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Review: Calibration and Validation of UV Visible Spectroscopy and Method Development of Letrosole by UV

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ABSTRACT

In this review we will explore UV spectroscopy, types of single beam and double beam UV spectroscopy instruments as well as various parameters of UV spectroscopy such as monochromator, amplifier, detector etc. and we will look at and review the calibration of UV spectroscopy with specific parameters. as a Control of wavelength.Control of Absorbance , limit of stray light and resolutrion power study with specific solvent.

UV-VIS Spectroscopy is the term used for the analytical evaluation of UV-VIS Spectroscopy is UV-VIS Spectroscopy. The method of analysis is based on assessing the absorption of colorless substances emitting monochromatic light in the near-ultraviolet path of the spectrum (200-400 nm). It's critical right now to create trustworthy analytical procedures for determining anticancer medicines obtain higher selectivity, sensitivity, and speed in the assay procedure than previously reported. and study of UV validation with parameters like accuracy precision etc. and also a study of the material and used in analysis of letrozole tablet by uv with specific wavelength absorbance and detector. We have developed a validated UV spectrometric method for Letrozole tablet. It is a simple, rapid, accurate, cost-effective and reproducible spectrophotometric method developed for the estimation of letrozole in pure and pharmaceutical forms. The UV instrument is a fast and specific result of the drug in a certain time. Absorption of UV light is introduced into the sample, which then passes through the detector and captures absorption and wavelength spectra. The developed method was validated according to the International Council for Harmonization (ICH guidelines) for accuracy, precision and range, etc.

Keywords :- Spectroscopy , letrozole , wavelength ,absorbance, resolution power

INTRODUCTION

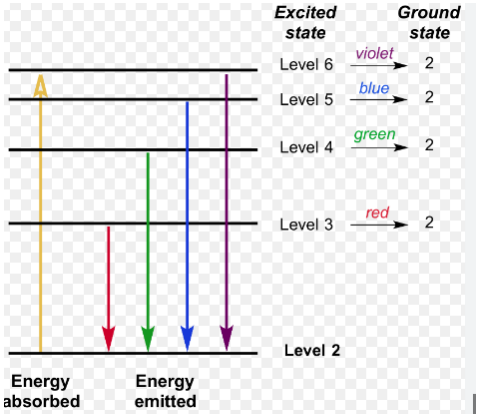
SPECTROSCOPY

Spectroscopy is the measurement and interpretation of electromagnetic radiation (EMR) that molecules, atoms and ions in a sample absorb and emit as they move from one energy level to another energy state. [1]

UV-VISIBLE SPECTROSCOPY

Ultraviolet and visible spectroscopy, also known as electronic spectroscopy, is used to measure the number of double bonds and aromatic conjugation in a molecule. Spectroscopy is the measurement and interpretation of electromagnetic radiation that is absorbed or emitted when molecules or atoms or ions in a sample move from one energy state to another.

UV spectroscopy is a type of absorption spectroscopy in which a molecule absorbs light in the ultraviolet range (200-400 nm), as a result of which electrons are excited from the ground state to a higher energy state. The ultraviolet range corresponds to 400-200 nm and the visible range to 800-400 nm.[2]



• UV-visible spectroscopy based on the Beer-Lambert law.

log Io I⁄= ε. *l*. *c* or ϵ = *A*/*cl*

where,

Io = incident light intensity

I = transmitted light intensity

ε = molar absorptivity l = length of test path

c = sample concentration

A = Absorption

• absorption (A): it is the inverse of transmittance [3]

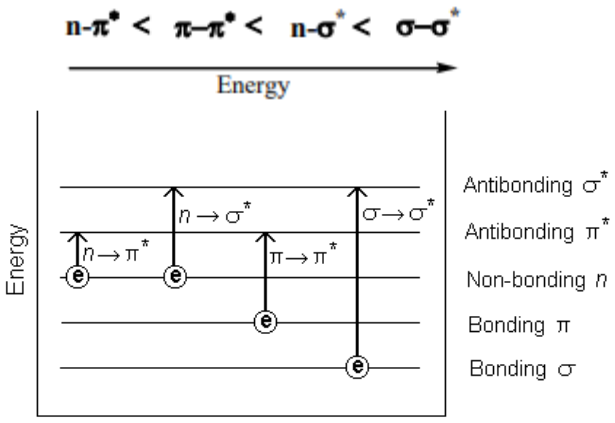
Absorbance (A) = optical density (D) = log 1 T or log Io I

• Transmission (T): The proportion of light passing through is called transmission.

Permeability (T) = I Io

ELECTRONIC TRANSITIONS

When a molecule is excited due to the absorption of electromagnetic radiation in the UV-visible region, its electrons move from the ground state to an excited state or from a bonding orbital to a non-bonding orbital.[4]



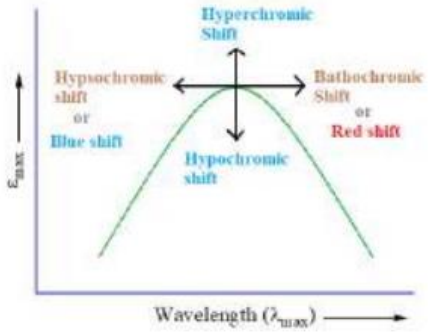
ABSORPTION, INTENSITY TRANSMISSION AND UV SPECTRUM

a) Bathochromic shift: also known as redshift, in this case the shift of absorption to a longer wavelength (*λ*max).

b) Hyprochromic shift: Also known as blue shift, in this case the shift of absorption to a shorter wavelength (*λ*max).

c) Hyperchromic transition: the intensity of the absorption maximum (εmax) increases.

d) Hypochromic change: decrease in intensity of absorption maximum (εmax). [5]



PRINCIPLES OF UV-VISIBLE SPECTROPHOTOMETER

1. Basically, spectroscopy deals with the interaction of light with matter.

2. When a substance absorbs light, the result is an increase in the energy content of the atoms or molecules.

3. Absorption of ultraviolet radiation leads to the excitation of electrons from the ground state to a higher energy state. [6]

4. Molecules that contain π-electrons or unbonded electrons (n-electrons) can absorb energy in the form of ultraviolet light to excite those electrons to higher unbonded molecular orbitals

5. The more easily electrons are excited, the longer wavelengths of light they can absorb. There are four transitions (π–π*, n–π*, σ–σ* and n–σ*) and they can be ordered as follows: σ–σ* > n–σ* > π– π* > n – π * [7]

There are two types of UV-Vis spectrophotometers

1. Single beam UV-Vis spectrometer

2. Two-beam UV-Vis spectrometer

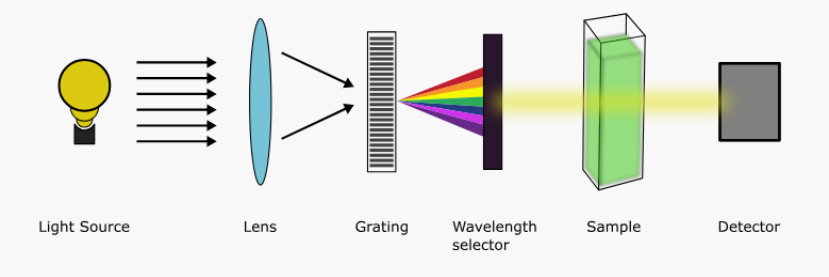
SINGLE BEAM UV-VISIBLE SPECTROPHOTOMETER

A single beam UV-vis spectrophotometer has a single beam, as the name suggests. The light from the source is directed through the monochromator so that the incoming monochromatic light passes through the slit. It then passes through the test solution. Part of the incident light is absorbed by the sample, while part is emitted.

The detector detects the emitted light. The detected light is then amplified, stored and then displayed on a suitable readout. The spectrum is plotted and λmax is located.

The single-beam UV-vis spectrophotometer consists of

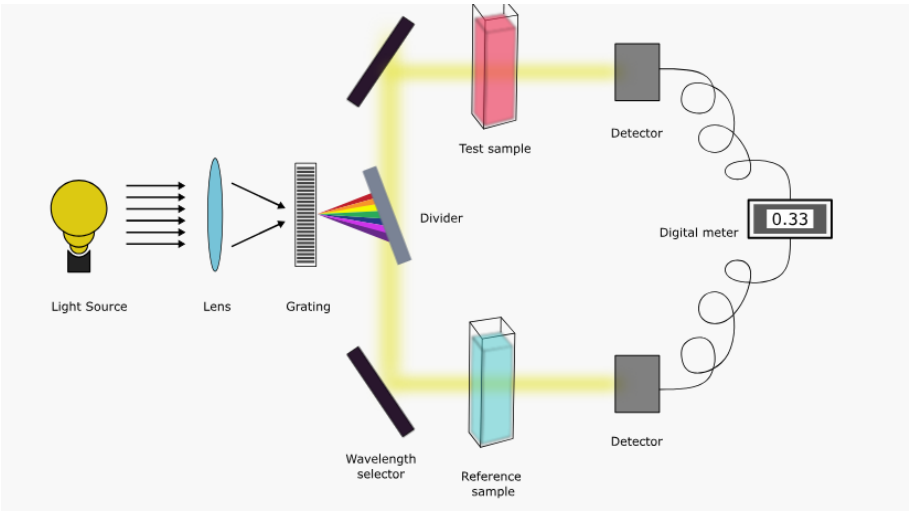
- light source
- Lens
- Gratings
- Wavelength selector
- Sample container/cuvette
- Detector
- Digital meter/recorder



DOUBLE BEAM UV-VISIBLE SPECTROPHOTOMETER

The instrumentation of single- and double -beam spectrophotometers is almost the same. The main difference with a single-beam UV-Vis spectrophotometer is that the incoming light beam passes through the sample and reference cells simultaneously. The incoming light is split and directed into both reference and sample cuvettes.

Detectors detect a refracted or transmitted beam. A two-ray UV-vis spectrophotometer requires two detectors that detect the ratio of electrons to measure or calculate the absorbance of the sample being studied. It also requires a stabilized voltage supply. [8]



INSTRUMENTATION OF UV-VISIBLE SPECTROSCOPY

The Main equipment of the UV-visible spectrometer consists of

1. Light source

2. Diffraction grating

3. Wavelength selector

4. Sample container or cuvette

5. Detector

LIGHT SOURCE

• tungsten Incandescent lamps and hydrogen deuterium lamps are the most used and suitable light sources because they cover the entire UV range.

MONOCHROMATOR

• Monochromators usually consist of prisms and slits

. • Most spectrophotometers are dual beam spectrophotometers.

• The radiation from the primary source is dispersed by rotating prisms

. • After that, the different wavelengths of the light source separated by the prism are selected using the slits, while the rotation of the prism leads to an increase in the wavelengths passing through the slits.

• The beam selected by the slit is monochromatic and split into two beams by another prism. [9]

CELLS AND COMPARISON

• One of the two split beams is passed through the sample solution and the other beam through the reference solution.

• The cells contain both sample and reference solution.

• These cells are made of either silica or quartz. Glass cannot be used for cells because it also absorbs UV light.

DETECTOR

• Usually two photocells serve the purpose of a detector in UV spectroscopy.

• One of the photocells receives the sample cell beam and the other detector receives the reference beam.

• The radiation intensity of the reference room is stronger than the radiation of the test room. As a result, a pulsed or alternating current is generated in the photocells. [10]

AMPLIFIER

• The alternating current generated in the photocells is transmitted to the amplifier.

• The amplifier is connected to a small servometer

. • In general, the current generated in the photocells is very low intensity, the main purpose of the amplifier is to multiply the signals so that we receive clear and recordable signals.

HOLDING DEVICES

• In most cases, the amplifier is connected to a pen that is connected to the computer.

• The computer stores all generated data and produces a spectrum of the desired compound. [11]

CALIBRATION

Calibration checks the accuracy of the device and determines the traceability of the measurement. Calibration of a UV-Vis spectrophotometer is done in four steps:-

1 Control of wavelength

2. Control of Absorbance

3. Limit of Stray Light

4. Resolution of Power

CALIBRATION PROCEDURE OF UV VISIBLE SPECTROSCOPY

Calibration of any instrument is the most important part of checking the accuracy of the results. Since you has not calibrated an instrument that does not give a precise and accurate result, calibration of any instrument is an important part.

UV-visible spectroscopy is calibrated every 3 months to check parameters such as absorbance, wavelength, etc., so we are investigating UV calibration with a specific solvent. [12]

1. Calibration of Wavelength

Holmium perchlorate solution:

Take 0.4 g of holmium(III) oxide in a 10 ml volumetric flask.

Add 1.4 M perchloric acid to this and heat gently until dissolved. Make up to 10 ml with 1.4 M perchloric acid.

Select SPECTRUM MODE on the device.

Select the wavelength range from 200 nm to 700 nm.

Press 1 to adjust the base level.

Fill the cuvette with 1.4 M perchloric acid and place it in the sample cuvette and press the auto reset button.

After autozero, replace the solution with holmium Perchlorate solution in sample cubicle and press start key.

. Scan and check the wavelength using the absorption maximum of the holmium perchlorate solution.

Acceptance criteria: Permitted tolerance is ±1 nm in the UV range and ±3 nm in the visible region.

2. Control of absorbance:

A certain amount of potassium dichromate is dried by heating to constant mass at 130°C.

To check the absorbance at 235 nm, 257 nm, 313 nm, and 350 nm, accurately weigh 57.0 to 63.0 mg of dried potassium dichromate and dissolve in 0.005 M sulfuric acid solution. Make up to 1000 ml with the same solvent.

For 430 nm, weigh out 57.0-63.0 mg of dried potassium dichromate and dissolve it in 0.005 sulfuric acid solution. Make up to 100 ml with the same solvent.

Fill the cuvette with 0.005 M sulfuric acid and place in the sample cuvettes and press automatic reset button.

Take the sample in the cuvette, place the cuvette in the cabinet and take the readings

Individually for five wavelengths.

S. No.	Wavelength (nm)	Specific absorbance A (1%1cm)	Maximum tolerance
1	235	124.5	122.9 to 126.2
2	257	144.5	142.8 to 146.2
3	313	48.6	47.0 to 50.3
4	350	107.3	105.6 to 109.0
5	430	15.9	15.7 to 16.1

Limit of Stray Light

To dry a certain amount of potassium m of chloride by heating to constant mass at 130°C.

Weigh accurately 1.2 g of dry potassium chloride into a 100 ml volumetric flask. Dissolve in 50 ml of water and make up to 100 ml with the same solvent.

Absorbance between 220 nm and 200 nm is measured. at 198 nm is greater than 2. Compared with water as a compensating liquid.

Resolution Power:

Prepare a 0.02% v/v solution of toluene in hexane.

Measure the absorbance of the above solution at 266 nm and 269 nm using hexane as a reference solution.

The ratio of absorbance at 269 nm to the ratio of absorbance at 266 nm should be greater than 1.5.[13]

ANALYTICAL ELEMENTS OF UV SPECTROSCOPY

Validation is a procedure involving laboratory testing to confirm or determine that a method, system or assay provides correct and reproducible results within the intended and defined range for the intended analytical application.

METHOD VALIDATION

Method validation is complete. As appropriate, ensure that the analytical process is accurate, specific, reproducible and reliable. The fact that the analysis of samples is studied to validate the method ensures the reliability of the method in daily use, for example known substances, they can be spectrophotometrically measured as compounds. [14] The reliability of the method in daily use is verified. Also known as a "procedure" that provides textual evidence that a license can do what it claims to do. Describe what had to be done and the parameters.

Precision

Precision refers to the degree to which an analytical technique can be repeated. In fact, under normal conditions, it is usually distributed as the standard deviation value of a given sample in centimeters. Since an operationally sufficient number of samples is a statistically significant precision, it should be done at three levels according to ICH. [15]

Accuracy

Accuracy is a measure of the accuracy or uniformity of an analytical method. between values accepted as normal value, actual value or accepted control value and values accepted as standard value. , the real value or the generally accepted set point It is calculated as a percentage of the analytes obtained in the test when the samples are included in the blind study. The set of components, the accuracy of the quantification of impurities is determined by examining the samples (drugs)[16].

Specificity

The ability to correctly and precisely quantify the analyzes of interest in the laboratory is called specificity. Presence of other expected components in the sample matrix. It is a measure used to determine the size of a group of people. Confounding factors such as excipients, contaminants and degradation are some of the other active ingredients. The products ensure that only one component causes the maximum response.

Detection limit Impurity

Tests/Limits of Determination - The lowest detectable concentration of a material is called the detection limit. As concentration range (LOD). An analyte that is detectable but not quantifiable in a sample. It is a kind of limit test that determines whether something is possible or not. Examples of non-instrumental visual methods, titration or chromatography (TLC) determine LOD values using techniques such as thin layer imaging.[17] LOD values can be determined by the standard deviation of the data. According to the formula LOD = 3.3 (SD/S), the height (S) and response (SD) of the calibration curve are at levels close to the LOD. The standard deviation of the response can be calculated using the standard deviation of the blank and the standard deviation of the residual.

Quantitative limits

The limit of measurement refers to the lowest concentration of the analytical method that can be determined with sufficient sensitivity and efficiency under laboratory-confirmed conditions of the product. Quantitation (LOQ), like LOD, is expressed as a concentration, but with precision and accuracy. Measurement accuracy was also reported. Linearity and range The ability of the method to provide test results directly related to the method, inversely proportional to the linearity of the test dose over a specific range, is presented. The ability of the method to provide proportional test results. The ability of a method to provide a concentration within a given range is called linear. Consistency USP defines consistency as the degree to which information obtained under different conditions is consistent. RSD is presented as a percentage. These conditions include some laboratories, analyzers and equipment. [18].

Linearity and Range

The ability of a method to produce test results that are directly related to the method inversely proportionate to the analyses linearity of dosage within a certain range is reported. The method's ability to produce test findings that are proportionate analysis. The capacity of the method to provide concentration within a particular range is known as linearity.[19]

Robustness

It refers to the ability of a process to remain unchanged with small changes. strength Robustness refers to the ability to handle conscious changes in input. Method parameters Variable Organisms, pH values and other method characteristics are used to measure the robustness of the technique. Determining the effect of ionic strength, temperature, and other variables (if any) on method robustness should be considered early in the development of a method that meets ICH standards. [20]

MATERIALS AND METHODS USED IN LETROZOLE TABLET:

Equipment, reagents and chemicals:

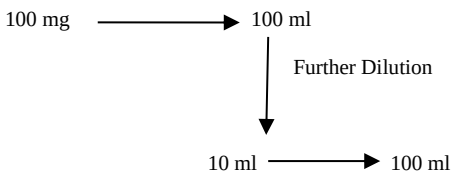
INSTRUMENT:

The instrument used was: UV-visible Single-ray spectrophotometer Cary 60 Uv - Vis with one cm matched quartz elements. Electronic balance, Shimadzu. The glassware used in each procedure was soaked overnight in a mixture of chromic acid and sulfuric acid, thoroughly washed twice with distilled water, and dried in a hot-air oven before use. Absorption spectra of reference and test solutions were determined in a 1 cm quartz cell between 200 and 400 nm. Material: [21]

PURE SAMPLE: Letrozole was kindly purchased from DM Pharma bhaddi Himachal Pradesh India.

REAGENTS AND CHEMICALS: Acetonitrile methyl cyanide, and chemicals used were of analytical grade [22]

PREPARATION OF THE STANDARD VERSION: To prepare a standard solution of letrozole, 100 mg of pure letrozole waswas transferred to a 100ml standard volumetric flask dissolved in a small amount of acetonitrile then the volume was adjusted with acetonitrile. The solution was then further diluted to 10 mL and then diluted to 100 mL with the same solvent in a volumetric flask.



Sample preparation: Twenty tablets were weighed and finely ground powder equivalent to 100 mg of letrozole was transferred to a 100 ml standard volumetric flask. Add a small amount of absolute acetonitrile and make up with acetonitrile to make a stock solution. Another 10 ml of solution was taken from the stock solution and then diluted to 100 ml with the same solvent in a volumetric flask. [23]

$$\text{SAMPLE WEIGHT} = \frac{\text{Average weight of Tablet} \times \text{Equivalent Weight}}{\text{Claim ot Tablet}}$$

Method Development: Aliquots of this standard stock solution were taken and diluted to obtain different concentrations such as 1, 2, 4, 6, 8 and 10 µg/ml. Standard solutions were scanned between 200-400 nm, they showed λ-max at 240 nm which was used for further measurement. The solutions obey Beer's law in the concentration range 1-10 µg/ml, with a regression of 0.9998. The optical property of the solution is given in. The same procedure was followed for the evaluation of letrozole tablets using the prepared sample solution.

Sr.No.	No. Concentration (µg/ml)	Absorbance at 240 nm ± standard deviation
1	1	0.161±0.002
2	2	0.272±.0026
3	4	0.570±0.0025
4	6	0.853±0.0036
5	8	1.133±0.0029
6	10	1.459±0.00264

MATERIALS AND METHODS:

Equipment, reagents and chemicals: A standard calibration curve was obtained for concentration v/s absorbance data; standard calibration curve data.[24]

VALIDATION OF LETROZOLE TABLET BY UV-VIS SPECTROSCOPY

Linearity and Range: Linearity of response was obtained from 01 to 24 µg/ml. A calibration curve was obtained by plotting absorbance and concentration data and was processed by linear regression analysis [25] The equation for the calibration curve for letrozole was y = 0.146x - 0. 024 and the calibration curve was found to be linear. in the concentrations mentioned above. The correlation coefficient (R2) was 0.999,

PRECISION:

The intra-day and inter-day accuracies of the proposed spectrophotometric methods were determined by evaluating the respective response three times on the same day and on three different days within one week at three different concentrations of letrozole (2, 5, 5, 0 . and 10. 0 µgml-1) and results were reported as relative standard deviation.

S. NO	Conc (mg/ml)	Wavelength	Absorbance	Mean ± S.D.	% RSD
1	6	240	0.853	0.856± 0.00275	0.321
2	6	240	0.854	0.856± 0.00275	0.321
3	6	240	0.855	0.856± 0.00275	0.321
4	6	240	0.857	0.856± 0.00275	0.321
5	6	240	0.861	0.856± 0.00275	0.321
6	6	240	0.857	0.856± 0.00275	0.321

Accuracy

This parameter was evaluated by percent recovery studies at 80, 100, and 120% concentration levels involving the addition of a known amount of letrozole reference material to a prequantitated sample solution. An aliquot of the test solution containing 5.0 µgml-1 letrozole was transferred to three 10 ml volumetric flasks containing 4.0, 5.0 and 6.0 µgml-1 letrozole reference solutions, respectively. The contents were mixed and diluted to volume to give final concentrations of 9.0, 10.0 and 11.0 µgml-1 letrozole. Achievements were verified by evaluating drugs in triplicate preparations at each prescribed concentration level. Spectra were recorded in the UV region and then analyzed. Results were presented as percent recovery.[26]

Specificity

The results of the tablet solution showed that the excipients did not interfere compared to the working standard solution. Thus, the methods were said to be specific.

Robustness

The robustness of the proposed methods was tested by varying parameters such as wavelength range and slit width. None of these variables significantly affected drug absorption, suggesting that the proposed methods can be considered robust..

Limit of detection and quantification (LOD and LOQ)

Limits of detection and quantification were determined based on the standard deviation of the y-intercept and the slope of the least-squares parameters defined by the International Conference on Harmonization (ICH). Q2 instructions. The LOD and LOQ were 0.0470 µg/mL and 0. 1424 µg/mL, respectively, and the data are reported in [27]

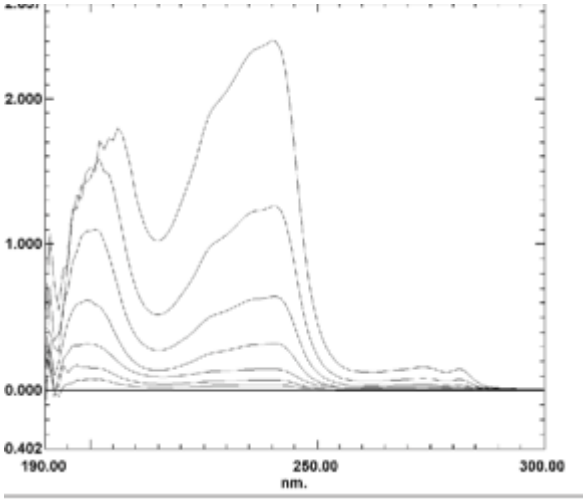
LOD = limit of detection,

LOQ = limit of quantification,

Memory study: To analyze the accuracy of the developed method, it was applied to the analysis of a commercially available letrozole tablet (FEMPRO 5.0 mg). Fifty tablets were weighed and ground. The amount of tablet powder corresponding to 100 mg of letrozole was accurately weighed and transferred to a 100 ml volumetric flask, then 10 ml of acetonitrile was added as a solvent and kept for 15-20 minutes with frequent shaking and the volume was filled. . given solvent The solution was then filtered through Whattman filter paper. This filtrate was properly diluted with solvent to obtain 05 µg/ml solution. Absorbance was measured relative to zero. [28]

Memory tests were performed at three different levels, which are 80%, 100%, 120%. A known amount of standard drug solution was added to the pre-analyzed sample solution at three different levels and the absorbance was recorded. The drug content of the formulation was calculated using a standard calibration curve. [29]

Drug	Tablet amount	Level of Addition (%)	Amount added	Amount Recovered	% recovery	Average % recovery
Letrozole	05	80	8	12.98	99.83	99.80
Letrozole	05	100	10	14.97	99.82	99.80
Letrozole	05	120	12	16.59	99.81	99.80



RESULTS AND DISCUSSION:

An attempt was made to develop a rapid, sensitive, economical, accurate and precise analytical method for letrozole in pure and pharmaceutical form. The maximum absorbance of the proposed method at 240 nm LOD and LOQ was found to be 0.0470 respectively, by UV spectrometric absorbance using acetonitrile as solvent. [30] A calibration curve for letrozole was plotted at 240 nm. and a linear relationship between 01 and 24 µg/ml was obtained. The accuracy of the method was determined by calculating the average yield, which was 99.80%. Additional precision was calculated based on repeatability, continuous variability and percentage of RSD below a certain value.[31]

CONCLUSION:

Calibration of UV result was accurate and specific range. A UV spectrophotometric method was developed and validated for pure and formulated letrozole. The UV spectrophotometric methodpd was an accurate and cost-effective method and it is a fast and accurate result. The method is accurate, selective, precise and linear in the studied concentration range. The proposed method is suitable for quality control, routine analysis and determination of letrozole in pure and pharmaceutical dosage forms.

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