

**EVALUATION AND VALIDATION OF A UPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ALBENDAZOLE AND IVERMECTIN IN BULK VETERINARY DOSAGE FORM**

Ashraf Unnisa\*<sup>1</sup>, Dr. Osman Ahmed<sup>1</sup>, Meher Afrin<sup>1</sup>, Mohammed Akthar Sulthana<sup>1</sup> and Dr. Anas Rasheed<sup>2</sup>

<sup>1</sup>Department of Pharmaceutical Analysis, Deccan School of Pharmacy, Hyderabad.

<sup>2</sup>CSO, Gaelib Medications Private Limited, Hyderabad.

**Corresponding Author: Ashraf Unnisa**

Department of Pharmaceutical Analysis, Deccan School of Pharmacy, Hyderabad.

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**ABSTRACT**

In order to achieve the separation under the optimized conditions after experimental trials that can be summarized. Stationary phase like Hypersil BDS C18 (100 mm x 2.1 mm, 1.7 μm) column was most suitable one, since it produced symmetrical peaks with high resolution and a very good sensitivity and with good resolution. The flow rate was maintained 1.2 mL min<sup>-1</sup> shows good resolution. The PDA detector response of Albendazole and Ivermectin, veterinary dosage form was studied and the best wavelength was found to be 215 nm showing highest sensitivity.

**KEYWORDS:** Veterinary dosage form, Albendazole and Ivermectin.

**INTRODUCTION**

**Veterinary Pharmaceutical**

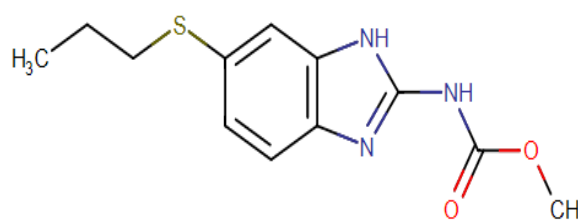
Veterinary pharmaceutical could be a field of pharmaceutical that's concerned with the avoidance, control, conclusion, and treatment of sickness, clutter, and harm in animals. It is additionally known as veterinary therapeutic hone. This can be in expansion to creature raising, cultivation, breeding, sustenance investigate, and item improvement being secured by the association. A wide assortment of creature species, both tamed and wild, are secured by the field of veterinary medication, which too incorporates a different range of illnesses that will influence different species.

**Albendazole**

Albendazole, commonly known as albendazolum could be a pharmaceutical that's utilized to treat a number of parasitic worm diseases, counting roundworms. It may be utilized to treat a assortment of sicknesses, counting giardiasis, trichuriasis, filariasis, neurocysticercosis, hydatid malady, pinworm infection, and ascariasis, among others. It is managed orally Nausea, stomach torments, and cerebral pains are all common antagonistic impacts of this medication. One of the possibly serious antagonistic impacts is bone marrow concealment, which regularly recuperates when the medicate is ceased for a whereas. It has been watched that the liver is kindled, and people who have had past liver issues are at higher risk. It is classified as pregnancy category C within the

Joined together States and category D in Australia, showing that it may be hurtful to a pregnant lady in the event that devoured. Albendazole may be a benzimidazole antihelminthic sedate with a wide run of action against an assortment of helminths.

**Chemical Structure**



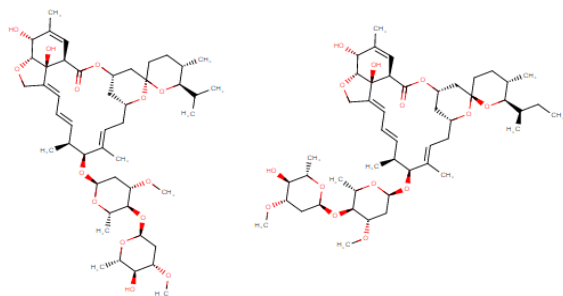
**Fig. 1: Chemical Structure of Albendazole.**

**Ivermectin**

Ivermectin is an anti-parasite medicate with a wide extend of movement. It was at first promoted beneath the brand title Stromectol® and was aiming for utilize against worms (with the special case of tapeworms). Be that as it may, in 2012, it was endorsed for the topical treatment of head lice invasions in patients 6 months of age and more seasoned, and was in this way showcased beneath the brand title Sklice™. Ivermectin could be a medicate that's mostly utilized in people to treat onchocerciasis, in spite of the fact that it is additionally

successful against other sorts of worm invasions (such as strongyloidiasis, ascariasis, trichuriasis and enterobiasis).

### Chemical Structure



**Fig. 2 Chemical Structure of Ivermectin.**

### Validation of Analytical Methods (USP/ICH)

Method validation, according to the United States Pharmacopeia (USP), is performed to ensure that an analytical methodology is accurate, specific, reproducible, and rugged over the specified range that an analyte will be analyzed. Regulated laboratories must perform method validation in order to be in compliance with FDA regulations. In a 1987 guideline (Guideline for Submitting Samples and Analytical Data for Methods Validation), the FDA designated the specifications in the current edition of the USP as those legally recognized when determining compliance with the Federal Food, Drug and Cosmetic Act can be referred to as the “eight steps of method validation”

### Experimental

#### Methodology

#### Method Validation

The analytical procedure refers to the way of performing the analysis. It should describe in detail the steps necessary to perform each analytical test. This may include but is not limited to: the sample, the reference standard and the reagents preparations, use of the apparatus, generation of the calibration curve, use of the formulae for the calculation, etc. The described method extensively validated in terms of specificity, system suitability, linearity, accuracy, precision, limit of detection, limit of quantification and robustness.

### ALBENDAZOLE and IVERMECTIN in UPLC System

ALBENDAZOLE and IVERMECTIN	
<b>System</b>	UPLC
<b>Stationary Phase</b>	C18 column
<b>“Mobile Phase”</b>	“Alcohol and acetonitrile in the ratio of 60:40%v/v”
<b>Diluents</b>	Methanol
<b>Injection volume</b>	20µl
<b>Temperature</b>	Ambient
<b>Flow rate</b>	1.2 ml/min
<b>UV detection</b>	215nm
<b>Retention Time</b>	ALBENDAZOLE – 2.016mins; IVERMECTIN – 6.217 mins
<b>Inference</b>	“High column pressure were observed”

## RESULTS

### Preparation of Standard Stock Solution

#### Preparation of Diluent

In order to achieve the separation under the optimized conditions after experimental trials that can be summarized. Stationary phase like Hypersil BDS C18 (100 mm x 2.1 mm, 1.7 µm) column was most suitable one, since it produced symmetrical peaks with high resolution and a very good sensitivity and with good resolution. The flow rate was maintained 1.2 mL min<sup>-1</sup> shows good resolution. The PDA detector response of ALBENDAZOLE and IVERMECTIN was studied and the best wavelength was found to be 215 nm showing highest sensitivity.

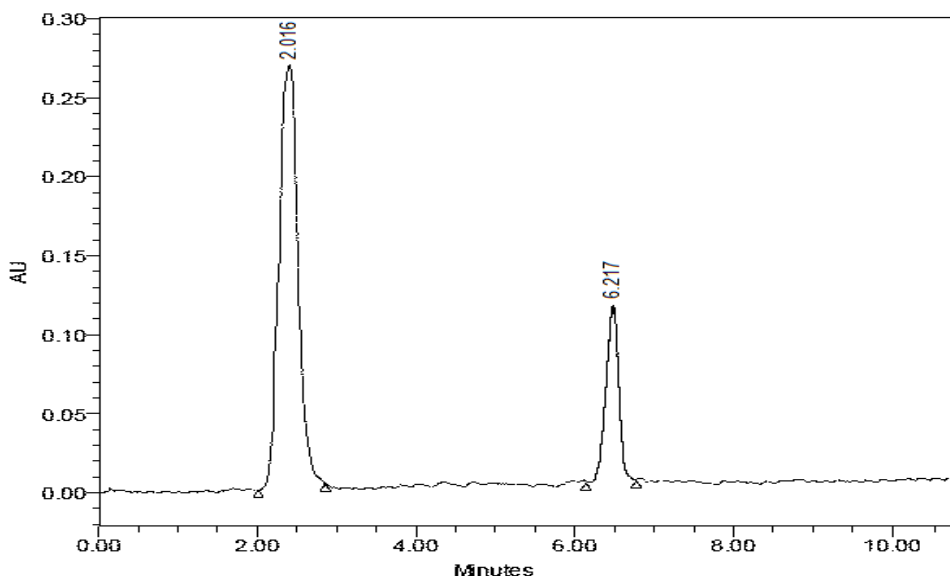
The mixture of two solutions Alcohol and acetonitrile in the ratio of 60:40%v/v”. Finally, the pH was adjusted to 7.65 by sodium hydroxide with gradient programming was used as mobile phase at 1.2mL/min was found to be an appropriate mobile phase for separation of ALBENDAZOLE and IVERMECTIN. The column was maintained at ambient temperature.

#### Preparation of internal standard solution

Weighed accurately about 10 mg of ALBENDAZOLE and IVERMECTIN working standard and transfer to 100 ml volumetric flask, add 50 ml of mobile phase and sonicate to dissolve it completely and then volume was made up to the mark with mobile phase to get 100 µg/ml of standard stock solution of working standard. Then it was ultrasonicated for 10 minutes and filtered through 0.20 µ membrane filter.

#### Preparation of ALBENDAZOLE and IVERMECTIN standard solution

Weighed accurately about 10 mg of ALBENDAZOLE and IVERMECTIN and transfer to 100 ml volumetric flask, add 50 ml of mobile phase and sonicate to dissolve it completely and then volume was made up to the mark with mobile phase to get 100 µg/ml of standard stock solution of working standard. Then it was ultrasonicated for 10 minutes and filtered through 0.20 µ membrane filter.



Chromatogram of standard preparation of ALBENDAZOLE and IVERMECTIN ("Alcohol and acetonitrile in the ratio of 60:40%v/v")

### Validation

#### Accuracy

##### Results of accuracy study

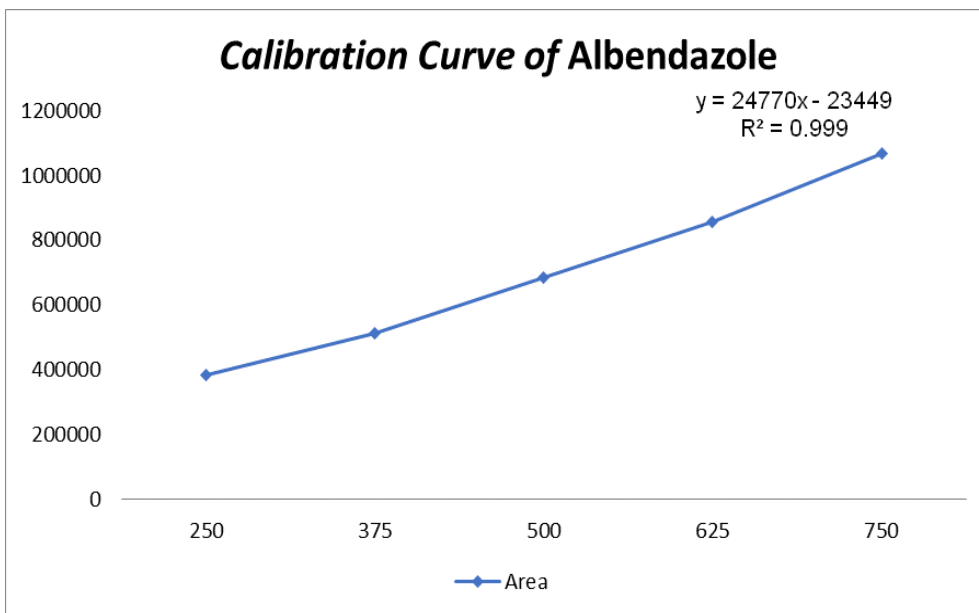
Drug	Level %	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery	Mean recovery (%)	Std. Dev	% RSD
Albendazole	50	5.64	5.55	98.40	98.97%	0.306	0.78%
	100	11.88	11.72	98.65			
	150	16.43	16.41	99.87			
Ivermectin	50	1.25	1.24	99.20	98.98%	0.194	0.20%
	100	2.5	2.47	98.82			
	150	3.78	3.74	98.94			

### Precision Study

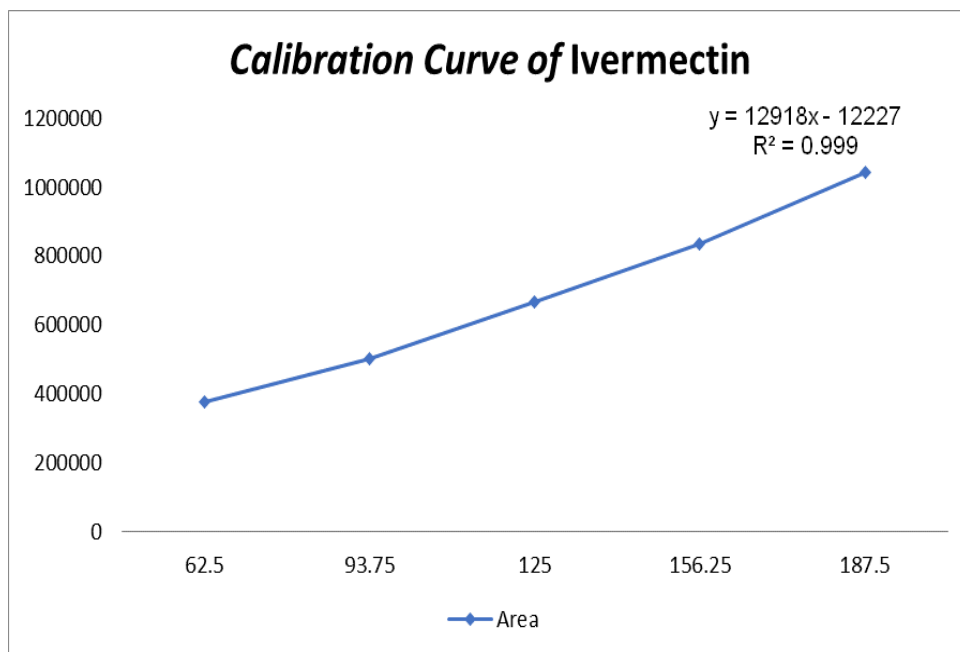
#### Method Precision

Replicate	ALBENDAZOLE		IVERMECTIN
S. No.	Injection volume (µl)	Area	Area
1	10 ul	684559	667881
2		684672	667754
3		684601	667969
4		684521	667894
5		684519	667909
6		684607	667856
Average	684579.83	667877.16	
Std.Dev	58.782	71.22	
% RSD	0.01%	0.02%	
Standard potency	99.98%	99.98%	

**Method Precision  
Linearity**



Calibration Curve of Albendazole



Calibration Curve of Ivermectin

Linearity level	ALBENDAZOLE		IVERMECTIN	
	Level	Concentration (µg/ml)	Area	Concentration (µg/ml)
1	250	385064	62.5	375690
2	375	513419	93.75	500920
3	500	684559	125	667894
4	625	855698	156.25	834867
5	750	1069622	187.5	1043583
Correlation co-efficient	0.9992		0.9994	
Slope	247703		129188	
Intercept	234494		122276	

**Robustness**

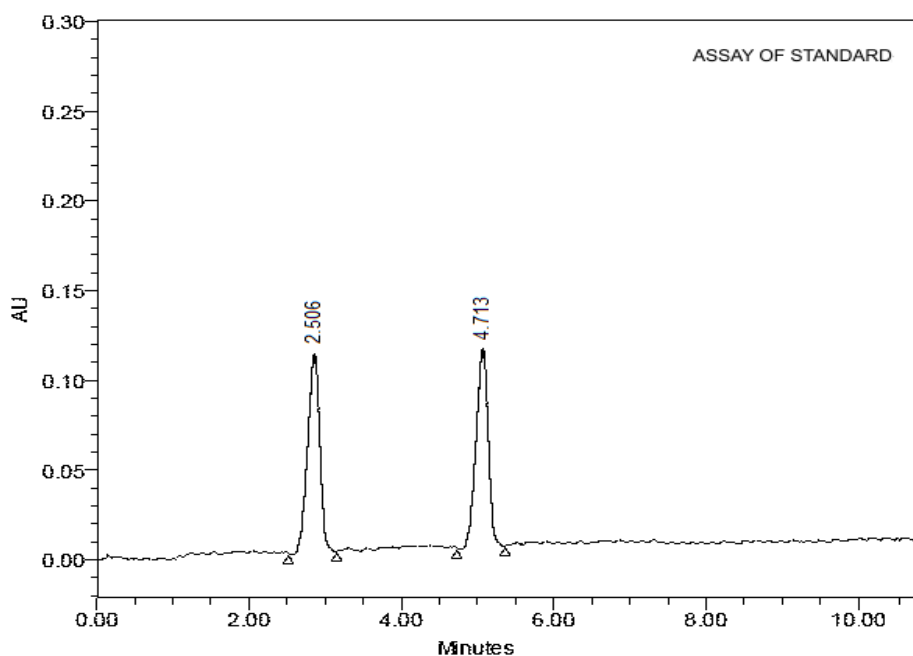
Parameter	<i>ALBENDAZOLE</i>		<i>IVERMECTIN</i>	
	Peak Area	% RSD	Peak Area	%RSD
Low	684654	0.03%	667849	0.04%
	684662			
	684717			
Actual	684756	0.02%	667741	0.02%
	684649			
	684687			
High	684758	0.02%	667734	0.03%
	684690			
	684699			

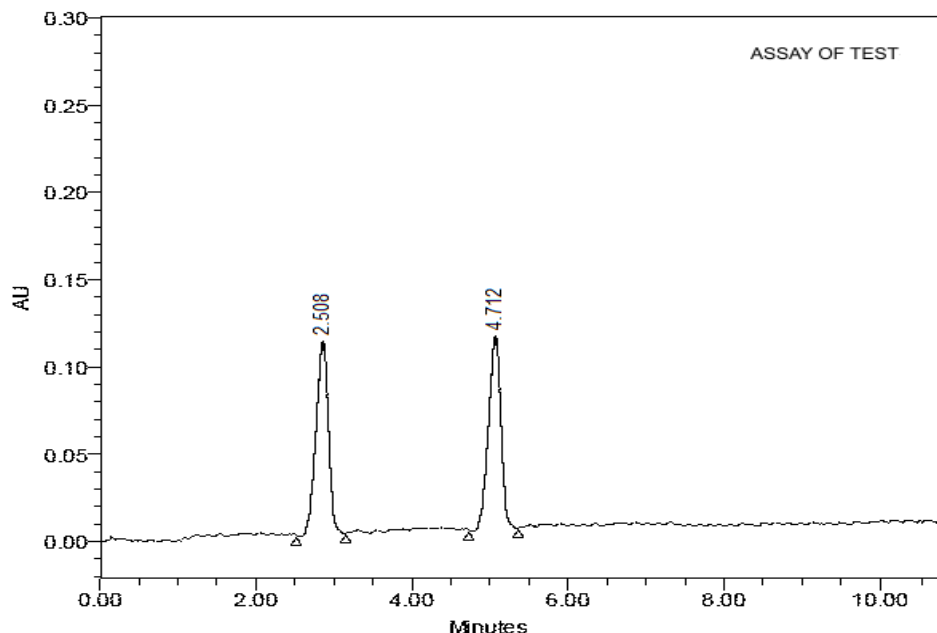
**Robustness  
Ruggedness**

Sr. No.	<i>ALBENDAZOLE</i>	<i>IVERMECTIN</i>
1	684756	667703
2	684852	667775
3	684762	667867
Mean	684790	667781
Std. Dev.	53.77	82.20
%RSD	0.02%	0.04%

**Analysis of Formulation**

Assay studies for the analysis of formulation of Albendazole and Ivermectin. Fixed chromatographic conditions were made use for the analysis of formulation.

**ASSAY OF STD**



### ASSAY OF TEST

$$\% \text{ Assay} = \frac{AT}{AS} \times \frac{W1}{100} \times \frac{1}{25} \times \frac{100}{W2} \times \frac{25}{1} \times \frac{AW}{LC} \times P$$

### Albendazole

$$\% \text{ Assay} = \frac{684756}{684239} \times \frac{10.32}{100} \times \frac{1}{25} \times \frac{100}{10.32} \times \frac{25}{1} \times \frac{500.40}{500} \times 99.98 = 99.28\%$$

### Calculation formula for IVERMECTIN

$$\% \text{ Assay} = \frac{AT}{AS} \times \frac{W1}{100} \times \frac{1}{25} \times \frac{100}{W2} \times \frac{25}{1} \times \frac{AW}{LC} \times P$$

### Ivermectin

$$\% \text{ Assay} = \frac{667703}{667321} \times \frac{2.5}{100} \times \frac{1}{25} \times \frac{100}{2.5} \times \frac{25}{1} \times \frac{125.65}{125} \times 99.98 = 99.41\%$$

### CONCLUSION

The RP-UPLC strategies utilized here fulfil all the gauges required. In further development, the methods suggested offer an increased enthusiasm for the evaluation of insufficient medicinal products and scheduling of jobs. The unexpected recoveries were discovered on all occasions and it was reported in the Convention that strategies might be used for examinations using the vet dosage methods.

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