# Analytical Method Development and Validation of Isatin API By UV- Visible Spectroscopy

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## Abstract
A simple , rapid and sensitive UV-VISIBLE Spectrophotometric method has been developed for the estimation of Isatin in bulk form. The Spectrophotometric detection was carried out on absorption maxima of 295nm using methanol as solvent. The method was validated for specificity, linearity, precision and robustness. The detector response for the Isatin was linear over the selected concentration range 5-25µ/ml correlation coefficient of 0.999 .The precision [ RSD] among six sample preparations was 0.005[intraday] and 0.026 [interday]. The LOD and LOQ are 1.3 and 4.00 respectively.

**Key words: Isatin,** UV-Visible spectroscopy, absorption maxima, correlation coefficient, Limit of detection, limit of quantification.

## 1. Introduction
Isatin is a heterocyclic organic compound with chemical formula $C_8H_5NO_2$, IUPAC name1H-indole-2,3-dione and the structure of Isatin fig1.1 the compound is derivative of indole and it is also known as tribulin The compound was found in many plants. Isatin was first discovered by Otto Linne Erdman and August Laurent in 1841 as a product from the oxidation of indigo dye by nitric acid and chromic acid which resulted in bright orange colored momoclinic crystals of Isatin as a product

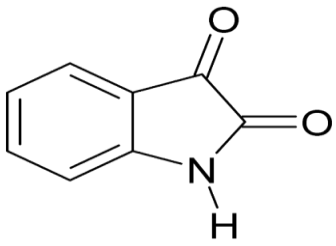



**Fig 1.1 IUPAC name 1H-Indole-2,3-dione**

Isatin and its derivatives possess several Biological activities such as antiviral[1,2], antidiabetic[3], anti inflammatory[4,5] , sedative[6], hypnotic[7], anticonvulsant[8], fungicidal[9,10], antibacterial[11] , antidepressant[12] , anticancer[13] and antiparkinsonian[14] etc. Isatin is an endogenous indole acts by inhibits monoamine oxidase (MAO), being more selective for MAO-B than MAO-A. p-substituted isatin semicarbazones have anticonvulsant activity, Isatin-N-mannich bases of Isatin -3- thiosemicarbazones have shown antiviral and antituberculostatic activity. isatin (3-o-nitrophenyl hydrazone ) shows activity against walker carcinoma-256,N-mannich bases of 3-(N-sulphadoximino) isatin and its methyl derivatives shows antimicrobial activity, Schiff and mannich bases of isatin and its derivatives with triazole have antibacterial, antifungal and anti-HIV activities.

## 1.2 Analytical chemistry

Analytical chemistry is a branch of chemistry focused on the identification and quantification of the chemical composition of materials. It involves various techniques and methods for separating, identifying, and quantifying the chemical components of natural and artificial materials.

### 1.2.1 Qualitative Analysis:

Identifies the components of a sample (e.g., what elements or compounds are present).Techniques include spectroscopy, chromatography, and microscopy.

### 1.2.2 Quantitative Analysis:

Measures the exact amounts or concentrations of components within a sample. Methods include titration, gravimetry, and instrumental techniques like mass spectrometry.

### 1.2.3 Instrumental Techniques:

**Spectroscopy**: Measures the interaction between light and matter (e.g., UV-Vis, IR, NMR).

**Chromatography**: Separates mixtures into their components (e.g., HPLC, GC).

**Electrochemical Methods**: Measures electrical properties related to the chemical composition (e.g., potentiometry, voltammetry).

## 1.3 Spectroscopy

Spectroscopy involves the measurement of light (or other electromagnetic radiation) absorbed, emitted, or scattered by materials. The interaction provides information about the structure, composition, and properties of the material.

## 1.4 UV-Visible spectroscopy

UV-Visible spectroscopy (UV-Vis) is a widely used analytical technique in chemistry that involves measuring the absorption of ultraviolet (UV) and visible light by a sample. It is particularly useful for studying the electronic transitions in molecules and can provide valuable information about the concentration and structure of a substance.

### 1.4.1 Principle of UV-Visible Spectroscopy:

UV-Vis spectroscopy is based on the principle that molecules absorb light at specific wavelengths corresponding to the energy differences between their electronic states. When a molecule absorbs UV or visible light, electrons are excited from a lower energy level (ground state) to a higher energy level (excited state).

The energy absorbed corresponds to a specific wavelength of light, and this absorption is detected and recorded as an absorption spectrum.

**Wavelength Range**:
Ultraviolet Region: 200-400 nm
Visible Region: 400-700 nm

**1.4.2 Components of a UV-Vis Spectrophotometer**:

1. Light Source: Typically a combination of a deuterium lamp (for UV) and a tungsten or halogen lamp (for visible light).
2 .Monochromator: Separates light into its component wavelengths.
3. Sample Holder: Contains the sample, usually in a quartz cuvette (for UV) or glass cuvette (for visible light).
4. Detector: Measures the intensity of light passing through the sample.

**1.4.3 Instrumentation**

**UV- Visible spectrophotometer Instrument**

**1.5 Method development**

Method development involves designing an analytical procedure tailored to measure specific analytes within a sample matrix. The goal is to create a method that is robust, accurate, precise, and suitable for its intended use.

**1.5.1 Steps involved in method development**:

1.Selection of standard drug
2.Literature search and prior methodology
3.Choosing analytical method
4.Instrumental setup
5.Method optimization
6.Documentation of analytical result
7.Evaluate the method performance
8.Determination of percent recovery of actual samples

### 1.6 Method Validation:

Method validation is the process of proving that an analytical method is suitable for its intended purpose. It provides documented evidence that the method performs consistently within predefined criteria.

### 1.6.1 Key validation parameters

Accuracy, precision, linearity, specificity, range, robustness, limit of detection, limit of quantification, sensitivity, stability, ruggedness.

## 2. Materials And Methods

### 2.1 Instruments

Labindia 2000 UV –visible double beam spectrophotometer with spectral band width of 2nm, Digital balance Wensar-PGB20,ultrasonicator

### 2.2 Materials

Isatin API purchased from sigma Aldrich,
methanol SD FINE -CHEM

**Preparation Of Standard Stock Solution:100µg/ml**

100mg of Isatin was accurately weighed and transferred it into the 100 ml volumetric flask. dissolved in 10ml with methanol and made up to 100ml with the same to obtain the concentration of 100µg/ml

**Selection Of Analytical Wavelength**

The 100 µg/ml isatin solution was scanned in the range of 200 nm to 800 nm using methanol as a blank. The absorption maximum was found to be at 295 nm, which was selected as the analytical wavelength.

**Preparation of Dilute Solutions from Stock**

A series of working standard solutions with concentrations ranging from 5 to 25 µg/ml were prepared by pipetting aliquots from the standard stock solution of isatin into 10 ml volumetric flasks and diluting to volume with methanol. The absorbance of these solutions was measured at 295 nm.

**Preparation of 5µg/ml Solution**

Withdraw 0.5 ml from the standard stock solution and transfer it into a 10 ml volumetric flask. Dilute to the mark with methanol**.**

**Preparation Of 10µg/ml Solution**

Withdraw 1 ml from the standard the stock solution and transfer it into a 10 mL volumetric flask. Dilute to the mark with methanol.

**Preparation of 15µg/ml Solution**

Withdraw 1.5 ml from the standard the stock solution and transfer it into a 10 mL volumetric flask. Dilute to the mark with methanol

**Preparation of 20µg/ml Solution**

Withdraw 1 ml from the standard stock solution and transfer it into a 10 ml volumetric flask. Dilute to the mark with methanol

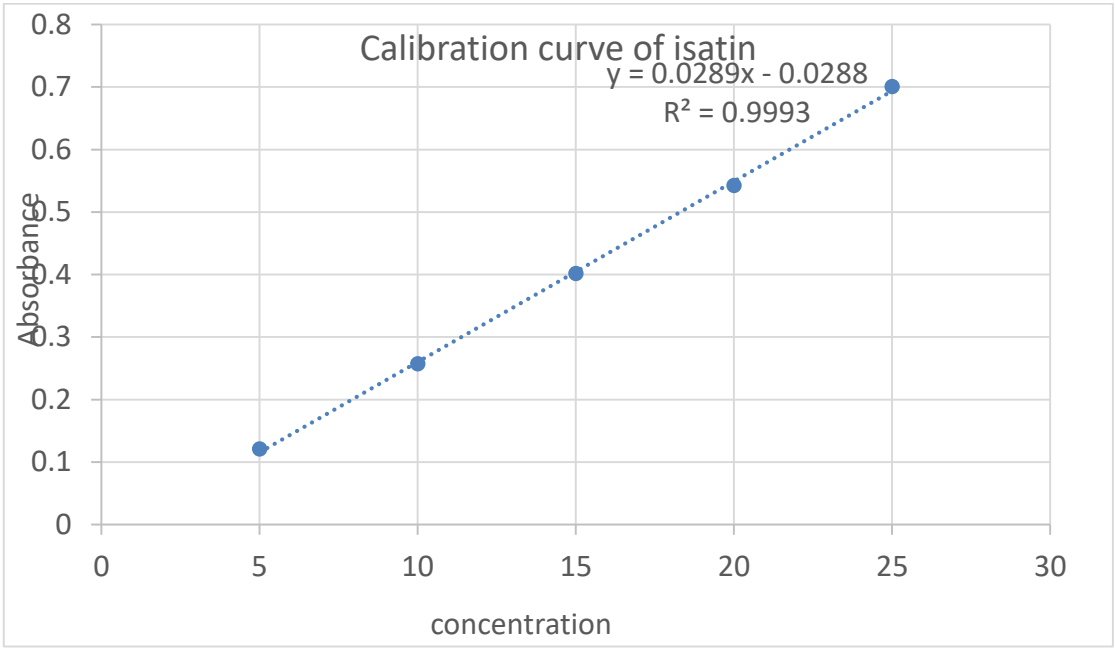
**Preparation of 25µg/ml Solution**

Withdraw 2.5 ml from the standard stock solution and transfer it into a 10 ml volumetric flask. Dilute to the mark with methanol

# Results And Discussion

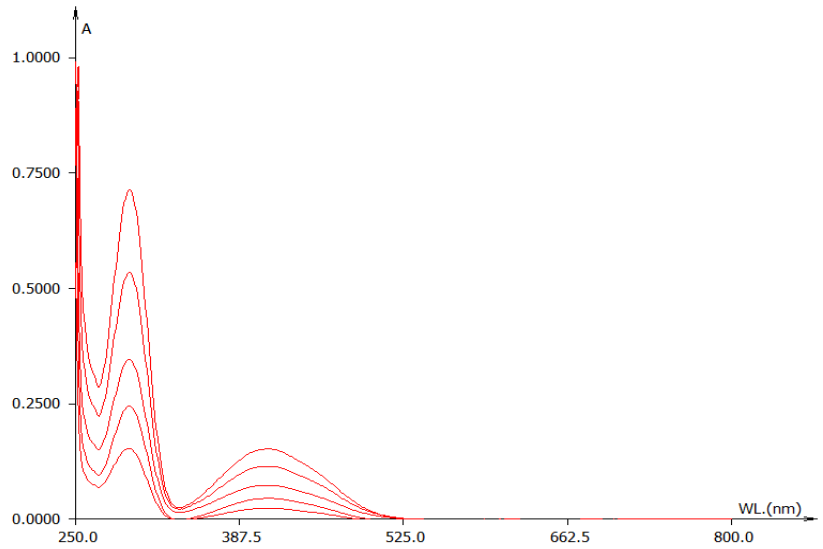
## Results of Linearity of Isatin

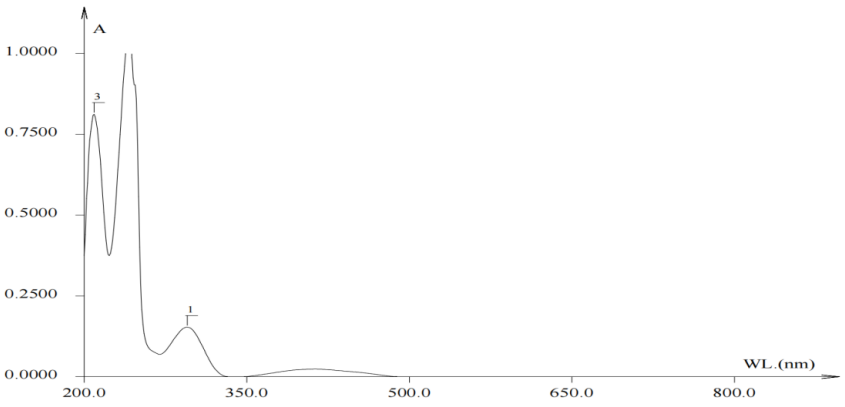
| SL.NO | CONCENTRATION (µg/ ml) | ABSORBANCE |
|---|---|---|
| 1 | 5µg/ml | 0.1211 |
| 2 | 10 µg / ml | 0.2571 |
| 3 | 15 µg / ml | 0.4018 |
| 4 | 20 µg / ml | 0.5426 |
| 5 | 25 µg / ml | 0.7008 |




**Calibration curve of Isatin**

## OVERLAY SPECTRUM

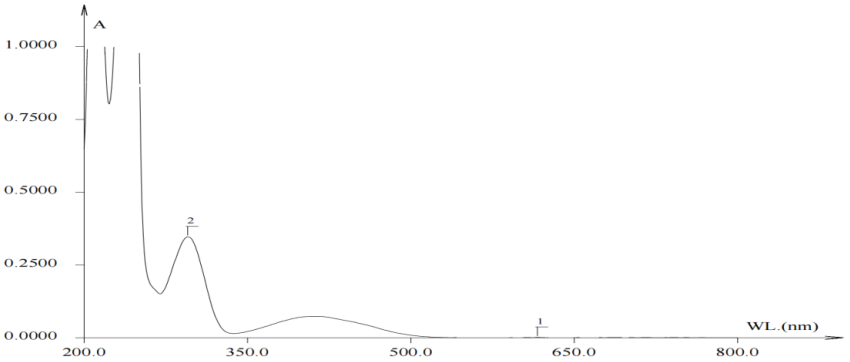



Peak List

| ID | WL(nm) | Abs. | %T |
|---|---|---|---|
| 1 | 295.0 | 0.1534 | 70.24 |
| 2 | 242.0 | 1.0922 | 8.09 |
| 3 | 209.0 | 0.8124 | 15.40 |

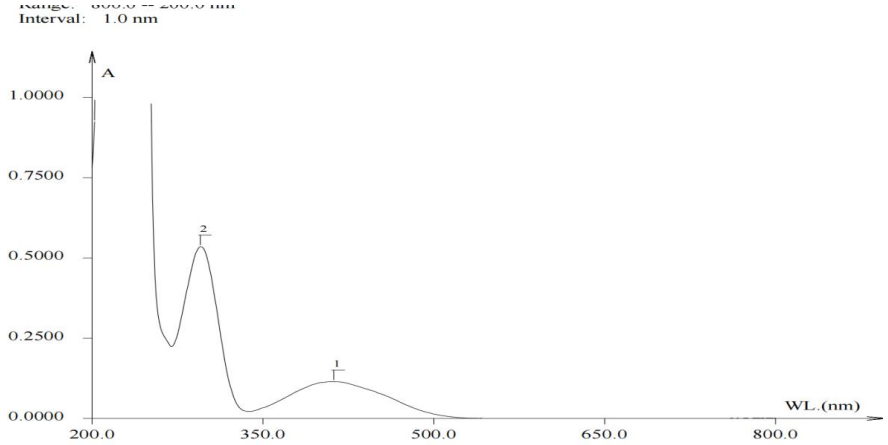
**Spectrum of 5µg/ml of Isatin**




Peak List

| ID | WL(nm) | Abs. | %T |
|---|---|---|---|
| 1 | 616.0 | 0.0014 | 99.67 |
| 2 | 295.0 | 0.3468 | 45.00 |
| 3 | 242.0 | 2.3258 | 0.47 |
| 4 | 209.0 | 1.6341 | 2.32 |

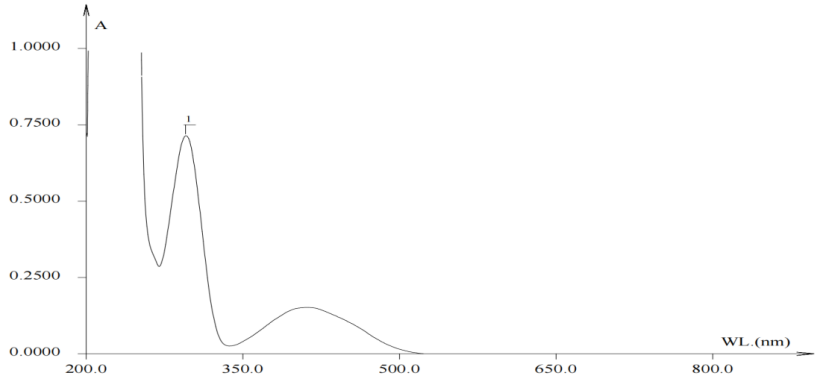
**Spectrum of 10µg/ml of Isatin**




Peak List

| ID | WL(nm) | Abs. | %T |
|---|---|---|---|
| 1 | 412.0 | 0.1147 | 76.79 |
| 2 | 295.0 | 0.5354 | 29.15 |
| 3 | 241.0 | 3.0531 | 0.09 |

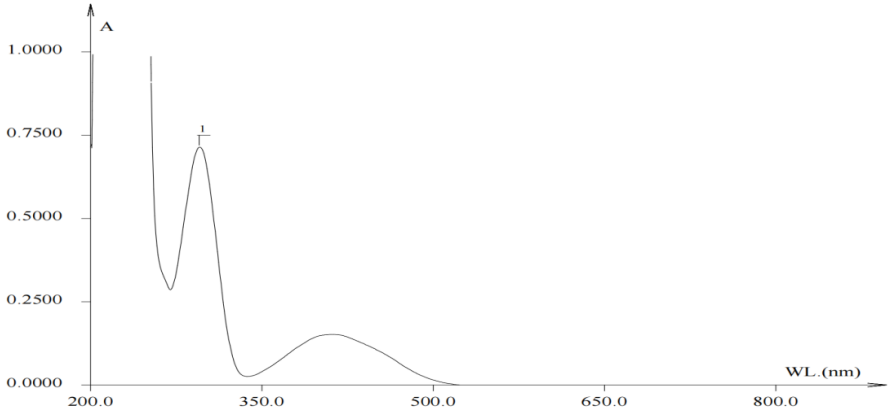
**Spectrum 15µg/ml of Isatin**




Peak List

| ID | WL(nm) | Abs. | %T |
|---|---|---|---|
| 1 | 295.0 | 0.7141 | 19.32 |
| 2 | 247.0 | 3.0191 | 0.10 |
| 3 | 245.0 | 3.0964 | 0.08 |

**Spectrum 20µg/ml of Isatin**




Peak List

| ID | WL(nm) | Abs. | %T |
|---|---|---|---|
| 1 | 295.0 | 0.7141 | 19.32 |
| 2 | 247.0 | 3.0191 | 0.10 |
| 3 | 245.0 | 3.0964 | 0.08 |

**Spectrum 25µg/ ml of Isatin**

**Results Of Interday And Intraday Precision**

| S.NO | Interday (Absorbance) | Intra day |
|---|---|---|
| 1 | O.4012 | 0.4111 |
| 2 | 0.4121 | 0.4123 |
| 3 | 0.4212 | 0.4134 |
| 4 | 0.4312 | 0.4153 |
| 5 | 0.4128 | 0.4162 |
| mean | 0.4157 | 0.41366 |
| Std Dev | 0.011204 | 0.002098 |
| % RSD | 0.026952 | 0.005073 |

**Results of Robustness Studies**

| S.No | Robust Condition | Parameters | % Rsd |
|---|---|---|---|
| 1 | Wavelength ±3nm | 292nm | 0.05824 |
| 2 | | 295nm | 0.500258 |
| 3 | | 298nm | 0.033643 |

**Results Of LOD and LOQ**

| S.NO | Range(µg/ml) | Standard deviation | Linear regression | $R^2$ | LOD (µg/ml) | LOQ (µg/ml) |
|---|---|---|---|---|---|---|
| 1 | 5-25µg/ml | 0.011204 | y = 0.0289x - 0.0288 | O.9996 | 1.3 | 4.00 |

## Summary and Conclusion

### SUMMARY

Isatin, an active pharmaceutical ingredient, was quantified using UV-Visible spectrophotometry. The analytical method developed for its determination underwent thorough validation, encompassing assessments of linearity, limit of detection (LOD), limit of quantification (LOQ), precision, and robustness.

The method's linearity was confirmed as isatin followed Beer-Lambert's Law within the concentration range of 5-25 μg/mL. The calibration curve demonstrated excellent linearity, evidenced by a high correlation coefficient of 0.9996. This indicates a strong, direct proportionality between the absorbance and concentration of isatin within this range.

In terms of precision, the method showed reproducibility with relative standard deviations (%RSD) consistently below 2%, aligning with acceptable criteria for analytical precision. The robustness of the method was also validated, ensuring that small, deliberate variations in experimental conditions did not significantly affect the results, thus confirming the method's reliability under varied conditions.

Overall, the validated UV-Visible spectrophotometric method proved to be accurate and reliable for the quantification of Isatin, meeting all necessary validation parameters including linearity, precision, and robustness.

## CONCLUSION

The UV-Visible spectrophotometric method developed for the estimation of Isatin as an active pharmaceutical ingredient (API) has been shown to be both effective and reliable. This method underwent rigorous validation according to the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines, ensuring its suitability for routine analytical purposes.

Validation Parameters:

1. Linearity: The method demonstrated a strong linear relationship between absorbance and concentration of Isatin over a specific range. This confirms the method’s ability to provide consistent results across different concentrations, a critical aspect for quantitative analysis.
2. Limit of Detection (LOD) and Limit of Quantification (LOQ): The LOD and LOQ were determined to assess the sensitivity of the method. The low values for both parameters indicate the method’s capability to detect and quantify even minimal amounts of Isatin with high accuracy.
3. Precision: The precision of the method was evaluated by analyzing multiple samples under the same conditions. Both intra-day and inter-day precision tests yielded consistent results, demonstrating the method’s reproducibility.
4. Accuracy: The method’s accuracy was validated by comparing the results obtained from the spectrophotometric analysis with a known reference method. The high level of agreement between the results confirms that the method provides reliable quantitative measurements.
5. Robustness: The robustness of the method was assessed by making small, deliberate variations in analytical conditions, such as changes in wavelength or temperature.

The method maintained its performance, indicating that it is robust and can withstand slight variations without compromising the accuracy or precision of the results.
The developed UV-Visible spectrophotometric method is advantageous for several reasons:
Rapid and Simple : The method's straightforward procedure allows for quick analysis, which is particularly beneficial in high-throughput laboratory settings.
Cost-effective : The economic efficiency of the method makes it an attractive choice for routine quality control (QC) analyses, especially in environments with budget constraints.
Ease of Use : The simplicity of the method, combined with its reliability, ensures that it can be easily adopted in laboratories without the need for extensive training or specialized equipment.
the UV-Visible spectrophotometric method for estimating Isatin is not only scientifically robust but also practically advantageous, making it an excellent choice for routine quality control in pharmaceutical settings. The method's alignment with ICH guidelines further underscores its credibility and reliability for consistent and accurate analysis of Isatin as an API.

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