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Research Article

MULTIVARIATE CALIBRATION TECHNIQUE FOR THE SPECTROPHOTOMETRIC QUANTIFICATION OF IVERMECTIN IN PHARMACEUTICAL FORMULATION

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ABSTRACT

Objective: The present abstract makes the use of multivariate calibration technique for the quantification of ivermectin in pharmaceutical dosage form.

Methods: Multivariate calibration technique is based on the use of linear regression equations, by correlating the relation between concentration and absorbance at seven different selected wavelengths. The λ_{max} of ivermectin was found to be 245 nm. The results were treated statistically. This statistical approach gives optimum results by eliminating the fluctuations arising from the instrumental or experimental conditions.

Results: The developed method was validated as per the ICH guidelines and was found to be simple, linear, accurate, precise, and reproducible. The method was found to be linear over a concentration range of 5–15 µg/mL with a correlation coefficient (r^2) value of about 0.9998. The limit of detection and quantification were found to be 0.029 and 0.087 µg/mL, respectively. The percentage relative standard deviation for intraday and interday precision was found to be in the range of 0.473–1.373 and 0.301–1.617, respectively. The percentage recovery was found within the range of 97.60–101.80% w/w.

Conclusion: The results evidence that a simple, linear, precise, accurate, sensitive, and reproducible multivariate calibration technique has been developed and validated for the quantification of ivermectin in bulk and pharmaceutical formulation.

Keywords: Ivermectin, ICH guidelines, Multivariate calibration technique, Pharmaceutical formulation, Validation.

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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 hypnotic and selectively binds to the α 1 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 $C_{17}H_{15}N_5O$ 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 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 spectral multivariate 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.

$$A\lambda_{226} = aXC_x+k_1 \quad (1)$$

$$A\lambda_{228} = bXC_x+k_2 \quad (2)$$

$$A\lambda_{230} = cXC_x+k_3 \quad (3)$$

$$A\lambda_{232} = dXC_x+k_4 \quad (4)$$

$$A\lambda_{234} = eXC_x+k_5 \quad (5)$$

Where, Aλ 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_x represents the concentration of the analyte. The above five equation systems (1-5) can be summarized as follows:

$$A_T = axC_x+bxC_x+cxC_x+dxC_x+exC_x+K_T \quad (6)$$

Which can be further simplified to

$$A_T = C_x\,(a+b+c+d+e)+K_T \quad (7)$$

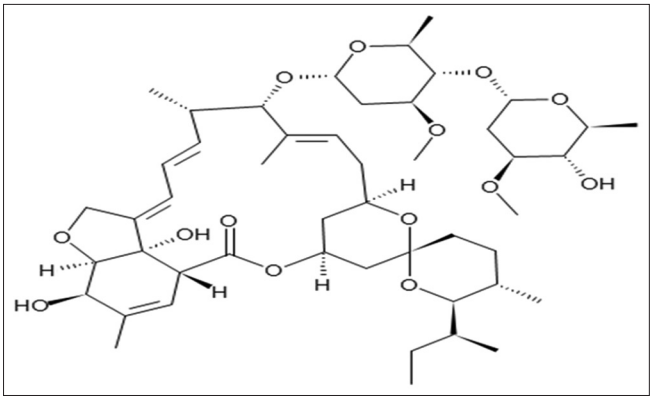

Fig. 1: Chemical structure of ivermectin

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:

$$C_x = \frac{A_T - K_T}{(a+b+c+d+e)} \quad (8)$$

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 λ_{max}

The stock solution of ivermectin was diluted with the solvent to get a concentration of 10 µg/mL. This solution was scanned in the UV region from 400 to 200 nm. The λ_{max} was found to be 245 nm (Fig. 2).

Preparation of sample solution

A total of 20 tablets of ivermectin were weighed and the contents were transferred into a mortar, powdered, and mixed well. A weight equivalent to 10 mg of ivermectin was weighed and dissolved in 5 mL of ethanol. The above solution was sonicated for 15 min and finally made up to the mark with ethanol, filtered, and diluted for further analysis.

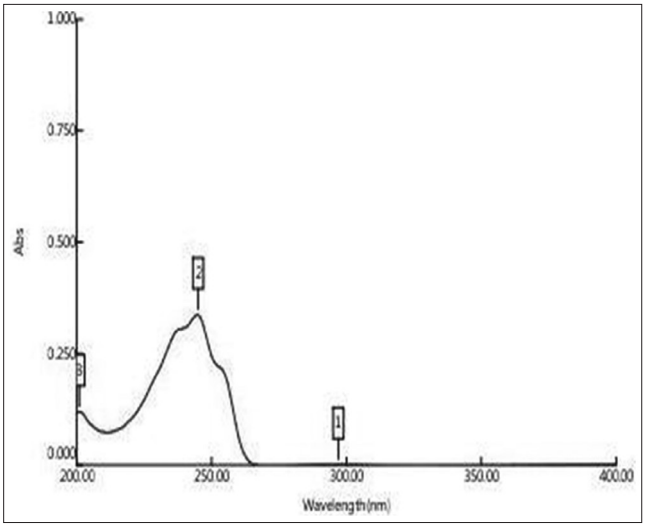

Fig. 2: UV spectrum of ivermectin

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 λ_{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 λ_{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.

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).

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

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^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

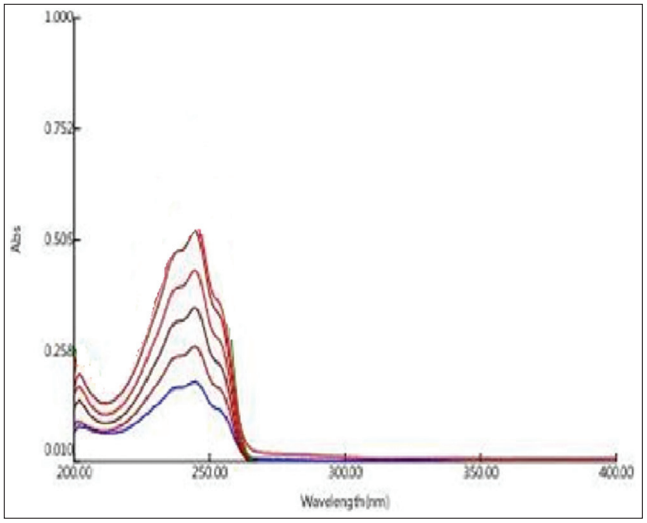

Fig. 3: Overlay UV spectrum showing linearity of ivermectin

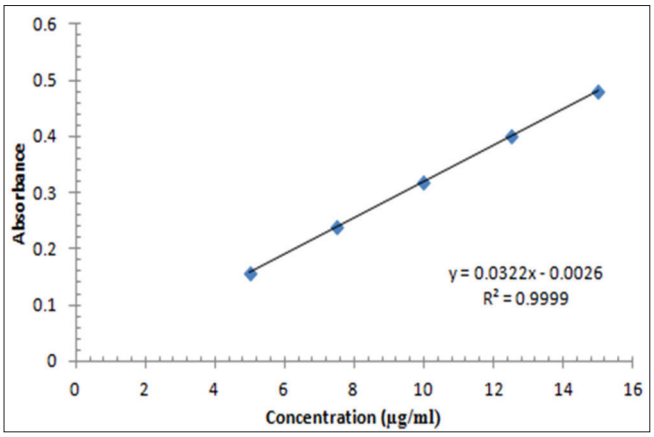

Fig. 4: Calibration graph at 239 nm

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.

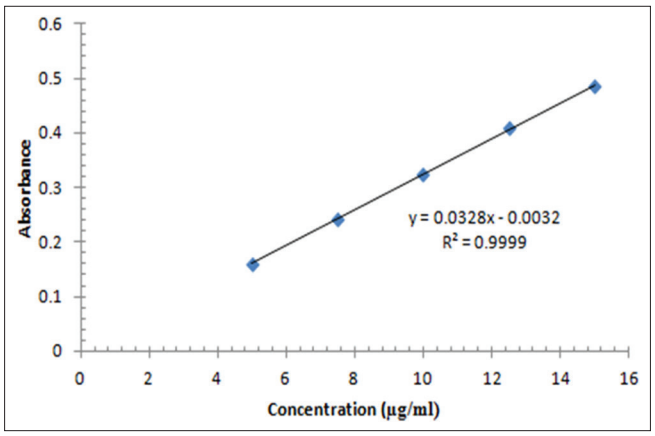

Fig. 5: Calibration graph at 241 nm

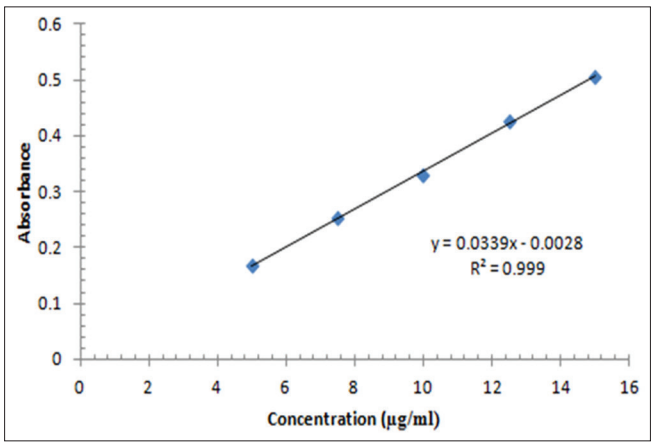

Fig. 6: Calibration graph at 243 nm

RESULTS AND DISCUSSION

The λ_{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 3: Absorbance values for intraday precision

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 4: Absorbance values for interday 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 5: Intraday precision

Concentration (µg/mL)	Description	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	0.001	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	0.002	0.004	0.004	0.004	0.003	0.003
	% RSD	1.018	0.473	1.073	1.105	1.137	0.937	1.136
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

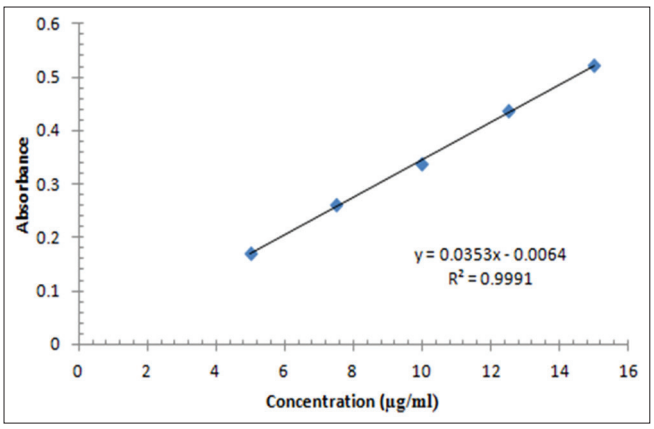

Fig. 7: Calibration graph at 245 nm

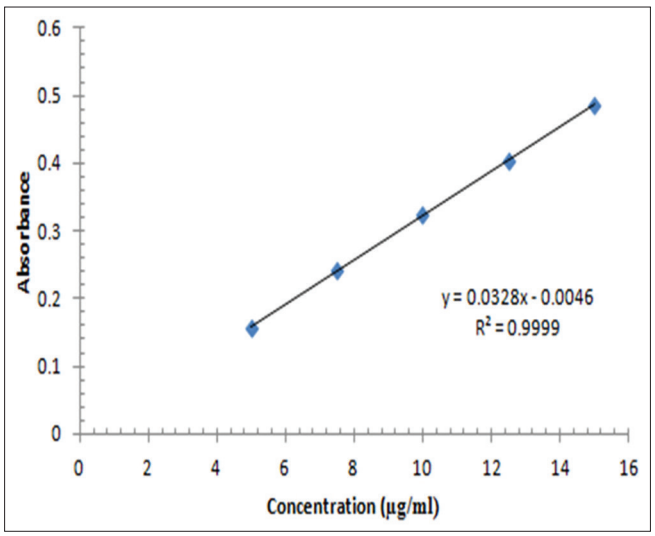

Fig. 8: Calibration graph at 247 nm

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

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

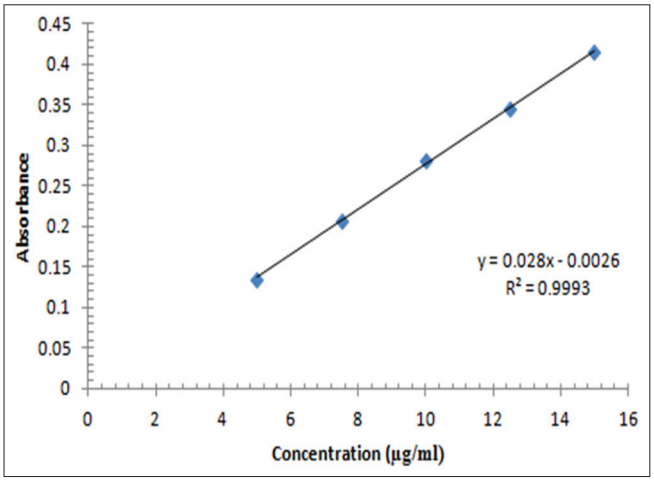

Fig. 9: Calibration graph at 249 nm

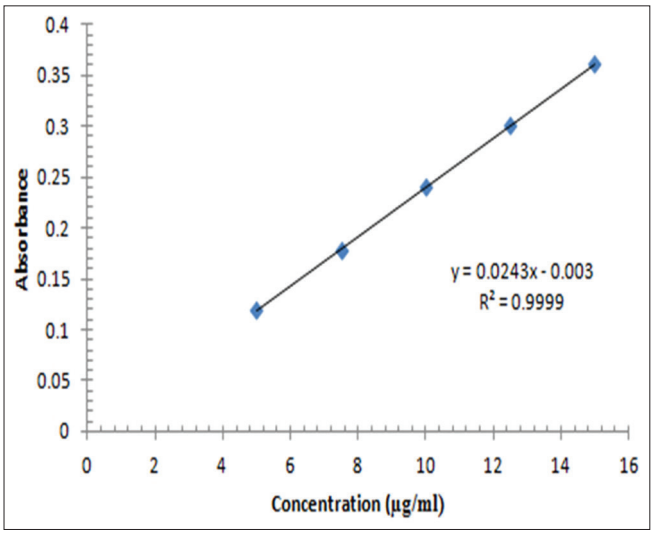

Fig. 10: Calibration graph at 251 nm

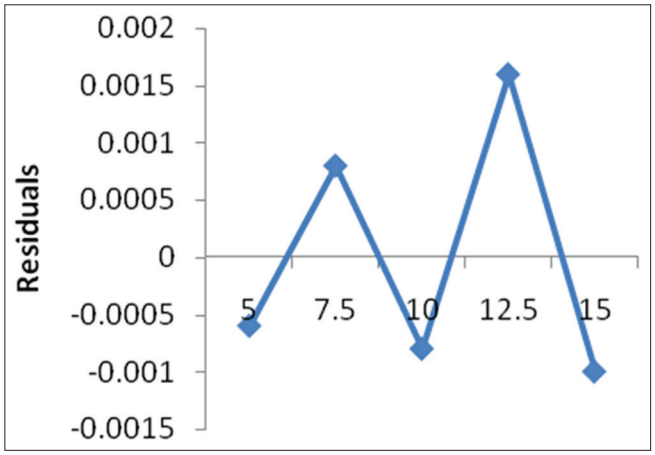

Fig. 11: Residual plot at 239 nm

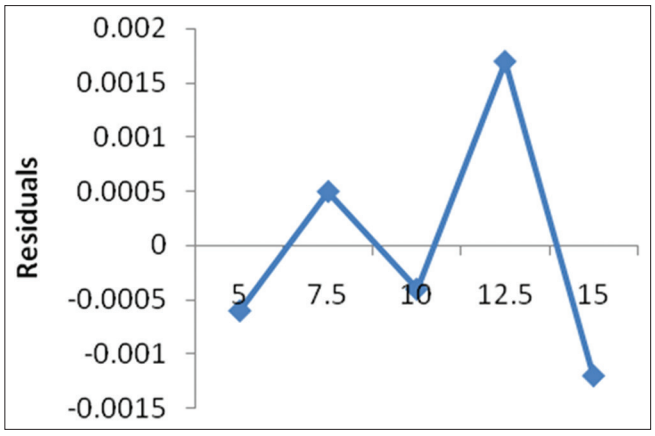

Fig. 12: Residual plot at 241 nm

graphs and the system suitability parameters were presented in Figs. 4-17 and Table 2, respectively.

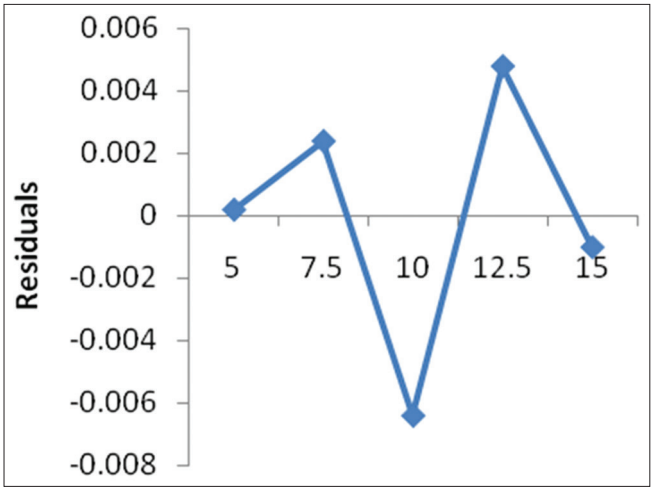

Fig. 13: Residual plot at 243 nm

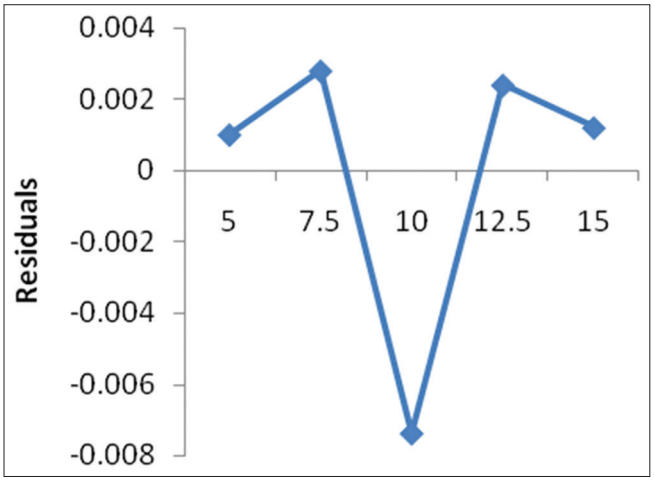

Fig. 14: Residual plot at 245 nm

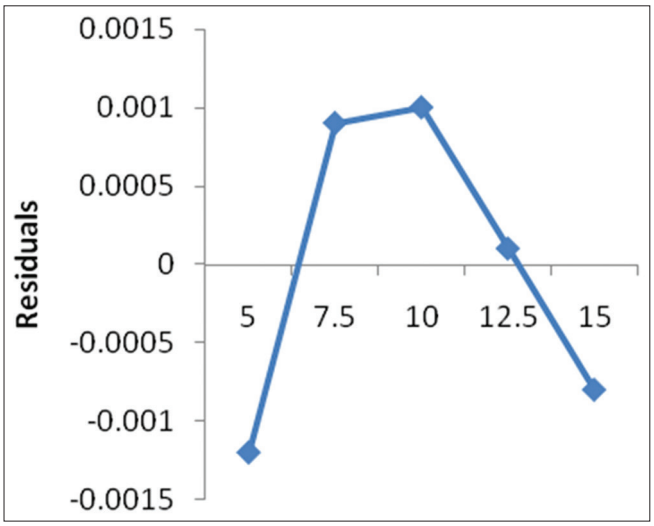

Fig. 15: Residual plot at 247 nm

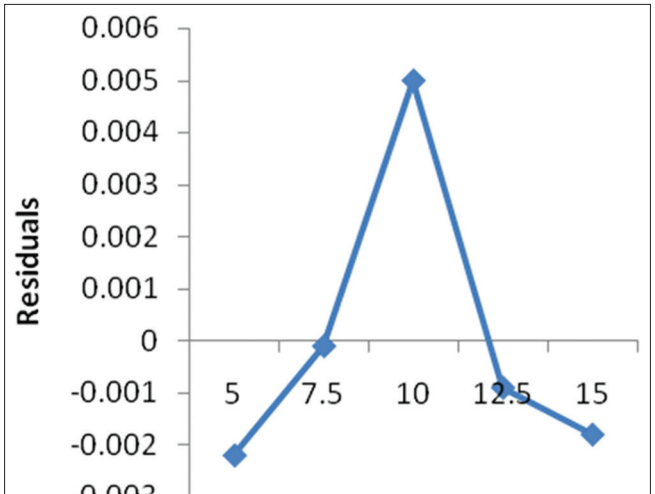

Fig. 16: Residual plot at 249 nm

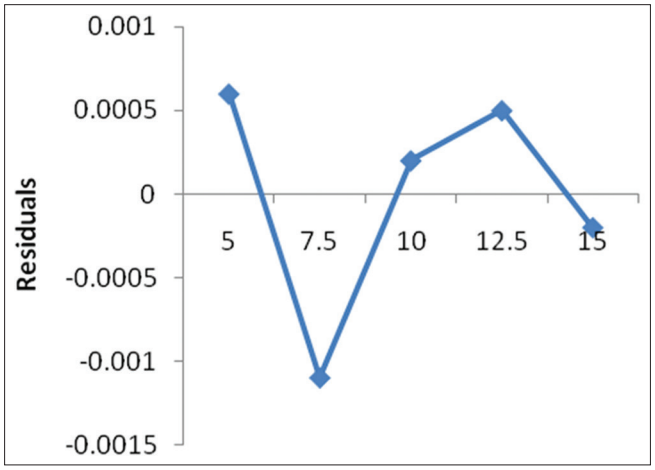

Fig. 17: Residual plot at 251 nm

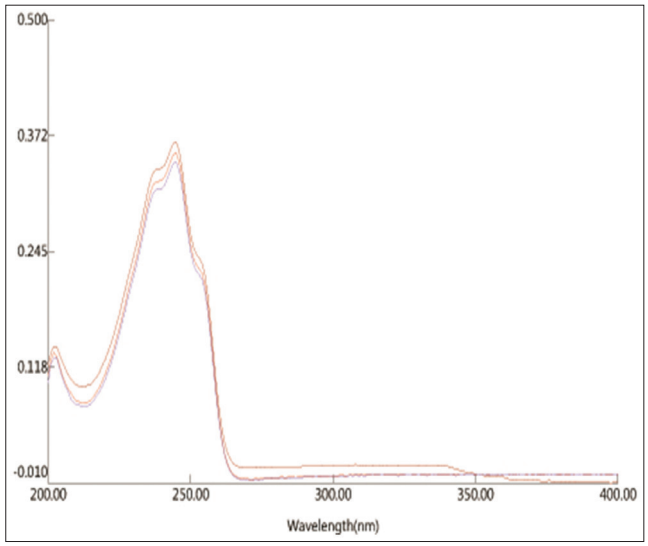

Fig. 18: Overlay UV spectrum showing intraday precision

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.

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

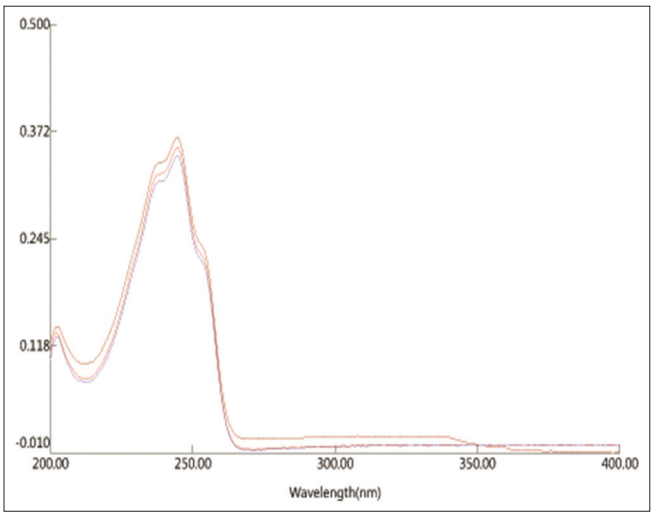

Fig. 19: Overlay UV spectrum showing interday precision

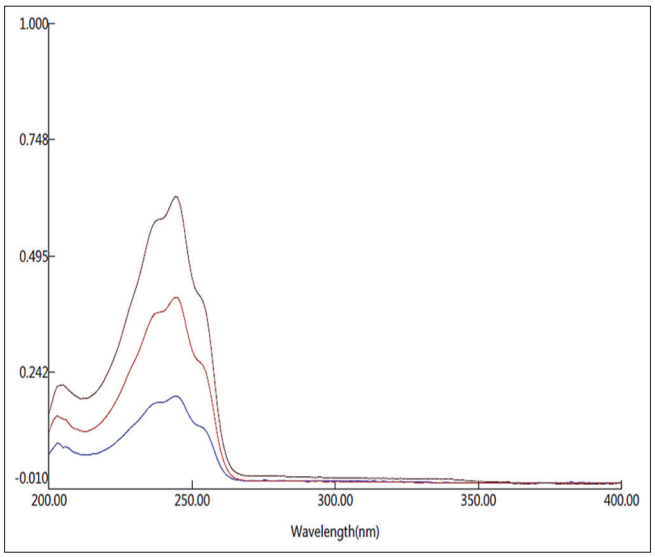

Fig. 20: Overlay UV spectrum displaying accuracy of ivermectin

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. 20 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.

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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.

CONFLICTS OF INTEREST

The authors report on conflicts of interest on the study.

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