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Analytical Method Development and Validation for Simultaneous Estimation of Albendazole and Ivermectin Tablet of Dosage Form by RP-HPLC

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A B S T R A C T

A reverse phased liquid chromatography (LC) method was developed and validated for simultaneous estimation of albendazole and ivermectin in tablet dosage form. The isocratic LC analysis was performed on INERTSIL C18 BDS column (250 x 4.6 mm, 5 µ) using mobile phase composed of acetonitrile, methanol and water in ratio of 40:60 (v/v) at a flow rate of 1 mL/min. Quantitation was performed using UV detector at 280 nm and the run time was 10 min. The retention times were found to be 3.9 min for albendazole and 2 min for ivermectin. The analytical method was validated according to ICH guidelines. The linearity was observed in the range of 133.7-293.4 with correlation coefficient, r=1.0 and 1 for albendazole and ivermectin respectively. The relative standard deviation values for repeatability and intermediate precision studies were less than 2%, and the accuracy (% recovery) was greater than 98% for both the drugs. The method was successfully applied for market sample analysis and mean percentage assay values were 99.99 and 100.01s for albendazole and ivermectin respectively. The present method is precise and accurate and can be used for the routine estimation of albendazole and ivermectin in tablet dosage forms.

Keywords: Albendazole, Ivermectin, RP-HPLC, validation

A R T I C L E I N F O

CONTENTS

1. Introduction 194
2. Materials and Methods 195
3. Results and discussion 196
4. Conclusion 201
5. Acknowledgement. 201
6. References 201

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1. Introduction

Need for Drug Analysis [5]

The number of drugs introduced into the market is increasing every year. These drugs may be either new entities or partial structural modification of the existing one. Very often there is a time delay from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, reports of new toxicities (resulting in their withdrawal from the market), development of long-suffering opposition and introduction of better drugs by competitors. Under these conditions, standards and analytical procedures for these drugs may not be available in the pharmacopoeias. It becomes necessary, therefore to develop newer analytical methods for such drugs. Also quality is important in every product or service but it is vital in medicines as it involves life. Quality control is a concept, which strives to produce a perfect product by series of measures designed to prevent and eliminate errors at different stage of production. The decision to release or reject a product is based on one or more type of control action. Providing simple analytical procedure for complex formulation is a matter of most important.

Instrumentation:

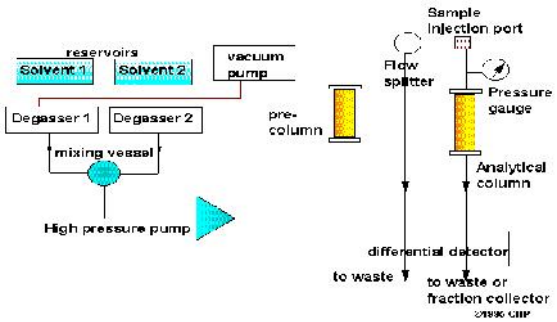


Figure 1: Schematic Diagram of an HPLC instrument

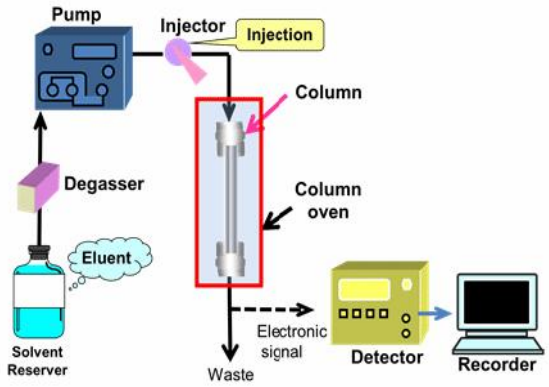


Figure 2: Typical Diagram of HPLC

Table 1

Goal	Comment
Resolution	Precise and rugged quantitative analysis requires that R_s be greater than 1.5.
Separation time	<5-10 min is desirable for routine procedures.
Quantization	≤2% for assays; ≤5% for less-demanding analyses; ≤15% for trace analyses.
Pressure	<150 bar is desirable, <200 bar is usually essential (new column assumed).
Peak height	Narrow peaks are desirable for large signal/noise ratios.
Solvent consumption	Minimum mobile-phase use per run is desirable.

Antihelmenthic[10]

Antihelminthics are drugs that expel parasitic worms (helminthes) from the body by either stunning or killing them and without causing significant damage to the host. They may also be called verifies (those which stun) or vermicides (those which kill). Anthelminthics are agents used to eradicate intestinal worms (helminthes) from the body .Tapeworms, roundworms and flukes are classified as helminthes. Anthelmintics are effective in eradicating worms but proper hygiene is necessary to prevent re-infection. Washing hands properly before meals and after visiting the toilet is essential. Members of the family or household need to be treated.

Treatment:

Anthelmintic**,** any drug that acts against infections caused by parasitic worms (helminthes). Helminthes can be divided into three groups: custodies, or tapeworms; nematodes, or roundworms; and treaties, or flukes. The helminthes differ from other infectious organisms in that they have a complex body structure. They are multicellular and have partial or complete organ systems (e.g., muscular, nervous, digestive, and reproductive). Several of the drugs used to treat worm infections affect the nervous of the parasite and result in muscle paralysis. Other drugs affect the uptake of glucose and thus energy stores. All are chemical agents and are generally administered orally, and many are used in both human and veterinary medicine. No anthelmintic, however, is completely effective, completely without toxic effect upon the host, or equally active against all worms.

Antiphrastic:

Antiparasitics, drugs which kill or inhibit the growth of parasitic organisms, may be subdivided into the following therapeutic categories:

a. Antinematodal
b. Anticestodal
c. antilog worm
d. antitrematodal
e. ant filarial
f. antiprotozoal
g. insecticide

Treatment:

Ant parasitic treatment is indicated for all cases of acute or reactivated Changes disease and for chronic *Trypanosome cruse* infection in children up to age 18. Congenital infections are considered acute disease. Treatment is strongly recommended for adults up to 50 years old with chronic infection who do not already have advanced Changes cardiomyopathy. For adults older than 50 years with chronic *T. cruse* infection, the decision to treat with ant parasitic drugs should be individualized, weighing the potential benefits and risks for the patient. Physicians should consider factors such as the patient's age, clinical status, preference, and overall health.

Rationale for Selecting the Drugs:

Albendazole:

It is widely used for the treatment of worm infestations (such as parenchyma neurocysticercosis and cystic hydrated disease of the liver, lung, and peritoneum and) and a number of other conditions.

Ivermectin:

It is used to treat certain parasitic roundworm infections. Curing parasitic infections helps to improve your quality of life. In people with weakened defense (immune) systems, curing roundworm infections can reduce the risk of developing a severe or life-threatening infection. Ivermectin belongs to a class of drugs known as antihelmintics. It works by paralyzing and killing parasites.

2. Materials and Methods

Chromatographic Conditions:

Flow rate	: 1ml/min
Column	: Inertsil-C18, BDS column
Detector wavelength	: 280nm
Column temp	: Ambient
Injection volume	: 20µl
Run time	: 10min
Retention time for abz	: 2.7min for ivmctn and 4.0min
Run time	: 10min
Retention time	: 2.6min for ivr and 4.7 for albz

Observation: Two peaks are merged and not separated completely. The trial 1 chromatogram result was shown in Fig: 1.

Optimized Method Parameters

Optimized chromatographic conditions:

Reference solution: The solution was prepared by dissolving 20.0 mg of accurately weighed Albendazole and 25.0 mg Ivermectin in Mobile phase, in two 100.0 mL volumetric flasks separately and sonicate for 20min. From the above solutions take 10.0 mL from each solution into a 50.0 mL volumetric flask and then makeup with mobile phase and sonicate for 10min.

Dosage form Procedure

Twenty tablets were weighed accurately and a quantity of tablet powder equivalent to 20 mg Albendazole and 25 mg Albendazole was weighed and dissolved in the 70 ml mobile phase with the aid of ultrasonication for 20 min. The content was diluted to 100 mL with mobile phase to furnish a stock test solution. The stock solution was filtered through a 0.45 µm Nylon syringe filter and 10.0 mL of the filtrate was diluted into a 50.0 mL volumetric flask to give a test solution containing 40 µg/mL Albendazole and 50 µg/mL Ivermectin.

Assay

Preparation of standard solution:

The stock solutions equivalent to 20ppm to 80ppm with respect to both drugs were prepared in combination of Albendazole and Ivermectin above, sonicated and filtered through 0.45µ membrane.

Preparation of stock solution:

Procedure for calibration curve:

The contents of the mobile phase were filtered before use through 0.45micron membrane and pumped from the respective solvent reservoirs to the column at a specified flow rate. Prior to injection of the drug solutions, the column was equilibrated for at least 30min with the mobile phase flowing through the system. The chromatographic

separation was achieved using a mobile phase consisting of Methanol : Water at 80:20V/V the eluent was monitored using Pda detector at a wavelength of 232nm.The column was maintained at ambient temperature (27^0c) and an injection volume of 20µl of each of standard and sample solutions were injected into the HPLC system to get the chromatograms. The retention time, peak areas of drug was recorded graph was plotted by taking concentration of the drug on x-axis and peak area on y-axis. A typical chromatogram of Albendazole and Ivermectin combination was shown in Fig

Observation:

Individual %assays and %RSD of Assay are within limit and passes the intermediate precision, Refer table: 4. The mean % recovery of the Albendazole and Ivermectin at each spike level should be a study was conducted to determine the effect of variation in flow rate. Standard solution prepared as per the test method was injected into the HPLC system using flow rates, 1.0ml/min and 1.2ml/min. The system suitability parameters were evaluated and found to be within the limits for 1.0ml/min and 1.2ml/min flow. Albendazole and Ivermectin and was resolved from all other peaks and the retention times were comparable with those obtained for mobile phase having flow rates 1.0ml/min.

Acceptance Criteria:

The Tailing Factor of Albendazole and Ivermectin standards should be NMT 2.0 for Variation in Flow.

Observation:

The tailing factor for Albendazole and Ivermectin was found to be within the limits. As shown in table 10.

b) Effect of variation of temperature:

A study was conducted to determine the effect of variation in temperature. Standard solution prepared as per the test method was injected into the HPLC system at 20ºC temperature. The system suitability parameters were evaluated and found to be within the limits for a temperature change of 20ºc.Similarly sample solution was chromatographic at 25ºC temperature. Albendazole and Ivermectin were resolved from all other peaks and the retention times were comparable with those

Acceptance Criteria:

The Tailing Factor of Albendazole and Ivermectin standard and sample solutions should be NMT 2.0 for Variation in temperature.

Observation: The tailing factor for Albendazole and Ivermectin

x is found to be within the limits. As shown in table 11.

5.3.8 Limit Of Detection and Quantitation (LOD and LOQ):

From the linearity data calculate the limit of detection and quantization, using the following formula.

$$LOD = \frac{3.3\ \sigma}{S}$$

σ = standard deviation of the response

S = slope of the calibration curve of the analyte.

$$LOQ = \frac{10\ \sigma}{S}$$

σ = standard deviation of the response

S = slope of the calibration curve of the analyte.

Observation:

Test results are showing that the test method is precise. Refer tables 2 and 3 for system precision and for method precision.

Intermediate precision (analyst to analyst variability):

A study was conducted by two analysts as per test method.

Acceptence Criteria:

The individual assays of Albendazole and Ivermectin should be not less than 98% and not more than 102% and %RSD of assays should be NMT2.0% by both analysts.

Table 2

Parameters	Method
Stationary phase (column)	Inertsil -BDS C_{18}(250 x 4.6 mm, 5 µ)
Mobile Phase	Methanol : ACN (60:40)
Flow rate (ml/min)	1.0 ml/min
Run time (minutes)	10 min
Column temperature (°C)	Ambient
Volume of injection loop (µl)	20
Detection wavelength (nm)	280nm
Drug RT (min)	2.min for ivm and 3.9 for Abz

3. Results and Discussion

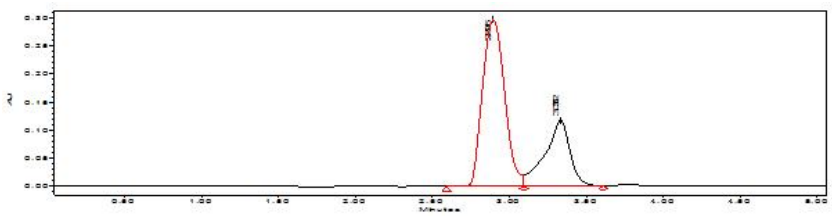


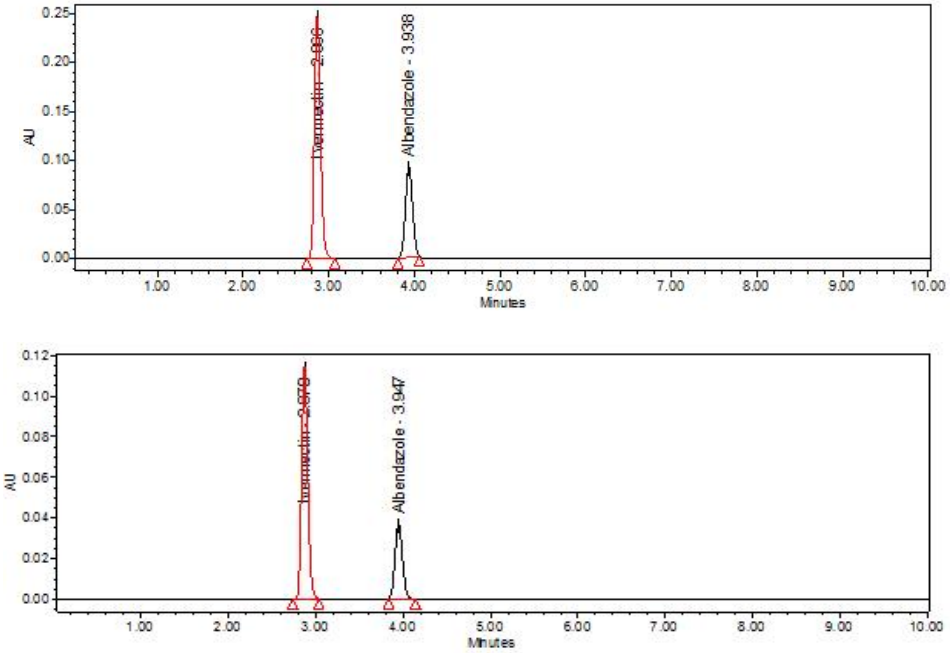
Figure 1: Chromatogram of Trial 1

Inference : Albendazole and Ivermectin are not seperated.

Table 3

S.No	Name of the peak	Retention time(min)
1	Ivermectin	2.8min
2	Albendazole	3.3min

Chromatograms of system precision



Inference: Chromatogram for system precision (standard - 5)

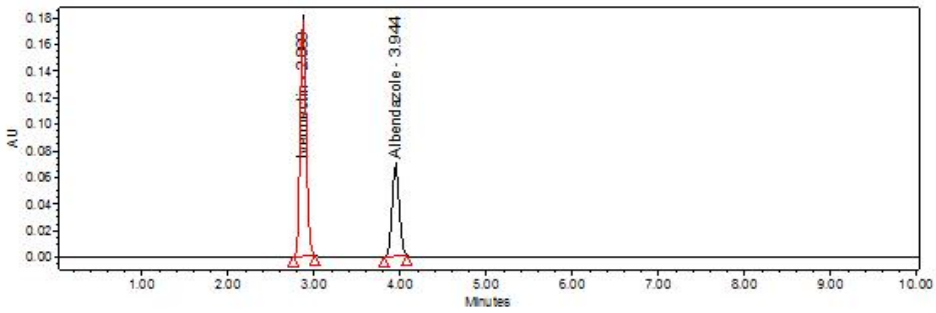
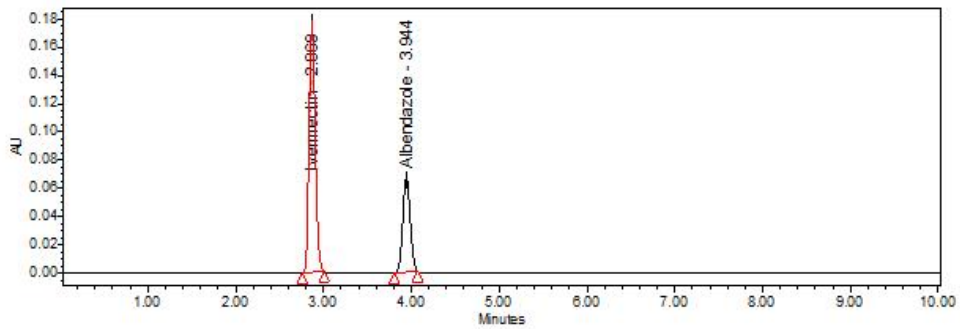
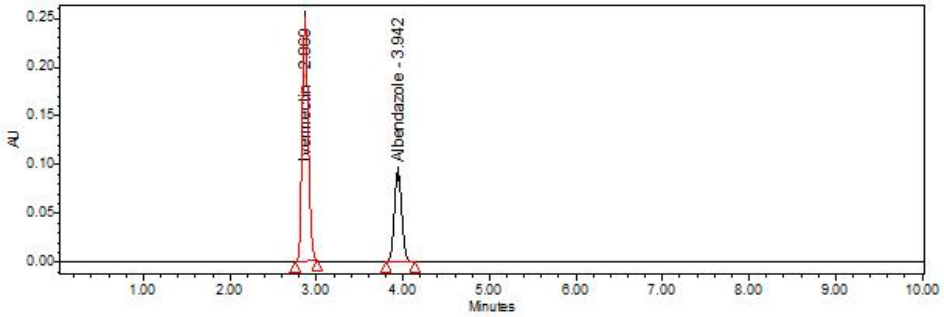
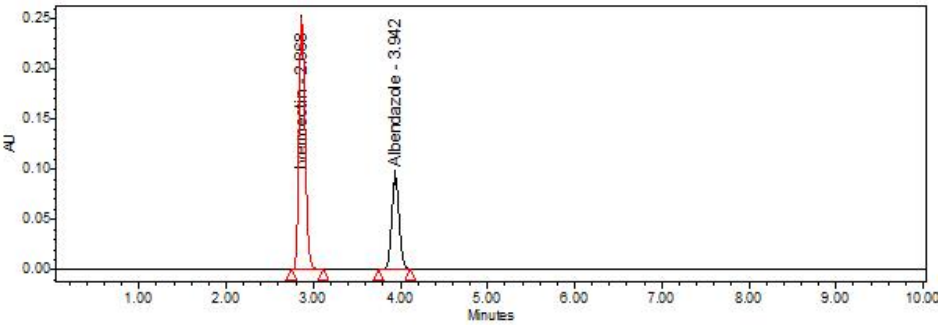


Figure 15-17: Chromatograms of Repeatability

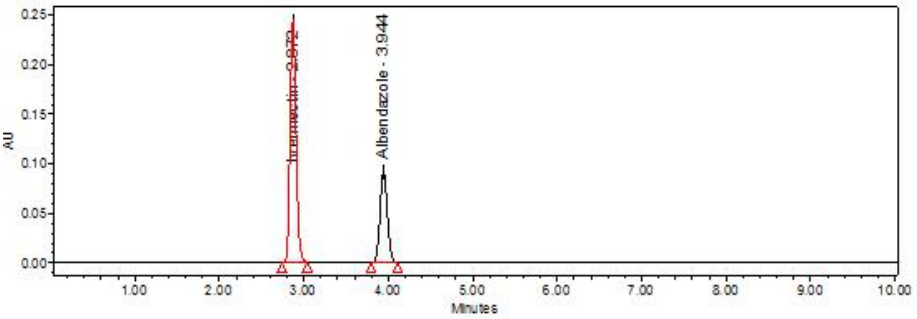




Inference: Chromatogram for Repeatability (standard-3)



Inference: Chromatogram for Repeatability (standard-4)



Intermediate precision:

For **Analyst 1** ref: Table3(I) & 3(II).

(Analyst 2)

Inference: Chromatogram for Intermediate Pre

Inference: Chromatogram for Intermediate Precision

Accuracy (Recovery)

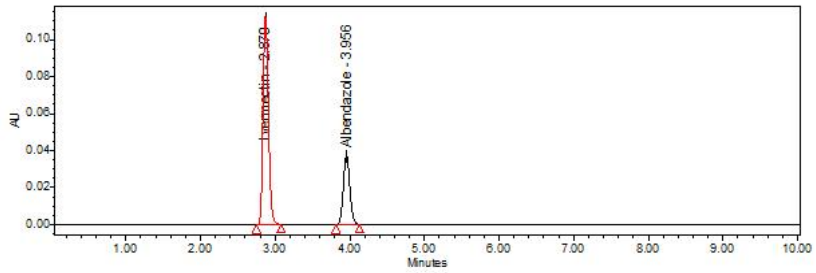
Table-5(a): Data of Accuracy of Ivermectin:

Concentration % of spiked level	Amount added (ppm)	**Amount found (ppm)**	**% Recovery**	**Statistical Analysis of % Recovery**	
50% Sample 1	20	20.00	100.01	**MEAN**	100.026
50% Sample 2	20	20.00	100.03		
50% Sample 3	20	20.00	100.04	**%RSD**	0.015
100 % Sample 1	40	39.99	99.99		
100 % Sample 2	40	40.00	100.00	**MEAN**	99.99
100% Sample 3	40	39.99	99.99	**%RSD**	0.005
150% Sample 1	60	59.99	99.99		
150% Sample 2	60	60.00	100.00	**MEAN**	100.00
150% Sample 3	60	60.00	100.01	**%RSD**	0.009

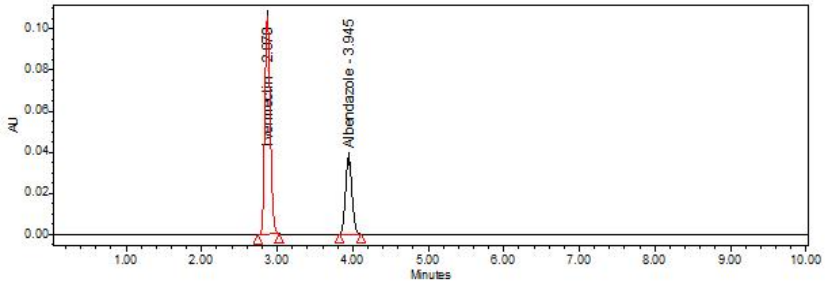
Table-5(b): Data of Accuracy of Albendazole:

Concentration % of spiked level	Amount added (ppm)	**Amount found (ppm)**	**% Recovery**	**Statistical Analysis of % Recovery**	
50% Sample 1	20	20.01	100.06	**MEAN**	100.05
50% Sample 2	20	20.02	100.10		
50% Sample 3	20	19.99	99.99	**%RSD**	0.055
100 % Sample 1	40	39.98	99.98	**MEAN**	100.02
100 % Sample 2	40	40.00	100.02	**%RSD**	0.045
100% Sample 3	40	40.03	100.07		
150% Sample 1	60	60.00	100.00	**MEAN**	99.97
150% Sample 2	60	59.98	99.96	**%RSD**	0.020
150% Sample 3	60	59.99	99.97		

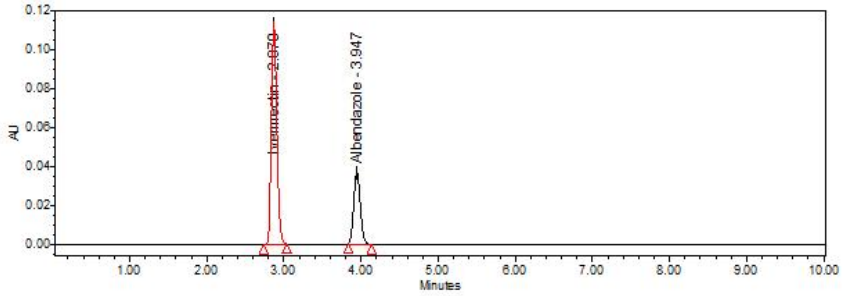
Figure 21 -22 Chromatograms for accuracy (50%)



Inference: Chromatogram for standard 1

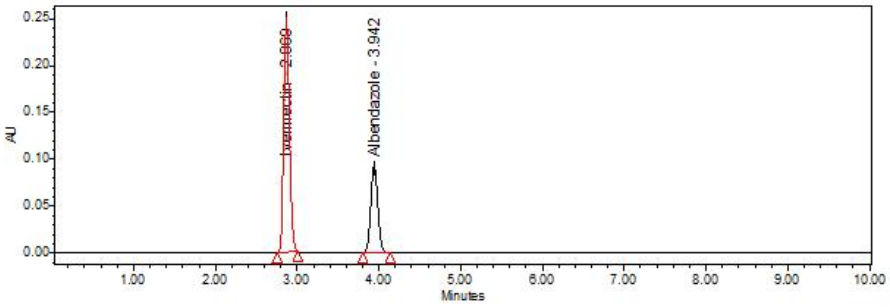


Inference: Chromatogram for standard 2

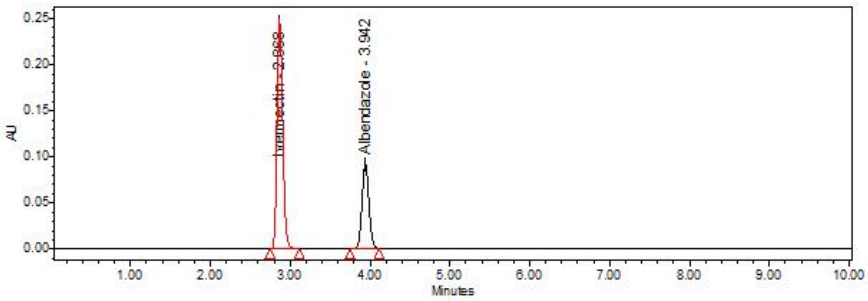


Inference: Chromatogram for standard 3

Figure 23-24: Chromatograms for accuracy (100%)

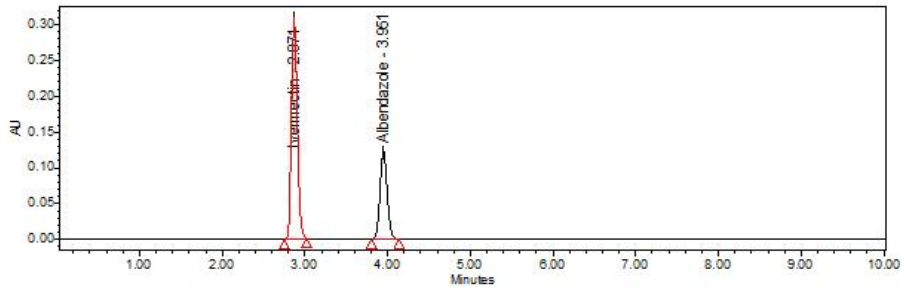


Inference: Chromatogram for standard 1

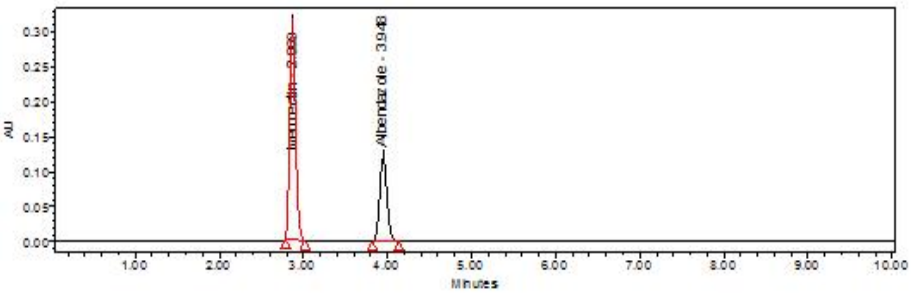


Inference: Chromatogram for standard 2

Fig 25-26: chromatograms For Accuracy (150%)



Inference:
Chromatogram for standard 1



Inference: Chromatogram for standard 2
Linearity:

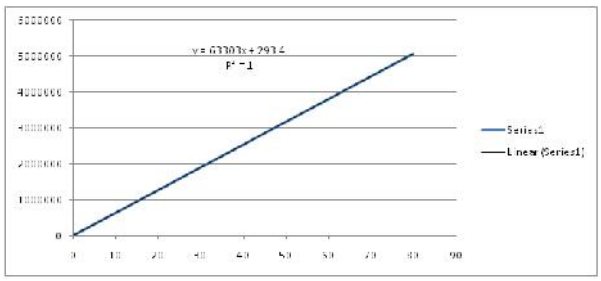


Figure: 41 Linearity Plot (Concentration Vs Response) for Albendazole

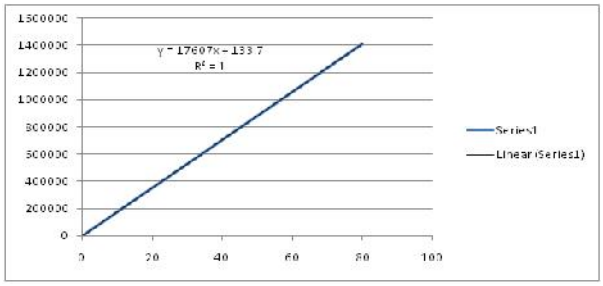


Table 6: Data of Linearity of Albendazole

Concentration (ppm)	Average Area	Statistical Analysis	
0	0	Slope	17607
20	352465	y-Intercept	133.7
30	528324	Correlation Coefficient	1
40	704495		
50	880406		
60	1056400		
70	1232551		
80	1408738		

Limit of Detection and Limit of Quantitation (LOD and LOQ):

From the linearity plot the LOD and LOQ are calculated:

Ivermectin:

$$\textbf{LOD} = \frac{3.3\,\sigma}{S}$$

$$= \frac{3.3\times 244.595}{63303} = 0.012$$

$$\textbf{LOQ} = \frac{10\,\sigma}{S}$$

$$= \frac{10\times 244.595}{63303} = 0.038$$

Albendazole:

$$\textbf{LOD} = \frac{3.3\,\sigma}{S}$$

$$= \frac{3.3\times 299.4912}{17607} = 0.056$$

$$\textbf{LOQ} = \frac{10\,\sigma}{S}$$

$$= \frac{10\times 299.4912}{17607} = 0.17$$

4. Acknowledgements

Authors are thankful to active pharma labs Hyderabad.

5. Conclusion

The proposed HPLC method was found to be precise, specific, accurate, rapid and economical for simultaneous estimation of Albendazole and Ivermectin in tablet dosage form. The sample recoveries in all formulations were in good agreement with their respective Label Claims and this method can be used for routine analysis. It can be applied for routine analysis in laboratories and is suitable for the quality control of the raw materials, formulations, dissolution studies and can be employed for bioequivalence studies for the same formulation.

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