

QUANTITATIVE ESTIMATION OF IVERMECTIN BY COLORIMETRY

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Abstract

Colorimetry of Ivermectin was carried out by derivatizing the ester group by using hydroxylamine and ferric chloride in alkaline pH, here yellowish orange color was obtained. In this method the estimation was carried out at 540 nm (λ_{max}). Beer's law range was 10 – 50 $\mu\text{g/ml}$. This method was statistically validated and LOD & LOQ were found to be 3.5329 & 9.7737 respectively.

Key Words : Ivermectin, Ivectin

1. Introduction

Objective of present work is to develop a colorimetric method for the estimation of Ivermectin in bulk and pharmaceutical dosage form. In colorimetric analysis the objective is to convert a colorless sample compound in to a derivative that absorbs in the visible region; the reactions used are called as chromogenic reactions. Here color intensity plays a main role in the analysis. The absorbance increases with the increase of intensity. Derivatization is very much essential step in colorimetric. Here functional groups are derivatized in to a form in which they exhibit color which can be used for the analysis.

In case of Ivermectin it was achieved by derivatization of ester group of the drug by using Hydroxylamine solution and ferric chloride solution in alkaline media. After derivatization yellowish orange colour was observed.

2. Materials and Methods

Analytical grade chemicals and reagents were used through out the work. Ivermectin manufactured by Indian Immunologicals Ltd, Hyderabad, used as standard. Ethanol manufactured by SD Fine Chemicals, Mumbai was used for the dilutions. Commercially available Pharmaceutical dosage form IVECTIN a veterinary preparation, manufactured by Indian Immunologicals Ltd, Hyderabad, used as test sample.

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Instrument:

SYSTRONICS-2201 UV Double Beam Spectrophotometer with 1cm matched glass cells were used for the analysis.

Method

Primary stock solution of Ivermectin was prepared by using ethanol. From this different dilutions were prepared to determine λ_{max} and beer's law range. Calibration curve was by using different concentrations of standard solution. Ivermectin in dosage form was estimated by calibration curve. Developed method was validated as per ICH guidelines with the help of several parameters like accuracy, precision, LOD, LOQ and stability.

2.1. Preparation of standard Ivermectin solution

1mg / ml solution was used as primary stock solution. The working solution of 0.1 mg / ml prepared by transferring 5ml from respective stock solution to a 50 ml volumetric flask and completing to volume with the mixture of Ethanol.

2.2. Preparation of sample

To 1 ml of 2.5 M Hydroxylamine, 1 ml of 2.7 M NaOH, Ivermectin solution (x ml) and water 10 ml were added then it was heated (75°C) and cooled to room temperature then 5 ml. Ferric Chloride then volume was made up to 25ml and stored in a dark place for 30 min. By this way sample was turned to yellowish orange color.

3. Results and Discussion

In this study yellowish orange color was developed by derivatizing the ester group of Ivermectin by using hydroxylamine solution and ferric chloride solution in alkaline media and that was scanned for λ_{max} against blank solution and λ_{max} was found to be 540 nm (Fig 1). Calibration curve was prepared by using the standard ivermectin solution at different concentrations. The beer's law

range was 10 – 50 mcg /ml (Fig 2) (Table 1). The color intensity of the sample after derivatization was checked for several hours after the preparation and it was found that it was stable for 6 hours (Table 2). The experiment preparation of calibration curve was repeated six times in a day for intra day and six different days for inter day precision. The average % RSD of intra day and inter day measurements were recorded (Table 3-4). The values of LOD and LOQ for both the drugs at selected wavelength are noted (Table 5). Accuracy of the proposed method was determined by performing recovery studies. A fixed amount of each drug from dosage form was taken and pure standard drug at three different concentrations within Beer's range was added, the total concentration was found by the proposed method. The determination with each concentration was repeated for three times and average percentage recovery of the added standard was calculated (Table 6). In formulations ivermectin was estimated by making the solution to beer's law range and recording the absorbance at 540 nm against blank solution. The result is shown in (Table 7)

4. Conclusion

This method was found to be suitable for the estimation of Ivermectin in dosage forms. By results, % RSD was always less than $\pm 2\%$ it indicates higher accuracy. This method is easy to perform and it can be applied for routine analysis and it is low at cost.

5. Acknowledgement

I sincerely thank Mr. Prabhakar Rao, Indian Immunologicals Ltd., Hyderabad for providing me the gift sample of Ivermectin and I Thank Mrs. Krishna Manjari Pawar, Asst. Proff., Andhra University for her support.

Fig 1: Absorbance maxima of Ivermectin

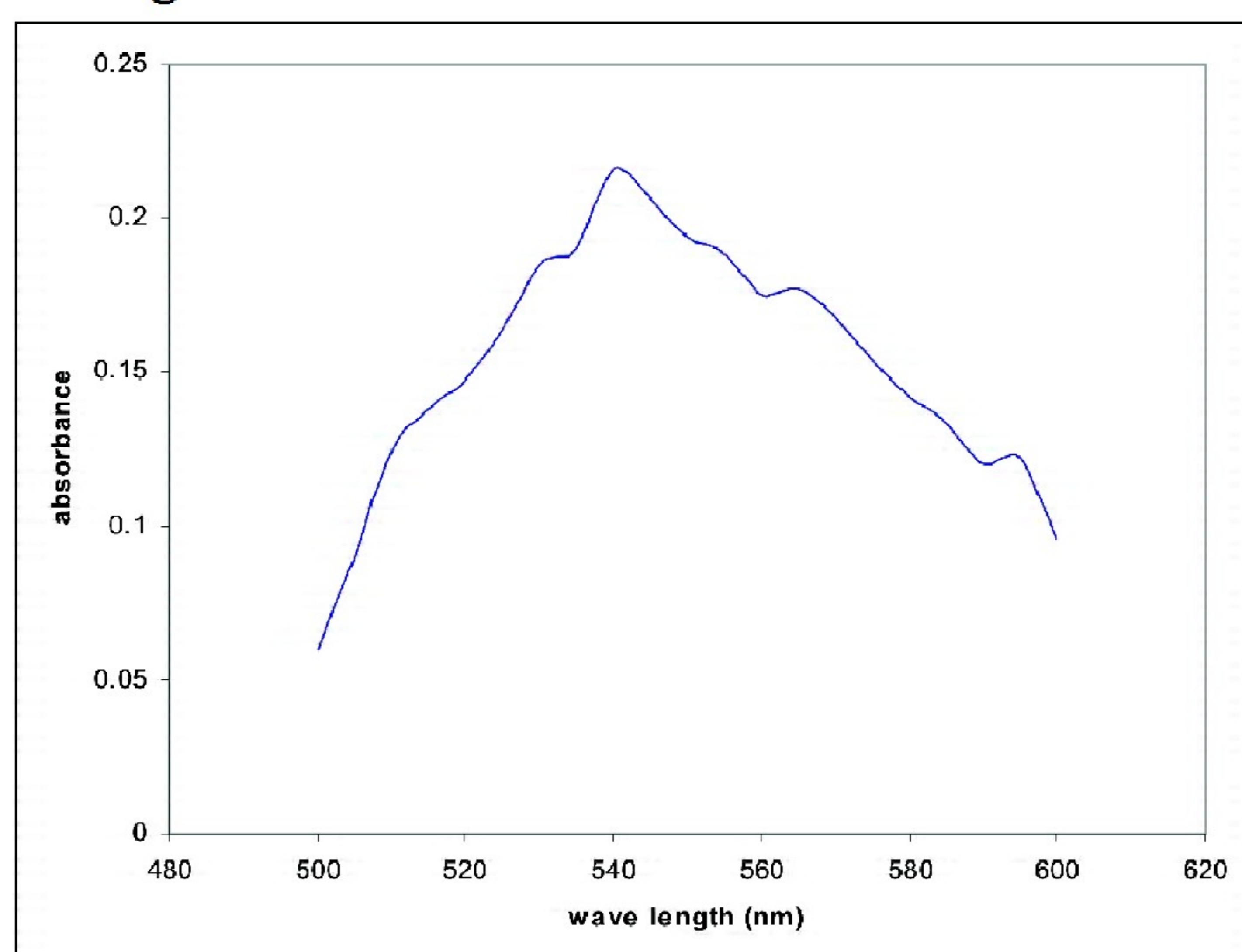


Fig 2: Calibration plot of Ivermectin at 540 nm

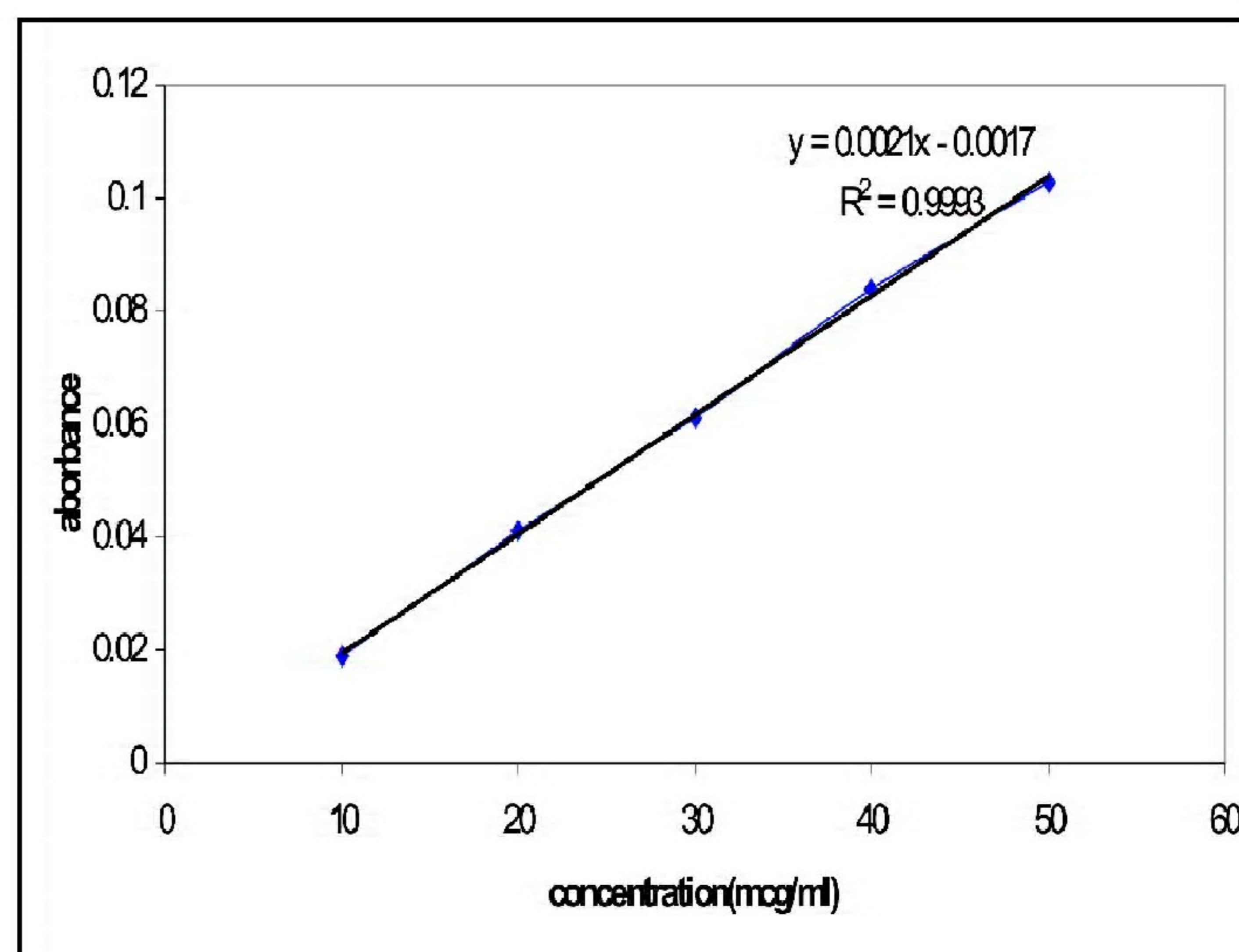


Table 1: Colorimetric observations

S. No.	Concentration of ivermectin ($\mu\text{g} / \text{ml}$)	Absorbance at 540 nm
01	10	0.019
02	20	0.041
03	30	0.061
04	40	0.084
05	50	0.103

Table 2: Stability studies of Ivermectin

S. No.	Concentration of Ivermectin ($\mu\text{g} / \text{ml}$)	Time in hours	Absorbance at 540 nm
1	30	30 min	0.064
2	30	02	0.066
3	30	04	0.062
4	30	06	0.067

Table 3: Intra day precision

S. No.	Concentration of Ivermectin ($\mu\text{g} / \text{ml}$)	* Mean	% RSD
01	6	0.0313	1.8426
02	8	0.0377	1.5327
03	10	0.0443	1.3022
Average of % RSD			1.5592

* Average of six trials

Table 4: Interday precision

S. No.	Concentration of Ivermectin	* Mean	%RSD
01	6	0.0333	1.7320
02	8	0.0373	1.5464
03	10	0.0413	1.3968
Average of % RSD			1.5584

* Average of six trials

Table 5: Limit of detection and limit of quantification

S. No	*Std.Dev	Slope	Limit of Detection (µg/ml)	Limit of Quantification (µg/ml)
01	0.002248	0.0023	3.5325	9.7737

* Average of six trials

Table 6: Recovery study from formulation

S.No.	Concentration of sample (µg/ml)	Concentration of Standard (µg/ml)	*Average amplitude at 540 nm	% Recovery of Ivermectin
01	10	6	0.0343 ± 0.0006	102.8
02	10	8	0.0377 ± 0.0015	99.647
03	10	10	0.0413 ± 0.0013	98.413

Table 7: Estimation of ivermectin in formulation

Name	Label Claimed (mg/ml)	Concentration found	% Recovery (mg/ml)
IVECTIN	10	9.862	98.6 ± 1.684

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