INTRODUCTION

Ivermectin (Fig. 1) (N-[3-(3-cyanopyrazolo[1,5-a]pyrimidin-7-yl)phenyl]-N-ethylacetamide) is a pyrazolopyrimidine derivative that acts as an effective hypnentic and selectively binds to the α1 benzodiazepine receptors [1]. It is being used for treating certain worm infections, onchocerciasis (river blindness), and some types of diarrhea (strongyloidiasis). It paralyzes and kills the offspring of adult worms which slowdowns the rate of reproduction of the adult worm that results in fewer worms in skin, eyes, and blood [2]. Its molecular formula is C_{15}H_{15}N_{5}O and has a molecular weight of about 305.33 g/mol [3]. The drug is official in Indian, British, and the United States Pharmacopoeia. The literature survey reveals on about 305.33 g/mol [3]. The drug is official in Indian, British, and the United States Pharmacopoeia. The literature survey reveals on the reported methods for the determination of ivermectin which includes spectrophotometry [4], capillary electrophoresis [5], high-performance thin-layer chromatography [6], high-performance liquid chromatography (LC) [7], LC–tandem mass spectrometry (MS) [8], and a gas chromatography–MS [9] either in pharmaceutical formulations or in biological fluids.

Spectrophotometric methods due to their inherent rapidity, simplicity of procedures, and low cost of the technique are employed as the favorable method in most of the laboratories [10,11]. The opted method is based on the direct determination of ivermectin with high range of accuracy and precision. The method is simple, economical and can be applied to estimate ivermectin in bulk drug and pharmaceuticals. The proposed method characterizes the use of UV spectr multi variate calibration technique employing simple mathematical contents for the estimation of ivermectin in pharmaceutical dosage form.

Multivariate calibration represents the conversion of single common species analysis from one dependent variable to “m” dependent variables, for example, wavelengths or sensors, which can be simultaneously included in the calibration model [12].

Under optimized conditions, the functional statistical method gives appreciable resolving power, sensitivity, rapidity and low cost for the quantitative analysis, quality control, and routine analysis of the investing compounds [13].

The absorbance of an analyte (X) is measured at five wavelengths set (λ=226, 228, 230, 232, and 234 nm), the following equation can be written for individual selected wavelength.

\[ A_{\lambda} = aX + k_{1} \]  \hspace{1cm} (1)

\[ A_{\lambda} = bX + k_{2} \]  \hspace{1cm} (2)

\[ A_{\lambda} = cX + k_{3} \]  \hspace{1cm} (3)

\[ A_{\lambda} = dX + k_{4} \]  \hspace{1cm} (4)

\[ A_{\lambda} = eX + k_{5} \]  \hspace{1cm} (5)

Where, \( A_{\lambda} \) represents the absorbance of the analyte; \( a, b, c, d, \) and \( e \) are the slopes of the linear regression functions of the analyte; \( k_{1}, k_{2}, k_{3}, k_{4}, \) and \( k_{5} \) are the intercepts of the linear regression functions at the five selected bandwidth and \( C_{i} \) represents the concentration of the analyte.

The above five equation systems (1-5) can be summarized as follows:

\[ A_{\lambda} = a_{1}C_{1} + b_{1}C_{2} + c_{1}C_{3} + d_{1}C_{4} + e_{1}C_{5} + K_{1} \]  \hspace{1cm} (6)

Which can be further simplified to

\[ A_{\lambda} = C_{i}(a+b+c+d+e)+K_{1} \]  \hspace{1cm} (7)
Where, $A_T$ and $K_T$ represent the sum of the absorbance obtained and the sum of intercepts of regression equations at five wavelength set, respectively. The concentration of the analyte $X$ in a solution can be calculated using the equation:

$$C_X = \frac{A_T - K_T}{(a + b + c + d + e)}$$  \hspace{1cm}  (8)

**Experimental**

**Chemicals and solvents**
- Distilled water
- Ethanol
- The reference standard of ivermectin was obtained as gift sample from Pondchy Pharmaceuticals, Puducherry. The marketed tablet formulation used was rapimec (label claim - 10 mg of Ivermectin), from the same pharmaceutical company.

**Solubility**
- Very freely soluble in methanol, ethanol, acetone, ethyl acetate, and acetonitrile.

**Instrumentation**
- Labindia UV-visible double beam spectrophotometer
- Sonicator
- Electric balance.

**Method development**

Selection of solvent
Ivermectin was freely soluble in ethanol which was used as the solvent to solubilize the standard drug and the sample as well.

Preparation of standard stock solution
The standard stock solution of ivermectin was prepared by dissolving 10 mg of the drug in 10 mL of the solvent to obtain a concentration of 1 mg/mL. The above solution was further diluted to get concentrations in the range of 5–15 µg/mL.

Determination of $\lambda_{\text{max}}$
The stock solution of ivermectin was diluted with the solvent to get a concentration in the range of 5–15 µg/mL. Now, to develop correlation and to reduce the instrumental fluctuations, absorbance of the above solutions was recorded in several selected wavelengths around the $\lambda_{\text{max}}$ of the drug (245 nm), that is, 239, 241, 243, 245, 247, 249, and 251 nm.

The overlay UV spectra showing linearity at the $\lambda_{\text{max}}$ (245 nm) were represented in Fig. 3. The absorbance values of the five different selected concentrations were recorded and represented in Table 1. Linearity data showing system suitability parameters at the selected wavelengths are shown in Table 2.

**Precision**
Intraday and interday precision was performed by measuring the absorbance of the solutions of concentration 5, 10, and 15 µg/mL at all the seven selected wavelengths. Each concentration was scanned 3 times a day (intraday precision) and for three different days (interday precision). The absorbance values recorded at the selected wavelengths for intraday and interday precision were provided in Tables 3 and 4. The SD and percentage relative standard deviation (RSD) values obtained at different wavelengths were calculated and represented in Tables 5 and 6. The overlay UV spectra for intra- and inter-day precision are shown in Figs. 18 and 19.

**Assay**
The absorbance of the extracted sample solution was recorded at 245 nm and the amount of drug present in the formulation was estimated (Table 7).

**Method validation**
The developed method was validated as per the ICH Q2 R1 guidelines to check validation parameters such as linearity, sensitivity, precision, and accuracy [14].

**Linearity**
Stock solution of ivermectin was diluted with the solvent to get a concentration in the range of 5–15 µg/mL. Now, to develop correlation and to reduce the instrumental fluctuations, absorbance of the above solutions was recorded in several selected wavelengths around the $\lambda_{\text{max}}$ of the drug (245 nm), that is, 239, 241, 243, 245, 247, 249, and 251 nm.

The overlay UV spectra showing linearity at the $\lambda_{\text{max}}$ (245 nm) were represented in Fig. 3. The absorbance values of the five different selected concentrations were recorded and represented in Table 1. Linearity data showing system suitability parameters at the selected wavelengths are shown in Table 2.

The calibrations graphs and the residual plots at the seven selected wavelengths were represented in Figs. 4-10 and Figs. 11-17, respectively.

**Accuracy (recovery studies)**
The accuracy of the developed method was evaluated by standard addition method at 80%, 100%, and 120% of the selected concentration levels. From the prepared stock solutions of standard and sample, 0.4 mL of standard solution was pipetted into three different volumetric flasks and 0.1, 0.6, and 1.1 mL of the sample solution were added to the
above volumetric flasks and the volume was made up to 10 mL with ethanol. The percentage recovery values were calculated. The results representing recovery studies are shown in Fig. 20 and provided in Table 8.

**RESULTS AND DISCUSSION**

The λ\textsubscript{max} of ivermectin was found to be 245 nm employing ethanol as the solvent (Fig. 2).

**Linearity**

The UV spectra showing linearity at 245 nm are represented in Fig. 3. The linearity for different prepared concentration of 5–15 µg/mL was

Table 1: Multivariate UV calibration at seven selected wavelengths

| Concentration (µg/mL) | Absorbance (nm) |
|-----------------------|-----------------|
|                       | 239  | 241  | 243  | 245  | 247  | 249  | 251  |
| 5                     | 0.158| 0.160| 0.167| 0.171| 0.158| 0.135| 0.119|
| 7.5                   | 0.240| 0.243| 0.254| 0.261| 0.242| 0.207| 0.181|
| 10                    | 0.319| 0.324| 0.340| 0.349| 0.324| 0.276| 0.240|
| 12.5                  | 0.402| 0.408| 0.426| 0.437| 0.405| 0.346| 0.301|
| 15                    | 0.480| 0.487| 0.510| 0.524| 0.486| 0.415| 0.361|

Table 2: Linearity data showing system suitability parameters at the selected wavelengths

| Wave length (nm) | Regression equation  | Slope | Intercept | % intercept | r\textsuperscript{2} | LOD (µg/mL) | LOQ (µg/mL) |
|------------------|-----------------------|-------|-----------|-------------|----------------|-------------|-------------|
| 239              | y=0.0322x−0.0026      | 0.0322| 0.0026    | 0.26        | 0.9999        | 0.039       | 0.117       |
| 241              | y=0.0328x−0.0032      | 0.0328| 0.0032    | 0.32        | 0.9999        | 0.105       | 0.319       |
| 243              | y=0.0339x−0.0028      | 0.0339| 0.0028    | 0.28        | 0.9990        | 0.068       | 0.206       |
| 245              | y=0.0353x−0.0064      | 0.0353| 0.0064    | 0.64        | 0.9991        | 0.029       | 0.087       |
| 247              | y=0.0328x−0.0046      | 0.0328| 0.0046    | 0.46        | 0.9999        | 0.019       | 0.058       |
| 249              | y=0.028x−0.0026       | 0.028 | 0.0026    | 0.26        | 0.9993        | 0.036       | 0.110       |
| 251              | y=0.0243x−0.003       | 0.0243| 0.003     | 0.3         | 0.9999        | 0.034       | 0.104       |
recorded at the selected wavelengths of about 239, 241, 243, 245, 247, 249, and 251 nm. The observed results were tabulated in Table 1.

All the calibration curves were found to be linear over the selected concentration range of about 5–15 µg/mL. The linear regression analysis data of the constructed calibration plots showed good linear relation with a correlation coefficient ($r^2$) > 0.998. The calibration

| Concentration (µg/mL) | Number of repetitions | Absorbance (nm) |
|-----------------------|-----------------------|-----------------|
|                       |                       | 239 | 241 | 243 | 245 | 247 | 249 | 251 |
| 5                     | 1                     | 0.151| 0.154| 0.151| 0.165| 0.151| 0.121| 0.112|
|                       | 2                     | 0.15 | 0.152| 0.153| 0.166| 0.15  | 0.123| 0.111|
|                       | 3                     | 0.154| 0.154| 0.154| 0.168| 0.154 | 0.121| 0.113|
| 10                    | 1                     | 0.317| 0.324| 0.337| 0.341| 0.318 | 0.269| 0.232|
|                       | 2                     | 0.318| 0.323| 0.339| 0.347| 0.32  | 0.271| 0.236|
|                       | 3                     | 0.312| 0.321| 0.332| 0.34  | 0.313 | 0.266| 0.231|
| 15                    | 1                     | 0.464| 0.47 | 0.494| 0.505| 0.466 | 0.395| 0.345|
|                       | 2                     | 0.473| 0.48 | 0.504| 0.515| 0.475 | 0.403| 0.352|
|                       | 3                     | 0.464| 0.471| 0.494| 0.509| 0.466 | 0.395| 0.344|

Table 3: Absorbance values for intraday precision

| Concentration (µg/mL) | Number of repetitions | Absorbance (nm) |
|-----------------------|-----------------------|-----------------|
|                       |                       | 239 | 241 | 243 | 245 | 247 | 249 | 251 |
| 5                     | 1                     | 0.141| 0.142| 0.150| 0.157| 0.141| 0.120| 0.101|
|                       | 2                     | 0.143| 0.142| 0.152| 0.153| 0.143| 0.122| 0.101|
|                       | 3                     | 0.140| 0.141| 0.151| 0.153| 0.142| 0.121| 0.102|
| 10                    | 1                     | 0.300| 0.303| 0.332| 0.331| 0.310| 0.255| 0.220|
|                       | 2                     | 0.305| 0.301| 0.333| 0.334| 0.315| 0.261| 0.222|
|                       | 3                     | 0.300| 0.302| 0.331| 0.340| 0.310| 0.255| 0.221|
| 15                    | 1                     | 0.460| 0.465| 0.490| 0.500| 0.460| 0.392| 0.343|
|                       | 2                     | 0.472| 0.462| 0.500| 0.515| 0.475| 0.403| 0.352|
|                       | 3                     | 0.460| 0.451| 0.492| 0.509| 0.466| 0.395| 0.344|

Table 4: Absorbance values for interday precision

| Concentration (µg/mL) | Description | Absorbance (nm) |
|-----------------------|-------------|-----------------|
|                       |             | 239 nm | 241 nm | 243 nm | 245 nm | 247 nm | 249 nm | 251 nm |
| 5                     | Mean        | 0.152 | 0.153 | 0.153 | 0.166 | 0.152 | 0.122 | 0.112 |
|                       | SD          | 0.002 | 0.002 | 0.002 | 0.002 | 0.002 | 0.001 | 0.001 |
|                       | % RSD       | 1.373 | 0.753 | 1.001 | 0.918 | 1.373 | 0.949 | 0.893 |
| 10                    | Mean        | 0.316 | 0.323 | 0.336 | 0.343 | 0.317 | 0.269 | 0.233 |
|                       | SD          | 0.003 | 0.004 | 0.004 | 0.004 | 0.004 | 0.003 | 0.003 |
|                       | % RSD       | 1.018 | 0.474 | 0.947 | 0.510 | 0.469 | 0.398 | 0.347 |
| 15                    | Mean        | 0.467 | 0.474 | 0.497 | 0.510 | 0.469 | 0.398 | 0.347 |
|                       | SD          | 0.005 | 0.006 | 0.006 | 0.005 | 0.005 | 0.005 | 0.004 |
|                       | % RSD       | 1.113 | 1.163 | 1.161 | 0.988 | 1.108 | 1.161 | 1.256 |

Fig. 7: Calibration graph at 245 nm

Fig. 8: Calibration graph at 247 nm
Table 6: Interday precision

| Concentration (µg/mL) | Description | 239 nm | 241 nm | 243 nm | 245 nm | 247 nm | 249 nm | 251 nm |
|-----------------------|-------------|--------|--------|--------|--------|--------|--------|--------|
| 5                     | Mean        | 0.141  | 0.142  | 0.151  | 0.154  | 0.142  | 0.121  | 0.101  |
|                       | SD          | 0.002  | 0.001  | 0.001  | 0.002  | 0.001  | 0.001  | 0.001  |
|                       | % RSD       | 1.081  | 0.408  | 0.662  | 1.496  | 0.704  | 0.826  | 0.570  |
| 10                    | Mean        | 0.302  | 0.302  | 0.332  | 0.335  | 0.312  | 0.257  | 0.221  |
|                       | SD          | 0.003  | 0.001  | 0.001  | 0.005  | 0.003  | 0.003  | 0.001  |
|                       | % RSD       | 0.957  | 0.331  | 0.301  | 1.368  | 0.926  | 1.348  | 0.452  |
| 15                    | Mean        | 0.464  | 0.459  | 0.494  | 0.508  | 0.467  | 0.397  | 0.346  |
|                       | SD          | 0.007  | 0.007  | 0.005  | 0.008  | 0.008  | 0.006  | 0.005  |
|                       | % RSD       | 1.493  | 1.605  | 1.071  | 1.486  | 1.617  | 1.434  | 1.424  |

Table 7: Assay of ivermectin

| Label claim (mg) | Amount estimated (mg) | % assay |
|------------------|-----------------------|--------|
| 10               | 9.86                  | 98.6   |
|                  | 9.87                  | 98.7   |
|                  | 9.89                  | 98.9   |
| Average          |                       | 98.7   |
| SD               |                       | 0.153  |
| % RSD            |                       | 0.154  |

Fig. 9: Calibration graph at 249 nm

Fig. 11: Residual plot at 239 nm

Fig. 10: Calibration graph at 251 nm

Fig. 12: Residual plot at 241 nm

graphs and the system suitability parameters were presented in Figs. 4-17 and Table 2, respectively.
Precision
Intra- and inter-day precision studies were carried out. The percentage RSD values for intraday and interday precision were found to lie within the range of 0.473–1.373 and 0.301–1.617 which was found well within the acceptance criteria of <2% at all the selected wavelengths. The low percentage RSD values indicate that the suggested method was precise. The results of precision study were represented in Figs. 18-19 and tabulated in Tables 3-6.
Assay
The absorbance of the extracted sample solution was recorded at 245 nm and the amount of drug present in the formulation was estimated. The assay percentage of ivermectin (rapimec tablets) was found to be 98.7% w/w. The amount estimated in the formulation was found to be 9.87 mg and the percentage RSD value was found to be <2% (Table 7).

Recovery
The percentage recovery of the drug by standard addition method was calculated and was found to be in the range of 97.60–101.80% w/w, which was well within the acceptance limit of 97–103% w/w as per the ICH guidelines. Hence, the full-fledged method was found to be accurate. The reports of accuracy study were shown in Fig. 19 and Table 8.

CONCLUSION
The newly developed spectrophotometric multivariate calibration technique was validated by employing various validation parameters as per the ICH guidelines and was found to lie within the acceptance limits. The method developed in the present study was found to be sensitive, accurate, precise, and reproducible for the estimation of ivermectin in its tablet formulation. Therefore, a simple and rapid method using mathematical contents was developed, which was found more predictable than the other spectrophotometric methods and is strongly recommended for the routine quality control analysis of ivermectin in pharmaceutical formulations.

ACKNOWLEDGMENT
Authors are thankful to the Chancellor, SRM Institute of Science and Technology and the management of SRM College of Pharmacy, SRM Institute of Science and Technology, Kattankulathur; and Pondicherry Pharmaceuticals, Puducherry, for providing various reprographic sources for carrying out this research work successfully.

AUTHORS’ CONTRIBUTION
All the authors have contributed equally in designing the analysis, for the collection of data, in performing the analysis, and to write the research work in the instructed manner to frame the final manuscript in a successful manner.

Table 8: Recovery studies

| Wavelength (nm) | Concentration levels (%) | Final concentration (µg/mL) | Amount present (µg/mL) | Amount added (µg/mL) | Amount recovered (µg/mL) | % recovery |
|-----------------|--------------------------|----------------------------|-----------------------|----------------------|--------------------------|------------|
| 239             | 80                       | 5                          | 4                     | 1                    | 4.98                     | 99.60      |
|                 | 100                      | 10                         | 4                     | 6                    | 9.84                     | 98.40      |
|                 | 120                      | 15                         | 4                     | 11                   | 14.86                    | 99.07      |
| 241             | 80                       | 5                          | 4                     | 1                    | 4.99                     | 99.80      |
|                 | 100                      | 10                         | 4                     | 6                    | 9.85                     | 98.50      |
|                 | 120                      | 15                         | 4                     | 11                   | 14.72                    | 98.13      |
| 243             | 80                       | 5                          | 4                     | 1                    | 4.88                     | 97.60      |
|                 | 100                      | 10                         | 4                     | 6                    | 10.01                    | 100.10     |
|                 | 120                      | 15                         | 4                     | 11                   | 15.07                    | 100.47     |
| 245             | 80                       | 5                          | 4                     | 1                    | 5.09                     | 101.80     |
|                 | 100                      | 10                         | 4                     | 6                    | 9.87                     | 98.70      |
|                 | 120                      | 15                         | 4                     | 11                   | 14.96                    | 99.73      |
| 247             | 80                       | 5                          | 4                     | 1                    | 4.96                     | 99.20      |
|                 | 100                      | 10                         | 4                     | 6                    | 9.88                     | 98.80      |
|                 | 120                      | 15                         | 4                     | 11                   | 15.09                    | 100.60     |
| 249             | 80                       | 5                          | 4                     | 1                    | 4.88                     | 97.60      |
|                 | 100                      | 10                         | 4                     | 6                    | 9.99                     | 99.90      |
|                 | 120                      | 15                         | 4                     | 11                   | 15.07                    | 100.47     |
| 251             | 80                       | 5                          | 4                     | 1                    | 5.03                     | 100.60     |
|                 | 100                      | 10                         | 4                     | 6                    | 9.85                     | 98.50      |
|                 | 120                      | 15                         | 4                     | 11                   | 14.95                    | 99.67      |

Fig. 19: Overlay UV spectrum showing interday precision

Fig. 20: Overlay UV spectrum displaying accuracy of ivermectin
CONFLICTS OF INTEREST
The authors report on conflicts of interest on the study.

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