

# Simultaneous equation method for the estimation of fluconazole and Ivermectin by UV spectrophotometer

Vishal U Thodsare<sup>1\*</sup>, Sager S Kale<sup>2</sup>.

Department of Pharmaceutical Chemistry, Sahyadri College of Pharmacy, Methwade, Sangola, Solapur, Maharashtra, India-413307.

## Abstract:

The simple, accurate, and precise UV spectrophotometric method has developed for simultaneous estimation of Fluconazole and Ivermectin in bulk tablet formulation. Two wavelength 273 nm and 253 nm were selected for estimation of Fluconazole and Ivermectin by simultaneous equation method respectively. UV spectrophotometric method was developed as per ICH guideline using O-phosphoric acid and water Mobile phase. Fluconazole and Ivermectin individually follows the Beer-Lamberts Law over concentration range 120-180 and 4.8-7.2 $\mu$ g/mL, regression of coefficient was found to be  $r^2=0.9966$  and  $r^2=0.9954$  respectively. The percentage recovery was found to be 98 to 101 % at three different levels. The proposed method was successfully applied for determination of Fluconazole and Ivermectin in tablet dosage form as per ICH guideline the result of the analysis were validated statistically and were found to be satisfactory.

**Key Words:** Fluconazole, Ivermectin, Simultaneous equation, UV Spectrophotometer, Validation.

## INTRODUCTION:

Fluconazole IUPAC name 2-(2,4-difluorophenyl)-1,3-bis(1,2,4-triazol-1-yl)propan-2-ol<sup>1</sup> It was patented by Pfizer in 1981 in United Kingdom and came into commercial use in 1988. Patent expirations occurred in 2004 and 2005. It is on the World Health Organization's List of Essential Medicines. Fluconazole is an antifungal medication used for a number of fungal infection. This includes candidiasis, blastomycosis, coccidiomycosis, cryptococcosis, histoplasmosis, dermatophytosis, and pityriasis versicolor<sup>2</sup>. It is also used to prevent candidiasis in those who are at high risk such as organ transplantation, low birth weight babies and low blood neutrophil counts.

It is a first-generation triazole antifungal medication. Like other imidazole's and triazole's –class antifungal, fluconazole inhibits the fungal cytochrome p450 enzyme 14 $\alpha$  – demethylase<sup>3</sup>. This inhibition prevents the conversion of lanosterol to ergosterol, an essential component of the fungal cytoplasmic membrane<sup>4</sup>.

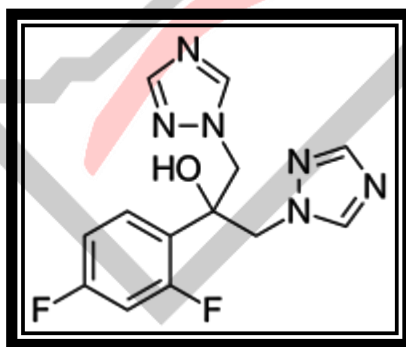


Fig.1 –Structure of Fluconazole

Molecular formula:- C<sub>13</sub>H<sub>12</sub>F<sub>2</sub>N<sub>6</sub>O

Molecular weight:- 306.271g/mol

Ivermectin IUPAC name 22,23-dihydroavermectin B<sub>1a</sub> + 22,23 dihydroavermectin B<sub>1b</sub><sup>5</sup>. Ivermectin is an antiparasitic drug. 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 in infestations including head lice, scabies, river blindness, trichuriasis, acariasis, and lymphatic filariasis. It works through many mechanisms to kill the targeted parasites, and can be taken orally or applied to the skin for external infestation<sup>5</sup>.

This lead to an increase in the permeability of the cell membrane to chloride ions with hyper polarization of the never or muscle cell, resulting in paralysis and death of parasite.

During the covid-19 pandemic misinformation has been widely spread claiming that ivermectin is beneficial for treating and preventing covid-19<sup>6789</sup>.

Molecular Formula:-  $C_{47}H_{72}O_{14}(H_2B_{1b})$

Molecular weight:- 875.1g/mol

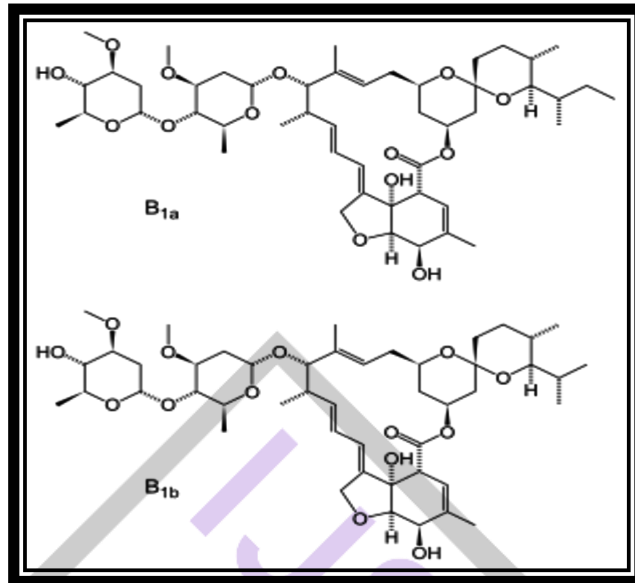


Fig .2- Structure of Ivermactin

## MATERIAL AND METHODS:

### INSTRUMENTS-

For weighing a calibrated weighing balance was used. A double beam UV spectrophotometer (shimadza-1800) was used. All the glasswares which was used made up of Borosilicate glass and they was calibrated.

### Chemicals –

Analytical pure sample fluconazole and ivermectin were received as a gift sample from Mankind Pharma LTD. New Delhi. A pharmaceutical tablet dosage from used in this study in this purchased “Nuforce plus” labelled to contain Fluconazole and Ivermectin 150:6mg per tablet. O-phosphoric acid (AR grade) got from Research lab fine chem. Industry; Mumbai and Distilled Water were used as a mobile phase in this work

### Selection of Wavelength-

UV spectra of Fluconazole and Ivermectin at 260nm and 232nm respectively Mobile phase O-phosphoric acid and water was used for good peak, good absorbance and better sensitivity.

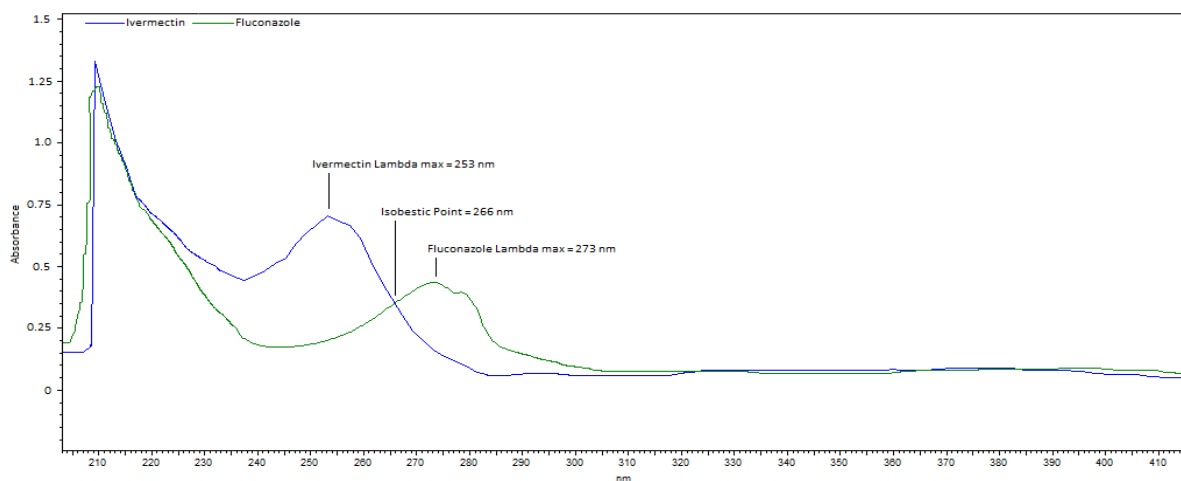


Fig 3- overlain spectra of Fluconazole and Ivermactin

**Preparation of Mobile phase (Diluent)**

ml O-phosphoric acid added in 100 ml of water, mix and Filtered

**Preparation of Standard Stock Solution of Fluconazole-**

Preparation standard stock solution of Fluconazole by adding 10mg of Fluconazole 15 ml volumetric flask and add 5ml diluent and mix and sonicate for 5 minutes. Make up to volume to 10ml with diluent. (Conc.1500µg/ml).Pipette out 1.0ml of standard stock solution in 10ml volumetric flask and make up the volume with diluent. (Conc.150µg/ml)

**Preparation of Standard Stock Solution of Ivermectin-**

Preparation of standard stock solution of Ivermectin by adding 6mg of ivermectin 10ml volumetric flask and adds 5 ml diluent and mix and sonicate for 5 minutes. Make up to volume to 10ml with diluent. (600µg/ml).Pipette out 1.0ml of standard stock solution in 10ml volumetric flask and make up the volume with diluent. (Conc.6µg/ml)

**Simultaneous equation method:-**

In order to observe feasibility of proposed method for simultaneous estimation of Fluconazole and Ivermectin in formulations, the method was tried on standard mixture of different concentration of both the drug were prepared in mobile phase. Absorbance of Fluconazole (150µg/mL) and Ivermectin (6µg/mL) were recorded at wavelength 273 nm and 253 nm respectively using simultaneous estimation method.

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

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

Where,

C<sub>x</sub> = Concentration of Fluconazole

C<sub>y</sub> = Concentration of Ivermectin

a<sub>x1</sub>= Absorptivity value of Fluconazole at 273nm

a<sub>x2</sub>= Absorptivity value of Fluconazole at 253nm

a<sub>y1</sub>= absorptivity value of Ivermectin at 273nm

a<sub>y2</sub> = absorptivity value of Ivermectin at 253nm

A<sub>1</sub>= absorbance of standard sample at 273nm

A<sub>2</sub>= absorbance of standard sample at 253nm

**Analysis of marketed formulation:-**

Ten tablets of brand name “Nuforce plus” were accurately weighed to calculate the average weight, and crush the tablets in mortar and pestle and the powder equivalent to 15mg Fluconazole and 0.6mg Ivermectin was weighed accurately and transferred to 10ml volumetric flask and 5-6 ml of mobile phase was added and sonicate for 2 minutes and make up to the mark with mobile phase. (Conc.of Fluconazole =1500µg/mL and Ivermectin =60µg/mL).Further 1ml of the above solution was pipetted out in 10ml volumetric flask and mix with 5 ml mobile phase and make up to the mark with diluent.(Conc.of Fluconazole 150µg/ml and Ivermectin 6µg/ml)

**Method Validation:-**

Validation of an analytical method is a process to establish to performance characteristics of the developed method to meet the requirement of the intended analytical application. The UV method is validated in terms of linearity, accuracy, precision, LOD and LOQ.

**Linearity:-**

Linearity was studied by plotting graph of absorbance v/s concentration and was found to be directly proportional Fluconazole was found to be linear in range of 120-180µg/mL and Ivermectin was found to be linear in range of 4.8-7.2µg/mL.so series of standard stock solution of Fluconazole were prepared in the concentration range about 120-180µg/mL and Ivermectin were prepared in concentration in rang of 4.8-7.2µg/mL is shown in below table no.(3).

Table No.1. Concentration Range and Absorbance of Ivermectin:-

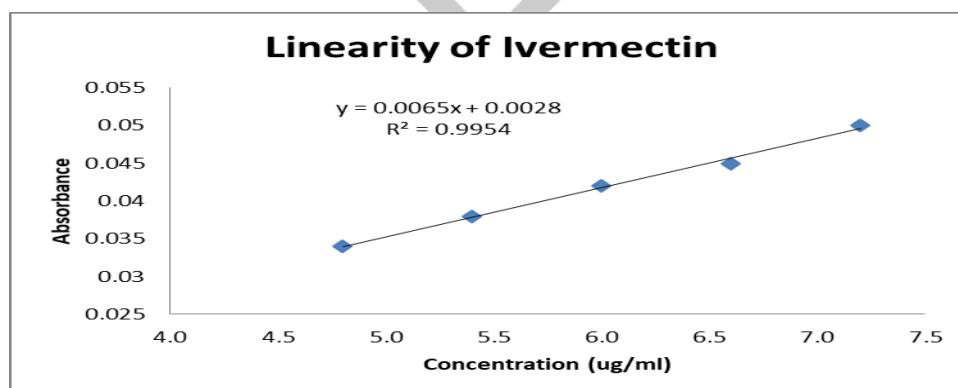
| Ivermectin |                       |            |
|------------|-----------------------|------------|
| % Level    | Concentration (ug/ml) | Absorbance |
| 80         | 4.8                   | 0.034      |
| 90         | 5.4                   | 0.038      |
| 100        | 6.0                   | 0.042      |
| 110        | 6.6                   | 0.045      |
| 120        | 7.2                   | 0.05       |

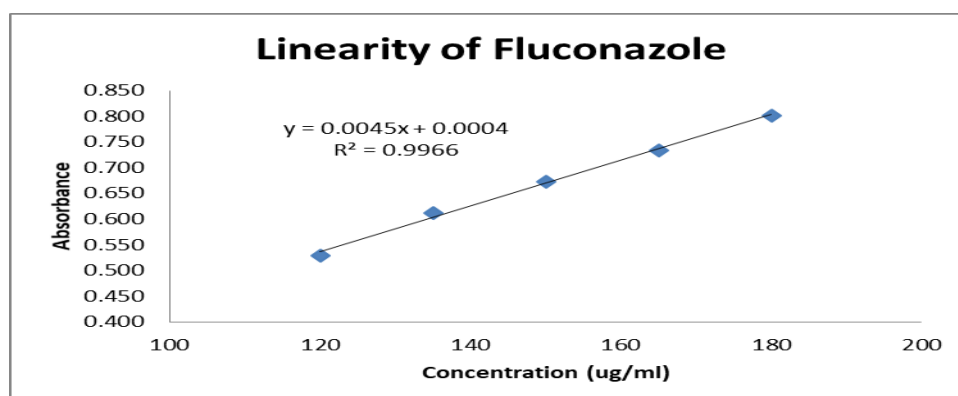
Table No. 2. Concentration Range and Absorbance of Fluconazole:-

| Fluconazole |                       |            |
|-------------|-----------------------|------------|
| % Level     | Concentration (ug/ml) | Absorbance |
| 80          | 120                   | 0.528      |
| 90          | 135                   | 0.611      |
| 100         | 150                   | 0.673      |
| 110         | 165                   | 0.734      |
| 120         | 180                   | 0.801      |

Table no.3. Linearity values of Fluconazole and Ivermectin

| Parameter               | Ivermectin         | Fluconazole        |
|-------------------------|--------------------|--------------------|
| Rang                    | 4.8-7.2 $\mu$ g/mL | 120-180 $\mu$ g/mL |
| Slope                   | 0.0065             | 0.0045             |
| Intercept               | 0.0028             | 0.0004             |
| Correlation Coefficient | 0.9954             | 0.9966             |





### Precision / Repeatability:-

A single sample was prepared as described and 6 injection were made from same sample checked for RSD. The result for Repeatability is shown in below (table No.8)

Precision studies were carried in term of intra-day and inter day. The %relative standard deviation (%RSD) values were found to be less than 2 which indicate the method is accurate. The result for intra-day precision is show in below (table No.4&5) and for inter-day precision is shown (table no.6&7)

Table No.4. Intra-day precision study Ivermactin

| Ivermactin |            |            |        |         |            |            |            |
|------------|------------|------------|--------|---------|------------|------------|------------|
| % level    | Conc.µg/mL | Absorbance |        |         | Mean       | SD         | %RSD       |
|            |            | Trial 1    | Trial2 | trial 3 |            |            |            |
| 90         | 5.4        | 0.037      | 0.038  | 0.037   | 0.03733333 | 0.00057735 | 1.54647394 |
| 100        | 6          | 0.043      | 0.042  | 0.043   | 0.04266667 | 0.00057735 | 1.35316469 |
| 110        | 6.6        | 0.044      | 0.045  | 0.044   | 0.04433333 | 0.00057735 | 1.30229384 |

Table No.5. Intra-day precision study Fluconazole

| Fluconazole |            |            |         |         |            |            |            |
|-------------|------------|------------|---------|---------|------------|------------|------------|
| %level      | Conc.µg/mL | Absorbance |         |         | Mean       | SD         | %RSD       |
|             |            | Trial 1    | Trial 2 | Trial 3 |            |            |            |
| 90          | 135        | 0.615      | 0.611   | 0.61    | 0.612      | 0.00264575 | 0.43231231 |
| 100         | 150        | 0.67       | 0.673   | 0.676   | 0.673      | 0.003      | 0.44576523 |
| 110         | 165        | 0.73       | 0.734   | 0.737   | 0.73366667 | 0.00351188 | 0.47867577 |

Table No.6. Inter-day precision study Ivermactin.

| Ivermactin |            |            |         |         |          |          |          |
|------------|------------|------------|---------|---------|----------|----------|----------|
| % level    | Conc.µg/mL | Absorbance |         |         | mean     | SD       | %RSD     |
|            |            | Trial 1    | Trial 2 | Trial 3 |          |          |          |
| 90         | 5.4        | 0.031      | 0.032   | 0.032   | 0.031667 | 0.000577 | 1.823211 |
| 100        | 6          | 0.037      | 0.038   | 0.037   | 0.037333 | 0.000577 | 1.546474 |
| 110        | 6.6        | 0.042      | 0.042   | 0.041   | 0.041667 | 0.000577 | 1.385641 |

Table No.7. Inter-day precision study Fluconazole.

| Fluconazole |            |            |         |         |          |          |          |
|-------------|------------|------------|---------|---------|----------|----------|----------|
| %level      | Conc.µg/mL | Absorbance |         |         | Mean     | SD       | %RSD     |
|             |            | Trial 1    | Trial 2 | Trial 3 |          |          |          |
| 90          | 135        | 0.599      | 0.602   | 0.607   | 0.602667 | 0.004041 | 0.670595 |
| 100         | 150        | 0.66       | 0.662   | 0.668   | 0.663333 | 0.004163 | 0.627638 |
| 110         | 175        | 0.72       | 0.725   | 0.728   | 0.724333 | 0.004041 | 0.557955 |

Table no.8. Repeatability Ivermectin and Fluconazole

| Sample ID    | Ivermectin ABS | Fluconazole ABS |
|--------------|----------------|-----------------|
| 100% Rep 1   | 0.042          | 0.673           |
| 100% Rep 2   | 0.041          | 0.675           |
| 100% Rep 3   | 0.042          | 0.674           |
| 100% Rep 4   | 0.043          | 0.672           |
| 100% Rep 5   | 0.042          | 0.671           |
| 100% Rep 6   | 0.043          | 0.672           |
| <b>AVG</b>   | 0.042          | 0.673           |
| <b>STDEV</b> | 0.001          | 0.00            |
| <b>RSD</b>   | 1.785          | 0.22            |

**Accuracy (recovery study)-**

To check the accuracy of the developed method and to study the interference of formulation additives analytical recovery experiment was carried out by standard addition method. The recovery studies were carried out in three levels i.e. 80% 100% 120%, to assure the reliability of the above method recovery studies were carried out by mixing a known quantity of the standard drug with the penalized sample formulation and the contents were reanalysed by the proposed method. The recovery values were within the limits indicating that the method is accurate. The % recovery values were shown in the below (table no.9&10)

Table no.9. Accuracy of Ivermectin

| Ivermectin |       |                     |       |                          |            |        |       |      |
|------------|-------|---------------------|-------|--------------------------|------------|--------|-------|------|
| % Level    | Reps  | Spiked Conc (ug/ml) | Abs   | Amount Recovered (ug/ml) | % Recovery | AVG    | STDEV | RSD  |
| 80         | Rep 1 | 4.80                | 0.034 | 4.86                     | 101.19     | 100.20 | 1.72  | 1.71 |
|            | Rep 2 | 4.80                | 0.033 | 4.71                     | 98.21      |        |       |      |
|            | Rep 3 | 4.80                | 0.034 | 4.86                     | 101.19     |        |       |      |
| 100        | Rep 1 | 6.00                | 0.042 | 6.00                     | 100.00     | 99.21  | 1.37  | 1.39 |
|            | Rep 2 | 6.00                | 0.041 | 5.86                     | 97.62      |        |       |      |
|            | Rep 3 | 6.00                | 0.042 | 6.00                     | 100.00     |        |       |      |
| 120        | Rep 1 | 7.20                | 0.05  | 7.14                     | 99.21      | 99.87  | 1.15  | 1.15 |
|            | Rep 2 | 7.20                | 0.051 | 7.29                     | 101.19     |        |       |      |
|            | Rep 3 | 7.20                | 0.05  | 7.14                     | 99.21      |        |       |      |

Table no. 10. Accuracy of Fluconazole

| Fluconazole |       |                     |       |                          |            |        |       |      |
|-------------|-------|---------------------|-------|--------------------------|------------|--------|-------|------|
| % Level     | Reps  | Spiked Conc (ug/ml) | Abs   | Amount Recovered (ug/ml) | % Recovery | AVG    | STDEV | RSD  |
| 80          | Rep 1 | 120.00              | 0.528 | 117.68                   | 98.07      | 97.88  | 0.19  | 0.19 |
|             | Rep 2 | 120.00              | 0.527 | 117.46                   | 97.88      |        |       |      |
|             | Rep 3 | 120.00              | 0.526 | 117.24                   | 97.70      |        |       |      |
| 100         | Rep 1 | 150.00              | 0.673 | 150.00                   | 100.00     | 100.15 | 0.15  | 0.15 |
|             | Rep 2 | 150.00              | 0.675 | 150.45                   | 100.30     |        |       |      |
|             | Rep 3 | 150.00              | 0.674 | 150.22                   | 100.15     |        |       |      |
| 120         | Rep 1 | 180.00              | 0.801 | 178.53                   | 99.18      | 99.22  | 0.19  | 0.19 |
|             | Rep 2 | 180.00              | 0.800 | 178.31                   | 99.06      |        |       |      |
|             | Rep 3 | 180.00              | 0.803 | 178.97                   | 99.43      |        |       |      |

**Robustness:-**

The analytical technique's robustness is a measure of its ability to remain unaffected by tiny but deliberate modification in method of parameters, and it gives an indicator of its dependability in routine use.

Table no. 11. Assay of Ivermectin and Fluconazole:-

| Sample       | Ivermectin   |         | Fluconazole  |         |
|--------------|--------------|---------|--------------|---------|
|              | Conc (ug/ml) | % Assay | Conc (ug/ml) | % Assay |
| DP-1         | 5.81         | 96.83   | 149.57       | 99.71   |
| DP-2         | 5.96         | 99.33   | 149.35       | 99.57   |
| DP-3         | 5.92         | 98.67   | 148.92       | 99.28   |
| DP-4         | 5.99         | 99.83   | 148.21       | 98.81   |
| DP-5         | 5.89         | 98.17   | 149.35       | 99.57   |
| <b>AVG</b>   |              | 98.57   | <b>AVG</b>   | 99.39   |
| <b>STDEV</b> |              | 1.16    | <b>STDEV</b> | 0.36    |
| <b>RSD</b>   |              | 1.18    | <b>RSD</b>   | 0.36    |

**LOD & LOQ:-**

LOD & LOQ were calculated by using AVONA Technique.

$$LOD = \frac{3.3 \times \text{Std Error of Intercept}}{\text{Coefficient of X variable 1}}$$

$$LOD = \frac{10 \times \text{Std Error of Intercept}}{\text{Coefficient of X variable 1}}$$

Table no.12: LOD&amp;LOQ Values

| Sr.no | Name of Drug | LOD in µg/mL | LOQ in µg/mL |
|-------|--------------|--------------|--------------|
| 1     | Fluconazole  | 16.88µg/mL   | 51.16µg/mL   |
| 2     | Ivermactin   | 0.78µg/mL    | 2.37µg/mL    |

Table no: - LOD&LOQ values

## RESULT & DISCUSSION:

The present work provides an accurate, rapid sensitive method for simultaneous estimation of Fluconazole and Ivermactin in bulk tablet formulation. Linear relationship between drug concentrations was obtained over the range of at 120-180& 4.8-7.2 µg/mL for Fluconazole and Ivermactin respectively. The correlation coefficient, slop and intercept obtained for each drug is shown in table no.(3) The proposed method was also successfully applied to a pharmaceutical formulation. The precision of the method with intra- day and inter-day was found to good %RSD less than 2 and Repeatability 6 injection were made from same sample to checked %RSD indicates that the method was precise and the the results presented in table no (4&5) and (6&7)and repeatability to result present in table no (8) for Fluconazole % recovery ranges from 98.07 to 99.43 with % RSD 0.19 to 0.19 for Ivermactin % recovery ranges from to 100.20 to99.87 with %RSD 1.71 to 1.15. The % assay was found to be 99.39 for Fluconazole and 98.57 for Ivermactin for the result is present in table no.(11 ). The LOD values of fluconazole and Ivermactin was found to be 16.88µg/mL and 0.78µg/mL. & LOQ values of Fluconazole and Ivermactin was found to be 51.16µg/mL and 2.37µg/mL respectively. No interference was found to spectrogram of formulation within the absorbance indicating that excipients used in tablet formulation did not interfere with simultaneous estimation of Fluconazole and Ivermactin in tablet formulation.

## CONCLISION:-

The recently developed UV spectrometric method for determination of Fluconazole and Ivermactin simultaneously is simple, specific accurate, precise rapid and economical which indicates its competence for routine pharmaceutical analysis of Fluconazole and Ivermactinin bulk tabletfomulation. It is conclude that HPLC method is successfully utilize for estimation of Fluconazole and Ivermactin this new method has been successfully applied for routine analysis.

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