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DEVELOPMENTAND VALIDATION OF SPECTROSCOPIC SIMULATANEOUS EQUATION METHOD FOR SIMULTANEOUS ESTIMATION OF AMLODIPINE AND VALSARTAN IN PURE AND PHARMACEUTICAL DOSAGE FORM

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Abstract:

The Analytical method was developed by Studying Different Parameters. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)).First of all, Maximum absorbance was found to be At 220 nm for Amlodipine, 252nm for Valsartan. And peaks were excellent.The UV spectrum of Amlodipine, Valsartan were obtained by using Methanol as a solvent and then validated.Concentration was selected to be 10µg/ml for Amlodipine, Valsartan which gave good Peaks.The linearity study for Amlodipine, Valsartan were found in concentration range of 2µg-10µg and correlation coefficient (r) was found to be 0.99 for amlodipine, Valsartan. And Regression coefficient (r^2) 0.99 for amlodipine, Valsartan.%Recovery studies were carried out and the percentage recovery was found to be in the range of 100% - 101% for Amlodipine, 100.65% to 101% for Valsarn %RSD of Absorbance for Intraday, Inter day precision was found to be 0.66 and 0.99 for Amlodipine, 0.88,0.88 for Valsartan respectively % RSD for Ruggedness was found to be less than 2. LOD and LOQ of calibration curve of drug prepared in Methanol .And concentration was found to be 1 and 3.14µg/ml respectively for Amlodipine.1 and 3.15 µg/ml respectively for Valsartan Hence the suggested UV Spectrophotometric method can be used for routine analysis of Glimepiride in API and Pharmaceutical dosage form

Key Words; *ICH guidelines (ICH, Q2 (R1)), Amlodipine, Valsartan,Regression coefficient (r^2) and calibration curve of drug prepared in Methanol.*

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INTRODUCTION [1-6]:

Analytical chemistry is often described as the area of chemistry responsible for characterizing the composition of matter, both qualitatively (what is present) and quantitatively (how much is present). Analytical chemistry is not a separate branch of chemistry, but simply the application of chemical knowledge.

Pharmaceutical Analysis may be defined as the application of analytical procedures used to determine the purity, safety and quality of drugs and chemicals. The term "Pharmaceutical analysis" is otherwise called quantitative pharmaceutical chemistry. Pharmaceutical analysis includes both qualitative and quantitative analysis of drugs and pharmaceutical substances starts from bulk drugs to the finished dosage forms. The technique employed in quantitative analysis is based upon the quantitative performance of suitable chemical reactions and either measuring the amount of reagent needed to complete the reaction, or ascertaining the amount of reaction product obtained. In the modern practice of medicine, the analytical methods are used in the analysis of chemical constituents found in human body whose altered concentrations during disease states serve as diagnostic aids and also used to analyze the medical agents and their metabolites found in biological system.

The term "quality" as applied to a drug product has been defined as the sum of all factors, which contribute directly or indirectly to the safety, effectiveness and reliability of the product. These properties are built into drug products through research and during process by procedures collectively referred to as "**quality control**". Quality is important in every product or service but it is vital in medicine as it involves life. Unlike ordinary consumer goods there can be no "second quality" in drugs. Quality control is a concept, which strives to produce a perfect product by series of measures designed to prevent and eliminate errors at different stages of production.

Quality control guarantees within reasonable limits that a drug products

- Is free of impurities.
- Is physically and chemically stable.
- Contains the amount of active ingredients as stated on the label and
- Provides optimal release of active ingredients when the product is administered.

Physico-chemical methods are used to study the physical phenomenon that occurs as a result of chemical reactions. Among the Physico-chemical methods, the most important are optical (Refractometry, Polarimetry, Emission, Fluorescence methods of analysis, Photometry including Photocolorimetry and Spectrophotometry covering UV-Visible and IR regions and Nephelometry or Turbidimetry) and chromatographic (Column, Paper, TLC, GLC, HPLC) methods. Methods such as Nuclear Magnetic Resonance and Para Magnetic Resonance are becoming more and more popular. The combination of Mass Spectroscopy with Gas Chromatography and Liquid Chromatography are the most powerful tools available. The chemical methods include the gravimetric and volumetric procedures which are based on complex formation; acid-base, precipitation and redox reactions. Titrations in non-aqueous media and complexometry have also been used in pharmaceutical analysis.

The number of new drugs is constantly growing. This requires new methods for controlling their quality. Modern pharmaceutical analysis must need the following requirements.

1. The analysis should take minimal time.
2. The accuracy of the analysis should meet the demands of Pharmacopoeia.
3. The analysis should be economical.
4. The selected method should be precise and selective.

These requirements are met by the Physico-chemical methods of analysis, a merit of which is their universal nature that can be employed for analyzing organic compounds with a diverse structure. Of them, Visible Spectrophotometry is generally preferred especially by small scale industries as the cost of the equipment is less and the maintenance problems are minimal.

The modern Pharmaceutical analytical instrumentation will include

1. UV Spectrophotometer
2. Infra-Red Spectroscopy
3. Differential Thermal Spectroscopy

4. X-Ray Spectroscopy
5. Mass Spectroscopy
6. NMR
7. Atomic Absorption Spectroscopy
8. Liquid Chromatography
9. Gas Chromatography
10. Ion Exchange Chromatography
11. Size Exclusion Chromatography etc.

These instruments vary in their principal method of detection as well in their objectives like quantitative and qualitative analysis. The method of analysis will also depend on their physical and chemical characters. Thermal sensitive products are not analyzed by thermal methods of analysis. Out of all these methods UV Spectroscopy, FTIR, Liquid Chromatography, Mass Spectroscopy, etc. is used most frequently because of they are easy for in method development, operation, etc. Along with the method development its validation should be done for determination of analytical method consistency.

Instrumental methods of Chemical analysis:
Instrumental method is an exciting and fascinating part of chemical analysis that interacts with all areas of chemistry and with many other areas of pure and applied sciences. Analytical instrumentation plays an important role in the production and evaluation of new products and in the protection of consumers and environment. This instrumentation provides lower detection limits required to assure safe foods, drugs, and water air. Instrumental methods are widely used by Analytical chemists to save time, to avoid chemical separation and to obtain increased accuracy.
Other chapters such as objectives, methodology, results, discussion and conclusion have been divided into two parts,

- UV Spectrophotometry
- Colorimetry

SPECTROPHOTOMETRY[7-9]:

In chemistry, **spectrophotometry** is the quantitative measurement of the reflection or transmission properties of a material as a function of wavelength. It is more specific than the general term electromagnetic spectroscopy in that spectrophotometry deals with visible light, near- ultraviolet, and near-infrared, but does not cover time-resolved spectroscopic techniques.

Spectrophotometry uses photometers that can measure a light beam's intensity as a function of its color (wavelength) known as spectrophotometers. Important features of spectrophotometers are spectral bandwidth, (the range of colors it can transmit through the test sample), and the percentage of sample-transmission, and the logarithmic range of sample-absorption and sometimes a percentage of reflectance measurement. A spectrophotometer is commonly used for the measurement of transmittance or reflectance of solutions, transparent or opaque solids, such as polished glass, or gases. However they can also be designed to measure the diffusivity on any of the listed light ranges that usually cover around 200 nm - 2500 nm using different controls and calibrations. Within these ranges of light, calibrations are needed on the machine using standards that vary in type depending on the wavelength of the photometric determination.

An example of an experiment in which spectrophotometry is used is the determination of the equilibrium constant of a solution. A certain chemical reaction within a solution may occur in a forward and reverse direction where reactants form products and products break down into reactants. At some point, this chemical reaction will reach a point of balance called an equilibrium point. In order to determine the respective concentrations of reactants and products at this point, the light transmittance of the solution can be tested using spectrophotometry. The amount of light that passes through the solution is indicative of the concentration of certain chemicals that do not allow light to pass through.

The use of spectrophotometers spans various scientific fields, such as physics, materials science, chemistry, biochemistry, and molecular biology. They are widely used in many industries including semiconductors, laser and optical manufacturing, printing and forensic examination, as well in laboratories for the study of chemical substances. Ultimately, a spectrophotometer is able to determine, depending on the control or calibration, what substances are present in a target and exactly how much through calculations of observed wavelengths

Ultraviolet Spectroscopy:

Ultraviolet Spectroscopy is concerned with the study of absorption of UV radiation which ranges from 200 nm to 400 nm. Compounds which are colorless absorb radiation in the UV region. In both UV as well as visible spectroscopy, only the valence electrons absorb the energy, thereby the molecule undergoes transition from ground state to excited state. This absorption is characteristic and depends on the concentration and path length as given by Beer – Lambert‟s law. Any molecule has n, π or σ or a combination of these electrons. These bonding (σ and π) and non-bonding
(n) electrons absorb the characteristic radiation and undergoes transition from ground state to excited state.

The electrons in a molecule can be of one of three types: namely σ (single bond), π (multiple-bond), or non-bonding (n- caused by lone pairs). These electrons when imparted with energy in the form of light radiation get excited from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO) and the resulting species is known as the excited state or anti-bonding state.

σ-bond electrons: These are the ones present in saturated compounds. Such electrons have the lowest energy level and are the most stable electrons. These would require a lot of energy to be displaced to higher energy levels. As a result these electrons generally absorb light in the lower wavelengths of the ultraviolet light (Vacuum UV radiation, <200nm) and these transitions are
rare.

Π-bond electrons: These electrons are present in unsaturated compounds eg. Double or triple bonds. These have much higher energy levels for the ground state. These electrons are therefore relatively unstable and can be excited more easily and would require lesser energy for excitation. These electrons would therefore absorb energy in the ultraviolet and visible light radiations.

n-electrons or non-bonding electrons are generally electrons belonging to lone pairs of atoms. These are not involved in any bonding. These are of higher energy levels than π-electrons and can be excited by ultraviolet and visible light as well.

Electronic transitions:

σ→σ" Transitions

An electron in a bonding σ orbital is excited to the corresponding anti bonding orbital. The energy required is large. This is observe d with saturated compounds. For example, methane (which has only C-H bonds, and can only undergo *transitions shows an absorbance maximum at 125 nm. Absorption maxima due to *transitions are not seen in typical UV-Vis. spectra (200 - 700 nm), but occur in vacuum UV or far UV region, i.e. 125 – 135nm.

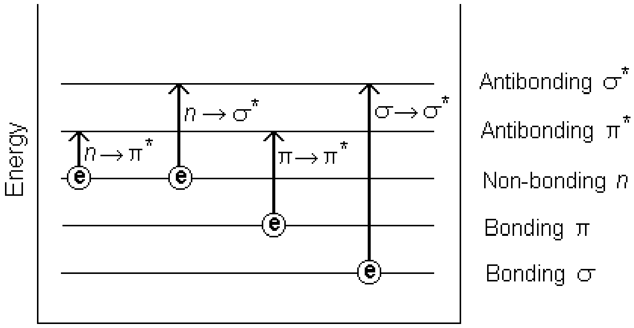
n□ □* *Transitions*

Saturated compounds containing atoms with lone pairs (non-bonding electrons) are capable of n* transitions. These transitions usually need less energy than * transitions. They can be initiated by light whose wavelength is in the range 150 - 250 nm.

n → □* and □ □ □* and * Transitions:

Most absorption spectroscopy of organic compounds is based on transitions of *n* electrons to the □* 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 unsaturated electrons group in the molecule to provide theMolar absorptivities from ***n*** → □* transitions are relatively low, and range from 10 to 100Lmol^{-1}cm^{-1}. □ □ □* transitions normally give molar absorptivities between 1000 and 10,000Lmol^{-1} cm^{-1}. The solvent in which the absorbing species is dissolved also has an effect on the spectrum of the species. Peaks resulting from ***n*** → ***p**** transitions are shifted to shorter wavelengths (*blue shift*) with increasing solvent polarity. This arises from increased solvation of the lone pair, which lowers the energy of the *n* orbital. Often (but *not* always), the reverse (i.e. *red shift*) is seen for □ □ □* transitions. This is caused by attractive polarization forces between the solvent and the absorber, which lower the energy levels of both the excited and unexcited states.

Electronic transitions



VISIBLE SPECTROSCOPY (COLORIMETRY) [10]:

Colorimetry is concerned with the study of absorption of visible radiation whose wavelength ranges from 400 nm to 800 nm. Any colored substance will absorb radiation in this wavelength region. Colored substances absorb light of different wavelength in different manner and hence we get an absorption curve (absorbance V/s wavelength). In this absorption curve, the wavelength at which maximum absorption of radiation takes place is called as λmax. This λmax is characteristic or unique for every colored substance and this is a qualitative aspect, useful in identifying the substance. λmaxis not usually affected by concentration of the substance. The absorbance of a solution increases with concentration of a substance. But there is no change in λmax when concentration changes. When we plot a graph of Concentration v/s Absorbance, we get a Calibration Curve or Standard Curve. This Calibration Curve is useful in determining the concentration or amount of a drug substance in the given sample solution or a formulation, by extrapolation or interpolation and calculation. Ultraviolet and Visible (UV-Vis) absorption spectroscopy is the measurement of the attenuation of a beam of light after it passes through a sample or after reflection from a sample surface. Absorption measurements can be at a single wavelength or over an extended spectral range. Ultraviolet and visible light are energetic enough to promote outer electrons to higher energy levels, and UV-Vis spectroscopy is usually applied to molecules or inorganic complexes in solution. The UV-Vis spectra have broad features that are of limited use for sample identification but are very useful for quantitative measurements. The concentration of an analyte in solution can be determined by measuring the absorbance at some wavelength and applying the Beer-Lambert Law.

Since the UV-Vis range spans the range of human visual acuity of approximately 400 - 750 nm, UV-Vis spectroscopy is useful to characterize the absorption, transmission, and reflectivity of a variety of technologically important materials, such as pigments, coatings, windows, and filters.

This more qualitative application usually requires recording at least a portion of the UV-Visible spectrum for characterization of the optical or electronic properties of materials.

Laws governing absorption of radiation:

Beer's law (related to concentration of absorbing species)

„The intensity of a beam of monochromatic light decreases exponentially with increase in the concentration of absorbing species arithmetically.

Lambert's law (related to thickness / path length of absorbing species)

„The rate of decrease of intensity (monochromatic light) with the thickness of the medium is directly proportional to the intensity of incident light".

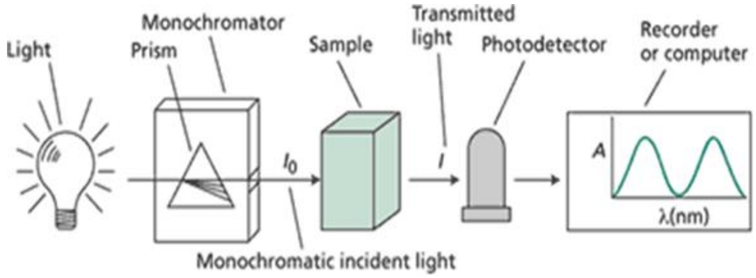
$$A = \varepsilon l c$$

A - absorbance at a specific wavelength
ε - absorption coefficient characteristic of the species at a given frequency
l - thickness of the sample
c - concentration

Limitations of the Beer-Lambert law:
The linearityof the Beer-Lambert law is limited by chemical and instrumental factors. Causes of nonlinearity include:
Deviations in absorptivity coefficients at high concentrations (>0.01M) due to electrostatic interactions between molecules in close proximity
Scattering of light due to particulates in the sample
Fluorescence or phosphorescence of the sample
Changes in refractive index at high analyte concentration
Shifts in chemical equilibria as a function of concentration
Non-monochromatic radiation, deviations can be minimized by using a relatively flat part of the absorption spectrum such as the maximum of an absorption band

Deviations from Beer's Law*:*
According to Beer‟s law, a straight line passing through the origin should be obtained, when a graph is plotted between absorbance (A) and concentration (C). Deviation from the law may be positive or negative, according to whether the resulting curve is concave upwards or concave downwards. The deviations from the Beer‟s law may be due to interaction of the solute molecules with each other or with the solvent or may be due to instrumental factors. The most important reasons that cause deviations are,

Negative deviation can always be expected when the illumination is not monochromatic.
The presence of impurities that fluoresce or absorb at the required absorption wavelength.
Environmental errors such as solvent, temperature and pressure Chemical factors such as change in pH and chemical equilibrium, presence of complexing agent, competitive metal ion reactions and concentration dependence.
Refractive index of sample.
Instrumental errors such as radiation, stability of radiation source, stability of slit control and electronics and reliability of the optical parts.



INSTRUMENTATION [11-12]:

A spectrophotometer is an instrument for measuring the transmittance or absorbance of a sample as a function of the wavelength of electromagnetic radiation.
A colorimeter is an instrument designed to read either transmittance or absorbance, normally single beam instruments and is of non-recording type.
UV-Vis Spectrophotometer

COMPONENTS OF A SPECTROPHOTOMETER:

Instruments for measuring the absorption of U.V. or visible radiation are made up of the following components;

1. Sources (UV and visible)
2. Wavelength selector (monochromator and filters)
3. Sample containers (cuvettes)

4. Solvents
5. Detector
6. Signal processor and readout

Radiation Source:

Any lamp source which gives adequate intensity of radiation over the entire wavelength region can be used. The ideal light source would yield a constant intensity over all wavelengths with low noise and long-term stability.

Unfortunately, however, such a source does not exist. The requirements of a source of light are:

a. It should provide continuous radiation
b. It should provide adequate intensity
c. It should be stable and free from fluctuations
d. Various UV radiation sources are as follows
e. Deuterium lamp
f. Hydrogen lamp
g. Xenon discharge lamp
h. Mercury arc lamp
i. Various Visible radiation sources are as follows
j. Tungsten lamp
k. Carbon arc lamp

Wavelength selector:

All monochromators contain the following component parts;

- An entrance slit
- A collimating lens
- A dispersing device (a prism or a grating)
- A focusing lens
- An exit slit

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.

Sample Containers:

In practice, by far the greater part of all measurements will be made on samples in solution. Vapours and solids can be accommodated, but most instruments are designed with a standard cell (or cuvette) as the normal sample container. The design, construction and material of the cuvette are all important to accurate measurements as are operator practice and sample preparation.

Cuvettes are typically made of glass or UV grade silica (according to the wavelength range of interest), are fused rather than cemented (to resist the action of some solvents), and have the following characteristics:

a. Optical windows (the sides through which the beam passes) are highly polished, parallel and flat.
b. Entrance and exit surfaces are exactly parallel and orthogonal
c. Light path (distance between inner surfaces of windows) is tightly controlled.

The holder that locates the cuvette in the light beam must ensure precise and reproducible location with respect to the beam. The most commonly used cuvette has a light path length of 10 mm, but longer or shorter path lengths are useful if concentration or absorbance fall outside normal ranges without further processing - e.g. solvent extraction or dilution. The cell holding the sample should be transparent to the wavelength region to be recorded. Quartz or fused silica cuvettes are required for spectroscopy in the UV region. Silicate glasses can be used for the manufacture of cuvettes for use between 350 and 2000 nm. The thickness of the cell is generally 1 cm. cells may be rectangular in shape or cylindrical with flat ends.

Solvents:

Solvent plays an important role in UV spectra, since compound peak could be obscured by solvent peak. Hence the solvent for a sample is selected in such a way that the solvent neither absorbs in the region of measurement nor affects the absorption of the sample. Some common solvents used and their absorption regions are:

TABLE List of commonly used solvents and their 'cut-off' wavelengths.

Solvents	Cut-off (nm)
Water	191nm
Cyclohexane	195nm
Methanol	203nm
Ethanol	204nm
Ether	215nm
Chloroform	237nm
Carbon tetrachloride	257nm

Detector:

Detectors used in UV/ Visible spectrophotometers can be called as photometric detectors. When a radiation is passed through a sample cell, part of it is being absorbed by the sample solution and rest is being transmitted. The transmitted radiation falls on the detector and the intensity of absorbed radiation can be determined or displayed. In these detectors, the light energy is converted to electrical signal which can be read or recorded. Ideally, it should give a linear response over a wide range with low noise and high sensitivity.

In order to detect radiation, three types of photosensitive devices are:

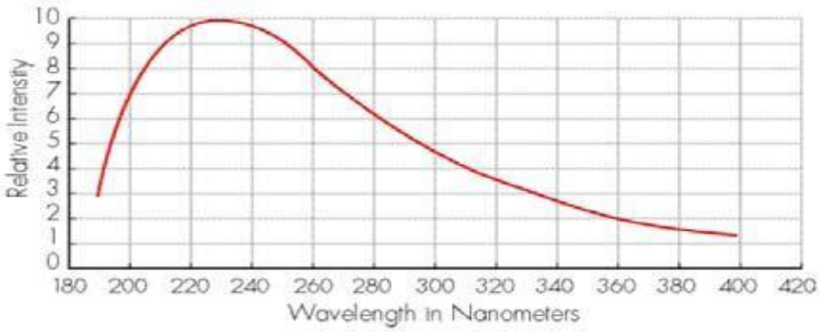
a. Photovoltaic cells or Barrier-layer cell
b. Phototubes or Photo emissive tubes
c. Photomultiplier tubes

Signal processor and readout:

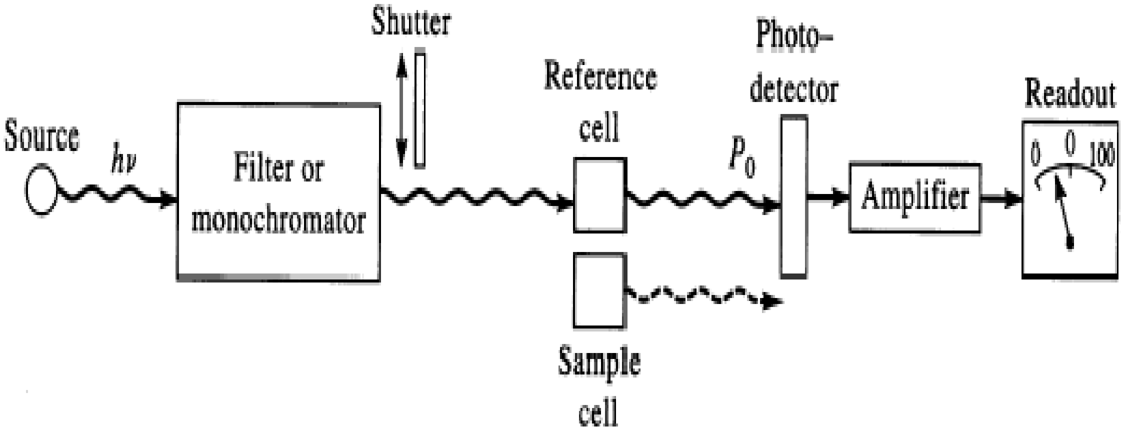
The primary function of a spectrophotometer ends with the provision of a signal (normally an electrical voltage) that is proportional to the absorption by a sample at a given wavelength. The signal handling and measuring systems can be as simple as an amplifier and a meter or as elaborate as a personal computer and printer, depending on the application. In simplest form, a meter will serve either to indicate the absolute value of the output signal, or in some instances, the null point in a back-off circuit. Digital readouts (LED or LCD) are favored for clarity and lack of ambiguity and may be linked with a microprocessor such that readout is in any preferred terms - directly in concentration units for example.

Once the operator has defined the parameters (e.g. wavelength, output mode and relevant computing factors) the system will ensure the correct and optimum combination of all the variables available. Selection of source and detector are automatically determined, any filters (e.g. order suppressing filters) or other components will be introduced into the optical train at appropriate points and sample and reference cells correctly managed in the sample area. Output in the required terms (transmittance, absorbance, concentration etc.) will be presented and the relevant sample identified.

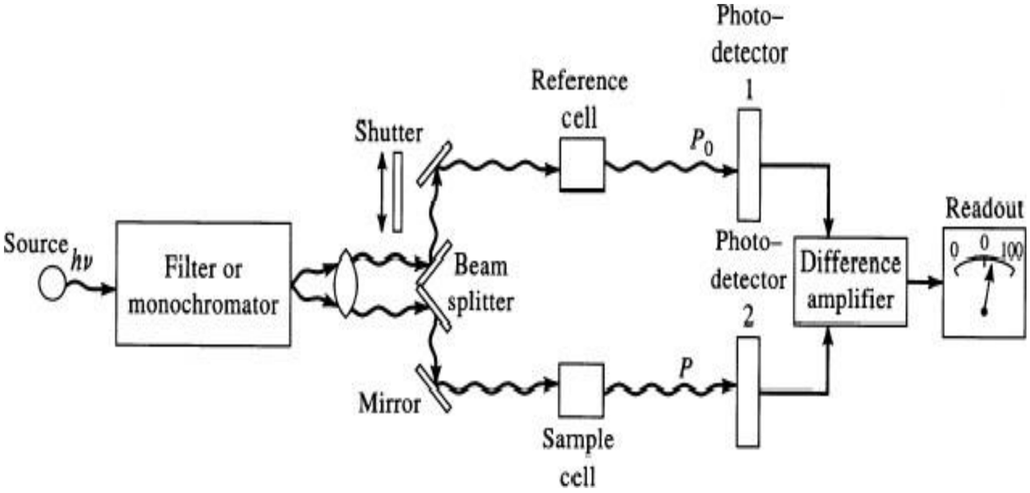
Absorption spectrum



Different types of instruments:



Single beam spectrometer



Double beam spectrometer

STATISTICAL ANALYSIS:

Statistical procedures and representative calculations:

The consistency and suitability of the developed method are substantiated through the statistical analysis like standard deviation, relative standard deviation and theoretical plates per meter.

Standard Deviation (SD) :

$$\mathrm{SD} = \sqrt{\frac{\sum_{i=1}^{n} (x_i - x)^2}{n - 1}}$$

Where, x = Sample, xi = Mean value of samples, n= Number of samples

Relative Standard Deviation (RSD):

Molar extinction coefficient (mol^{-1} cm^{-1}) = A / C × L

Where, A= Absorbance of drug, C= Concentration of drug, L= Path length

Sandell's sensitivity (mcg / cm^2 / 0.001 absorbance units) = C / A × 0.001

Where, C = Concentration of drug, A = Absorbance of drug

Units - (µg / cm^2 = 0.001 absorbance)

Coefficient of variance (σ) = $\sum(x - xi)^2 / n-1$

Regression equation y = a + bx

Slope = y / x

Where, x = Concentration y = Absorbance

a = Intercept

Limit of detection: (LOD) = 3.3 × / S

Units - (mcg / ml)

Limit of quantitation (LOQ) = 10 × / S

Units - (mcg / ml) Where,

 = Standard deviation of the response. S = Slope of the calibration curve.

The slope S may be estimated from the calibration curve of the analyte. The estimate of may be carried out in a variety of ways.

METHOD VALIDATION: [13-23]

According to USP General Chapter <1225> "Validation is the process of providing documented evidence that the method does what it is intended to do". In other words the process of method validation ensures that the proposed analytical methodology is accurate, specific, reproducible and rugged for its intended use.

According to the **FDA** Guidelines on General Principles of Process Validation, process validation is defined, „„„as establishing documented evidence, which provides a high degree of assurance, that a specific process will consistently produce a product meeting its predetermined specifications and quality characteristics.

According to **ICH Guideline** Validation of an Analytical procedure is to demonstrate that it is suitable for its intended purpose. Method validation can be defined as (ICHQ.2B) "Establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics".

Method validation is an integral part of the method development; it is the process of demonstrating that analytical procedures are suitable for their intended use and that they support the identity, quality, purity, and potency of the drug substances and drug products. Simply, method validation is the process of proving that an analytical method is acceptable for its intended purpose.

Method Validation, however, is generally a one-time process performed after the method has been developed to demonstrate that the method is scientifically sound and that it serves the intended analytical purpose.

Validation is documented evidence, which is completed to ensure that an analytical method is accurate, reproducible and robust over the specific range. The quality of the analytical data is a key factor in the success of a drug development program. The process of method development and validation has a direct impact on the quality of these data.

Method validation:

Method validation is the process to confirm that analytical procedure employed for a specific test is suitable for its intended use. Method needs to be validated or revalidated

- Before their introduction into routine use
- Whenever the conditions changes for which the method has been validated, e.g., instrument with different characteristics
- Whenever the method is changed, and the change is outside the original scope of the method.

TYPES OF ANALYTICAL PROCEL LIDATED:

$$RSD\ (\%) = \frac{100\ SD}{x}$$

- Ide
- Quantitative tests for impurities content;
- 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.

Method Validation is required for the following:

- Method validation required for assuring the quality identity purity.
- Achieving the acceptance of the product by the international agencies.
- Mandatory requirements for the registration of Pharmaceuticals pesticides and formations.
- Validation methods are only acceptable for under taking sufficiency testing.

Identification tests are indented to ensure the identity of analyte in a sample. This is normally achieved by comparison of sample chromatograms to that of a reference standard. Testing for impurities can be either a qualitative or quantitative limit test for the impurity in the sample.

Purpose of Validation:

1. Enable the scientists to communicate scientifically and effectively on technical matter.
2. Setting the standards of evaluation procedures for checking
3. compliance and taking remedial action.
4. Economic: Reduction in cost associated with process sampling and testing.
5. As quality of the product cannot always be assured by routine quality control because of testing of statistically insignificant number of samples.
6. Retrospective validation is useful for trend comparison of results compliance to CGMP/CGLP.
7. Closure interaction with Pharmacopoeial forum to address analytical problems.
8. International Pharmacopoeial harmonization particularly in respect of impurities determination and their limits.

Depending on the use of the assay, different parameters will have to be measured during the assay validation. ICH and several regulatory bodies and Pharmacopoeia have published information on the validation of analytical procedures.

The goal of the validation process is to challenge the method and determine the limit of allowed variability for the conditions needed to run the method.

All the variables of the method should be considered, including sampling procedure, sample preparation, chromatographic separation, detection and data evaluation. For chromatographic methods used in analytical applications there is more consistency in validation practice with key analytical parameters including

a) Recovery
b) Response of function
c) Sensitivity / Selectivity/ Specificity
d) Precision
e) Accuracy
f) Calibration
g) Linearity and sensitivity of the method
h) Range
i) Limit of detection
j) Limit of quantitation
k) Ruggedness
l) Robustness
m) Stability
n) System suitability

Recovery:

The absolute recovery of analytical method is measured as the response of a processed spiked matrix standard expressed as a percentage of the response of pure standard, which has not been subjected to sample pre-treatment and indicates whether the method provides a response for the entire amount of analyte that is present in the sample. It is best established by comparing the responses of extracted samples at low, medium and high concentrations in replicates of at least 6 with those non-extracted standards, which represent 100 % recovery.

Absolute recovery:

<u>Response of analyte spike into matrix (processed)</u> x 100 Response of analyte of pure standard (unprocessed)

If an internal standard is used, its recovery should be determined independently at the concentration levels used in the method.

Response of function:

In chromatographic methods of analysis, peak area or peak height may be used as response function to define the linear relationship with concentration known as the calibration model. It is essential to verify the calibration model selected to ensure that it adequately describes the relationship between response function (Y) and concentration (X).

Sensitivity:

The method is said to be sensitive if small changes in concentration cause large changes in response function. The sensitivity of an analytical method is determined from the slope of the calibration line. The limits of quantification (LOQ) or working dynamic range of bio analytical method are defined as the highest and lowest concentrations, which can determined with acceptable accuracy. It is suggested that, this be set at ± 15% for both the upper and lower limit of quantification respectively. Any sample concentration that falls outside the calibration range cannot be interpolated from the calibration line and extrapolation of the calibration curve is discouraged. If the concentration is over range, the sample should be diluted in drug-free matrix and re-assayed.

Selectivity (specificity):
The ability of analytical method accurately to measure the analyte response in a sample in the presence of other potential components like excipients , degradants, impurities etc. This parameter is measured for identity tests, for content or potency tests, and for purity tests to ensure that the assay provides an accurate statement of the identity, potency or purity of a product. Selectivity (specificity), like accuracy, is expressed as the bias or the % error between the measured and known value.

Precision:
The closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

$$\mathrm{SD} = \sqrt{\frac{\sum_{i=1}^{n} (x_i - x)^2}{n - 1}}$$

The purpose of carrying out a determination is to obtain a valid estimate of a „true‟ value. When one considers the criteria according to which an analytical procedure is selected, precision and accuracy are usually the first time to come to mind. Precision and accuracy together determine the error of an individual determination. They are among the most important criteria for judging analytical procedures by their results.Precision should be obtained preferably using authentic samples. As parameters, the standard deviation (SD), the relative standard deviation (coefficient of variation) and the confidence interval should be calculated for each level of precision.

$$\mathrm{RSD}\ (\%) = \frac{100\ \mathrm{SD}}{x}$$

Repeatability expresses the analytical variability under the same operating conditions over a short interval of time (within-assay, intra-assay). At least nine determinations covering the specified range or six determinations at 100 % test concentration should be performed. Intermediate precision includes the influence of additional random effects within laboratories, according to the intended use of the procedure, for example, different days, analysts or equipment, etc.

Reproducibility, i.e., the precision between laboratories (collaborative or inter laboratory Studies), is not required for submission, but can be taken into account for standardization of analytical procedures.

Precision refers to the reproducibility of measurement within a set, that is, to the scatter of dispersion of a set about its central value. The term „set‟ is defined as referring to a number (n) of independent replicate measurements of some property. One of the most common statistical terms employed is the standard deviation of a population of observation. Standard deviation is the square root of the sum of squares of deviations of individual results for the mean, divided by one less than the number of results in the set. The standard deviation S, is given by

Standard deviation has the same units as the property being measured.

The square of standard deviation is called variance (S^2). Relative standard deviation is the standard deviation expressed as a fraction of the mean, i.e., S/x. It is sometimes multiplied by 100 and expressed as a percent relative standard deviation. It becomes a more reliable expression of precision.
Acceptance: %RSD should be not more than 2% for assay.

Accuracy:
Accuracy of analytical method is „Measure of how close the experimental value to the true value.‟
Accuracy normally refers to the difference between the mean x, of the set of results and the true or correct value for the quantity

measured. According to IUPAC accuracy relates to the difference between results (or mean) and the true value. For analytical methods, there are two possible ways of determining the accuracy, absolute method and comparative method.

Accuracy is best reported as percentage bias or percentage error, which is calculated from the expression.

Since for real samples the true value is not known, an approximation is obtained based on spiking
drug – free matrix to a nominal concentration. The accuracy of analytical method is then determined at each concentration by assessing the agreement between the measured and nominal concentrations of the analytes in the spiked drug – free matrix sampler.

Accuracy studies is evaluated by recovery studies or spike studies in which known amount of the drug substances is added to the previously analyzed pharmaceutical preparations of the drug and tested for the added drug. Accuracy can be demonstrated by the following approaches:Inferred from precision, linearity and specificity

$$\%Bias = \frac{(measured\ value\ -\ true\ value)}{true\ value}\ X\ 100$$

- Comparison of the results with those of a well characterized, independent procedure
- Application to a reference material (for drug substance)
- Recovery of drug substance spiked to placebo or drug product (for drug product)
- Recovery of the impurity spiked to drug substance or drug product (for impurities)

For the quantitative approaches, at least nine determinations across the specified range should be obtained, for example, three replicates at three concentration levels each. The percentage recovery or the difference between the mean and the accepted true value together with the confidence intervals are recommended.

$$s_b = \frac{s_{y/z}}{\sqrt{\sum(x_i - \bar{x})^2}}$$

It is important to use the same quantitation method (calibration model) in the accuracy studies as used in the control test procedure. Sometimes in the literature, the data from linearity studies are simply used to calculate the content of spiked samples. However, the validation linearity study is usually not identical to the calibration applied in routine analysis. Again, validation has to demonstrate the suitability of the routine analytical procedure. Deviations from the theoretical recovery values, while performing a calibration with a drug substance alone, may indicate interferences between the analyte and placebo components, incomplete extraction, etc. In such a case, the calibration should be done with a synthetic mixture of placebo and drug substance standard. Such interferences will also be detected by comparing the linearity‟s of diluted drug substance and of spiked placebo, but the evaluation is more complex. In contrast, recovery studies usually concentrate directly on the working range and are simpler (but not always easy) to evaluate.

Acceptance: The method is considered as accurate if average recovery is in between 98.0% to 102.0% for assay.

Calibration

Calibration is the most important step in bioactive compound analysis. A good precision and accuracy can only be obtained when a good calibration procedure is adopted. In the spectrophotometric methods, the concentration of a sample cannot be measured directly, but is determined using another physical measuring quantity „y‟ (absorbance of a solution). An unambiguous empirical or theoretical relationship can be shown between this quantity and the concentration of an analyte. The calibration between y = g (x) is directly useful and yields by inversion of the analytical calculation function.

The calibration function can be obtained by fitting an adequate mathematical model through the experimental data. The most convenient calibration function is linear, goes through the origin and is applicable over a wide dynamic range. In practice, however, many deviations from the ideal calibration line may occur. For the majority of analytical techniques the analyst uses the calibration equation.

Y = a + bx.

In calibration, univariate regression is applied, which means that all observations are dependent upon a single variable X.

Standard deviation of slope (Sb)

The standard deviation of slope is proportional to standard error of estimate and inversely proportional to the range and square root of the number of data points.

Where, Xi is the arithmetic mean of Xi values.

$$s_{y/x} = \sqrt{\frac{\sum(y_i - \hat{y}_i)^2}{n-2}}$$

Standard deviation of intercept, (Sa)

Intercept values of least squares fits of data are often to evaluate additive errors between or among different methods.

Where, Xi denotes the arithmetic mean of xi, values.

$$s_a = s_{y/x}\sqrt{\frac{\sum x_i^2}{n\sum(x_i - \bar{x})^2}}$$

Correlation Coefficient, (r)

The correlation coefficient r(x, y) is more useful to express the relationship of the chosen scales. To obtain a correlation coefficient the covariance is divided by the product of the standard deviation of x and y.

$$r = \frac{n\sum xy - \left(\sum x\right)\left(\sum y\right)}{\sqrt{n\left(\sum x^2\right) - \left(\sum x\right)^2}\sqrt{n\left(\sum y^2\right) - \left(\sum y\right)^2}}$$

Linearity and sensitivity of the method

Knowledge of the sensitivity of the color is important and the following terms are commonly employed for expressing sensitivity. According to Bouger- Lambert – Beer‟s law, log intensity of incident radiations

$$A \quad Log\frac{Intensity of\ incident light}{Intensity of\ transmitted light}$$

The absorbance (A) is proportional to the concentration (c) of the absorbing species, if absorptivity (Î) and thickness of the medium (t) are constant. When c is in moles per liter, the constant is called molar absorptivity. Beer‟s law limits and Îmax values are expressed as µg ml-^{1} and mole^{-1} cm^{-1} respectively.

Sandell‟s sensitivity refers to the number of µg of the drug to be determining, converted to the colored product, which in a column solution of cross section 1cm^2 shows an absorbance of 0.001 (expressed as µg cm^{-2}).

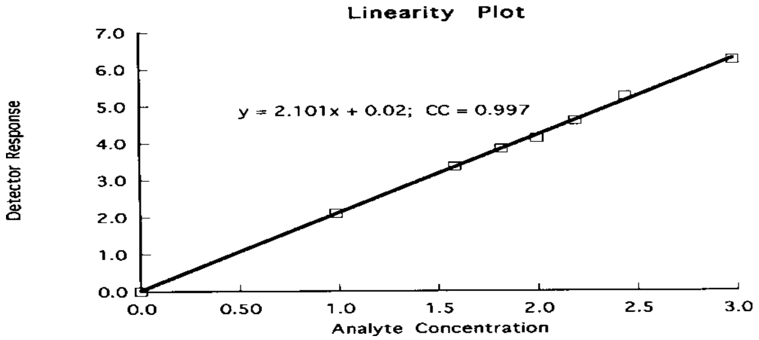
The ability (within specified range) to obtain test results are directly proportional to the concentration of analyte in the sample.

Prepare serial dilutions of minimum 5 concentration of standard stock solutions usually drug product and analyzed six times for the higher and the lower concentrations, two times other concentrations. Take the average area of each injection and plot the graph of average peak area versus actual concentration of each solution in µg/ml.

Linearity evaluated by visual inspection of plot of signal as a function of analyte concentration. If there is a linear relationship, Test results calculated by regression line by method of least squares.

Recommended data: The correlation coefficient, Y-intercept slope of the regression line and residual sum of squares should be submitted. A graphical presentation of the data and the residuals is recommended.

Fig. Linearity plot



Acceptance Criteria: The correlation coefficient should not be less than 0.999.

Range:

"The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity."

It is defined as the range of concentrations in which method provides acceptable degree of linear, precise and accurate. For the range data shall be considered from linearity and accurate section. The precise shall be calculated for lower and higher concentrations calculated in linearity sections.

Acceptance Criteria:

Linearity: The correlation coefficient should be not less than 0.999.

Precision: The relative standard deviation of 6 replicate injections made in linearity section of lower and higher concentrations should not be more than 2.0%.

Accuracy: A method is considered "Accurate" if the average recovery is between 98.0% and 102.0%.

Limit of Detection:

It is the lowest quantity of a substance that can be distinguished from the absence of that substance within a stated confidence limit. It is the lowest amount of the analyte in a sample that can be detected but not necessarily be quantitated as an exact concentration or amount.

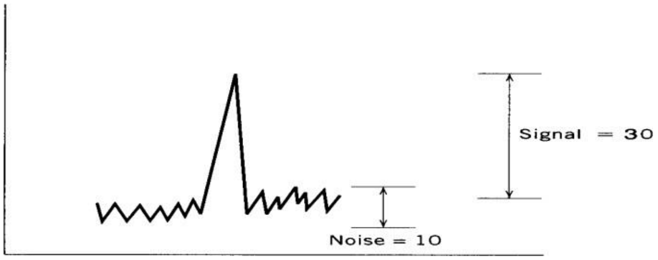


Fig. Limit of Detection (LOD) = 3: 1 (Signal-to-Noise ratio)

Limit of Quantitation:

It is the lowest quantity of a substance that can be distinguished from the absence of that substance within a stated confidence limit. It is the lowest amount of the analyte in a sample that can be detected but not necessary be quantified as an exact concentration or amount.

LOD = 3.3 σ/S σ = the standard deviation of the response

LOQ = 10 σ/S S = the slope of the calibration curve

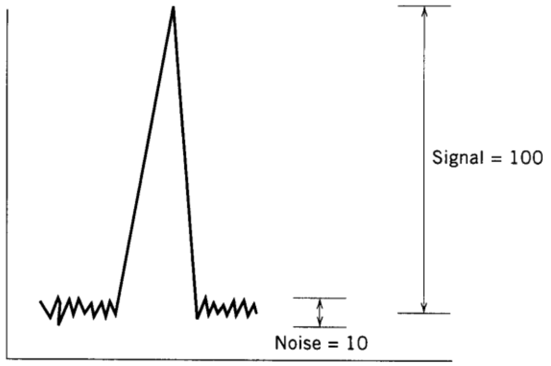


Fig. Limit of Quantitation (LOQ) = 10: 1 (Signal-to-Noise ratio)

Table Robustness parameters

Parameter	**Variation to be checked**
Flow rate	± 0.2 mL/min
Column temperature	±5˚C
pH variation	±0.2
Mobile phase composition	±5%
Filter validation	Different filter

Stability:

To generate reproducible and reliable results, the samples, standards and reagents used for the HPLC method must be stable for a reasonable time (e.g. one day, one week, one month etc, depending upon need).

System suitability

According to USP, system suitability is an integral part of the chromatographic system.

AIM AND OBJECTIVES

1. Understand theory of Simultaneous estimation of drugs by UV
2. Determine the concentration of Amlodipine, Valsartan by Simultaneous equation method
3. The present study aimed to develop simple, precise and accurate methods for the Simultaneous estimation of Amlodipine, Valsartan in Pure and pharmaceutical dosage forms by UV Spectrophotometry.
4. To develop Analytical method for Amlodipine, Valsartan by UV Spectrophotometry.
5. To validate the Analytical method developed according to ICH guidelines

PLAN OF WORK

Selection and collection of literature for drugs for analysis .

Method development by UV Spectrophotometry method: The plan of the proposed work includes the following steps:

- The extensive survey of literature for drugs regarding their physio- chemical properties, pharmacological properties and analytical methods. This formed the basis for the development of methods.
- To undertake solubility studies for the drugs identification,
- Selection of suitable solvent for quantitative extraction of drug present in the formulations. The solvent should be readily available, economical and of analytical grade.
- Selection of analytical wavelength.
- Setting up of initial spectrophotometric conditions for the assay of Amlodipine, Valsartan by simultaneous equation methods

- Preparation of standard calibration curve of Amlodipine, Valsartan.
- Assay of pure mixed standards and formulation.

Validation of the developed UV Spectrophotometry Analytical method according to ICH method validation parameters.

MATERIALS AND METHODS:

Table -Chemicals used for the Study

S.NO	CHEMICALS	COMPANY NAME
1.	Valsartan-160mg Amlodipine- 10mg	Valvex
2.	Valsartan (pure drug)	Micro Labs Ltd
3.	Amlodipine (Pure drug)	Micro Labs Ltd
4	Distilled Water	MERCK
5.	Methanol	MERCK
5.	Ethanol	MERCK

Table Equipment's and Glassware's used for the study

S.NO.	EQUIPMENT AND GLASSWARES	COMPANY NAME
1.	Double beam UV spectrophotometer	SHIMADZU Toshvin Analytical pvt. ltd
2.	Analytical balance	WENSAR
3.	Sonicator	ELERTECH
4.	Standard flask 50ml	Borosil
5.	Standard flasks 10ml	Borosil
6.	Pipette 2ml	Borosil
7.	Beakers	Borosil
8.	Measuring cylinder	Borosil

UV Spectrophotometry Method Development: Solvent selection:

In the start of the method development for this drug, different solvents were tested such as water, methanol, and Ethanol. In order to select suitable solvent for determination of Amlodipine, Valsartan,in various solvents were selected for the solubility studies and it was found that Amlodipine,Valsartan was freely soluble in Water, Methanol Ethanol. In the present investigation, Methanol was used for all the dilutions. Due to greater solubility and reproducible readings of maximum absorbance, Methanol was taken under consideration for further work.

Selection of wavelength:

The absorbance of the solution containing Amlodipine, Valsartan at 10 µg/ml was determined in the UV range 200-400 nm using an appropriate blank.

The maximum absorbance was found to be 220nm of Amlodipine 252nm of Valsartan.

Assay of Amlodipine Valsartan by simultaneous equation method Preparation of Amlodipine standard solution:

Weigh accurately1 mg of standard substances of Amlodipine [1mg=1000µg]
Dissolve 1 mg of Amlodipine in to a 100 ml of Methanol Solution [Stock solution of Amlodipine 10µg/ml]
1mg/100ml 1000µg/100ml=10 µg/ml
The 10 µg/ml Amlodipine standard solution was observed and found absorban

Preparation of Valsartan standard solution:
Weigh accurately1 mg of standard substances of Valsartan [1mg=1000µg]
Dissolve 1 mg of Valsartan in to a 100 ml of Methanol Solution [stock solution of Valsartan [Stock solution of Valsartan 10µg/ml]
1mg/100ml
1000µg/100ml=10 µg/ml
The 10 µg/ml Valsartan standard solution was observed and found absorbance in UV at 222nm, 252nm respectively. Calculate absorptivity

Preparation of (sample) stock solution of Tablet formulation: Weigh accurately 20 tablets of marketed formulation Valvex Calculate average weight
Weigh accurately 10 mg equivalent weight of Amlodipine powder in to a100 ml of Methanol solution [Stock solution of mixture 10µg/ml]
1mg/100ml 000µg/100ml=10 µg/ml
The10 µg/ml solution was observed and found absorbance in UV at 220nm

Repeat the same Procedure for the Valsartn and Found absorbane in UV at 252 nm.

The necessary information is required for this purpose

1. The absorptivity of Amlodipine at 220nm and 252nm are ax1, ax2
2. The absorptivity of Valsartan at 220nm and 252nm are ay1, ay2
3. The absorbance of Sample (Amlodipine) at 220 nm, The absorbance of Sample (Valsartan) at 252 nm are A1, A2 respectively 4, Cx and Cy are Concentration of Amlodipine and Valsartan respectively in the diluted sample
4. Using below equation Concentration of Amlodipine and Valsartan are Calculated

$$Cx = \frac{A_2\, a_{y1} - A_1\, a_{y2}}{a_{x2}\, a_{y1} - a_{x1}\, a_{y2}}$$

$$Cy = \frac{A_1\, a_{x2} - A_2\, a_{x1}}{a_{x2}\, a_{y1} - a_{x1}\, a_{y2}}$$

Calculation for absorptivity:

a=Absorbance/Concentration in µg /ml

Calculation For Dilution Factor(Assay)
DF=Final Volume /Transferred Volume
Table 10µgm/ml Amlodipine solution Preparation

Particulars	Dilution Volume	Concentration	Dilution Factor=
10mg Amlodipine	10	µg/ml1000	10
1 ml Solution	10	100 µg/ml	10
1 ml Solution	10	100 µg/ml	10

Final Dilution Factor=10x10x10=1000

Table 10µgm/ml Valsartan solution Preparation

Particulars	Dilution Volume	Concentration	Dilution Factor
10mg Valsartan	10	1000 µg/ml	10
1 ml Solution	10	100 µg/ml	10
1 ml Solution	10	100 mg/ml	10

Final Dilution Factor=10x10x10=1000

METHOD VALIDATION PARAMETERS:

LINEARITY OF TEST METHOD:

Preparation of Amlodipine standard solution:

Weigh accurately10 mg of standard substances of Amlodipine
[1mg=1000µg/ml]
Dissolve 10 mg of Amlodipine in to a 10 ml of Mehanol [From Stock solution of Amlodipine 1000µg/ml]
10mg/10ml=mg/ml=1000 µg/ml
Take above 1ml stock solution and dilute up to 10ml with Methanol. [From Stock solution of Amlodipine 1000µg/ml]
1ml/10ml* 1000 µg/10ml = 100 µg/ml

Preparation of Valsartan standard solution:

Weigh accurately10 mg of standard substances of Valsartan
[1mg=1000µg/ml]
Dissolve 10 mg of Valsartan in to a 10 ml of Methanol [stock solution of Valsartan [Stock solution of Valsartan 1000µg/ml]
10mg/10ml=mg/ml=1000 µg/ml
Take above1ml stock solution and dilute up to 10ml with Methanol. [100µg/ml
1ml/10ml* 1000 µg/10ml = 100 µg/ml

Preparation of standard curve:

A series of Standard solutions (Not Less than five) were prepared by taking Different dilutions of standard drug Amlodipine having the 2,4,6,8,10
Valsartan having the concentration 2,4,6,8,10µg/ml were prepared and measure the absorbance of Amlodipine at 220nm, absorbance of Valsartan at 252nm against Solvent blank

Table 2, 4,6,8, 10µg/ml concentration Amlodipine solution preparation:

Name of the Drug	Standard solution Stock Amlodipine of 100µg/ml]	Dilute up to	Total Concentration we get
Amlodipine	0.2 ml from 100µg/ml	10ml	2µg/ml
	0.4 ml from 100µg/ml	10ml	4µg/ml
	0.6 ml from 100µg/ml	10ml	6µg/ml
	0.8ml from 100µg/ml	10ml	8µg/ml
	1 ml from 100µg/ml	10ml	10µg/ml

Table 2, 4,6,8,10µg/ml concentration Valsartan solution preparation

Name of the Drug	Standard Stock solution of Valsartan 100µg/ml]	Dilute up to	Total Concentration we get
Valsartan	0.2 ml from 100µg/ml	10ml	2µg/ml
	0.4 ml from 100µg/ml	10ml	4µg/ml
	0.6 ml from 100µg/ml	10ml	6µg/ml
	0.8 ml from 100µg/ml	10ml	8µg/ml
	1.0ml from 100µg/ml	10ml	10µg/ml

Procedure: Each Concentration was observed into the UV spectrophotometric system and Absorbance Amlodipine, valsartan were measured at 220nm, 252nm against solvent blank. Plot a graph of Absorbance versus concentration (on X-axis concentration and on Y-axis Absorbance) and the correlation coefficient was calculated. And the absorbance values were shown in Table no 20,21. The obtained absorbance values were plotted against the concentration of Amlodipine, Valsartan to get the calibration graph and were represented as Fig no:10,11. The regression equation and correlation coefficient were determined and are given in above Table.

Acceptance criteria-Correlation coefficient should be not less than 0.999.

Accuracy:Preparation of Standard stock Solution of Amlodipine,Valsartan

Weigh accurately 10 mg of standard substances of Amlodipine **[**1mg=1000µg/ml] Dissolve in to a10 ml of Methanol solution [Stock solution of Amlodipine 1000µg/ml]

10mg/10ml=mg/ml=1000 µg/ml

Take above1ml stock solution and dilute up to 10ml with Methanol Solution [100µg/ml]

1ml/10ml* 1000 µg/ml = 100 µg/ml

Repeat the same Procedure for Valsartan. And prepare the 100µg/ml valsartan standard solution Prepared

Preparation of stock solution of Tablet formulation:

Weigh accurately 20 tablets of marketed formulation [Valvex] Calculate average weight.

Weigh accurately 10 mg equivalent weight of Amlodipine powder in to a10 ml of Methanol solution [Stock solution of mixture 1000µg/ml] Take above 1ml stock solution of mixture and dilute up to 10ml with Methanol [100µg/ml]. Repeat the same Procedure for Valsartan. And prepare the 100µg/ml valsartan Sample solution.

Table Preparation of 50,100,150% Amlodipine solution of Tablet Formulation:

% level	Stock solution of Tablet formulations	Standard stock Solution of Amlodipine	Dilute Upto	Total conc we get
80%	1 ml from 100µg/ml	0.5. ml from 100µg/ml	10ml	15µg/ml
100%	1 ml from 100µg/ml	1 ml from 100µg/ml	10ml	20 µg/ml
120%	1 ml from 100µg/ml	1.5 ml from 100µg/ml	10ml	25 µg/ml
Make three same dilution at each % level				

Table Preparation of 50,100,150% Valsartan solution of Tablet Formulation:

% level	Stock solution of Tablet formulations	Standard stock Solution of Valsartan	Dilute Upto	Total conc we get
80%	1 ml from 100µg/ml	0.5 ml from 100µg/ml	10ml	15 µg/ml
100%	1 ml from 100µg/ml	1 ml from 100µg/ml	10ml	20 µg/ml
120%	1 ml from 100µg/ml	1.5 ml from 100µg/ml	10ml	25 µg/ml

Make three same dilution at each % level

Procedure: The accuracy study was performed for 50%,100%,150% for Amlodipine, Valsartan. Each level was observed in triplicate into UV spectrophotometric System.

The Concentrationof each level was used for calculation of % recovery. Results are tabulated in above Table.

Acceptance criteria: The Percentage recovery was found to be within the limits (98-102%).

PRECISION:

To evaluate Precision of the method, Precision was performed on Different days by maintaining same conditions.

Preparation of Standard stock Solution of Amlodipine, Valsartan:

Weigh accurately10 mg of standard substances of Amlodipine [1mg=1000µg/ml]

Dissolve in to a10 ml of Methanol solution [Stock solution of Valsartan 1000µg/ml]

10mg/10ml=mg/ml=1000 µg/ml

Take above1ml stock solution and dilute up to 10ml with Methanol [100µg/ml]

1ml/10ml* 1000 µg/ml = 100 µg/ml

Repeat the same Procedure for Valsartan. And Prepare the 100µg/ml valsartan Standard stock solution Prepared

Preparation of stock solution of Tablet formulation: Weigh accurately 20 tablets of marketed formulation (VALVEX) Calculate average weight

Weigh accurately 10 mg equivalent weight of powder in to a10 ml of Ethanol solution [Stock solution of mixture 1000µg/ml] Take above1ml stock solution of mixture and dilute up to 10ml with Ethanol [100µg/ml].

Repeat the same Procedure for Valsartan. And Prepare the 100µg/ml valsartan Sample solution Prepared

Table Preparation of 100% Amlodipine solution[10µg/ml] of Tablet Formulation

% level	Stock solution of Tablet formulations	Standard stock Solution of Amlodipine	Dilute Upto	Total conc we get
100%	5 ml from 100µg/ml	5 ml from 100µg/ml	100ml	10 µg/ml

Table Preparation of 100% Valsartan solution[10µg/ml]of Tablet Formulation:

% level	Stock solution of Tablet formulations	Standard stock Solution of Valsartan	Dilute Upto	Total conc we get
100%	5 ml from 100µg/ml	5 ml from 100µg/ml	100ml	10 µg/ml

Procedure:

Intra-day precision: The 10 µg/ml solutions of Amlodipine, Valsartan was observed for six times and found absorbance of each drug for all 6 times In UV

.The % RSD for absorbance, Concentration Found, % Assay for 6 Sample was found to be within the specified limits.

Inter-day precision: The10 µg/ml solutions of Amlodipine, Valsartan was observed for six times and found absorbance of each drug for all 6 times In UV

.The % RSD for absorbance, Concentration Found , % Assay of each drug For 6 Sample was found to be within the specified limits. Amlodipine Results are tabulated in Table no 26,27.Valsartan Results are tabulated in Table no 28,29. **Acceptance Criteria:**

The % Relative Standard Deviation of % Assay of Amlodipine Valsartan from the six sample preparations should be not more than 2.0%. The individual % Assay of Amlodipine Valsartan should not be less than 98.0% and not more than 102.0%.

Ruggedness:

Analyst to analyst variability:

To evaluate Ruggedness of the method, Ruggedness was performed by using two different analysts by maintaining same conditions

Preparation of Standard stock Solution of Amlodipine, Valsartan:

Weigh accurately 10 mg of standard substances of Amlodipine [1mg=1000µg/ml] Dissolve in to a10 ml of Methanol solution [Stock solution of Amlodipine 1000µg/ml]

10mg/10ml=mg/ml=1000 µg/ml

Take above 1ml stock solution and dilute up to 10ml with Methanol [100µg/ml]

1ml/10ml* 1000 µg/ml = 100 µg/ml

Repeat the same Procedure for Valsartan. And prepare the 100µg/ml valsartan standard solution Prepared

Preparation of stock solution of Tablet formulation :

Weigh accurately 20 tablets of marketed formulation [Valent 10mg] Calculate average weight.Weigh accurately 10 mg equivalent weight of powder in to a10 ml of Methanol solution [Stock solution of mixture 1000µg/ml] Take above1ml stock solution of mixture and dilute up to 10ml with Methanol [100µg/ml]. Repeat the same Procedure for Valsartan. And prepare the 100µg/ml valsartan sample solution Prepared

Table Preparation of 100% solution Amlodipinene ,[10µg/ml] of Tablet Formulation:

% level	Stock solution of Tablet formulations	Standard stock Solution of Amlodipine	Dilute Up to	Total concentration we get
100%	5 ml from 100µg/ml	5 ml from 100µg/ml	100ml	10 µg/ml

Table Preparation of 100% solution Valsartan ,[10µg/ml] of Tablet Formulation:

% level	Stock solution of Tablet formulations	Standard stock Solution of Valsartan	Dilute Up to	Total concentration we get
100%	5 ml from 100µg/ml	5 ml from 100µg/ml	100ml	10 µg/ml

Analyst 1: The10 µg/ml solution was observed for six times and found absorbance for all 6 times In UV .The % RSD for absorbance, Concentration Found, % Assay for 6 Sample was found to be within the specified limits.

Analyst 2: The10 µg/ml solution was observed for six times and found absorbance for all 6 times In

UV .The % RSD for absorbance, Concentration Found, % Assay For 6 Sample was found to be within the specified limits. Amlodipine Results are tabulated in Table no 30, 31. Valsartan Results are tabulated in Table no 32, 33

Acceptance Criteria:

The % Relative Standard Deviation of % Assay of Amlodipine, Valsartan from the six sample preparations should be not more than 2.0%. The individual % Assay of Amlodipine, Valsartan should not be less than 98.0% and not more than 102.0%.

Limit of detection and Limit of quantification:

Weigh accurately10 mg of standard substances of Amlodipine [1mg=1000µg/ml] dissolve in to a10 ml of Methanol solution [Stock solution of Amlodipine 1000µg/ml]

10mg/10ml=mg/ml=1000 µg/ml

Take above1ml stock solution and dilute up to 100ml with Methanol [10µg/ml]

1ml/100ml* 1000 µg/ml = 10 µg/ml

Finally pipette out 0.2ml, 0.4ml, 0.6ml, 0.8ml, 1.0 ml from 10 µg/ml Solution of Amlodipine and dilute up to 10ml by using Methanol. Finally Concentration we get 0.2 µg/ml, 0.4µg/ml, 0.6µg/ml, 0.8µg/ml, and 1.0µg/ml simultaneously

Repeat the same procedure for Valsartan

Procedure: Each Concentration was observed into the UV spectrophotometric system and Absorbance of Amlodipine was measured at 220nm. Absorbance of Valsartan was measured at 252nm against solvent blank.

Amlodipine Results are tabulated in Table no 35,36.Valsartan results are tabulated in table no 38,39

Limit of detection (LOD):

LOD's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) at levels approximating the LOD according to the formula. The standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Formula:

Limit of quantification:

σ LOD = 3. 3 X ─S

LOQ's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) according to the formula. Again, the standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Formula:

LOQ = 10 X $^{\sigma}S$ –

Where

σ - Standard deviation S - Slope

RESULTS AND DISCUSSION:

Table Optical Characteristic results

S. No	Parameter	Result
1	Absorption Maximum of Amlodipine Absorption Maximum of Valsartan	220nm 252 nm
2	Linearity Range[µg/ml] of Amlodipine ,Valsartan	2-10[µg/ml]
3	Standard Regression Equation of Amlodipne Standard Regression Equation of Valsartan	Y=0.047x+0.154 Y=0.0533x+0.0142
4	Slope of Amlodipine, Valsartan	0.047, 0.0533
5	Intercept Amlodipine, Valsartan	0.154,0.0142
6	Correlation coefficient(r) Amlodipine, Valsartan	0.99

7	Regression coefficient (r^2) Amlodipine, Valsartan		0.99
8	Accuracy(% Recovery) Amlodipine ,Valsartan	For 80%	100.5,100.65 ,
		For 100%	102,101.6
		For 120%	101.1,101
9	Precision(% RSD of Absorbance) 0f Amlodipine, Valsartan	Inter ,intraday of Amlodipine	0.66%,0.99%
		Inter ,intraday of Valsartan	0.8%,0.8%
10	Ruggedness(%RSD of Absorbance)	Amlodipine Analyst 1 Analyst 2	0.8% 1%
		Valsartan Analyst 1 Analyst 2	0.9% 1%
11	LOD, LOQ Of Amlodipine	1 µg/ml,3.14 µg/ml,	
12	LOD, LOQ Of Valsartan	1 µg/ml,3.15 µg/ml,	

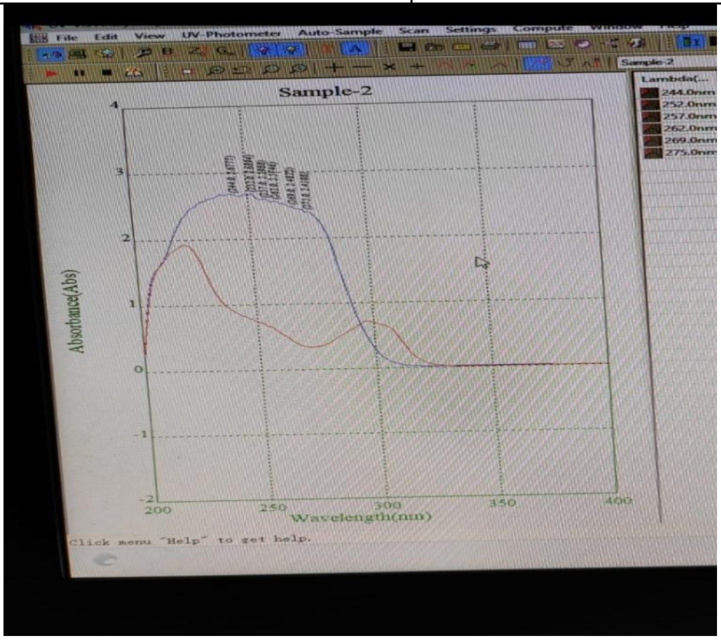


Fig UV spectrum of Amlodipine, Valsartan standard

Assay of Amlodipine and Valsartan by simultaneous equation method

Calculation:

Table Observation table for Amlodipine

Concentration Taken	Absorbance at 220nm	Absorbance at 252nm	Absorptivity at 220nm(ax1)	Absorptivity at 252nm(ax2)
10µg/ml	0.650	0.038	0.0650	0.0038

Table Observation table for Valsartan

Concentration taken	Absorbance at 220nm	Absorbance at 252nm	Absorptivity at 220nm(ay1)	Absorptivity at 252nm(ay2)
10µg/ml	0.187	0.54	0.0187	0.054

Table Observation table for sample solutions

Concentration Taken 10µg/ml	Absorbance at 220nm (A1)	Absorbance at 252nm(A2)
Amlodipine	1.16	-
Valsartan	-	0.61

Concentration Of Amlodipine Cx in µg/ml=

$$Cx = \frac{A_2\, a_{y1} - A_1\, a_{y2}}{a_{x2}\, a_{y1} - a_{x1}\, a_{y2}}$$

$$Cy = \frac{A_1\, a_{x2} - A_2\, a_{x1}}{a_{x2}\, a_{y1} - a_{x1}\, a_{y2}}$$

=0.61x0.0187-1.16x0.054/0.0038x0.0187-0.0650x0054
= 9.91µg/ml

Concentration of Amlodipine Cx in mg/ml=

Concentration Of Amlodipine Cx in µg/ml X Dilution Factor/1000
=9.91 X 1000/1000
= 9.91 mg/ml

Concentration in mg/ml X avg wt of Tablet
Assay of Amlodipine=--- 100
Wt of Powder equivalent to 10mg Amlodipine X label claim of Amlodipine
9.9 mg/ml X 700
= X100
700 X 10mg --------------------

= 99%

Concentration of Valsartan Cy µg/ml =

=1.16 X O.0038-0.62X0.0650/0.0038XO.O18-0.0650X0.054

= 10.11µg/ml

Concentration of Valsartan mg/ml=

Concentration of Valsartan Cy in mg/ml X Dilution Factor/1000

=10.01 X 1000/1000

= 10.11 mg/ml

Concentration in mg/ml X avg wt of Tablet
Assay of Valsartan=-- 100
Wt of Powder equivalent to 10mg Valsartan X label claim of Amlodipine

10.01 mg X 700
= X100
43.7 X 160 mg ---------------------
=101.1%

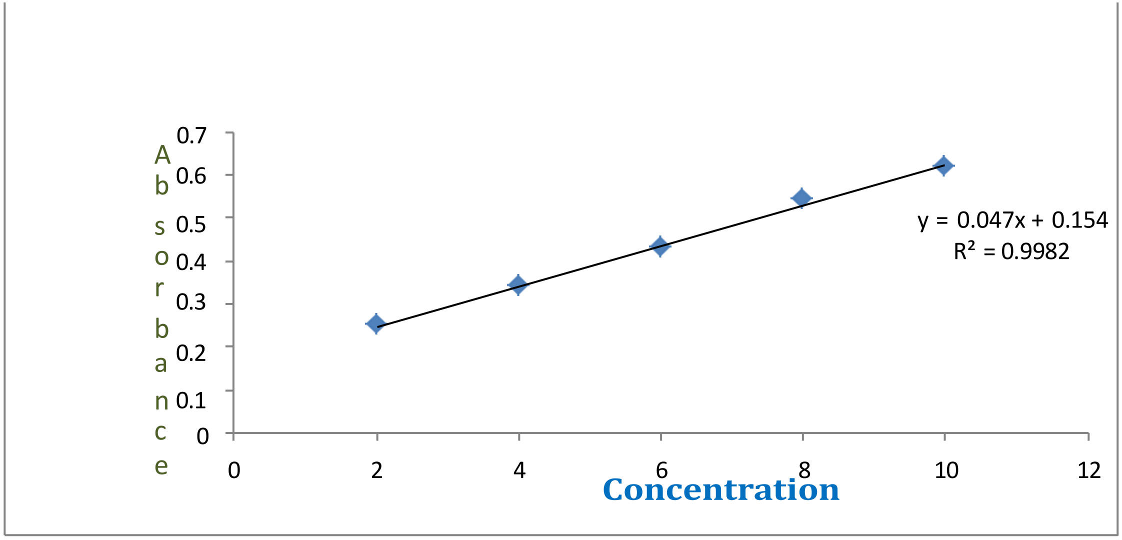
Linearity

Amlodipine Spectrophotometric results data for Linearity study:

Table Absorbance values for calibration curve of Amlodipine at 220nm

Name of the Drug	Actual concentration[µg/ml]	Absorbance	centration Found [µg/ml]
Amlodipine	2	0.25	2.04
	4	0.31	3.3
	6	0.43	5.87
	8	0.52	7.78
	10	0.61	1.9

Fig Calibration Curve of Amlodipine at 220nm



Valsartan Spectrophotometric results data for Linearity study:

Table Absorbance values for calibration curve of Valsartan at 252nm

Name of the Drug	Actual concentration[µg/ml]	Absorbance	ncentration Found [µg/ml]
Valsartan	2	0.1216	2.05
	4	0.2296	4.04
	6	0.3292	5.90
	8	0.4392	7.97
	10	0.5496	10.03

The plot of Concentration [x] versus the absorbance [y] data of Amlodipine, Valsartan are straight line Y= mx + c

Slope (m) of Amlodipine= 0.047 Slope (m) of Valsartan = 0.053 Intercept (c) of Amlodipine = 0.154 Intercept (c) Of Valsartan = 0.0142

Regression coefficient Of Amlodipine, Valsartan (r^2) =0.9982, 9997 Correlation coefficient(r) Amlodipine, Valsartan = 0.9

Validation Criteria:

The Correlation Coefficient should be not less than 0.99.

Conclusion: Correlation coefficient (r) of Amlodipine, Valsartan was 0.99.Regression Coefficient (r^2) of Amlodipine Valsartan was 0.99. These values meet the Validation Criteria. Hence these methods are applicable.

Accuracy

Amlodipine Spectrophotometric results data for Accuracy study:

Table Observation table for accuracy of Amlodipine

Concentration Taken[15ppm] 50%		**Concentration Taken[20ppm] 100%**		**Concentration Taken [25ppm] 150%**	
Absorbance	Concentration Found [Absorbance - Intercept/Slope]	Absorbance	Concentration Found [Absorbance-Intercept/Slope]	Absorbance	Concentration Found [Absorbance-Intercept/Slope]
0.850	14.80	1.09	19.91	1.350	25.44
0.871	15.23	1.10	20.12	1.321	24.80
0.880	15.44	1.12	20.55	1.360	25.65

Table: Results of Amlodipine Accuracy for concentration 50%, 100%, 150%

Sample no	Recovery at about (in %)	% Recovery [concentration Found/concentration taken*100]	Mean% recovery	Standard Deviation	% Relative standard deviation
1	50	98.66	100.55	0.71	0.70
2	50	101			
3	50	102			
1	100	104	102	2	1.96
2	100	100			
3	100	102			
1	150	101.76	101.1.	1.77	1.75
2	150	99.2			
3	150	102.6			

Acceptance criteria: The Percentage recovery of Amlodipine was found to be within the limits (98-99%).

Conclusion: The results obtained for recovery at 50%, 100%.150% are within the limits. Hence method is accurate.

Valsartan Spectrophotometric results data for Accuracy study:

Table Observation table for accuracy of Valsartan

Concentration Taken[15ppm] 50%		**Concentration Taken[20ppm] 100%**		**Concentration Taken [25ppm] 150%**	
Absorbance	Concentration Found [Absorbance - Intercept/Slope]	Absorbance	Concentration Found [Absorbance-Intercept/Slope]	Absorbance	Concentration Found [Absorbance-Intercept/Slope]
0.812	14.96	1.00	20.37	1.391	25.83
0.821	15.13	1.105	20.46	1.359	25.23
0.825	15.21	1.102	20.40	1.355	25.15

Table Results of Valsartan Accuracy for concentration 50%, 100%, 150%

Sample no	Recovery at about (in %)	% Recovery [concentration Found/concentration taken*100]	Mean% recovery	Standard Deviation	% Relative standard deviation
1	50	99.7	100.65	0.86	0.85
2	50	100.86			
3	50	101.4			
1	100	101	101.6	0.577	0.56
2	100	102			
3	100	102			
1	150	103	101	1.73	1.71
2	150	100			
3	150	100			

Acceptance criteria: The Percentage recovery was found to be within the limits (98-102%).
Conclusion: The results obtained for recovery at 50%, 100%.150% are within the limits. Hence method is accurate

Precision

Amlodipine, Spectrophotometric results data for Precision study:

Table Amlodipine Results of Method Precision (Day-1)

Actual concentration Taken	Sample Absorbance	Concentration found [Absorbance-Intercept/Slope]	% Assay
10 µg/ml	0.625	10.02	100.2
10 µg/ml	0.620	9.91	99.1
10 µg/ml	0.619	9.89	98.9
10 µg/ml	0.626	10.04	100.4
10 µg/ml	0.622	10.035	100.3
10 µg/ml	0.623	9.97	99.7
Average	0.6225	9.775	97.75
SD	0.002739	0.06525	0.65
%RSD	0.44	0.66	0.66

Inter-day precision: [Day-2]
Table Amlodipine Results of Method Precision (Day-2)

ual concentration Taken	Sample Absorbance	Concentration found [Absorbance-Intercept/Slope]	% Assay
10 µg/ml	0.625	10.021	100.21
10 µg/ml	0.621	9.93	99.3
10 µg/ml	0.619	9.89	98.9
10 µg/ml	0.622	9.95	99.5
10 µg/ml	0.615	9.80	98.0
10 µg/ml	0.614	9.78	97.8
Average	0.619	9.895	98.95
SD	0.00682	0.09	0.92
%RSD	0.6	0.9	0.9

Acceptance Criteria:
The % Relative Standard Deviation of % Assay of Amlodipine, Valsartan from the six sample preparations should be not more than 2.0%. The individual % Assay of Amlodipine, Valsartan should not be less than 98.0% and not more than 102.0%.
Conclusion: The %RSD obtained is within the limits .Hence the method is accurate.
Valsartan Spectrophotometric results data for Precision study

Table Valsartan Results of Method Precision (Day-1)

Actual concentration Taken	Sample Absorbance	Concentration found [Absorbance- Intercept/Slope]	% Assay
10 µg/ml	0.619	9.8	98
10 µg/ml	0.621	9.93	99.3
10 µg/ml	0.625	10.021	100.21
10 µg/ml	0.624	10	100
10 µg/ml	0.623	9.97	99.7
10 µg/ml	0.618	9.87	98.7
Average	0.62166	9.931	99.31
SD	0.0028	0.0839	0.83
%RSD	0.45	0.8	0.8

Inter-day precision: [Day-2]
Table Valsartan Results of Method Precision (Day-2)

Actual concentration Taken	Sample Absorbance	Concentration found [Absorbance- Intercept/Slope]	% Assay
10 µg/ml	0.621	9.93	98
10 µg/ml	0.619	9.8	99.3
10 µg/ml	0.623	9.97	100.21
10 µg/ml	0.624	10	100
10 µg/ml	0.625	10.021	99.7
10 µg/ml	0.618	9.87	98.7
Average	0.621	9.931	99.31
SD	0.0028	0.0839	0.83
%RSD	0.45	0.8	0.8

Acceptance Criteria:
The % Relative Standard Deviation of % Assay of Amlodipine, Valsartan from the six sample preparations should be not more than 2.0%. The individual % Assay of Amlodipine**,** Valsartan should not be less than 98.0% and not more than 102.0%.
Conclusion: The %RSD obtained is within the limits .Hence the method is accurate.
Ruggedness
Amlodipine Spectrophotometric results data for Ruggedness study:
Analyst 1:

Table Amlodipine Results of Method Ruggedness (Analyst-1)

Actual concentration Taken	Sample Absorbance	Concentration found [Absorbance- Intercept/Slope]	% Assay
10 µg/ml	0.619	9.8	98
10 µg/ml	0.621	9.93	99.3
10 µg/ml	0.625	10.021	100.21
10 µg/ml	0.624	10	100
10 µg/ml	0.623	9.97	99.7
10 µg/ml	0.618	9.87	98.7
Average	0.621	9.931	99.31
SD	0.0028	0.0839	0.83

%RSD	0.45	0.8	0.8

Analyst 2:

Table Amlodipine results of Method Ruggedness (Analyst-2)

Actual concentration Taken	Sample Absorbance	Concentration found [Absorbance-Intercept/Slope]	% Assay
10 µg/ml	0.619	9.8	98
10 µg/ml	0.619	9.8	98
10 µg/ml	0.625	10.021	100.2
10 µg/ml	0.621	9.931	99.3
10 µg/ml	0.623	9.97	99.7
10 µg/ml	0.615	9.80	98.0
Average	0.620333	9.887	98.87
SD	0.003502	0.099487	0.9
%RSD	0.5	1	1

Acceptance Criteria:

The % Relative Standard Deviation of % Assay of Amlodipine,Valsartan from the six sample preparations should be not more than 2.0%. The individual % Assay of Amlodipine, Valsartan should not be less than 98.0% and not more than 102.0%.

Conclusion: The %RSD obtained is within the limits .Hence the method is accurate

Valsartan Spectrophotometric results data for Ruggedness study

Table Valsartan results of Method Ruggedness (Analyst-1)

Actual concentration Taken	Sample Absorbance	Concentration found [Absorbance- Intercept/Slope]	% Assay
10 µg/ml	0.619	9.8	98
10 µg/ml	0.625	10.021	100.21
10 µg/ml	0.625	10.021	100.21
10 µg/ml	0.624	10	100
10 µg/ml	0.618	9.87	98.7
10 µg/ml	0.618	9.87	98.7
Average	0.6215	9.93033333	99.3033333
SD	0.00350714	0.09545819	0.95458193
%RSD	0.5	0.9	0.9

Table Valsartan results of Method Ruggedness (Analyst-2)

Actual concentration Taken	Sample Absorbance	Concentration found [Absorbance- Intercept/Slope]	% Assay
10 µg/ml	0.619	9.8	98
10 µg/ml	0.625	10.021	100.21
10 µg/ml	0.625	10.021	100.21
10 µg/ml	0.624	10	100
10 µg/ml	0.618	9.87	98.7
10 µg/ml	0.619	9.8	98
Average	0.62166667	9.91866667	99.1866667
SD	0.00332666	0.10778806	1.07788064
%RSD	0.5	1	1

Acceptance Criteria:
The % Relative Standard Deviation of % Assay of Amlodipine**,** Valsartan from the six sample preparations should be not more than 2.0%. The individual % Assay of Amlodipine, Valsartan should not be less than 98.0% and not more than 102.0%.

Conclusion: The %RSD obtained is within the limits .Hence the method is accurate.

LOD, LOQ study

Amlodipine Spectrophotometric results data for LOD, LOQ study

Table Absorbance values for calibration curve of Amlodipine at 220nm for LOD, LOQ Study

Name of the Drug	Actual concentration[µg/ml]	Absorbance
Amlodipine	0.2	0.023
	0.4	0.032
	0.6	0.043
	0.8	0.056
	1	0.065

Spectrophotometric results data for LOD (Amlodipine) study: Table Showing Amlodipine Results for Limit of Detection

Drug name	Standard deviation(σ)	Slope(s)	LOD(µg)
Amlodipine	0.017	0.054	1

σ LOD = 3. 3 X $-S$

LOD=3.3X0.017/0.054 = 1.03 µg/ml

Spectrophotometric results data for LOQ (Amlodipine) study:

TABLE: Showing Amlodipine Results for Limit of Quantification

Drug name	Standard deviation(σ)	Slope(s)	LOQ(µg)
Amlodipine	0.017	0.054	3.14

LOQ = 10 X ─ S

LOQ=10X0.017/0.054= 3.14µg/ml

Valsartan Spectrophotometric results data for LOD, LOQ study

Table Absorbance values for calibration curve of Valsartan at 220nm for LOD, LOQ Study

Name of the Drug	Actual concentration[µg/ml]	Absorbance
Valsartan	0.2	0.0111
	0.4	0.02196
	0.6	0.03294
	0.8	0.04192
	1.0	0.05496

Table Showing Valsartan Results for Limit of Detection

Drug name	Standard deviation(σ)	Slope(s)	LOD(µg)
Valsartan	0.017	0.0538	1

σ LOD = 3. 3 X ─ S

Where

LOD=3.3X0.017/0.053 =1.05 µg/ml

σ LOQ = 10 X ─ S

Table Showing Valsartan Results for Limit of quantification

Drug name	Standard deviation(σ)	Slope(s)	LOQ(µg)
Valsartan	0.017	0.0538	3.2

LOQ=10X0.017/0.053= 3.2µg/ml

CONCLUSION:

The method was developed using Methanol as solvent and the λmax was found to be 220 nm for Amlodipine 252nm for Valsartan. The method was validated with respect to linearity, precision, accuracy, sensitivity and ruggedness. The calibration plot for the method was constructed.

The method was established according to ICH guideline and definition. Accuracy was investigated by analyzing marketed formulations and percentage recovery was found to be within the limits. Therefore it can be said that the method were highly accurate.

The inter day and intraday relative standard deviation (RSD) values with low percentage RSD values were obtained. This indicated that the precision of the method was found to be good.

The proposed method based on UV spectrophotometer is precise, accurate, simple to perform and economy in practice. It do not require expensive or sophisticated and chemicals in contrast with Spectrophotometric method.

REFERENCES:

1. Sharma B. Instrumental methods of Chemical Analysis .7th ed. Meerut : Goel publishing house ; 2000. P. 1-2.
2. Beckett H., Stenlake JB., Practical pharmaceutical chemistry, 4th ed .New Delhi [Ind] :C.B.S Publications ; 2002 . Page.1.
3. Willard HH., Merritt LL., Dean JA., Frank A S., Instrumental methods of Analysis, 7th edition , CBS Publishers and Distributors: New Delhi , 1986 ; page 1-5
4. Basset J., Denny RC., jeffry GH, Mandham J., Vogel's Text book of quantitative inorganic analysis .4th ed. England : Longman group ; 1986 .
5. Hohat H. Willard ., Lunne L . Merrit , John A. Dean. Instrumental methods of analysis , 7th edition New Delhi : CBS Publishers ;1999.
6. Gurdeep R. Chatwal , Sham K. Anand, Instrumental Methods of Chemical Analysis , 5th edition New Delhi : Himalaya publishing House ; 2008.
7. David CL, Michael Webb. Pharmaceutical Analysis . London: Black well publishing ; 1994. P. 2-4.
8. Chatten LG. Pharmaceutical Chemistry . Vol .II . New York : Marcel Dekker Inc ; 1996.p. 23-25 .
9. Day RA Underwood AL. Quantitative Analysis. 4th edition .New Delhi : Prentice Hall ;1986 .p. 14-19.
10. Ravi Sankar S . Text book of pharmaceutical analysis .3rd ed . Tirunelveli: Rx publications ;2001.p.1-35.
11. Michael E, Schartz IS, Krull. Analysis method development and Validation . 3rd ed .London : John Wilyey & sons ;2004:p.25-46.
12. David Harvey . Modern Analytical Chemistry .1st ed . United states of America : Harcourt Brace & Company ; 1997 .p. 578-1093 .
13. Pharmacopeial Forum : Volume No. 31 (4) Page 1092 -1093.
14. International conference of Harmonization Guidelines on validation of Analytical procedure Definitions and Terminology Federal Register ; (March 1, 1995).
15. "Validation of compendia methods" USP23 1225 USPC Rockville Maryland USA1994.
16. United States Pharmacopoeia convention (USP -NF XXIV) , Rockville MD 20852; United States Pharmacopoeia Convention Inc.; 1985.p. 214951.
17. Ermer J, Miller McB JH. Method validation in pharmaceutical analysis
18. Federal Republic of Germany . WILEY-VCH Verlag GmbH & Co; 2005.p. 21-2,52-3,63-4,80,99,101,120-1.
19. Asian Guidelines for Validation of Analytical Procedure Adopted from ICH guideline Q2A27 Oct.1994 and ICH Q2B ,6th Nov .1994.
20. International Conference on Harmonization . Validation of Analytical Procedure . Text and Methodology Q2 (R1) ICH , IFPMA; Geneva : 2005 .
21. http://www.bcg-usa.com/regulatory/docs/ich/ICHQ2B.pdf.
22. United States Pharmacopeia (USP), XXVI. Validation of compendial methods. United States Pharmacopeia Convention Inc; Rockville ,MD, USA: 2003.
23. ICH: Q2B , Analytical validation - methodology ; 1996.
24. ICH: Q2A, Text on Validation of analytical procedure; 1994.
25. ICH: Q2 (R1) , Validation of analytical procedures : text and methodology ; 2005.
26. Simultaneous estimation for analytical method development and validation of amlodipine and valsartan by uv viscible spectroscopy.
27. Method development for simultaneous estimation of Amlodipine Besylate and Perindopril Tertbutyl amine in fixed-dose Volume 9, Issue 3, March 2023.
28. UV Spectrophotometric Method Development and Validation for Amlodipine Besylate in Bulk and Tablet Dosage Form. Volume - 12, Issue - 2,Year -2022
29. UV Spectrophotometric Method Development and Validation for the Estimation of Valsartan in Bulk and Pharmaceutical Dosage Form https://www.researchgate.net/publication/309270506_UV_Spectrophotometric_Method_Development_and_Validation_for_the_Estimation_of_Valsartan_in_B

ulk_and_Pharmaceutical_Dosage_Form

30. Development and validation of UV Spectrophoto metric Methods for Simultaneous Analysis of Amlodipine and Indapamide in Combined Dosage forms .volume 7,Issue 4
31. Development and Validation of a Novel UV Spectrophotometric Method for Simultaneous Analysis of Amlodipine, Indapamide and Perindopril https://www.rDevelopment and Validation of a Novel UV Spectrophotometric Method for Simultaneous Analysis of Amlodipine, Indapamide and Perindoprilz
32. Development and Validation of Spectrophotometric Method for the Simultaneous Estimation of Valsartan and HydrochlorothiazideVolume 4 Issue 1 January 2020.
33. Development and validation of analytical method for the simultaneous estimation of amlodipine and valsartan in bulk and pharmaceutical dosage form by uv-spectrophotometric method. https://researchgate.net/publication/327537369_DEVELOPMENT_AND_VALIDATION_OF_ANALYTICAL_METHOD_FOR_THE_SIMULTANEOUS_ESTIMATION
34. OF_AMLODIPINE_AND_VALSARTAN_IN_BULK_AND_PHARMACEUTICAL_DOS AGE_FORM_BY_UV-_SPECTROPHOTOMETRIC_METHOD
35. UV Spectrophotometric Method Development and Validation for the Estimation of Valsartan in Bulk and Pharmaceutical Dosage Form. Articles In Volume - 6, Issue – 3.
36. Estimation of Amlodipine Besylate, Valsartan and Hydrochlorothiazide in Bulk Mixture and Tablet by UV Spectrophotometry. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3507340/
37. Simultaneous Determination of Amlodipine and Valsartan https://ncbi.nlm.nih.gov/pmc/articles/PMC3169342/
38. Simultaneous estimation of valsartan, amlodipine besylate and hydrochlorthiazide by first order derivative uv spectrophotometric method https://www.researchgate.net/publication/259288954_Simultaneous_estimation_of_valsartan_amlodipine_besylate_and_hydrochlorthiazide_by_first_order_derivative_uv_spectrophotometric_method