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# METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF SOLANUM LYCOPERSICUM BY USING UV-VISIBLE SPECTROSCOPY

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**ABSTRACT:**

This study presents the development of an accurate, precise, and cost-effective UV-visible spectroscopy method for predicting phytochemical constituents in Solanum lycopersicum (tomatoes). The method quantifies key phytochemicals such as lycopene and carotenoids, known for their antioxidant properties and health benefits. Method optimization included solvent selection, determination of absorbance maxima, and refined sample preparation to minimize inaccuracies. Validation followed ICHQ2R1 guidelines, assessing linearity, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ). Linearity was confirmed with the equation Y = 0.0134X - 0.0737, demonstrating proportionality between concentration (20–80 µg/mL) and absorbance. The S/N ratio thresholds were set at 3 for LOD and 10 for LOQ, ensuring reliable detection and quantification. The developed method is robust, straightforward, and applicable for routine analysis in quality control and Solanum lycopersicum research. Its efficiency supports further investigations into tomato-based health promotion, nutraceuticals, and pharmaceuticals.

**Keywords**: Solanum Lycopersicum, UV-Visible Spectroscopy, Validation, Method development.

## INTRODUCTION:

Tomatoes which are scientifically known as solanum lycopersicum are known to contain lycopene, flavonoids, phenolics and vitamins with antioxidants. Tomatoes are one of the most widely consumed and versatile fruits worldwide. They belong to the Solanaceae family (night shade family). They are native to western south America. They come in different shapes, sizes and colors which includes red, yellow and orange.

**Fig:1 Solanum Lycopersicum**

UV-Visible Spectroscopy is the analytical technique applied to determine the absorption of Ultra Violet light of 200-400nm, and visible light,400-800nm.It depends upon the Beer-Lambert Law which explains that absorbance of light varies directly with concentration of absorbing species and the path length of sample. Common transitions include $\pi \rightarrow \pi^*$, $n \rightarrow \pi^*$, and $n \rightarrow \sigma^*$, depending on the molecular structure. The components involved include a light source like deuterium or tungsten lamp, monochromator for selecting a particular wavelength and detectors, such as a photodiode or photomultiplier.

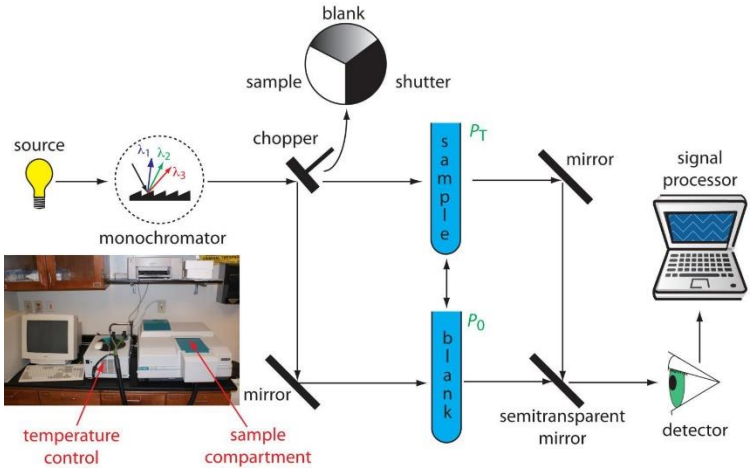



**Fig:2 UV-Visible Spectroscopy**

Method validation is like quality assurance for scientific methods. It's a way to prove that the method is reliable and consistent for its intended purpose. According to ICH The analytical parameters can be validated are accuracy, precision, detection of limit, quantitation limit, linearity, range, system suitability and robustness. Analytical Method Validation According to ICH Q2 (R1), method validation can be defined as, ''Establishing a documented proof, which provides a high degree of assurance that specific process consistently produce a desired result at it pre-arranged specifications and quality characteristics."

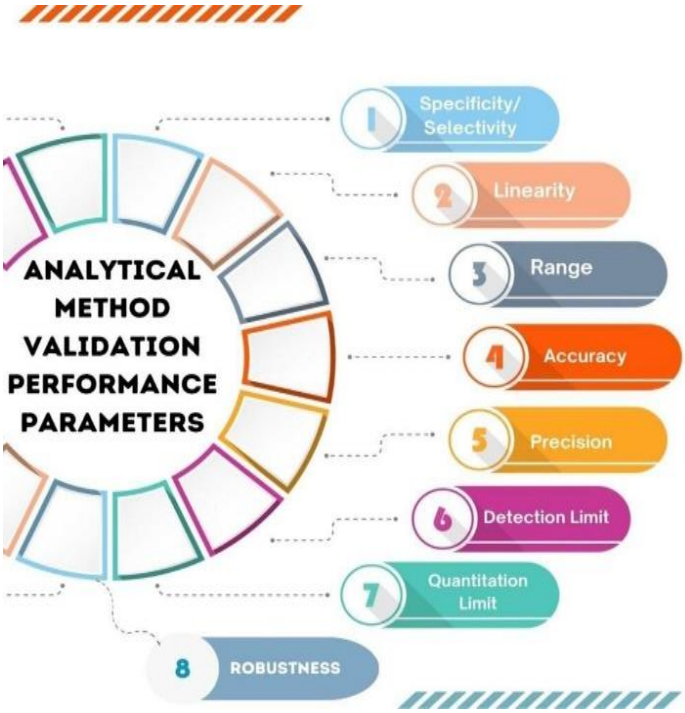



**Fig3: Method Validation**

## Methodology:

**Method For Extraction**:

The chopped tomatoes were crushed through a grinding machine, and the moisture from the pulp was removed by manual hand pressing. The pulp was then subjected to drying at 60 °C for a period of 6 hours. Following this stage, water saturated ethyl acetate was added to the pulp and left in contact for a maximum period of 1 hour. The lycopene extract was filtered through muslin cloth, to remove any large particles. The filtered extract was poured into a separating funnel, vigorously shaken, and set aside to allow layer separation. There were two layers a lower aqueous layer and an upper layer of ethyl acetate containing lycopene. The surface layer was collected in a conical flask, while the bottom layer was discarded into a beaker. The final product was purified and stored.

Fresh tomatos was thoroughly washed with portable water and fruits were chopped into piecesThe chopped tomato was ground in grinder and water from pulp is removed by hand pressed

Dried at 60 °C for 6hrs and then water saturated ethyl acetate added to the pulp and stained up to 1 hour

The lycopene extracts thus obtained were filtered through a musin cloth to remove coarse particles

After that filtered extract was transferred into separating funnel. Mixture was shaken vigorosly and kept aside for the layers to separate

Two layers were sperated one was an aqueous layers and other was an etyl acetate layer with lycopene

surface layer was collected in a conical flask and the bottom layer was discarded in beaker.The final product was purified and stored.

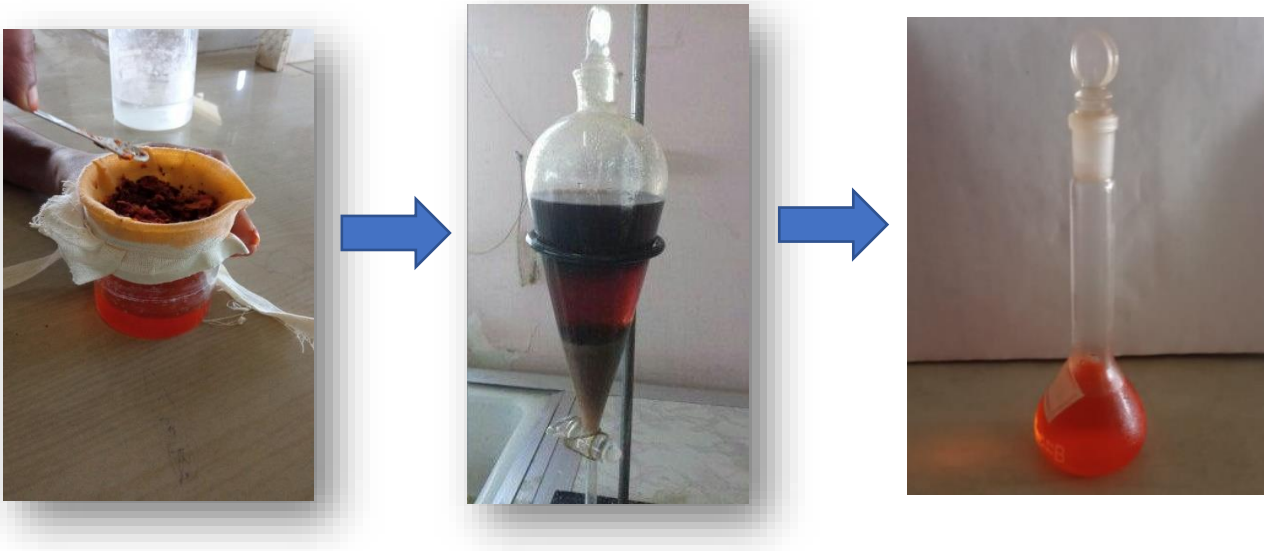
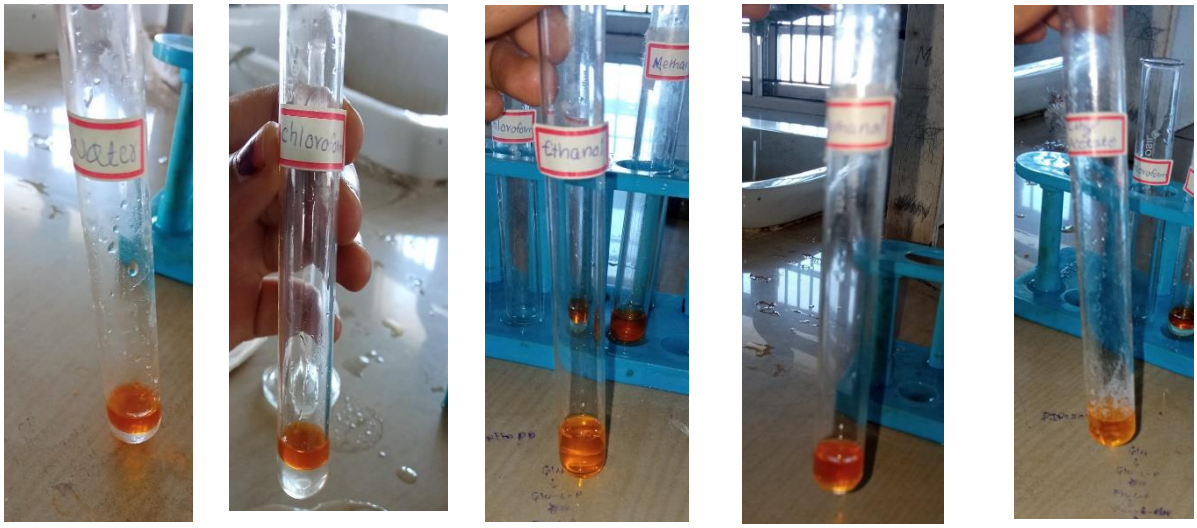


**Fig 4: Method For Extraction**

## Method Development:

***Solubility:***

The term 'solubility' is characterized as maximum amount of solute that dissolved in a given amount of solvent. Lycopene, A tomato phytoconstituent is soluble in selected solvents which includes ethyl acetate. partially soluble in methanol, ethanol and completely insoluble in chloroform and water.



**WATER** **CHLOROFORM** **ETHANOL** **METHANOL** **ETHYL ACETATE**

**Fig 5: Solubility Of Lycopene Extract In Different Solvents**

***Selection of solvent:***

Selection of solvents based on solubility of lycopene ,stability of lycopene,compatilbility with UV-Visible Spectroscopy. Lycopene is a non- polar compound , it is soluble in solvents like ethyl acetate . Lycopene extract of solanum lycopersicum was disolved in ratio of 1:1 ratio of ethyl acetate which shows complete solublity as above figure 4.

**Method development:**

**Trail-1:**

**Preparation Of Working Standard Solution:**

Accurately I ml of solanum lycopersicum extract were measured and mixed with ethyl acetate transferred into 10ml volumetric flask, keep it aside.
From the above, 1 ml of solution was pipetted out and transferred into 10ml volumetric flask and the volume was made up with 8:2 ethyl acetate and ethanol with a concentration of 10µg/ml extract respectively.

**Lycopene Extract In 8:2 Ethyl Acetate And Ethanol:**

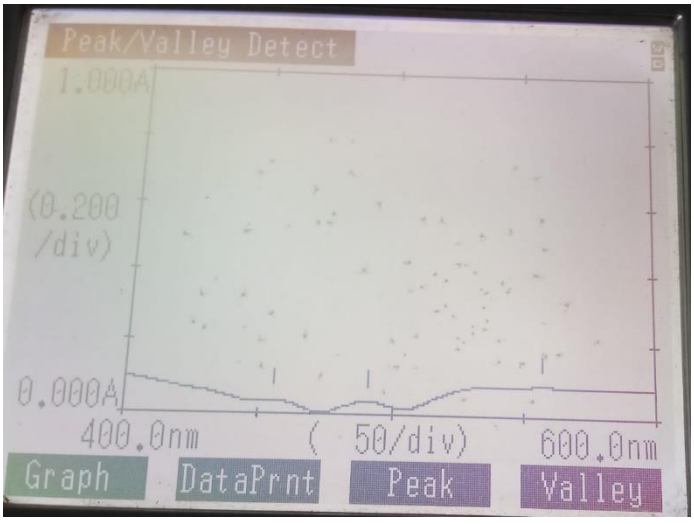



**Fig 6: The peak has not been observed.**

**TRAIL-2:**

Preparation of Standard solution:
Accurately 1λml of solanum lycopersicum extract were measured and mixed with ethyl acetate transferred into 10ml volumetric flask, keep it aside.
From this I ml of solution was pipetted out and transferred into 10ml volumetric flask and the volume was made up with 6:4 ethyl acetate and ethanol with a concentration of 10µg/ml extract respectively.

**Lycopene extract in 6:4 ethyl acetate and ethanol:**

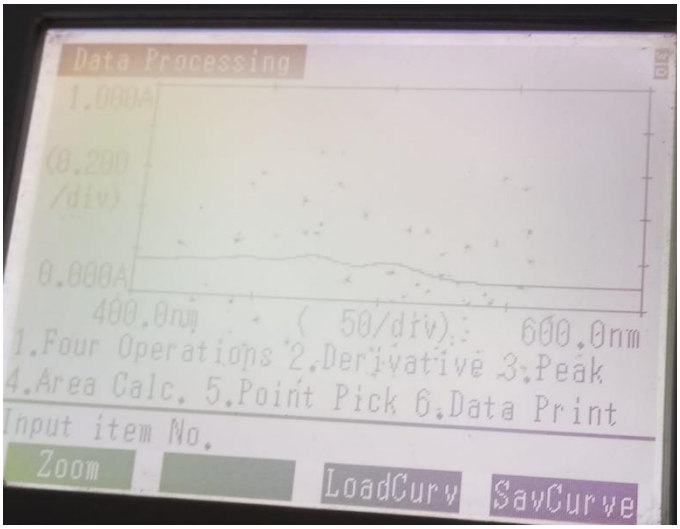



**Fig 7: The peak has not been observed.**

**TRAIL-3:**

Preparation of Standard solution:

Accurately 1 ml of solanum lycopersicum extract were measured and mixed with ethyl acetate transferred into 10ml volumetric flask, keep it aside. From this 1 ml of solution was pipetted out and transferred into 10ml volumetric flask and the volume was made up with ethyl acetate a concentration of 10µg/ml extract respectively.

**The Wavelength maxima was found to be – 426nm**

**1 ml Lycopene Extract In Ethyl Acetate**

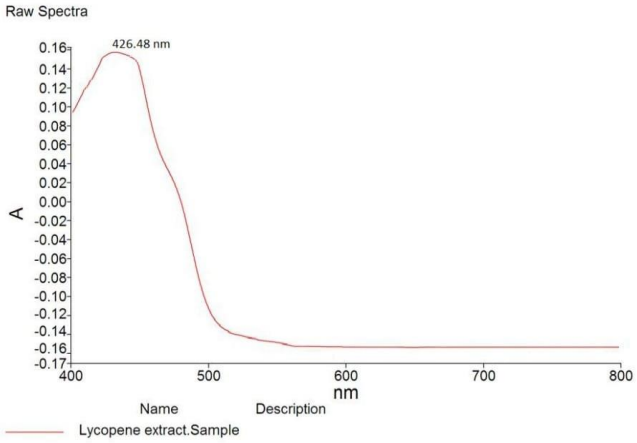



**Fig 8: The peak has been observed.**

**Preparation of stock solution:**100mg of drug was taken and transfer into clean, dry volumetric flask. the volume was made up to the mark with ethyl acetate, which is the standard solution.

**Determination of absorbance maxima:**The stock solution was diluted with ethyl acetate to get concentration of 100µg/ml . this solution was scanned in the range of 400 to 600nm. The wavelength of maximum absorbance was found at 426nm

**List of instruments used:**

**TABLE NO :1**

| **INSTRUMENTS** | **NAME** | **MODEL** |
|---|---|---|
| WEIGHING BALANCE | Shimadzu | ELB3OO |

| | | |
|---|---|---|
| UV- VISIBLE SPECTROPHOTOMETER | Shimadzu | UV-1800 |

**TABLE NO :2**

**Reagents and chemicals:**

| S.NO | CHEMICALS | GRADE | MANUFACTURE/SUPPLIER |
|---|---|---|---|
| **1.** | Solanum Lycopersicum | LR | Natural and organic |
| **2.** | Distilled water | LR | Himalaya chemicals |
| **3.** | Ethanol | LR | Himalaya chemicals |

**Validation:**

**Analytical Method Optimization:**

**Aim:**

The present study is to develop a new method and validation of lycopene extract by UV- Visible Spectroscopy.

**Quantitative Determination Of The Drug By Using The UV Visible Spectroscopy**

**Method**:

Sample: Lycopene Extract

Standard solution drug: accurately weigh lycopene powder equivalent to 1gm and mix with 10ml of ethanol transferred into 10ml volumetric flask and filtered, keep it aside.

From this 1ml of solution was pipetted out and transferred into 10ml volumetric flask and the volume was made up with 1:9 ethanol and water to give a concentration of 10µg/ml of lycopene extract respectively**.**

**Assay formula:**

$$\text{Average weight of powder} = \frac{\text{Weight of powder}}{\text{Bulk density}}$$

$$\text{Amount to be taken} = \frac{\text{Average weight} \times \text{Equivalent weight}}{\text{Labelled claim}}$$

$$\frac{A_1}{A_2} = \frac{C_1}{C_2}$$

$$C_2 = \frac{A_2}{A_1} \times C_1$$

$$\%\text{Purity} = \frac{\text{concentration}}{\text{Labelled claim}} \times 100$$

**Acceptance criteria:**

The limit of assay is in between the

Where,

A1=Peak area of sample solution

A2=peak area of standard solution

C1=weight of working standard solution

**Validation Parameters**

1.**Specificity:**

Definition: specificity is the ability to assess and unequivocally the analyte in the presence of impurities, degradants, matrix(components) which may be expected to be present. Lack of specificity of an individual analytical procedure may be contented by other supporting analytical procedure. The analytical procedure results in wavelength maxima of 426nm.

**ICH Requirement:**

The ICH document states that when chromatographic procedure are used representive peaks should be presented to demonstrate the degree of labelled.

peaks purity test (example; using selectivity and peaks should be approximately diode array or mass spectroscopy) may be useful to show that the analyte UV peak is not attributable to more than one component.

**Specificity:** At 50 µg/ml against ethyl acetate

**Table no :3**

| S. No | No of observation | absorbance |
|---|---|---|
| **1** | **1** | **0.534** |
| **2** | **2** | **0.534** |
| **3** | **3** | **0.534** |
| **4** | **4** | **0.534** |
| **5** | **5** | **0.534** |
| **6** | **6** | **0.534** |

**2. LINEARITY AND RANGE**

**Linearity**:

Linearity is the ability of the method to obtain test results that are directly proportional to the analyte concentration within a given range. The analytical procedure results in wavelength maxima of 426nm.

**Range:**

Range of analytical procedure is the interval between the upper and lower concentration of analyte in the sample (including concentration) for which it has been demonstrated that the analytical has a suitable level of precision, accuracy and linearity. The analytical procedure results in wavelength maxima of 426nm.

**Procedure:**

Preparation of Standard Stock Solution:

Accurately 1ml of solanum lycopersicum were measured and transferred into volumetric flask and make up to the mark by using solvent ethyl acetate

Preparation of (0.2, 0.3, 0.4, 0.5,0.6,0.7,0.8µg/ml) sample solutions:

From the above stock solution pipette out 0.2, 0.3, 0.4, 0.5,0.6,0.7 and 0.8ml respectively into individual 10ml of volumetric flasks and made up to 10ml with ethyl acetate mark with to prepare 0.2, 0.3, 0.4, 0.5,0.6,0.7and 0.8 µg/ml of sample solutions respectively.

Acceptance criteria for Range:

Correlation co-efficient should not be less than 0.999.

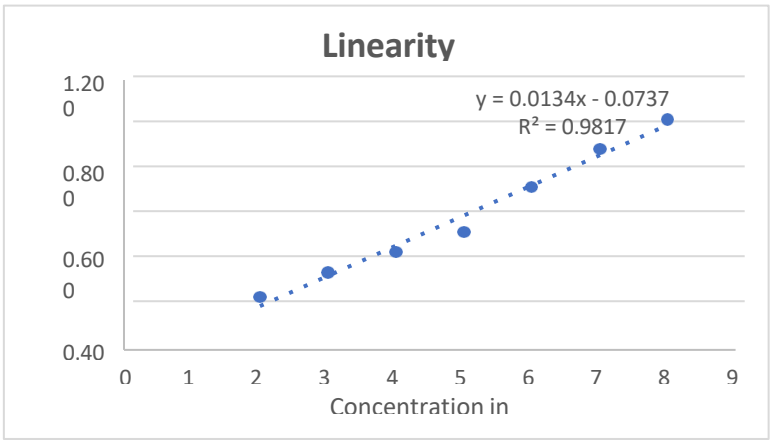
**Linearity and Range:**

**Linearity:**

**Table no :4**

| | Concentration in µg/ml | Observation 1 | Observation 2 | Observation 3 | Average |
|---|---|---|---|---|---|
| **1** | 20 | 0.234 | 0.234 | 0.234 | 0.234 |
| **2** | 30 | 0.345 | 0.344 | 0.344 | 0.344 |
| **3** | 40 | 0.436 | 0.436 | 0.436 | 0.436 |
| **4** | 50 | 0.522 | 0.522 | 0.526 | 0.523 |
| **5** | 60 | 0.727 | 0.724 | 0.724 | 0.725 |
| **6** | 70 | 0.893 | 0.895 | 0.895 | 0.894 |
| **7** | 80 | 1.022 | 1.022 | 1.026 | 1.023 |

**Linearity Graph:**



**Range:**

The concentration from 20 to 80 µg/ml is found to be the range of this sample. Since this concentration obey linearity. The analytical procedure results in wavelength maxima of 426nm.

**3. Precision**

Precision of an analytical method is the degree of agreement among individual test result procedure is applied repeatedly to multiple sampling of a homogenous sample. Precision method is usually expressed as the standard deviation and relative standard. The analytical procedure results in wavelength maxima of 426nm.

**Determination:**

The precision of the analytical method was determined by assaying sufficient of samples and relative standard deviation was calculated. The precision of the instrument determined by assaying the samples consecutively number of time and relative's deviation was calculated.

**Procedure:**

A**) System Precision**

Preparation of Standard solution:

Accurately 1ml of solanum lycopersicum extract were measured and mixed with ethyl acetate transferred into 10ml volumetric flask, keep it aside.

From this 1ml of solution was pipetted out and transferred into 10ml volumetric flask and the volume was made up with ethyl acetate a concentration of 10µg/ml extract respectively. The analytical procedure results in wavelength maxima of 426nm.

**Method Precision**

preparation of Sample solution:

Accurately 1ml of solanum lycopersicum extract were measured and transferred into 10ml volumetric flask and made up with 10ml ethyl acetate

%RSD Formula = $\sigma/\mu \times 100$

Acceptance criteria:

Relative standard deviation (RSD) for the areas of betel from the standard peaks should be more than 2.0. The method precision was determined by preparing the sample from the tablet formulation for five times and six successive injections of 10ul of working sample solution were injected and the peaks were recorded.

**PRECISION:**

**Intraday Precision at 50 µg/ml:**

**Table no :5**

| **No of observation** | **Absorbance** |
|---|---|
| 1 | 0.543 |
| 2 | 0.543 |
| 3 | 0.543 |
| 4 | 0.542 |
| 5 | 0.544 |
| 6 | 0.546 |
| **Average** | 0.5435 |
| **Standard deviation** | 0.0014 |
| **Relative standard deviation in % (RSD)** | **0.254** |

**Interday Precision at 50 µg/ml:**

**Table no :4**

| **No of observation** | **Absorbance** |
|---|---|
| 1 | 0.536 |
| 2 | 0.542 |
| 3 | 0.543 |
| 4 | 0.543 |
| 5 | 0.544 |
| 6 | 0.539 |

| | |
|---|---|
| **Average** | **0.541** |
| **Standard deviation** | **0.003** |
| **Relative standard deviation in % (RSD)** | **0.266** |

**8 4.Accuracy:**

The accuracy of an analytical method is the closeness of that results obtained by that method to be true value. Accuracy may often be expressed as percent recovery by the assay of known added amount of analyte.

**Determination:**

The accuracy of the analytical method was determined by applying the method the analysed samples, to which known amounts of analyte had been added, the accuracy was calculated from the results as the percentage of analyte recovered by the assay

**Procedure:**

**Preparation of Standard solution:**

Accurately 0.4, 0.5 and 0.6ml of Solanum Lycopersicum extract respectively were weighed and transferred into 10ml volumetric flask. From this 1ml of solution was pipetted out and transferred into 10ml of volumetric flask and the volume was made up with ethyl acetate water to a concentration of 0.4mg/ml, 0.5mg/ml and 0.6mg/ml of Solanum Lycopersicum extract respectively.

Preparation of 40% sample solution:

Accurately 0.4ml of Solanum Lycopersicum extract were measured and transferred into 10ml volumetric flask made up with ethyl acetate.

Preparation of 50% sample solution:

Accurately 0.5ml of Solanum Lycopersicum extract were measured and transferred into 10ml volumetric flask made up with ethyl acetate

Preparation of 60% sample solution:

Accurately 0.6ml of Solanum Lycopersicum extract were weighed and transferred into 10ml volumetric flask made up with ethyl acetate.

$$\%\text{Recovery} = \frac{\text{(Amount recovered)}}{\text{(Actual amount added)}} \times 100$$

Acceptance criteria:

Percentage recovery in all the cases should be between 98.0 and 102.0% w/v.

**Table showing results of accuracy:**

**Table no :6**

| **S.No** | **Absorbance** | | |
|---|---|---|---|
| | **4.0** | **5.0** | **6.0** |
| **1.** | **0.436** | **0.522** | **0.727** |
| **2.** | **0.436** | **0.522** | **0.724** |
| **3.** | **0.436** | **0.526** | **0.724** |
| **mean** | **0.436** | **0.523** | **0.725** |
| **STD** | **0.002** | **0.003** | **0.004** |
| **%RSD** | **0.526** | **0.566** | **0.568** |

$$\text{Formula: \% recovery} = \frac{\text{(amount recovered)}}{\text{(actual amount added)}} 100$$

$$\text{\% recovery for 40\%} = \frac{0.436}{0.490} \times 100 = 99.1\%\text{w/v}$$

$$\text{\% recovery for 50\%} = \frac{0.523}{0.538} \times 100 = 97\%\text{w/v}$$

$$\text{\% recovery for 60\%} = \frac{0.725}{0.730} \times 100 = 99.3\%\text{w/v}$$

**5. ROBUSTNESS**

It is a measure of ability to remain unaffected by small but deliberate variations parameters and provides an indication of its reliability during normal usage.

The robustness of an analytical method was determined by analysis of aliquots from homogenous lots by differing physical parameters of the assay for example change in physical parameters of the assay for example change in physical parameters like wavelength, temperature and concentration.

Preparation of Sample solution:

Accurately 1 ml of Solanum Lycopersicum extract were measured and transferred into 10ml volumetric flask and made up to the mark with ethyl acetate.

**Table no :7**

| S.no | Absorbance | λmax(nm) |
|---|---|---|
| **1.** | **0.536** | **426nm** |
| 2. | **0.542** | **426nm** |
| 3. | **0.543** | **426nm** |
| 4. | **0.541** | **426nm** |
| 5. | **0.542** | **426nm** |
| 6. | **0.566** | **426nm** |
| **mean** | **0.541** | |
| **STD** | **0.003** | |
| **%RSD** | **0.566** | |

**Table no :7.1**

| S.no | Absorbance | λmax(nm) |
|---|---|---|
| **1.** | **0.536** | **431nm** |
| 2. | **0.542** | **431nm** |
| 3. | **0.543** | **431nm** |
| 4. | **0.541** | **431nm** |
| 5. | **0.542** | **431nm** |
| 6. | **0.566** | **431nm** |
| **mean** | **0.541** | |
| **STD** | **0.003** | |
| **%RSD** | **0.566** | |

**Table no :7.2**

| S.no | Absorbance | λmax(nm) |
|---|---|---|
| **1.** | **0.536** | **421nm** |
| 2. | **0.542** | **421nm** |
| 3. | **0.543** | **421nm** |
| 4. | **0.541** | **421nm** |
| 5. | **0.542** | **421nm** |
| 6. | **0.566** | **421nm** |
| **mean** | **0.541** | |

| | | |
|---|---|---|
| **STD** | **0.003** | |
| **%RSD** | **0.566** | |

**Table no :7.3**

| S.no | Absorbance | Temperature($^0$c) | λmax(nm) |
|---|---|---|---|
| **1.** | **0.536** | **37$^0$c** | **426nm** |
| 2. | **0.542** | **37$^0$c** | **426nm** |
| 3. | **0.543** | **37$^0$c** | **426nm** |
| 4. | **0.541** | **37$^0$c** | **426nm** |
| 5. | **0.542** | **37$^0$c** | **426nm** |
| 6. | **0.566** | **37$^0$c** | **426nm** |
| **mean** | **0.541** | | |
| **STD** | **0.003** | | |
| **%RSD** | **0.566** | | |

**Table no: 7.4**

| S.no | Absorbance | Temperature($^0$c) | λmax(nm) |
|---|---|---|---|
| **1.** | **0.536** | **42$^0$c** | **426nm** |
| 2. | **0.542** | **42$^0$c** | **426nm** |
| 3. | **0.543** | **42$^0$c** | **426nm** |
| 4. | **0.541** | **42$^0$c** | **426nm** |
| 5. | **0.542** | **42$^0$c** | **426nm** |
| 6. | **0.566** | **42$^0$c** | **426nm** |
| **mean** | **0.541** | | |
| **STD** | **0.003** | | |
| **%RSD** | **0.566** | | |

**Table no:7.5**

| S.no | Absorbance | Temperature($^0$c) | λmax(nm) |
|---|---|---|---|
| **1.** | **0.536** | **32$^0$c** | **426nm** |
| 2. | **0.542** | **32$^0$c** | **426nm** |
| 3. | **0.543** | **32$^0$c** | **426nm** |
| 4. | **0.541** | **32$^0$c** | **426nm** |
| 5. | **0.542** | **32$^0$c** | **426nm** |
| 6. | **0.566** | **32$^0$c** | **426nm** |
| **mean** | **0.541** | | |

| | | | |
|---|---|---|---|
| **STD** | **0.003** | | |
| **%RSD** | **0.566** | | |

**6.Limit Of Detection**

It is determined by based on the standard deviation of response and the slope The Detection limit may be expressed as

$$DL = \frac{3.3\sigma}{S}$$

$$DL = \frac{3.3\times0.003}{0.0134}$$

$$= 0.73880\ \mu g/ml$$

LOD is based on the s/n ratio of the standard injection.

s/n ratio value shall be 3 for LOD.

**7. Limit Of Quantification**

It is determined by based on the standard deviation of response and the slope The Quantification limit may be expressed as

$$QL = \frac{10\sigma}{S}$$

$$QL = \frac{10\times0.003}{0.0134}$$

$$= 2.2388\ \mu g/ml$$

LOQ is based on the s/n ratio of the standard injection

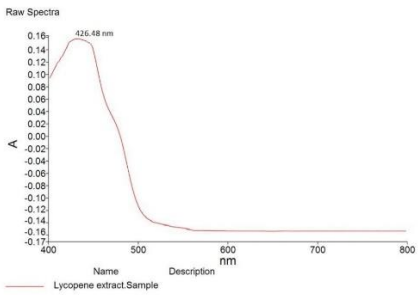
s/n ratio value shall be 10 for LOQ

## RESULT:

**Selection of solvent:** Solubility of drug was checked in the solvent like ethyl acetate and n-hexane. The drug shows good absorbance in ethyl acetate.

| S.no | Solvent | Solubility |
|---|---|---|
| 1. | Water | Insoluble |
| 2. | Chloroform | Insoluble |
| 3. | Ethanol | Partially Soluble |
| 4. | Methanol | Partially Soluble |
| 5. | Ethyl Acetate | Soluble |

**SPECTRUM OF SOLANUM LYCOPERSICUM EXTRACT:**



**Selection Of Detector Wavelength**

The wavelength selection is made at 400nm and 600nm maximum absorbance in uv spectrum as reported in the literature is in 400nm and 600nm respectively

**ASSAY OF SOLANIUM LYCOPERSICUM EXTRACT:**

Weight of extract = 15 mg

$$\text{Average weight} = \frac{15}{0.899} = 16.68\text{mg}$$

$$\text{Amount to be taken} = \frac{15 \times 1}{10} = 1.5\text{mg}$$

$$\frac{A_1}{A_2} = \frac{C_1}{C_2}$$

$$C_2 = \frac{A_2}{A} \times C_1$$

$$C_2 = \frac{0.515}{0.54} \times 10 = 9.5$$

$$\%\text{Purity} = \frac{\text{Concentration}}{\text{Labelled claim}} \times 100 = \frac{9.5}{10} \times 100$$

= 95% w/v

Acceptance Criteria:

The limit of assay is in between the 90 – 102% w/v

**1.SPECIFICITY**

The specificity of the method was confirmed by injecting he placebo and placebo standard and observed that the no shift in wavelength interference due to placebo. This confirms the specificity of the proposed method. The results are reported in table no:

**Specificity : at 50 µg/ml against ethylene acetate**

**Table no : 8**

| S. No | No of observation | absorbance |
|---|---|---|
| **1** | **1** | **0.534** |
| **2** | **2** | **0.534** |
| **3** | **3** | **0.534** |
| **4** | **4** | **0.534** |
| **5** | **5** | **0.534** |
| **6** | **6** | **0.534** |

**2. LINEARITY AND RANGE:**

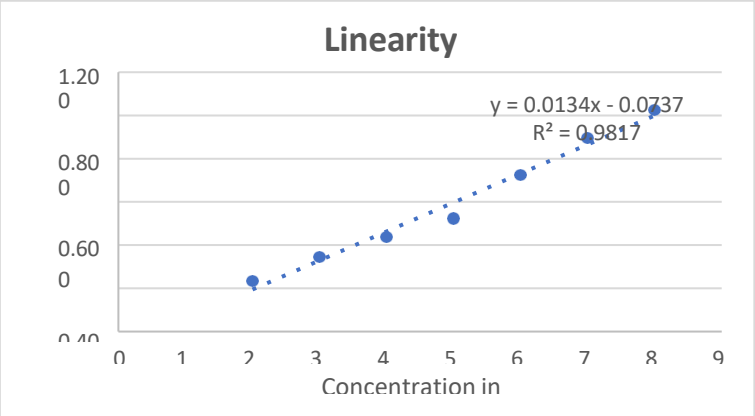
Linearity was evaluated by visual inspection of plot of peak are as a function of analyte concentrations for drug. From the linearity studies the specified range was determined for drug.

**Linearity:**

**Table no : 9**

| S.no | Concentration in µg/ml | Observation 1 | Observation 2 | Observation 3 | Average |
|---|---|---|---|---|---|
| **1** | 20 | 0.234 | 0.234 | 0.234 | 0.234 |
| **2** | 30 | 0.345 | 0.344 | 0.344 | 0.344 |
| **3** | 40 | 0.436 | 0.436 | 0.436 | 0.436 |
| **4** | 50 | 0.522 | 0.522 | 0.526 | 0.523 |
| **5** | 60 | 0.727 | 0.724 | 0.724 | 0.725 |
| **6** | 70 | 0.893 | 0.895 | 0.895 | 0.894 |
| **7** | 80 | 1.022 | 1.022 | 1.026 | 1.023 |

**LINEARITY GRAPH:**




**RANGE:**

The concentration from 20 to 80 µg/ml is found to be the range of this sample. Since this concentration obey linearity.

**3.PRECISION:**

**Intraday Precision at 50 µg/ml:**

**Table no:10**

| **No of observation** | **Absorbance** |
|---|---|
| 1 | 0.543 |
| 2 | 0.543 |
| 3 | 0.543 |
| 4 | 0.542 |
| 5 | 0.544 |
| 6 | 0.546 |
| **Average** | 0.5435 |
| **Standard deviation** | 0.0014 |
| **Relative standard deviation in % (RSD)** | **0.254** |

**Interday Precision at 50 µg/ml:**

**Table no :11**

| No of observation | Absorbance |
|---|---|
| 1 | 0.536 |
| 2 | 0.542 |
| 3 | 0.543 |
| 4 | 0.543 |
| 5 | 0.544 |
| 6 | 0.539 |
| **Average** | **0.541** |
| **Standard deviation** | **0.003** |
| **Relative standard deviation in % (RSD)** | **0.266** |

**4.ACCURACY**

**Table showing results of accuracy:**

**Table no :11**

| S. No | Absorbance | | |
|---|---|---|---|
| | **4.0** | **5.0** | **6.0** |
| **1.** | **0.436** | **0.522** | **0.727** |
| **2.** | **0.436** | **0.522** | **0.724** |
| **3.** | **0.436** | **0.526** | **0.724** |
| **mean** | **0.436** | **0.523** | **0.725** |
| **STD** | **0.002** | **0.003** | **0.004** |
| **%RSD** | **0.526** | **0.566** | **0.568** |

$$\text{Formula: \% recovery} = \frac{\text{(amount recovered)}}{\text{(actual amount added)}} \times 100$$

$$\text{\% recovery for 40\%} = \frac{0.436}{0.490} \times 100 = 99.1\%\text{w/v}$$

$$\text{\% recovery for 50\%} = \frac{0.523}{0.520} \times 100 = 97\%\text{w/v}$$

$$\text{\% recovery for 60\%} = \frac{0.725}{0.730} \times 100 = 99.3\%\text{w/v}$$

**5.ROBUSTNESS:** Robustness is carried out the assay during which free rate and temperature were altered slightly. The results are reported in the

**Table no :12**

| S.no | Absorbance | λmax(nm) |
|---|---|---|
| **1.** | **0.536** | **426nm** |
| 2. | **0.542** | **426nm** |
| 3. | **0.543** | **426nm** |

| | | |
|---|---|---|
| 4. | **0.541** | **426nm** |
| 5. | **0.542** | **426nm** |
| 6. | **0.566** | **426nm** |
| **mean** | **0.541** | |
| **STD** | **0.003** | |
| **%RSD** | **0.566** | |

**Table no :12.1**

| S.no | Absorbance | λmax(nm) |
|---|---|---|
| **1.** | **0.536** | **431nm** |
| 2. | **0.542** | **431nm** |
| 3. | **0.543** | **431nm** |
| 4. | **0.541** | **431nm** |
| 5. | **0.542** | **431nm** |
| 6. | **0.566** | **431nm** |
| **mean** | **0.541** | |
| **STD** | **0.003** | |
| **%RSD** | **0.566** | |

**Table no :12.2**

| S.no | Absorbance | λmax(nm) |
|---|---|---|
| **1.** | **0.536** | **421nm** |
| 2. | **0.542** | **421nm** |
| 3. | **0.543** | **421nm** |
| 4. | **0.541** | **421nm** |
| 5. | **0.542** | **421nm** |
| 6. | **0.566** | **421nm** |
| **mean** | **0.541** | |
| **STD** | **0.003** | |
| **%RSD** | **0.566** | |

**Table no :12.3**

| S.no | Absorbance | Temperature($^0$c) | λmax(nm) |
|---|---|---|---|
| **1.** | **0.536** | **37$^0$c** | **426nm** |
| 2. | **0.542** | **37$^0$c** | **426nm** |
| 3. | **0.543** | **37$^0$c** | **426nm** |
| 4. | **0.541** | **37$^0$c** | **426nm** |
| 5. | **0.542** | **37$^0$c** | **426nm** |

| | | | |
|---|---|---|---|
| 6. | **0.566** | **37$^0$c** | **426nm** |
| **mean** | **0.541** | | |
| **STD** | **0.003** | | |
| **%RSD** | **0.566** | | |

**Table no: 12.4**

| S.no | Absorbance | Temperature($^0$c) | λmax(nm) |
|---|---|---|---|
| **1.** | **0.536** | **42$^0$c** | **426nm** |
| 2. | **0.542** | **42$^0$c** | **426nm** |
| 3. | **0.543** | **42$^0$c** | **426nm** |
| 4. | **0.541** | **42$^0$c** | **426nm** |
| 5. | **0.542** | **42$^0$c** | **426nm** |
| 6. | **0.566** | **42$^0$c** | **426nm** |
| **mean** | **0.541** | | |
| **STD** | **0.003** | | |
| **%RSD** | **0.566** | | |

**Table no:12.5**

| S.no | Absorbance | Temperature($^0$c) | λmax(nm) |
|---|---|---|---|
| **1.** | **0.536** | **32$^0$c** | **426nm** |
| 2. | **0.542** | **32$^0$c** | **426nm** |
| 3. | **0.543** | **32$^0$c** | **426nm** |
| 4. | **0.541** | **32$^0$c** | **426nm** |
| 5. | **0.542** | **32$^0$c** | **426nm** |
| 6. | **0.566** | **32$^0$c** | **426nm** |
| **mean** | **0.541** | | |
| **STD** | **0.003** | | |
| **%RSD** | **0.566** | | |

**6.LIMIT OF DETECTION:**

It is determined by based on the standard deviation of response and the slope The Detection limit may be expressed as

$$DL = \frac{3.3\sigma}{S}$$

$$DL = \frac{3.3\times0.003}{0.0134}$$

$$= 0.73880\ \mu g/ml$$

LOD is based on the s/n ratio of the standard injection.

s/n ratio value shall be 3 for LOD.

**7.LIMIT OF QUANTIFICATION:**

It is determined by based on the standard deviation of response and the slope The Quantification limit may be expressed as

$$QL = \frac{10\sigma}{\text{-----}}$$

$$QL = \frac{S}{\frac{10 \times 0.003}{0.0134}} = 2.2388\ \mu g/ml$$

LOQ is based on the s/n ratio of the standard injection

s/n ratio value shall be 10 for LOQ

## CONCLUSION:

- A new method is developed for estimation in Solanum Lycopersicum extract by UV Visible spectrophotometry.
- The sample preparation is simple and analysis time is short.
- The analytical procedure is validated as per ICH Q2R1guidelines and shown to be accurate, precise, specific and obeys linearity within range.
- This method represents a fast-analytical procedure for the estimation of Solanum Lycopersium in its bulk and pharmaceutical dosage form by using UV Visible spectrophotometry.
- The methods enable to routine analysis of large number of samples with good precision and accuracy.
- The solvents used are ethyl acetate which is easily available and economical**.**

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