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# DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR ESTIMATION OF IVERMECTIN IN TABLET DOSAGE FORM

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## ABSTRACT

**Objective:** To develop and validate simple, rapid, linear, accurate, precise and economical UV Spectroscopic method for estimation of Ivermectin in tablet dosage form. **Methods:** The drug is freely soluble in analytical grade methanol. The drug was identified in terms of solubility studies and on the basis of melting point done on melting point apparatus of Equiptronics. It showed absorption maxima were determined in analytical grade methanol. The drug obeyed the Beer's law and showed good correlation of concentration with absorption which reflect in linearity. The UV spectroscopic method was developed for estimation of Ivermectin in tablet dosage form and also validated as per ICH guidelines. **Results:** The drug is freely soluble in analytical grade methanol, soluble in ethanol and very slightly soluble in water. So, the analytical grade methanol is used as a diluent in method. The melting point of Ivermectin was found to be 155-156˚C (uncorrected). It showed absorption maxima 260 nm in analytical grade methanol. On the basis of absorption spectrum the working concentration was set on 5 µg/ml (PPM). The linearity was observed between 1-9 µg/ml (PPM). The results of analysis were validated by recovery studies. The recovery was found to be 98.75, 99.00 and 99.17% for three levels respectively. The % RSD for precision was found to be 0.88% and for Ruggedness is 1.16%. **Conclusion:** A simple, rapid, linear, accurate, precise and economical UV Spectroscopic method has been developed for estimation of Ivermectin in tablet dosage form. The method could be considered for the determination of Ivermectin in quality control laboratories.

**KEYWORDS:** Ivermectin, UV Spectrophotometer, Melting Point, Assay Method, Validation, Accuracy, Linearity, Ruggedness, Precision.

## INTRODUCTION

Ivermectin has been the drug of choice to treat various diseases due to pathogenic parasites. This comprises head lice, scabies, river blindness (onchocerciasis), strongyloidiasis, trichuriasis, ascariasis and lymphatic filariasis.[1] Ivermectin IUPAC name 22, 23-dihydroavermetin B1a + 22, 23 dihydroavermetin B1b5. Ivermectin is a semisynthetic antiparasitic drug that is produced from a class of very effective broad spectrum antiparasitic compounds known as avermectins. Ivermectin is an antiphrastic drug.[2-3] After its discovery in 1975, its first uses were in veterinary medicine to prevent and treat heartworm and acariasis. Approved for human use in 1987, today it is used infestations including head lice, scabies, river, blindness trichuriasis acariasis and lymphatic filariasis.[4-5] It works through many mechanisms to kill the targeted parasites, and can be taken orally or applied to the skin for external infestation. This lead to an increase in the permeability The available literature studies describe utilization of few analytical methods for the evaluation of Ivermectin in tablet dosage form. These methods are based on various techniques such as TLC[7], Ultra Violet spectroscopy[8-9], of the cell membrane to chloride ions with hyper polarization of the never or muscle cell, resulting in paralysis and death of parasite.[6]

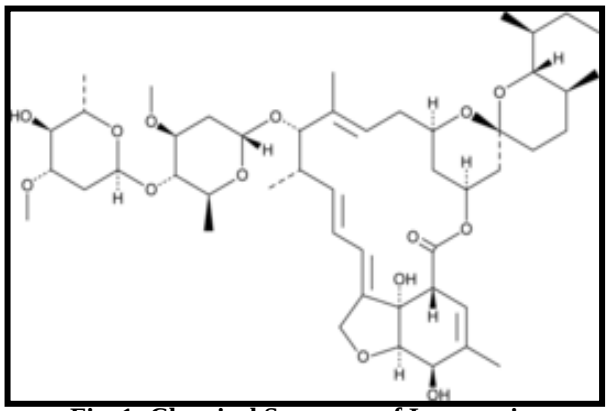

**Fig. 1: Chemical Structure of Ivermectin.**

RP-HPLC[10-12], HPLC with Tandem mass spectroscopy[13] and on Human plasma.[14] But, some of these methods lack adequate sensitivity, and some are expensive and time consuming. Therefore, it is important to develop new simple and sensitive methods for the UV spectrophotometric determination of Ivermectin in tablet dosage form. So, the aim of work was to develop and validate an analytical method by UV-Visible Spectrophotometer for the estimation of Ivermectin.

## MATERIALS AND METHODS

- **Instruments**

Shimadzu double beam UV-visible spectrophotometer 1700 Ultra with matched pair
Quartz cells corresponding to 1 cm path length and spectral bandwidth of 1 nm, Bath sonicator and citizen weighing balance.
Melting point apparatus of Equiptronics were used.

- **Materials**

Ivermectin was obtained as a gift sample. Ivermectin tablets were procured from local pharmacy. Methanol used was of analytical grade was used throughout the experiment. Freshly prepared solutions were employed.

**Method Development**

**A. Determination of λ max (10 PPM)[15, 16, 17]**

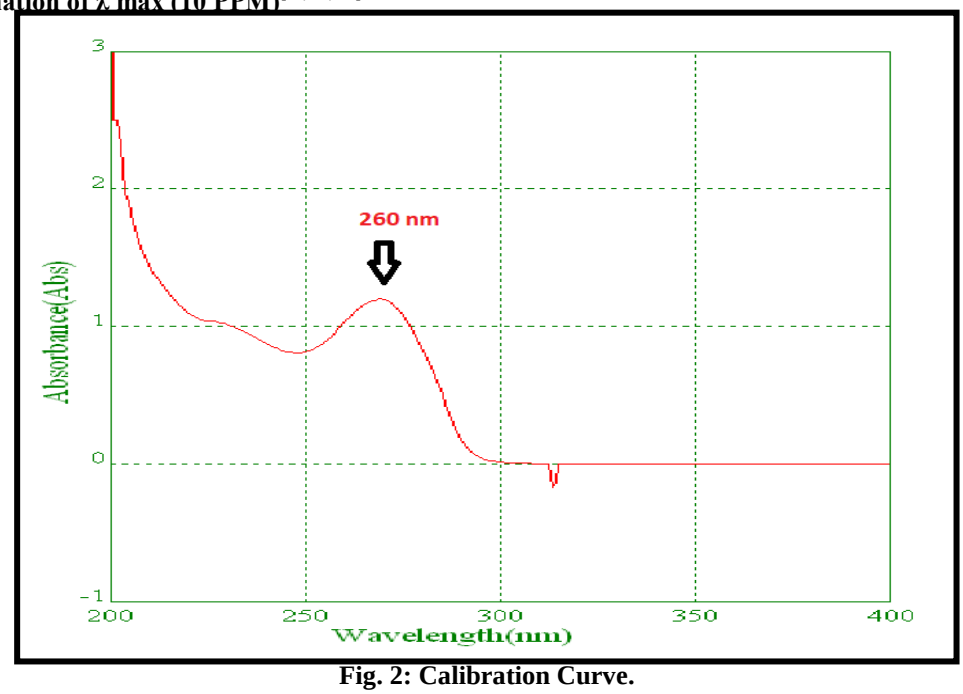


**Fig. 2: Calibration Curve.**

50 mg weighed amount of Ivermectin was dissolved into 100 ml of volumetric flask with analytical grade methanol. Pipette out 1 ml and added in 50 ml of volumetric flask dissolved and diluted up to the mark with analytical grade methanol. This solution was subjected to scanning between 200-400 nm and absorption maximum was determined.

**B. Preparation of Working concentration**

**Preparation of Standard stock solution**

Standard stock was prepared by dissolving 50 mg of Ivermectin in 100 ml of analytical grade methanol to get concentration of 500 µg/ml (PPM).

**Preparation of Standard solution**

Pipette out 0.5 ml from standard stock solution and diluted up to 50 ml with analytical grade methanol to get concentration of 5 µg/ml (PPM).

**C. Procedure for UV reading**

**Blank Solution:** (For Auto zero)

Fill the cuvette with analytical grade methanol. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

**Standard Solution**

Fill the cuvette with standard solution. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

**Sample Solution**

Fill the cuvette with sample solution. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

**D. Procedure for sample preparations[15,16,17]**

For analysis of commercial formulations; twenty tablets are taken weighed it and powdered. The powder equivalent to 50 mg of Tofactinib was accurately weighed and transferred into the 100 ml of volumetric flask, added 70 ml analytical grade methanol, the solution was sonicated for 20 min. After sonication cool the flask and diluted upto 100 ml with analytical grade

methanol. Filtered the solution through nylon syringe filter 0.45 µ. Pipette out 0.5 ml of the filtered solution and diluted up to 50 ml with analytical grade methanol. The absorbance was measured at 260 nm. The absorbance was recorded.

**Table 1: Absorbance of Dosage Form.**

| Ausmed Life Science Pvt. Ltd. (12 mg) | | |
|---|---|---|
| **Sr. no.** | **Sample** | **Absorbance** |
| 1 | Blank | 0.0000 |
| 2 | Standard | 0.5523 |
| 3 | Sample | 0.5478 |

**Table 2: Dosage Form Specifications.**

| No. | Brand / Company | M.D. | E.D. | Batch No. | Avg wt (g) | Assay (%) |
|---|---|---|---|---|---|---|
| 1 | IVERTAC - 12 Ausmed Life Science (12mg) | 05/2023 | 02/2026 | B001547 | 0.102 | 99.19 |

**E. Method of validation[14,16]**

The proposed method was developed by using linearity, accuracy, precision and ruggedness as per ICH guidelines, 1996.

**Linearity**

The linearity of the proposed assay was studied in the concentration range 1 - 9 PPM at 260 nm. The calibration data showed a linear relationship between concentrations.

**Table 3: Linearity Studies.**

| Sr. no. | Sample Concentration | Absorbance |
|---|---|---|
| 1 | 1 PPM | 0.1098 |
| 2 | 3 PPM | 0.3154 |
| 3 | 5 PPM | 0.5428 |
| 4 | 7 PPM | 0.7447 |
| 5 | 9 PPM | 0.9526 |
| **Correlation coefficient** | | 0.999 |

**Accuracy**

To ensure the accuracy of the method, recovery study was performed by preparing 3 sample solutions of 80, 100 and 120% of working concentration and adding a known amount of active drug to each sample solution and dissolved in 100 ml of volumetric flask with analytical grade methanol and measuring the absorbance at 260nm.

**Table 4: Accuracy Studies.**

| SPECTROPHOTOMETRIC METHOD | | | |
|---|---|---|---|
| **Accuracy (%)** | **Qty weighed (mg)** | **Qty found (mg)** | **Recovery (98-102%)** |
| 80 | 0.8 | 0.78 | 98.75 |
| 100 | 1 | 0.99 | 99.00 |
| 120 | 1.2 | 1.19 | 99.17 |

**Precision**

The precision of the method was demonstrated by inter-day and intra-day variation studies. Five sample solutions were made and the %RSD was calculated.

**Table 5: Precision studies.**

| Sr. No. | Sample Solution | Absorbance |
|---|---|---|
| 1 | Sample Solution 1 | 0.5414 |
| 2 | Sample Solution 2 | 0.5364 |
| 3 | Sample Solution 3 | 0.5421 |
| 4 | Sample Solution 4 | 0.5436 |
| 5 | Sample Solution 5 | 0.5322 |
| **MEAN** | | 0.5391 |
| **SD** | | 0.0047 |
| **% RSD** | | 0.8770 ~ 0.88 |

**Ruggedness**

Ruggedness is a measure of the reproducibility of a test result under normal, expected operating condition from instrument to instrument and from analyst to analyst.

**Table 6: Results for Ruggedness Studies.**

| Sr. No. | Analyst | Results | Mean | % Assay | % RSD |
|---|---|---|---|---|---|
| 1 | Analyst 1 | 0.5421 | 0.5425 | 98.22 | 1.1592 ~ 1.16 |
| | | 0.5428 | | | |
| 2 | Analyst 2 | 0.5447 | 0.5467 | 99.84 | |
| | | 0.5486 | | | |

## RESULTS

### 1. Solubility of Ivermectin

Solubility test was passed as per criteria.

**Table 7: Results for solubility studies.**

| Sr. no. | Title | Result |
|---|---|---|
| 1 | Methanol | Freely Soluble |
| 2 | Ethanol | Soluble |
| 3 | Water | Very Slightly Soluble |

### 2. Melting point of Ivermectin

The melting point of Ivermectin was found to be 155 - 156˚C (uncorrected).

### 3. Results for linearity for assay method of Ivermectin

The linearity of method was determined at concentration level ranging from 1 to 9 µg/ml (PPM). The correlation coefficient value was found to be **($R^2$) 0.999**

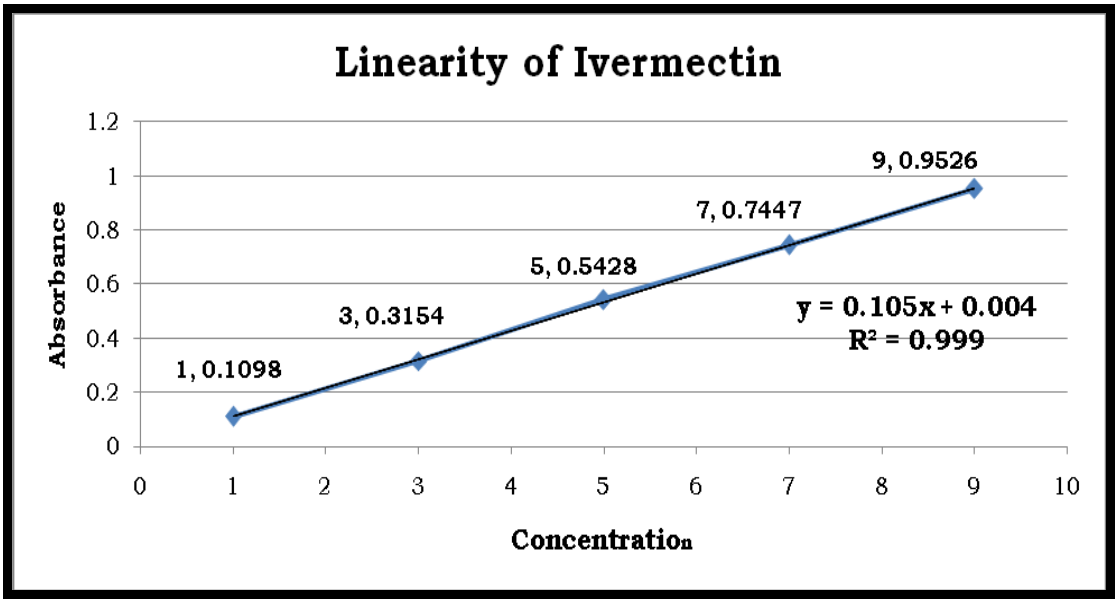


**Fig. 3: Ivermectin Standard Curve.**

### 4. Results for accuracy for assay method of Ivermectin

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out and the percentage recovery were calculated and represented in Table - 4. The high percentage of recovery indicates that the proposed method is highly accurate. Accuracy results were found within acceptance criteria that are within 98-102%.

**5. Results for precision for assay method of Ivermectin**

The % RSD for different sample of precision was found to be 1.4410 ~ 1.44 and it is within acceptance criteria represented in Table - 5.

**6. Results for ruggedness for assay method of Ivermectin**

The % RSD for different sample of ruggedness was found to be 0.9744 ~ 0.97 and it is within acceptance criteria represented in Table - 6.

## CONCLUSION

A method for the estimation of Ivermectin in tablet form has been developed. From the spectrum of Ivermectin, it was found that the maximum absorbance was 260 nm in analytical grade methanol. A good linear relationship was observed in the concentration range of 1 - 9 µg/ml (PPM). The high percentage recovery indicates high accuracy of the method. This demonstrates that the developed spectroscopic method is simple, linear, accurate, rugged and precise for the estimation of Ivermectin in solid dosage forms. Hence, the method could be considered for the determination of Ivermectin in quality control laboratories.

## ABBREVARTIONS

1. PPM - Parts per Million
2. nm - Nanometer
3. TLC - Thin Layer Chromatography
4. HPLC - High Performance Liquid Chromatography
5. UV - Ultra violet
6. MS - Mass Spectroscopy
7. LC - Liquid Chromatography
8. ICH - International Council for Harmonization
9. RSD - Relative Standard Deviation
10. SD - Standard Deviation
11. Qty - Quantity
12. °C - Degree Celsius
13. M.D. - Manufacturing Date
14. E.D. - Expiry Date
15. µg/ml - Microgram per milliliter
16. Avg - Average
17. Wt - Weight
18. g - gm

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