buffer solution. The formed complexes were extracted with chloroform and measured at 410 and 425 nm using BCP and MO, respectively. Beer's law was obeyed in the range 2.0–20 µg mL−1 with correlation coefficient (n = 6) ≥0.9996. The molar absorptivity, Sandell sensitivity, detection and quantification limits were also calculated. The composition of the ion pairs was found 1:1 by Job's method. The proposed methods have been applied successfully for the analysis of TDF in pure and in its dosage forms. These developed methods were validated for accuracy and precision.

**2.2 Analytical Profile of Valsartan**

Extensive literature survey has done on Valsartan and found spectroscopic methods, and HPLC methods have been reported for the estimation of Valsartan in bulk, other dosage forms.

1. **Mallegowda *et al.*,**[29] developed a simple, sensitive and extraction free Spectrophotometric method for the quantitative estimation of Valsartan in both pure and pharmaceutical preparation. The method is based on transfer complexation reaction between Valsartan as n- electron donor and p- chloranilic acid as π-acceptor. Valsartan reacted with p-chloranilic acid to produce bright pink colour complex with maximum absorbance at 530 nm. Beer's law obeyed in the concentration range of 5-50 µg/ml.

2. **Galande *et al.*,**[30] developed a simple UV Spectrophotometric method for the estimation of amlodipine besylate, Valsartan and hydrochlorothiazide in bulk mixture and tablet. The measurement of absorbance at absorbance maxima of 359 nm, 317 nm and 250 nm for amlodipine besylate, hydrochlorothiazide and Valsartan in methanol respectively. The Beer lambert's law obeyed concentration ranges of 5-25 µg/ml, 10-50 µg/ml and 5-25 µg/ml for amlodipine besylate, hydrochlorothiazide and Valsartan respectively.

3. **Deshpande *et al.*,**[31] reported a simple, accurate, precise UV spectrometric method for the estimation of Valsartan and hydrochlorothiazide in pharmaceutical formulation. Method-1 absorption ratio method (Q-analysis) using two wavelength 265 nm (isobestic point) and 249 nm (maximum absorbance of Valsartan) Method-II area under curve. The area under curve in the range of 249- 259 nm and 261-281 nm for the analysis of Valsartan and

hydrochlorothiazide respectively. Linearity for the detector response in the range of 2-24 µg/ml & 2-14µg/ml for the Valsartan and hydrochlorothiazide respectively. The two methods were validated as per ICH guidelines.

4. **Tarkase Kailash N *et al.,*** [32] developed two new simple and precise UV Spectrophotometric methods for estimation of Valsartan in bulk and tablet formulation in phosphate buffer 6.8. The drug obeyed the Beer's law with correlation coefficient 0.9995 and 0.9998 respectively for Method-I and Method-II. It showed absorbance maxima at 250 nm and 220 nm respectively for method-I and method-II in phosphate buffer 6.8. The linearity was observed between 5-30 µg/ml. The results of analysis were validated for recovery studies, accuracy, precision, LOD, LOQ and robustness.

5. **Narendra *et al.,*** [33] developed three simple, accurate spectroscopic methods for the determination of Valsartan in pharmaceutical formulations. Method A based on formation of colored species on binding of drug with ligand, 1-10 phenanthroline followed by ferric chloride and orthophosphoric acid. developed chromogen shows the maximum absorbance at wavelength 510 nm, linearity in the range of 1-6 µg/ml. Method B based on reaction between drug and 2-2' Bipyridyl with ferric chloride and orthophosphoric acid to form a coloured chromogen its shows maximum absorbance at wavelength 520 nm and linearity in the range 20-80 µg/ml. Method C based on oxidation of the drug in the presence of ferric chloride followed by complex with bathophenanthroline to form a coloured chromogen its shows the maximum absorbance at wavelength 620 nm, linearity in the range of 1-5 µg/ml.

6. **K. S. Nataraj *et al.,*** [34] reported simple, precise and accurate UV spectroscopic method for estimation of Valsartan in pure form and pharmaceutical dosage forms. This method is based on measurement of absorption in UV light, the spectra of Valsartan in methanol showed maximum wavelength at 250 nm and concentration ranging from 2-20 µg/ml with correlation coefficient 0.9998. The LOD and LOQ were found to be 0.15 and 0.449 respectively. Validation was performed as per ICH guidelines.

7. **G. Tuljarani *et al.,*** [35] performed a simple spectroscopic method for the estimation of Valsartan and benazepril hydrochloride in pure and pharmaceutical formulations. This method is based on formation of ion association complex by the drug with safranin-O, in buffer of $P^H$ 9.8. The ion association complex formed was quantitatively extracted under

the experimental conditions in to chloroform. The absorbance of the chloroform layer of each drug measured at its wavelength against reagent blank.

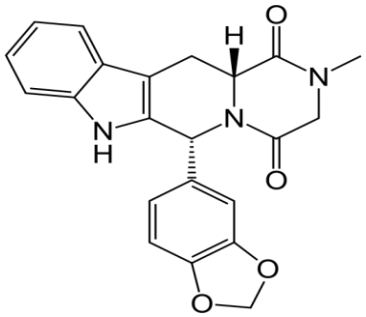
8. **Ramachandran *et al.,*** [36] developed a simple and extraction free Spectrophotometric method for the simultaneous estimation of Valsartan and Ezetimibe in pharmaceuticals. It is developed using acid dyes namely, bromophenol blue (BPB), bromocresol green (BCG). The method was based on ion-par formation between Valsartan and acid-dye. The yellow-coloured ion-pair induce bathochromic shift in the spectrum with maximum absorbance at 425 and 428 nm for BPB &BCG respectively. Its obeyed Beer's law in the range of 5-40 & 1-50 for BPB & BCG respectively. The developed method was validated as per ICH guidelines.

9. **K.R. Gupta *et al.,*** [37] developed a simple, accurate method for the simultaneous estimation Valsartan and amlodipine in tablet by UV spectrophotometry. Method A employs the estimation of drug by simultaneous equation method using 250 and 238 nm for VAL & AMD respectively. Method B employs the estimation of drug by absorption correction method at 360 nm i.e. wavelength of one drug and 326.0 nm an isobestic wavelength VAL and AMD individually and in mixture follows Beer's law over the concentration range 5-30 µg/ml.

10. **K.R. Gupta *et al.,*** [38] reported two simple, accurate UV- spectroscopic methods for the estimation of Valsartan in bulk and tablet dosage form. The zero order spectra of Valsartan in methanol showed maximum absorbance at 250 nm and estimation carried out A (1%, 1cm) and by comparison with standard (Method I). The second order spectra showed maximum absorbance at 241 nm where n=2 and estimation were carried out by comparison with standard (Method II) calibration graph were found to be linear ($r^2$ = 0.999) over the concentration range 10-50 µg/ml.

11. **Ankit *et al.,*** [39] developed a new absorption ratio method for the determination of Valsartan and hydrochlorothiazide in pharmaceutical dosage forms. Calibration curve for Valsartan and hydrochlorothiazide over concentration range of 2-20 µg/ml were plotted and molar absorptivity for both drugs was calculated at both the wavelength of 270.5 nm (wavelength of hydrochlorothiazide) 231.5 nm (isobestic point). Recovery studies ranging from 99.05-102.23% for Valsartan and 97.42–100.22% for hydrochlorothiazide.

## 3. DRUG PROFILE

| | | |
|---|---|---|
| **Drug Name** | **:** | **Tadalafil** |
| **Molecular Structure** | **:** |  |
| **Chemical Name** | **:** | (6R, 12aR)-2, 3, 6, 7, 12, 12a-hexahydro-2-methyl-6-(3, 4-methylene dioxyphenyl) pyrazino (1′, 2′: 1, 6) pyrido- (3,4-b) indole-1, 4- dione |
| **Molecular Formula** | **:** | $C_{22}H_{19}N_3O_4$ |
| **Molecular Weight** | **:** | 389.4 |
| **Category** | **:** | Oral phosphodiesterase type 5 inhibitor (PDE5) |
| **Description** | **:** | White crystalline powder |
| **Solubility** | **:** | Insoluble in water, slightly soluble in ethanol, Soluble in acetonitrile. |
| **Brand names** | **:** | Cialis, Adcirca |
| ***PKa*** | **:** | Acidic – 1.2; Basic-12.1 |
| **Melting Point** | **:** | 301°C – 302°C |
| **Mechanism of action** | **:** | |

Tadalafil is a selective inhibitor of (cGMP) - specific phosphodiesterase type-5 (PDE- 5). Through the inhibition on PDE-5, (*R, R*)-tadalafil increases the concentration of (cGMP), producing smooth muscle relaxation and increased blood flow to the corpus cavernosum, thereby enhancing erectile response following appropriate sexual stimulation.

### DESCRIPTION

Each tablet contains 40mg of Tadalafil and is formulated for oral administration. In addition to active ingredients Tadalafil each tablet also contains the following ingredients: Lactose, microcrystalline cellulose, sodium starch glycolate, povidone, sodium lauryl sulphate, magnesium stearate, hydroxy propyl cellulose talc, polyethylene glycol, titanium dioxide, iron oxide red.

### Clinical Pharmacology

**Adcirca:** Inhibiting the phosphodiesterase type 5(PDE5) using Tadalafil causes increase in concentrations of cyclic guanosine monophosphate (cGMP), which in turn results in relaxation of the pulmonary vascular smooth muscle cells and also the vasodilation of the pulmonary vascular bed.

**Cialis:** Enhanced effect of the nitric oxide at the nerve-ending and the endothelial cells in corpus cavernosum due to inhibition of the PDE5 in corpus cavernosum of penis. This leads to the vasodilation, an increased blood inflow into the corpus cavernosum and also in ensuing the penile erection upon sexual stimulation.

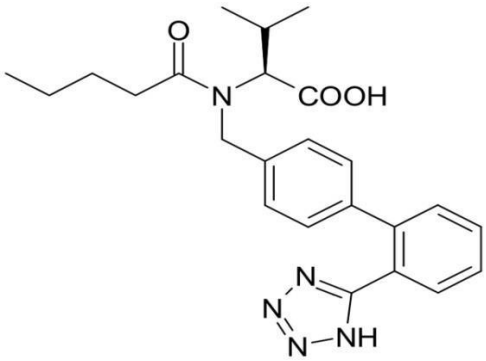
**Pharmacokinetic Data**

**Absorption:** Bioavailability undetermined. T max is 30min to 6 h (median, 2h) (Cialis) and 2 to 8 hrs. (Median, 4h) (Adcirca). The Steady state is attained within 5 days of once-daily dosing.

**Distribution:** VD of tadalafil is approximately 77L (Adcirca) and 63L (Cialis). Tadalafil is 94% protein bound.

**Metabolism:** It is metabolized by CYP3A4 to a catechol metabolite, which undergoes further metabolism to the methyl catechol glucuronide which is a major circulating metabolite.

**Elimination:** Mean oral Cl is 3.4 L/h (Adcirca) and 2.5 L/h (Cialis). The terminal half-life is 15 h (Adcirca) and 17.5 h (Cialis). Tadalafil is excreted major as metabolites, mainly in the feces (~ 61%) and to a lesser degree in the urine (~ 36%).

| | | |
|---|---|---|
| **Drug Name** | **:** | **Valsartan** |
| **Molecular Structure** | **:** |  |
| **IUPAC Name** | **:** | (2s)-3-methyl-2 - [pentanoyl – [[4 - [2-(2H-tetrazol -5-yl) phenyl] methyl] amino] butanoic acid. |
| **Molecular Formula** | **:** | C24H29N5O3 |
| **Molecular Weight** | **:** | 435.518 |
| **Category** | **:** | Anti-hypertensive |
| **Description** | **:** | A white to almost white powder |
| **Solubility** | **:** | very soluble in methanol, ethanol, practically insoluble in water |
| ***PKa*** | **:** | 3.6 |
| **Melting Point** | **:** | 116-117°C |
| **Dosage forms** | **:** | Tablets |
| **Brand names** | **:** | Valent-40 |

**Administration** : Oral administration
**Dose** : 80-160 mg daily

**Pharmacodynamics**

By blocking of AT1 receptor, the drug inhibits the actions of angiotensin II and produce following effects.

- Vasodilation and consequent decrease in peripheral vascular resistance.
- Decreased retention of sodium and water due to decrease in aldosterone synthesis.
- Decrease release of vasopressin which is an anti-diuretic hormone and a vasoconstrictor. All these effects lead to fall in elevated blood pressure.
- Decreases in cardiovascular hypertrophy
- They also reduce diabetic nephropathy and improve kidney function.

**Mechanism of action**

Valsartan is an Angiotensin-II receptor blocker that selectively inhibits the binding of angiotensin –II to angiotensin- I. which is found in many tissues such as adrenal glands and vascular smooth muscle. They act by blocking the angiotensin receptor of subtype I (AT1) which regulates the effects angiotensin-II on blood pressure, sodium and water balance.

**Pharmacokinetic data**

**Half-life:** The initial phase $t_{1/2\alpha}$ < 1 hour while terminal phase $t_{1/2\beta}$ is 5-9 hours
**Absorption:** Absolute bioavailability = 23% with high variability
**Distribution:** 17 L (low tissue distribution)
**Metabolism:** Valsartan in excreted largely as uncharged drug (80%) and minimally metabolized in humans. The circulating metabolite 4-OH- valsartan is pharmacologically inactive and produces by CYP2C9 4-OH Valsartan accounts for approximately 9% of the circulating of dosage of Valsartan.
**Excretion:** 83% of absorbed Valsartan is excreted in faces and 13% is excreted in Urine primarily as uncharged drug.

**Clearance**

- L/h (IV Administration)
- 4.5 L/h (Heart patients receiving oral administration 40 to 160 mg twice a day)

**Therapeutic uses**

- Treating of high blood pressure and heart failure
- It is also used for the improve the chance of living longer after a heart attack

**Side effects**

- Head ache, Excessive tiredness
- Nausea, Stomach pain
- Back pain, Joint pain
- Blurry vision
- Cough
- Rash
- Dizziness
- Upper respiratory infection
- Diarrhea
- Edema
- Pharyngitis

**Contra indications**

- Should not use with the rennin inhibitor aliskiren in people with diabetes mellitus
- Should not use in the people with kidney disease

**Drug interactions**

- Other inhibitors of the rennin – Angiotensin system may increase the risk of low blood pressure, kidney problems and hypercalcemia.
- Potassium sparing diuretics, potassium supplements and salt substitutes containing potassium may increase the risk of hypercalcemia.
- NSAIDS may increase the risk of kidney problems and may interfere blood pressure lowering effects.

## 4. AIM ANDOBJECTIVE

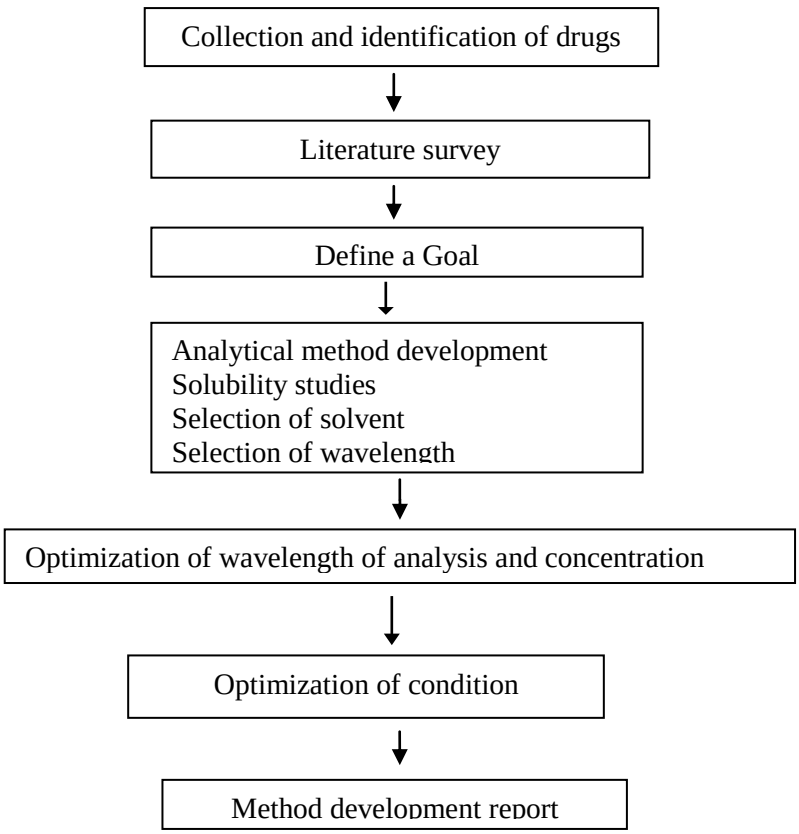
The drug analysis plays an important role in the development of new analytical methods, their manufacture and the therapeutic use. Pharmaceutical industries rely upon quantitative chemical analysis to make sure that the raw materials used and the final product obtained meets the required specifications. For the present study drugs Tadalafil and Valsartan were

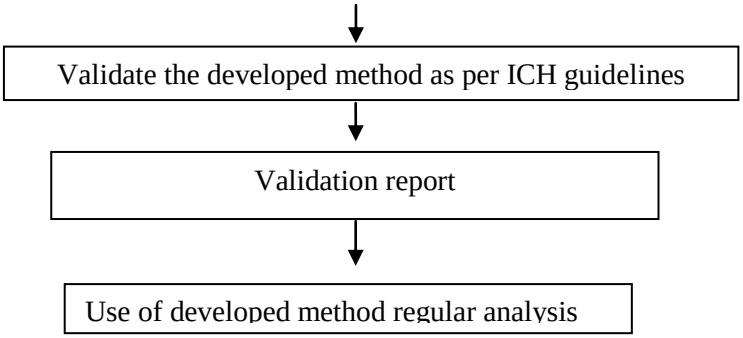
selected as the need of newer analytical method for the estimation of Tadalafil and Valsartan in pharmaceutical formulation. The current status of analytical profile of selected drug Tadalafil and Valsartan was thoroughly reviewed through journals and internet access. An attempt was made to develop and validated a specific, precise, accurate, linear simple UV spectroscopic methods for estimation of Tadalafil in tablet dosage form and Valsartan in tablet dosage form.

- The aim of the present work is to develop a novel, simple, rapid and accurate method using UV spectroscopic method for the assay of Tadalafil in bulk & tablet dosage form and the assay of Valsartan in bulk & tablet dosage form.
- In present project work, it is planned to
  - Develop UV method for quantitation of Tadalafil in bulk & tablet dosage form,
  - Develop UV method for quantitation of Valsartan in bulk & tablet dosage form,
  - Validate the developed methods as per ICH Guidelines.

## 5. PLAN OF WORK

Present work was planned to develop a new, simple, precise and accurate method for analysis in quantitation of Tadalafil in bulk & tablet dosage form and Valsartan in bulk & tablet dosage form.






## 6. EXPERIMENTAL WORK

### 6.1. Development and Validation of UV Spectroscopic Method for the Quantitation of Tadalafil in tablet dosage form

#### 6.1.1. MATERIALS AND METHODS

**Drug Samples:** Tadalafil was obtained as a gift sample from Hetero Drugs Pvt., Ltd., Hyderabad. The Tadalafil tablets (Megalis-10) obtained from local market.

**Instruments used**

The different instruments used for the present work were as follows:

- Electronic Weighing balance having single pan, (Model- Axis LC/GC).
- Ultra Sonicater (Model- Bandelin sonorex).
- Double Beam UV-Visible spectrophotometer.

Shimadzu UV-1800 - Double Beam UV-Visible spectrophotometer. By using 1cm quartz cells in the wavelength range of 200-400 nm, the solutions of both standard as well as sample solutions were recorded through scanning between 200-400 nm. Data acquisition was performed by UV-Probe software. Initially Instrument was allowed to initialize its take approximately 4 minutes. Then the method was created by entering parameters viz. start wavelength, end wave length, data interval, scan speed, slit width in the instrument. Then the baseline correction was performed by kept the blank in both the reference and sample compartments. Finally remove the blank solution from sample compartment and take the respective test solutions which are to be scanned.

**Chemicals used**

- Water - Milli Q water in house
- Methanol - GR/Merck
- Dichloromethane - GR/Merck
- Acetonitrile - GR/Merck

### 6.1.2. UV Spectroscopic Method development

The present work was made to develop and validate simple, precise and accurate method for the quantitation of Tadalafil in bulk and in tablet dosage form. It started with the choice of the appropriate technique viz. chromatography, spectroscopy or any other suitable analytical technique.

The new method for drug substances was established with the below described different steps

- Solubility studies are performed by dissolving the drug in polar and non – polar solvents for the selection of suitable solvent.
- Determination suitable $\lambda_{max}$
- Optimization of drug concentration

**Selection of solvent**

The solubility of drug substance was determined in different solvents. Solubility was carried out in polar to non-polar solvents. The common solvent was found as dichloromethane for the analysis of Tadalafil for proposed method.

**Preparation of Tadalafil standard stock solution**

Accurately 50mg of Tadalafil working standard was weighed and transferred into 50ml volumetric flask. 25ml of dichloromethane added and mixed well, then made up to the final volume. Further required concentrations were made by diluting with methanol, from this stock solution.

**Preparation of Tadalafil assay standard solution**

Accurately 5ml stock solution of Tadalafil standard was transferred to 50ml volumetric flask and it was diluted up to the mark with methanol. From this 2.0ml was transferred accurately to 10ml standard flask and it was dilute up to the mark with methanol (20ppm).

**Preparation of Tadalafil assay test solution**

Twenty tablets were accurately weighed and powered and Tadalafil content equivalent to 50mg was weighed and transferred to 50ml volumetric flask. The sample was dissolved by using solvent dichloromethane and made up to the mark. From this, accurately 5ml solution transferred to 50ml flask and the volume was made up to the mark with methanol. From this 2.0ml was transferred accurately to 10ml standard flask and it was dilute up to the mark with methanol. The resulting solution was scanned between 250nm - 310nm.

**Determination suitable λmax**

The selection of suitable λmax for the estimation Tadalafil was done by using a suitable diluted stock solution containing 20□g/ml of both standard and sample solutions were scanned between 250nm - 310nm by using methanol as blank. From the obtained spectra, by the observation of spectral characteristics and repetition of curve with same λmax values 284nm was selected for estimation of Tadalafil in dichloromethane, and dilutions made with methanol. UV spectrum of Sample & Standard solutions was shown in Fig. 7.1, 7.2.

**Optimization of drug concentration**

The series of concentrations were prepared from stock solutions and measured absorbance values at selected λmax. From the absorbance values, the concentration selected which has absorbance value between 0.40-0.50. In the present work 20□g/ml was selected as optimized concentration which shows absorbance values 0.404, 0.406, 0.406 among repetition for the estimation of Tadalafil in dichloromethane, and dilutions made with methanol.

**Preparation of calibration curve standards**

- **10ppm:** Accurately transferred 5ml of stock solution to 50ml volumetric flask and the volume made up to the mark with methanol, from this solution 1.0ml was transferred accurately to 10ml volumetric flask and made the volume to the mark with methanol.
- **15ppm:** Accurately transferred 5ml of stock solution to 50ml volumetric flask and the volume made up to the mark with methanol, from this solution 1.5ml was transferred accurately to 10ml volumetric flask and made the volume to the mark with methanol.
- **20ppm:** Accurately transferred 5ml of stock solution to 50ml volumetric flask and the volume made up to the mark with methanol, from this solution 2.0ml was transferred accurately to 10ml volumetric flask and made the volume to the mark with methanol.
- **25ppm:** Accurately transferred 5ml of stock solution to 50ml volumetric flask and the volume made up to the mark with methanol, from this solution 2.5ml was transferred accurately to 10ml volumetric flask and made the volume to the mark with methanol.
- **30ppm:** Accurately transferred 5ml of stock solution to 50ml volumetric flask and the volume made up to the mark with methanol, from this solution 3.0ml was transferred accurately to 10ml volumetric flask and made the volume to the mark with methanol.
- **35ppm:** Accurately transferred 5ml of stock solution to 50ml volumetric flask and the volume made up to the mark with methanol, from this solution 3.5ml was transferred accurately to 10ml volumetric flask and made the volume to the mark with methanol.

- **40ppm:** Accurately transferred 5ml of stock solution to 50ml volumetric flask and the volume made up to the mark with methanol, from this solution 4.0ml was transferred accurately to 10ml volumetric flask and made the volume to the mark with methanol.

**Calculation**

The content of Tadalafil or the percentage purity was calculated using the following formula,

$$\% \text{ Purity} = \frac{\text{Test Absorbance}}{\text{Std. Absorbance}} \times \frac{\text{Std. dilutions}}{\text{Test dilutions}} \times \text{Avg. Wt} \times \frac{\text{Label Claim}}{100}$$

**6.1. Method validation System Precision**

The system precision was performed by analyzing a standard solution of Tadalafil at working concentration level of 20µg/ml for 6 times and calculated mean, SD, % RSD values.

**Acceptance criteria:** The % relative standard deviation for absorbance values at λ max should be less than 2.0

Results were summarized in the table 7.1.

**Method Precision:** The method precision was performed by analyzing a sample solution of Tadalafil at working concentration level (20µg/ml) for 6 times.

**Acceptance criteria:** The % relative standard deviation of absorbance values at λ max should be less than 2.0

Results were summarized in the table 7.2.

**Linearity and Range:** A calibration curve was plotted between concentration and absorbance. Linearity was performed in various concentrations ranging from 10, 15, 20, 25, 30, 35 and 40µg/ml. It was found that the response was linear with the concentration range of 10-40 □g/ml at 284nm.

**Acceptance criteria:** The regression coefficient value from the plot should be not less than 0.995.

Results were summarized in the table 7.3 & Fig 7.3, 7.4.

**Accuracy**

The accuracy is calculated as the % recovery by spiking with the known amount of analyte in the sample. The accuracy of an analytical method must be established across the range.

The determination of content of Tadalafil was performed at three levels by spiking the known

calculated amount of Tadalafil to the blank solution. The sample was prepared in triplicate (9 determinations) i.e. 50%, 100%, 150% of the working concentrations for the method.

**Level –I (50%)**

Weigh accurately and transferred the powdered content which is equivalent to 50mg weight of Tadalafil to 50ml volumetric flask and dissolve in dichloromethane and made the volume to the mark with same solvent. From this accurately transferred 5ml of solution to 50ml of volumetric flask and made the volume to the mark with methanol. From this solution accurately transferred 1.0ml of solution to 10ml of volumetric flask and made up the volume up to mark with methanol. The final conc. of Tadalafil sample was 10□g/ml. The solution was scanned in the UV- Visible region i.e., from 200 to 400 nm.

**Level –II (100%)**

Weigh accurately and transferred the powdered content which is equivalent to 50mg weight of Tadalafil to 50ml volumetric flask and dissolve in dichloromethane and made the volume to the mark with same solvent. From this accurately transferred 5ml of solution to 50ml of volumetric flask and made the volume to the mark with methanol. From this solution accurately transferred 2.0ml of solution to 10ml of volumetric flask and made up the volume up to mark with methanol. The final conc. of Tadalafil sample was 10□g/ml. The solution was scanned in the UV- Visible region i.e., from 200 to 400 nm.

**Level –III (120%)**

Weigh accurately and transferred the powdered content which is equivalent to 50mg weight of Tadalafil to 50ml volumetric flask and dissolve in dichloromethane and made the volume to the mark with same solvent. From this accurately transferred 5ml of solution to 50ml of volumetric flask and made the volume to the mark with methanol. From this solution accurately transferred 3.0ml of solution to 10ml of volumetric flask and made up the volume up to mark with methanol. The final conc. of Tadalafil sample was 10□g/ml. The solution was scanned in the UV- Visible region i.e., from 200 to 400 nm.

**Acceptance criteria:** The % recovery of drug at three levels should be within the limits of 98% to 102%.

Results were summarized in the table 7.4.

**Ruggedness**

The ruggedness of an analytical method is to be determined by analyzing the standard and sample solution in duplicate with varying following parameters,

- Day to day : Day -1, Day -2
- Analyst to analyst : Analyst 1, Analyst 2

**Acceptance criteria:** The % relative standard deviation of % Assay values at λmax should be less than 2.0.

**Robustness**

The robustness of an analytical method is assessed of its capability to remain unaffected through small, but deliberate changes in method parameters and provides an indication of its consistency during normal usage.

 λ max variation for Tramadol HCl

| Actual | Low | High |
|---|---|---|
| 284nm | 282 nm | 286 nm |

**Acceptance criteria:** The % relative standard deviation of % Assay values at λmax should be less than 2.0.

## 6.2. Development and Validation of UV Spectroscopic Method for the Quantitation of Valsartan in tablet dosage form

### 6.2.1. MATERIALS AND METHODS

**Drug samples:** Valsartan was obtained as a gift sample from Reddy's Laboratories, Hyderabad. The tablet dosage form (valent-40) obtained from market.

**Instruments used**

The different instruments used for the present work were as follows:

- Electronic Weighing balance having single pan, (Model- Axis LC/GC).
- Ultra Sonicater (Model- Bandelin sonorex).
- Double Beam UV-Visible spectrophotometer.

Shimadzu UV-1800 - Double Beam UV-Visible spectrophotometer. By using 1cm quartz cells in the wavelength range of 200-400 nm, the solutions of both standard as well as sample solutions were recorded through scanning between 200-400 nm. Data acquisition was

performed by UV-Probe software. Initially Instrument was allowed to initialize its take approximately 4 minutes. Then the method was created by entering parameters viz. start wavelength, end wave length, data interval, scan speed, slit width in the instrument. Then the baseline correction was performed by kept the blank in both the reference and sample compartments. Finally remove the blank solution from sample compartment and take the respective test solutions which are to be scanned.

**Chemicals used**

- Water - Milli Q water in house
- Acetonitrile - GR/Merck

### 6.2.2. UV Spectroscopic Method development

The present work was made to develop and validate simple, precise and accurate UV method for the quantitation of Valsartan in pure form and in tablet dosage form. It started with the choice of the appropriate technique viz. chromatography, spectroscopy or any other suitable analytical technique.

The new method for drug substances was established with the below described different steps

- Solubility studies are performed by dissolving the drug in polar and non – polar solvents for the selection of suitable solvent.
- Determination suitable $\lambda_{max}$ Optimization of drug concentration

**Selection of solvent**

The solubility of the drug substance was determined in different. Solubility was carried out in polar to non-polar solvents. The common was found as acetonitrile for the analysis of Valsartan for proposed method.

**Preparation of Valsartan standard stock solution**

10mg of Valsartan was accurately weighed and transferred into 10ml volumetric flask. To this 5 ml of acetonitrile added, mixed well, then made up to the final volume. Further required concentrations were made by diluting with selected solvent, from this stock solution.

**Preparation of Valsartan assay standard solution**

1 ml of the above stock solution accurately transferred to 10 ml volumetric flask and it was diluted up to mark with suitable solvent. From this solution 1 ml transferred to 10ml

volumetric flask and make to the mark with suitable solvent (10ppm).

**Preparation of Valsartan assay test solution**

Five tablets were accurately weighed and powered and Valsartan content equivalent to 10mg was weighed and transferred to 10ml volumetric flask. The sample was dissolved by the solvent and made up the mark with solvent. Form this, accurately 1ml solution transferred to 10ml flask and the volume was made up to the mark with solvent. Form this 1ml was transferred to 10ml standard flask and volume made up to the mark with solvent. The resulting solution was scanned between 200- 400 nm.

**Determination of suitable λ max**

The selection of suitable λ max for the estimation of Valsartan was done by using a suitable diluted stock solution contain 10 µg/ml of both standard and sample solutions were scanned between 200-400 nm by using acetonitrile as blank. From the obtained spectra, by the observation of spectral characteristics and repetition of curve with same λmax values 230 nm was selected for estimation of Valsartan in acetonitrile solvent.

**Optimization of drug concentration**

The series of concentrations were prepared from stock solutions and measured absorbance values at selected λmax. from the absorbance values, the concentration selected which has absorbance value between 0.500 - 0.600. In the present work 10µg/ml was selected as optimized concentration which shows absorbance values 0.545, 0.546, 0.548 Among repetition for the estimation of Valsartan in acetonitrile.

**Preparation of calibration curve standards**

- **2.5ppm:** Accurately transferred 1ml of stock solution to 10ml volumetric flask and volume made up to the mark with solvent, from this solution 0.25ml was transferred accurately to 10ml volumetric flask and made the volume to the mark with solvent.
- **5ppm:** Accurately transferred 1ml of stock solution to 10ml volumetric flask and the volume made up to mark with solvent, from this solution 0.5ml was transferred accurately to 10ml volumetric flask and made the volume to the mark with solvent.
- **7.5ppm:** Accurately transferred 1ml of stock solution to 10ml volumetric flask and the volume made up to the mark with solvent, from this 0.75ml was transferred accurately to 10ml volumetric flask and made the volume to the mark with solvent.
- **10ppm:** Accurately transferred 1ml of stock solution to 10ml volumetric flask and the

volume made up to the mark with solvent, from this 1.0ml was transferred accurately to 10ml volumetric flask and made the volume to the mark with solvent.

- **12.5ppm:** Accurately transferred 1ml of stock solution to 10ml volumetric flask and the volume made up to the mark with solvent, from this 1.25ml was transferred accurately to 10ml volumetric flask and made the volume to the mark with solvent.
- **15ppm:** Accurately transferred 1ml of stock solution to 10ml volumetric flask and the volume made up to the mark with solvent, from this 1.5ml was transferred accurately to 10ml volumetric flask and made the volume to the mark with solvent.

**Calculation**

The content of Valsartan or the percentage purity was calculated using the following formula.

$$\% \text{ Purity} = \frac{\text{Test Absorbance}}{\text{Std. Absorbance}} \times \frac{\text{Std. dilutions}}{\text{Test dilutions}} \times \frac{\text{Avg. Wt.}}{\text{Label Claim}} \times 100$$

**6.2.1 Method validation**

**System Precision**

The system precision was performed by analyzing a standard solution of Valsartan (10µg/ml) for 6 times and calculate mean, SD, %RSD values.

***Acceptance criteria:*** The % relative standard deviation of assay values should be less than 2.0

**Standard preparation**

Weigh accurately about 10mg of standard Valsartan and dissolved in acetonitrile and made the volume to the mark with solvent. Finally make up the concentration 10µg/ml. The solution was scanned in the UV- Visible region i.e., from 200-400nm.

**Method Precision**

The method precision was performed by analyzing a sample solution of Valsartan at working concentration level (10µg/ml) for 6 times.

***Acceptance criteria:*** The % relative standard deviation of assay values should be less than 2.0.

**Sample preparation**

Weigh accurately transferred 0.032gr of tablet content which is equivalents to 10mg weight of valsartan drug to 10ml volumetric flask and dissolve in acetonitrile and made the volume to the mark with same solvent. Finally make up the concentration to 10µg/ml. The solution absorbance was measured by scanning in the UV- visible region i.e., 200-400nm.

**Linearity and Range**

A calibration curve was plotted between concentration and absorbance. For Valsartan Linearity was performed in various concentrations ranging from 2.5, 5, 7.5, 10, 12.5 and 15µg/ml. It was found that the response was linear with the concentration rang of 2.5-15 µg/ml at 230nm.

***Acceptance criteria:*** The regression coefficient value from the plot should be less than 0.995.

**Accuracy**

The accuracy is calculated as the % recovery by spiking with the known amount of analyte in the sample. The determination of content of Valsartan was performed at three levels by spiking the known calculated amount of Valsartan to blank solution. The sample was prepared in triplicates (9 determinations) i.e. 50%, 100%, 150% of the working concentrations for the method.

**Level – I (50%)**

The powdered content which is equivalent to 10mg of Valsartan transferred to 10ml volumetric flask and dissolved in acetonitrile and made the volume to the mark with same solvent. From this accurately 1ml of solution transferred to 10ml of volumetric flask and made the volume to the mark with solvent. From this solution accurately transferred 0.25ml of solution to the 10ml of volumetric flask and made up to the volume up to the mark with acetonitrile. The final concentration of Valsartan sample was 2.5µg/ml. The solution was scanned in the UV- Visible region i.e., from 200-400 nm.

**Level – II (100%)**

The powdered content which is equivalent to 10mg of Valsartan transferred to 10ml volumetric flask and dissolved in acetonitrile and made the volume to the mark with same solvent. From this accurately 1ml of solution transferred to 10ml of volumetric flask and made the volume to the mark with solvent. From this solution accurately transferred 0.5ml of

solution to the 10ml volumetric flask and made up to the volume up to the mark with acetonitrile. The final concentration of Valsartan sample was 5µg/ml. The solution was scanned in the UV- Visible region i.e., 200-400 nm.

**Level – III (150%)**

The powdered content which is equivalent to 10mg of Valsartan transferred to 10ml volumetric flask and dissolved in acetonitrile and made the volume to the mark with solvent. From this accurately 1ml solution transferred to 10ml of volumetric flask and made the volume to the mark with acetonitrile. From this solution accurately transferred 0.75ml of solution to 10ml of volumetric flask and made up the volume up to mark with acetonitrile. The final concentration of Valsartan sample was 7.5µg/ml. The solution was scanned in the UV- Visible region i.e., 200-400 nm.

***Acceptance criteria:*** The % recovery of drug at three levels should be within the limits of 98% to 102%

**Ruggedness**

The ruggedness of an analytical method is to be determined by analyzing the standard and sample solution in duplicate with varying following parameters,

- Day to day : Day – 1, Day – 2
- Analyst to analyst : Analyst – 1, Analyst - 2

***Acceptance criteria:*** The % relative standard deviation of % Assay values at λ max should be less than 2.0.

**Robustness**

The robustness of an analytical method is assessing of its capability to remain unaffected through small, but deliberate changes in the method parameters and provide an indication of its consistency during normal usage.

- λmax variation for Valsartan

| Actual | Low | High |
|---|---|---|
| 230nm | 228 nm | 232 nm |

***Acceptance criteria:*** The % relative standard deviation of % Assay values should be less than 2.0.

## 7. RESULTS AND DISCUSSION

### 7.1. Development and Validation of UV Spectroscopic Method for the Quantitation of Tadalafil in tablet dosage form

The solubility studies for Tadalafil were performed in number of polar and non–polar solvents to dissolve the drug. From the solubility profile dichloromethane was chosen as solvent for the estimation of Tadalafil.

The standard and sample solutions of 20□g/ml of Tadalafil in dichloromethane were prepared individually and resulting solutions were scanned in the UV-Visible region i.e., from 200 to 400 nm by using methanol as blank. From the spectrum 284nm was selected as wavelength of analysis for the estimation of Tadalafil since there is no interference from excipients. Standard and sample solution spectrums were shown in Fig. 7.1.1 and 7.1.2.

The optimum conc. of the Tadalafil was found as 20µg/ml. This conc. of Tadalafil was shown good absorbance value at respective wavelength was found as 0.404, 0.406 and 0.406 upon repetition.

The system precession was performed by using six replicates of standard solutions and % RSD value was found as0.6. The low % RSD values indicate that the system has good precision. The results were tabulated in Table No7.1.1.

Further the method precision was established by intra-day and inter-day analysis. The % RSD values of intraday analysis were found to be 0.4 and 0.9 respectively for Tadalafil (20□g/ml). The % RSD values of inter-day analysis were found to be 0.4 and 0.5 for Tadalafil. The results were tabulated in Table No7.1.2.

Different aliquots of Tadalafil solutions were prepared in the concentration range of 10 – 40 □g/ml. The absorbance values of solutions were measured at 284nm and shown in Table No7.1.1. The calibration curve for Tadalafil was plotted by taking concentration on x-axis against absorbance on y- axis. The calibration graph at 284nm for Tadalafil was shown in figure7.1.2. The preparation of calibration curve was repeated for three times for each solution at their selective wavelength. The correlation coefficient for the drug solutions of different was found as above 0.999. The results of Linearity study were summarized in Table no. 7.1.3.

The method accuracy was assessed by means of recovery studies. A known quantity of Tadalafil raw material solutions were added at three different levels (50,100 and 150%) and solution's absorbance was measured; finally, the calculated % recovery was found as 99.6 – 100.1% for Tadalafil. The recovery data was tabulated in table no7.1.4.

The proposed method was validated for ruggedness. It refers to the specific of one lab to multiple days which may include analysts, different instruments and different sources of reagents and so on. In the present work ruggedness was established by different analysts. The % RSD values by analyst-1 at two intervals were found to be 0.4 and 0.3. The %RSD values by analyst-2 at two intervals were found to be 0.5 and 0.4 for Tadalafil for concentration of 20□g/ml at 284nm. The results were tabulated in Table7.1.5.

The Robustness was performed at different wave length by using working standard solutions of Tadalafil. The % RSD values for wavelength variation were found to be 0.7, 0.2, and 0.4 for wavelength 282nm, 284nm and 286nm respectively. The results were tabulated in Table7.1.6

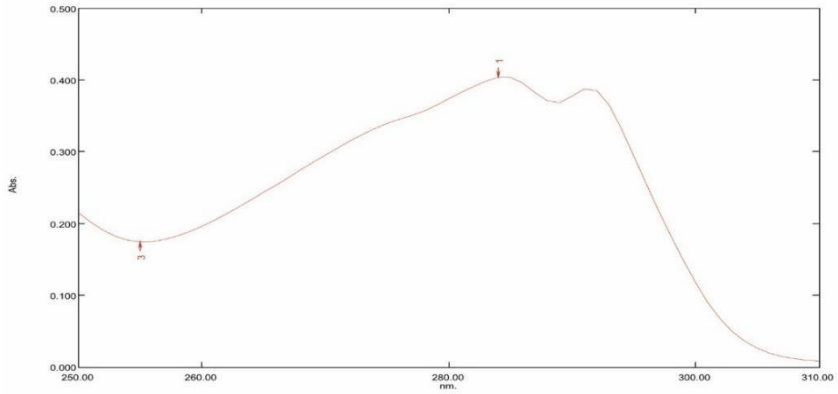



**Figure 7.1.1: UV spectrum of Standard Tadalafil.**

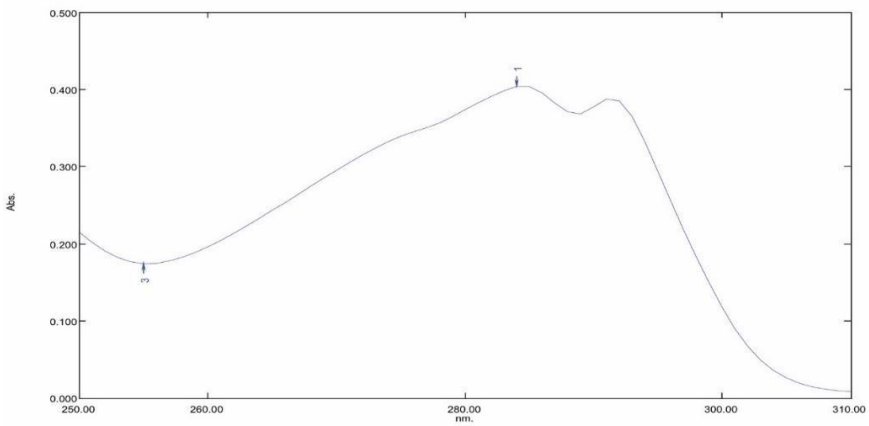



**Figure 7.1.2: UV spectrum of Tadalafil Tablet Dosage form.**

**Table 7.1.1.: System Precision.**

| S. No | Assay (%w/w) |
|---|---|
| 1 | 100.2 |
| 2 | 100.9 |
| 3 | 100 |
| 4 | 100.2 |
| 5 | 99.2 |
| 6 | 99.8 |
| **Mean** | **100.05** |
| **SD** | **0.3** |
| **% RSD** | **0.6** |

The % relative standard deviation values of the assay results for Tadalafil were within the limit, less than 2.0.

**Table 7.1.2: Method Precision.**

| S. No | Assay (%w/w) |
|---|---|
| 1 | 100.9 |
| 2 | 100.2 |
| 3 | 99.8 |
| 4 | 99.2 |
| 5 | 99.8 |
| 6 | 99.6 |
| **Mean** | **99.9** |
| **SD** | **0.3** |
| **% RSD** | **0.6** |

The % relative standard deviation values of the assay results for Tadalafil were within the limit, less than 2.0.

**Table 7.1.3: Results of Linearity study for Tadalafil.**

| S. No. | Concentration (µg/ml) | Absorbance | Regression Analysis |
|---|---|---|---|
| 1 | 10 | 0.206 | Regression equation: y = 0.01013x + 0.10100 $R^2$ =0.99940 |
| 2 | 15 | 0.301 | |
| 3 | 20 | 0.404 | |
| 4 | 25 | 0.509 | |
| 5 | 30 | 0.603 | |
| 6 | 35 | 0.701 | |
| 7 | 40 | 0.818 | |

The linearity curves for Tadalafil showed the regression coefficient value 0.99940 which complies that the limit of not less than 0.995.

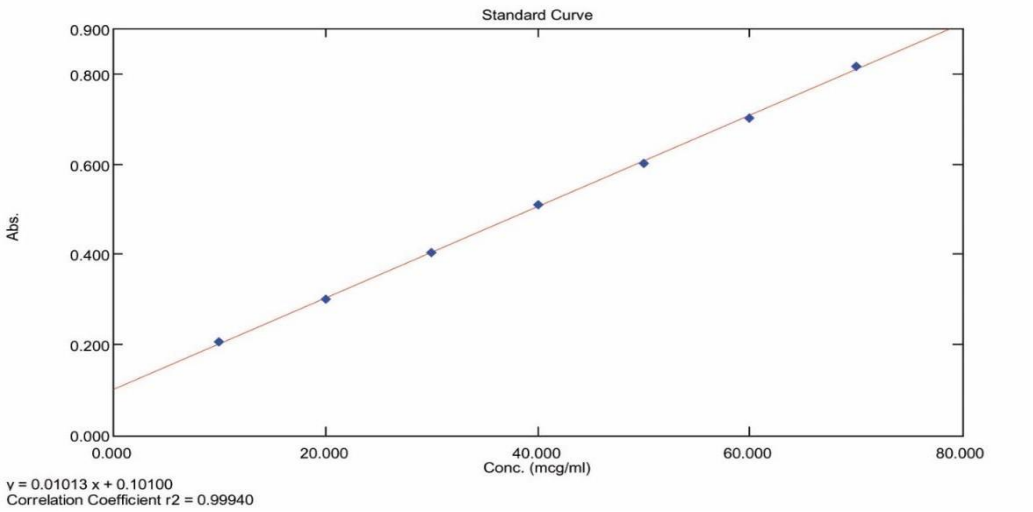



**Figure 7.1.3: calibration curve for Tadalafil (10-40 µg/ml).**

**Table 7.1.4: Intraday precession.**

| S. No | %Assay Interval-1 | %Assay Interval-2 |
|---|---|---|
| 1 | 100 | 100.9 |
| 2 | 100.2 | 99.6 |
| 3 | 99.8 | 100.9 |
| 4 | 99.6 | 101.3 |
| 5 | 100.2 | 99.8 |
| 6 | 99.2 | 99.2 |
| **Mean** | **99.8** | **100.3** |
| **SD** | **0.388** | **0.857** |
| **% RSD** | **0.4** | **0.9** |

The % relative standard deviation values of %Assay for Tadalafil sample was within the limit, i.e. less than 2.0.

**Table 7.1.6: Intraday precession.**

| S. No | %Assay Interval-1 | %Assay Interval-2 |
|---|---|---|
| 1 | 100 | 99.2 |
| 2 | 99.4 | 100.2 |
| 3 | 99 | 99 |
| 4 | 100 | 99.4 |
| 5 | 99.6 | 99.8 |
| 6 | 99.2 | 99.2 |
| **Mean** | **99.5** | **99.5** |
| **SD** | **0.413** | **0.45** |
| **% RSD** | **0.4** | **0.5** |

The % relative standard deviation values of %Assay for Tadalafil sample was within the limit, i.e. less than 2.0.

**Table 7.1.7: Accuracy studies.**

| Pre-analyzed sample | | % spike level | amount added (mcg/ml) | Abs after spiking | Abs for Added Amount | Amount recovered (mcg/ml) | Recovery (%) | Mean ± SE |
|---|---|---|---|---|---|---|---|---|
| Conc (mcg/ml) | Abs | | | | | | | |
| 25 | 0.509 | 50 | 5 | 0.61 | 0.101 | 4.95 | 98.96 | 100.29 ± 1.16 |
| 25 | 0.508 | 50 | 5 | 0.611 | 0.103 | 5.05 | 100.96 | |
| 25 | 0.509 | 50 | 5 | 0.612 | 0.103 | 5.05 | 100.96 | |
| 25 | 0.508 | 100 | 10 | 0.713 | 0.205 | 9.95 | 99.52 | 99.53 ± 0.98 |
| 25 | 0.509 | 100 | 10 | 0.716 | 0.207 | 10.05 | 100.53 | |
| 25 | 0.509 | 100 | 10 | 0.712 | 0.203 | 9.86 | 98.56 | |
| 25 | 0.508 | 150 | 15 | 0.811 | 0.303 | 15.10 | 100.65 | 100.31 ± 0.89 |
| 25 | 0.509 | 150 | 15 | 0.813 | 0.304 | 15.15 | 100.97 | |
| 25 | 0.509 | 150 | 15 | 0.808 | 0.299 | 14.89 | 99.30 | |

The % recovery of Tadalafil at three different levels were meet the Acceptance criteria i.e., 98% to 102% recovery.

**Table 7.1.8: Ruggedness data for analyst variation.**

| Parameter | Analyst-1 (%Assay) | | Analyst-2 (%Assay) | |
|---|---|---|---|---|
| | Interval-1 | Interval- 2 | Interval-1 | Interval-2 |
| Analyst to Analyst | 99.4 | 99.4 | 100.4 | 99.8 |
| | 100.2 | 100 | 99.4 | 99.6 |
| | 100 | 9.4 | 100 | 100.6 |
| | 99.8 | 99.6 | 99.8 | 100 |
| | 99.6 | 100 | 101 | 100.2 |
| | 100.4 | 99.8 | 100 | 100.4 |

| | | | | |
|---|---|---|---|---|
| **Mean** | 99.9 | 99.7 | 100.1 | 100.1 |
| **STDEV** | 0.374 | 0.276 | 0.548 | 0.374 |
| **%RSD** | 0.4 | 0.3 | 0.5 | 0.4 |

The % relative standard deviation values of %Assay for Tadalafil was within the limit i.e., less than 2.0.

**Table 7.1.9: Robustness data for wavelength variation.**

| Sl. NO | Tadalafil % Assay at different wavelength (nm) | | |
|---|---|---|---|
| | 282 | 284 | 286 |
| 1 | 99.8 | 99.9 | 100.2 |
| 2 | 100.2 | 100.2 | 100.4 |
| 3 | 100.9 | 100.1 | 99.8 |
| 4 | 99.4 | 99.8 | 100.9 |
| 5 | 99.6 | 100.9 | 100.2 |

| | | | |
|---|---|---|---|
| 6 | 100.4 | 100.2 | 99.9 |
| **Mean** | 100.05 | 100.18 | 100.23 |
| **SD** | 0.558 | 0.387 | 0.393 |
| **%RSD** | 0.557 | 0.386 | 0.392 |

The % relative standard deviation values of absorbance for Tadalafil were within the limit, i.e., less than.2.0.

### 7.2. Development and Validation of UV Spectroscopic Method for the Quantitation of Valsartan in tablet dosage form

The solubility studies for Valsartan were performed in number of polar and non-polar solvents to dissolve the drug. Form the solubility profile acetonitrile and was chosen as common solvent for the estimation of Valsartan. The solubility data is shown in table 7.2.1.

The standard and sample solution of 10µg/ml of Valsartan in acetonitrile were prepared individually and resulting solution water scanned in the UV- Visible region i.e., from 200-400nm by using acetonitrile as blank as shown in figure. From the spectrum 230nm was selected as maximum wavelength for the estimation of Valsartan.

The optimum concentration of the Valsartan was found as 10µg.ml. This concentration of Valsartan was shown good absorbance value at 230nm was found to be 0.545.

Different aliquots of Valsartan in acetonitrile were prepared in the concentration range of 2.5-15 µg/ml. The absorbance values of solutions were measured at 230nm and shown in Table 7.2.2. The calibration curve for Valsartan was plotted by taking concentration on x-axis against absorbance on y-axis. The calibration graph at 230nm for Valsartan was shown in figure 7.2.5. The preparation of calibration curve was repeated for six times for each solution at their selective wavelength. The correlation coefficient for the drug solutions of different was found to be 0.997.

The system precision was performed by using six replicates of standard solution and % RSD value was found as 0.8. The low % RSD values indicate that the system as good precision. The results were tabulated in Table 7.2.3.

The method precision was performed by using six replicates of standard solution and % RSD value was found as 0.8. The results were tabulated in Table 7.2.4.

The method accuracy was assessed by means of recovery studies. A known quantity of Valsartan raw material solution was added at three different levels (50. 100.and 150%) and solution's absorbance was measured; finally, the calculated % recovery was found to be 99.58-100.03 % for Valsartan in acetonitrile. The recovery data was tabulated in Table 7.2.5.

The proposed method was validated for ruggedness. It refers to the specific of one lab to multiple days which may include analysts, different instruments and different sources of reagents and so on. In the present work ruggedness was established by different analysts. The % RSD values by analyst-1 and analyst-2 were found to be 0.7 and 0.8. The % RSD values by day-1 and day-2 were found to be 0.4 and 0.9 for Valsartan in acetonitrile for concentration of 10µg/ml at 230nm wavelength.

The Robustness was performed at different wavelength by using working standard solutions of Valsartan. The % RSD values for wavelength variation were found to be 0.97, 0.86 and 0.82 for wavelength 228nm, 230nm and 232nm respectively. The results were tabulated in Table 7.2.8.

**Table 7.2.1: Solubility profile of Valsartan in different solvents.**

| S. No | Solvents | Solubility |
|---|---|---|
| 1 | Methanol | Soluble |
| 2 | Ethanol | Soluble |
| 3 | Acetonitrile | Freely soluble |
| 4 | Distilled Water | Partially insoluble |

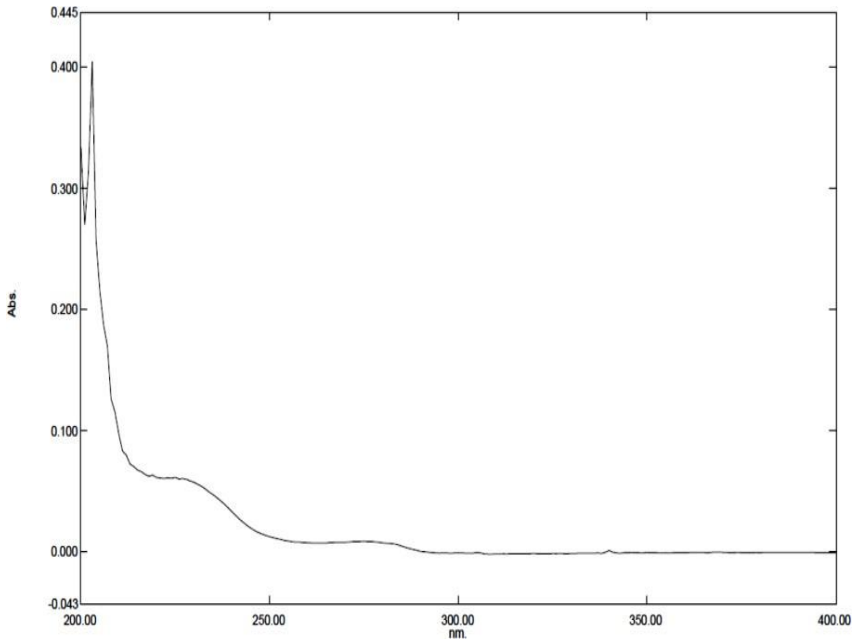



**Figure 7.2.1: UV spectrum of blank (Acetonitrile).**

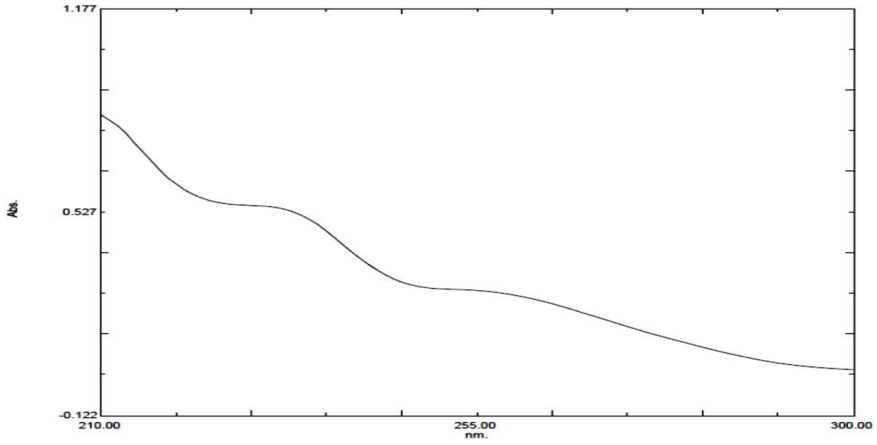



**Figure 7.2.2: UV spectrum of Standard valsartan in Acetonitrile.**

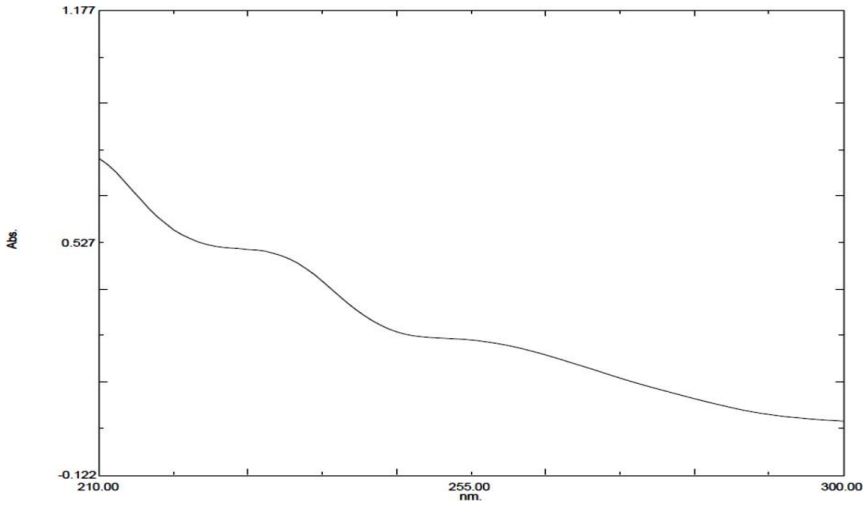



**Figure 7.2.3: UV spectrum of Valsartan Dosage form in Acetonitrile.**

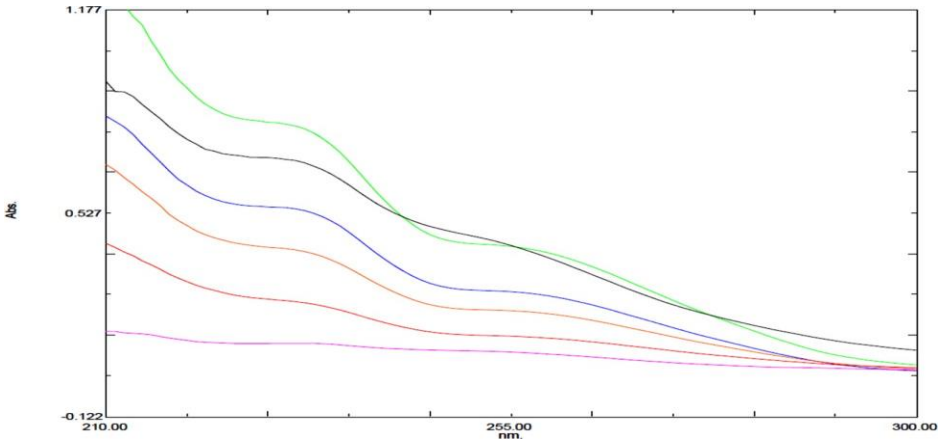



**Figure 7.2.4: Overlay spectrum of standard solution (2.5-15µg/ml).**

**Table 7.2.2: Calibration data for Valsartan in Acetonitrile.**

| S. No. | ncentration (µg/ml) | Absorbance | Regression Analysis |
|---|---|---|---|
| 1 | 2.5 | 0.111 | **y = 0.05702x+0.02740 $r^2$ = 0.997** |
| 2 | 5 | 0.248 | |

| | | | |
|---|---|---|---|
| 3 | 7.5 | 0.414 | |
| 4 | 10 | 0.545 | |
| 5 | 12.5 | 0.699 | |
| 6 | 15 | 0.812 | |

The linearity curve for Valsartan showed the regression coefficient value 0.997 which compiles that the limit of not less than 0.995.

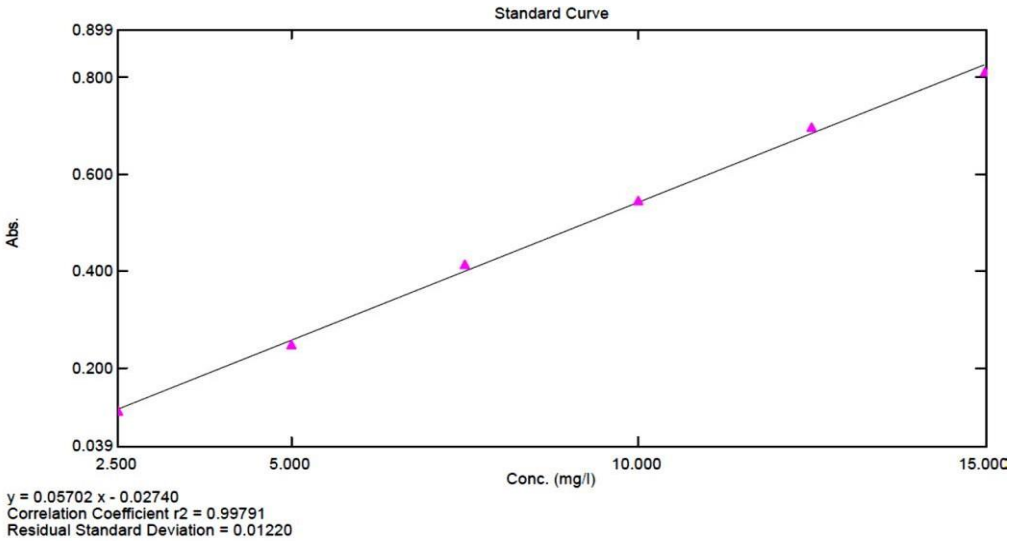



**Figure 7.2.5: Calibration Curve for Valsartan in Acetonitrile.**

**Table 7.2.3: System Precision.**

| S. No. | Absorbance values at 230nm |
|---|---|
| 1 | 0.545 |
| 2 | 0.546 |
| 3 | 0.548 |
| 4 | 0.550 |
| 5 | 0.554 |
| 6 | 0.540 |
| **Mean** | **0.54** |
| **SD** | **0.004** |
| **% RSD** | **0.8** |

The % relative standard deviation values of absorbance in system precision study for Valsartan in Acetonitrile were within the limit, less than 2.0.

**Table 7.2.4: Method Precision.**

| S. No. | Absorbance values at 230nm |
|---|---|
| 1 | 0.535 |
| 2 | 0.536 |
| 3 | 0.536 |
| 4 | 0.526 |

| | |
|---|---|
| 5 | 0.540 |
| 6 | 0.535 |
| **Mean** | **0.534** |
| **SD** | **0.004** |
| **%RSD** | **0.8** |

The % relative standard deviation values of absorbance in method precision study for Valsartan in Acetonitrile was within the limit, less than 2.0

**Table 7.2.5: Accuracy Studies.**

| Pre-analyzed sample | | % spike level | amount added (mcg/ml) | Abs after spiking | Abs for Added Amount | Amount recovered (mcg/ml) | Recovery (%) | Mean ± SE |
|---|---|---|---|---|---|---|---|---|
| Conc (mcg/ml) | Abs | | | | | | | |
| 25 | 0.509 | 50 | 5 | 0.61 | 0.101 | 4.95 | 98.96 | 100.29 ± 1.16 |
| 25 | 0.508 | 50 | 5 | 0.611 | 0.103 | 5.05 | 100.96 | |
| 25 | 0.509 | 50 | 5 | 0.612 | 0.103 | 5.05 | 100.96 | |
| 25 | 0.508 | 100 | 10 | 0.713 | 0.205 | 9.95 | 99.52 | 99.53 ± 0.98 |
| 25 | 0.509 | 100 | 10 | 0.716 | 0.207 | 10.05 | 100.53 | |
| 25 | 0.509 | 100 | 10 | 0.712 | 0.203 | 9.86 | 98.56 | |
| 25 | 0.508 | 150 | 15 | 0.811 | 0.303 | 15.10 | 100.65 | 100.31 ± 0.89 |
| 25 | 0.509 | 150 | 15 | 0.813 | 0.304 | 15.15 | 100.97 | |
| 25 | 0.509 | 150 | 15 | 0.808 | 0.299 | 14.89 | 99.30 | |

The % recovery of Valsartan at three different levels were meet the acceptance criteria i.e., 98% to 120% recovery.

**Table 7.1.6: Ruggedness data for analyst variation.**

| Parameter | Absorbance of Valsartan at 230nm | |
|---|---|---|
| | Analyst-1 | Analyst-2 |
| Analyst to Analyst | 0.526 | 0.535 |
| | 0.536 | 0.527 |
| | 0.536 | 0.527 |
| | 0.535 | 0.536 |
| | 0.535 | 0.536 |
| | 0.536 | 0.535 |
| **Mean** | 0.534 | 0.532 |
| **STDEV** | 0.003 | 0.004 |
| **%RSD** | 0.7 | 0.8 |

**Table 7.2.7: Ruggedness data for day variation.**

| Parameter | Absorbance of Valsartan at 230nm | |
|---|---|---|
| | Day-1 | Day-2 |
| Day to Day | 0.536 | 0.536 |
| | 0.535 | 0.526 |
| | 0.535 | 0.535 |

| | | |
|---|---|---|
| | 0.540 | 0.536 |
| | 0.540 | 0.536 |
| | 0.536 | 0.535 |
| **Mean** | 0.537 | 0.532 |
| **STDEV** | 0.002 | 0.004 |
| **%RSD** | 0.4 | 0.9 |

The % relative standard deviation values of absorbance for Valsartan in acetonitrile were within the limit I.e., less than 2.0.

**Table 7.1.8: Robustness data for wavelength variation.**

| S. No. | Valsartan absorbance at different wavelength(nm) | | |
|---|---|---|---|
| | **228** | **230** | **232** |
| 1 | 0.535 | 0.535 | 0.532 |
| 2 | 0.534 | 0.536 | 0.531 |
| 3 | 0.537 | 0.36 | 0.530 |
| 4 | 0.526 | 0.526 | 0.521 |
| 5 | 0.542 | 0.540 | 0.533 |
| 6 | 0.535 | 0.535 | 0.528 |
| **Mean** | 0.5348 | 0.5346 | 0.5291 |
| **SD** | 0.0051 | 0.0046 | 0.0043 |
| **%RSD** | 0.97 | 0.86 | 0.82 |

The % relative standard deviation values of absorbance for Valsartan in acetonitrile, was within the limit, less than 2.0.

## 8. CONCLUSION

The UV spectroscopic method for the quantitation of Tadalafil is based on dissolving the drug in dichloromethane and further dilutions with methanol and the measurement of absorbance of the Tadalafil at 284nm using methanol as blank. Beer's law was obeyed in concentration range 10-40mcg/ml having line equation y = 0.01013x + 0.10100with correlation coefficient value 0.99940.

The calculated % recovery was found as 99.697 – 100.27% for Tadalafil at three different levels (50,100 and 150%) which confirms the accuracy of the developed method. The % relative standard deviation values in the precision studies, intra and Interday analysis, analyst-to-analyst variation, reveals that the % RSD of assay results for Tadalafil were within the limit, i.e. less than 2.0. Hence the developed method was found to be precise.

The quantitation of Valsartan through UV- spectroscopic method accomplished with the requirements of specificity, precision and accuracy in order to be used as a method for the

quantity control of pharmaceuticals. The method has been evaluated for Linearity, Precision and ruggedness in order to ascertain the suitability of the analytical method. The UV spectroscopic method which is based on the measurement of ultraviolet absorbance maxima of the above drug at 230nm using Acetonitrile as diluents. Beer's law was obeyed in concentration range 2.5-25mcg/ml having line equation y = 0.05702 x - 0.02740 with correlation coefficient value 0.997. The drug obeyed Beer's law in the concentration range of 2.5-15 mcg/ml.

Both the methods are very simple and rapid as they do not include any complicated procedure for preparation of sample etc., and economic nature, which makes it especially suitable for routine quantity control work.

To conclude, the developed method can be successfully used for estimation of selected drugs in bulk and tablet dosage form.

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