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SPIKED FORCE DEGRADATION ASSAY METHOD EVALUATION FOR ESTIMATION OF ALBENDAOLE IN VETERINARY DOSAGE FORM

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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 1.5 mL min-1 shows good resolution. The PDA detector response of ALBENDAOLE was studied and the best wavelength was found to be 225 nm showing highest sensitivity. The mixture of two solutions Water -acetonitrile in the ratio of 70:30%v/v". Finally, the pH was adjusted to 7.65 by sodium hydroxide. with gradient programming was used as mobile phase at 1.5mL/min was found to be an appropriate mobile phase for separation of ALBENDAOLE. The column was maintained at ambient temperature.

KEYWORDS: veterinary dosage form, ALBENDAOLE and Hypersil BDS C18.

INTRODUCTION

Veterinary Pharmaceutical

Veterinary research about incorporate the anticipation, control, conclusion, and treatment of animal sicknesses, as well as the ponder of animal science, welfare, and care, among other things. Perspectives of veterinary investigate that rise above species boundaries incorporate the ponder of actually happening and tentatively actuated models of both human and animal maladies, as well as inquire about at the human-animal interface in ranges such as nourishment security, natural life and biological system wellbeing, zoonotic infections, open arrangement, and open wellbeing.

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

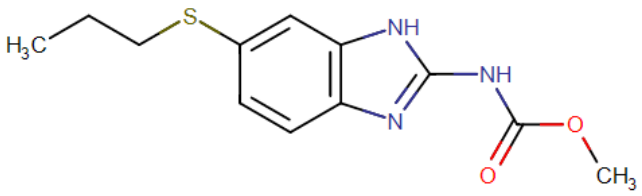


Fig. 1 Chemical Structure of Albendazole.

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 SkliceTM. 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

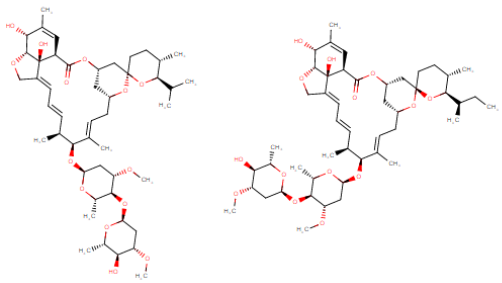


Fig. 2 Chemical Structure of Ivermectin.

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"

- **Forced degradation studies of our selected pharmaceutical drugs**

In order to establish the analytical method for a stability indicating method, the drugs are subjected to various stress conditions to conduct forced degradation studies. Stress studies were carried out under the conditions of acid/base hydrolysis, oxidation, reduction, in accordance with ICH Q1A (R2). Several trials with different severity of each stressed condition are to be conducted, so that upto 10-30% degradation is to be achieved.

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.

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 1.5 mL min-1 shows good resolution. The PDA detector response of ALBENDAOLE was studied and the best wavelength was found to be 225 nm showing highest sensitivity.

The mixture of two solutions Water -acetonitrile in the ratio of 70:30%v/v". Finally, the pH was adjusted to 7.65 by sodium hydroxide. with gradient programming was used as mobile phase at 1.5mL/min was found to be an appropriate mobile phase for separation of ALBENDAOLE. The column was maintained at ambient temperature.

Preparation of internal standard solution

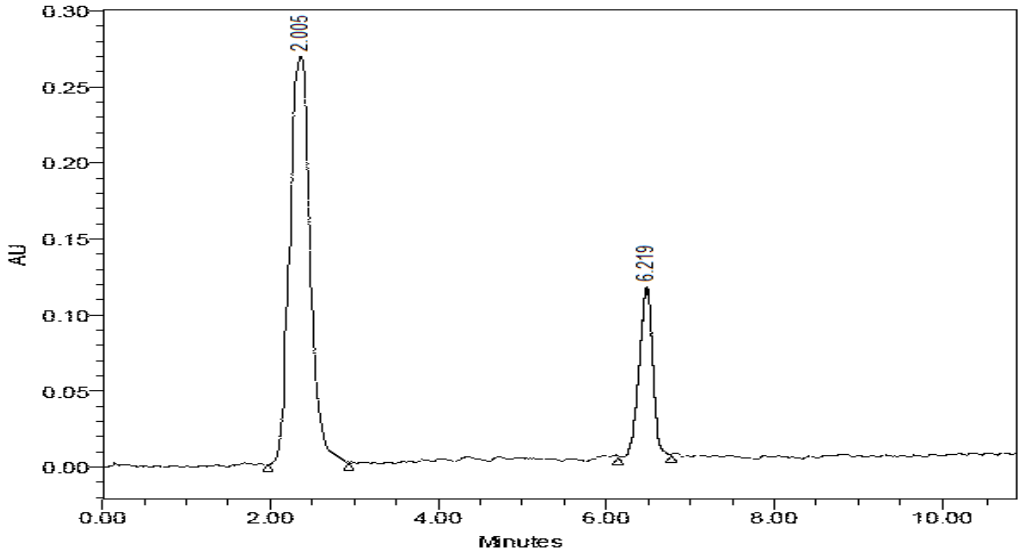
Weighed accurately about 10 mg of ALBENDAOLE working standard and transfer to 100 ml volumetric flask, add 50 ml of mobile phase and sonicate to dissolve it completely and then volume was made up to the mark with mobile phase to get 100 µg/ml of standard stock solution of working standard. Then it was ultrasonicated

for 10 minutes and filtered through 0.20 µ membrane filter.

Preparation of ALBENDAOLE standard solution
Weighed accurately about 10 mg of ALBENDAOLE 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.

ALBENDAOLE	
System	UPLC
Stationary Phase	C18 column
"***Mobile Phase***"	"Water -acetonitrile in the ratio of 70:30%v/v"
Diluents	Acetonitrile
Injection volume	20µl
Temperature	Ambient
Flow rate	1.5 ml/min
UV detection	225nm
Retention Time	ALBENDAZOLE– 2.005 mins; IVERMECTIN – 6.219 mins
Inference	"Satisfactory separation of the drugs was achieved with good resolution and minimal tailing."

ALBENDAOLE in UPLC System



Chromatogram of standard preparation of ALBENDAOLE (Water and acetonitrile in the ratio of 70:30%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	5.66	5.56	98.42	98.98%	0.307	0.79%
	100	11.89	11.73	98.67			
	150	16.46	16.43	99.89			
Ivermectin	50	1.27	1.25	99.22	98.99%	0.195	0.21%
	100	2.52	2.48	98.84			
	150	3.79	3.75	98.95			

PRECISION STUDY
METHOD PRECISION

Replicate	*ALBENDAZOLE*		IVERMECTIN
S.No.	**Injection volume (µl)**	**Area**	**Area**
1	10 ul	684558	667883
2		684675	667752
3		684606	667963
4		684525	667895
5		684516	667904
6		684608	667853
% RSD	0.01%		0.02%
Standard potency	99.98%		99.98%

METHOD PRECISION
LINEARITY

Linearity level	*ALBENDAZOLE*		*IVERMECTIN*	
Level	**Concentration (µg/ml)**	**Area**	**Concentration (µg/ml)**	**Area**
1	250	385065	62.5	375694
2	375	513414	93.75	500925
3	500	684555	125	667894
4	625	855696	156.25	834865
5	750	1069623	187.5	1043589
Correlation co-efficient	0.9992		0.9994	
Slope	247703		129188	
Intercept	234494		122276	

ROBUSTNESS

Parameter	*ALBENDAZOLE*		*IVERMECTIN*	
	Peak Area	**% RSD**	**Peak Area**	**%RSD**
Low	684655	0.03%	667844	0.04%
	684663		667795	
	684714		667784	
Actual	684755	0.02%	667743	0.02%
	684645		667854	
	684686		667846	
High	684757	0.02%	667732	0.03%
	684698		667811	
	684694		667705	

Robustness

RUGGEDNESS

Sr. No.	*ALBENDAZOLE*	*IVERMECTIN*
1	684758	667704
2	684853	667776
3	684764	667864
Mean	684795	667783
%RSD	0.02%	0.04%

Ruggedness

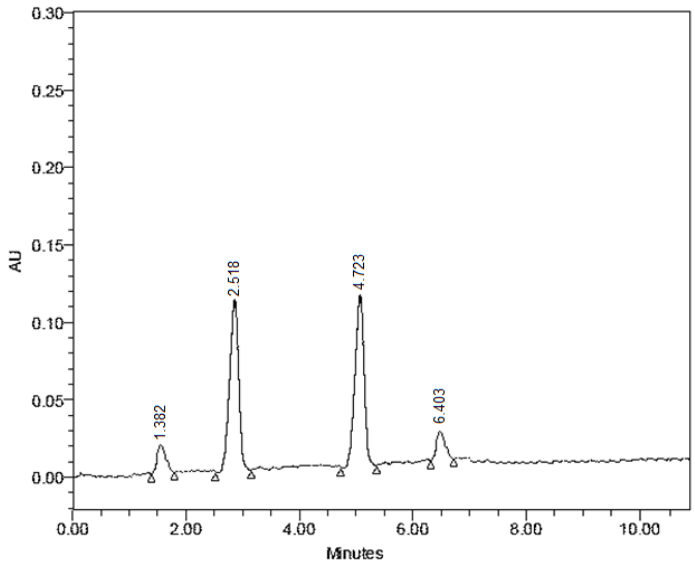
ANALYSIS OF FORMULATION

Assay studies for the analysis of formulation of ALBENDAOLE. Fixed chromatographic conditions were made use for the analysis of formulation.

Acidic Degradation

An accurate 10 ml of pure drug sample solution was transferred to a clean and dry round bottom flask (RBF). 30 ml of 0.1 N HCl was added to it. It was refluxed in a

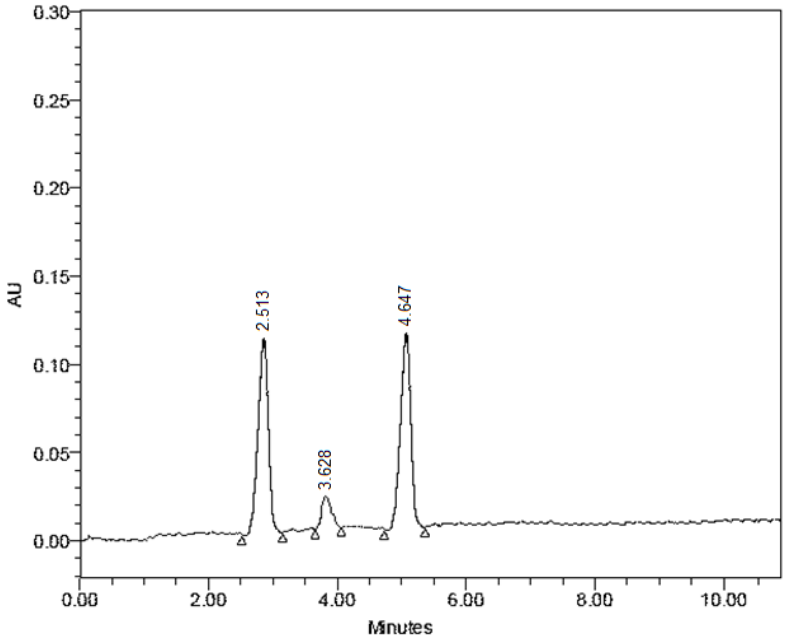
water bath at 60°C for 6 hours. Drug became soluble after reflux which was insoluble initially. Allowed to cool at room temperature. The sample was then neutralized using 2N NaOH solution and final volume of the sample was made up to 100ml with water to prepare 100ppm solution. It was injected into the UPLC system against a blank of mobile phase after optimizing the mobile phase composition, chromatogram was recorded."



Acidic Degradation

Basic Degradation

"An accurate 10 ml of pure drug sample solution was transferred to a clean and dry RBF. 30 ml of 0.1N NaOH was added to it. It was refluxed in a water bath at 60°C for 6 hours. Drug became soluble after reflux which was insoluble initially. It was allowed to cool at room temperature. The sample was then neutralized using 2N HCl solution and final volume of the sample was made up to 100ml with water to prepare 100ppm solution. It was injected into the UPLC system against a blank of mobile phase after optimizing the mobile phase composition, chromatogram was recorded."

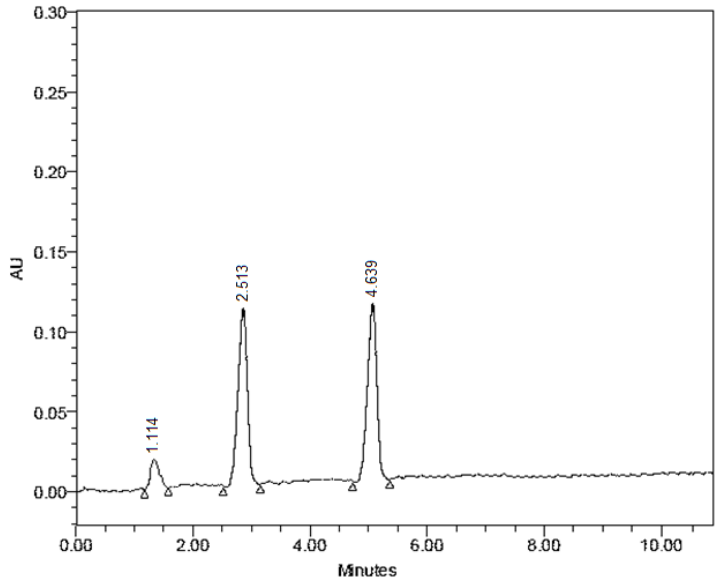


Basic Degradation

Oxidative

Approximately 10 ml of pure drug sample was transferred in a clean and dry 100 ml volumetric flask. 30 ml of 3% H2O2 and a little methanol was added to it to make it soluble and then kept as such in dark for 6 hours. Final volume was made up to 100 ml using water

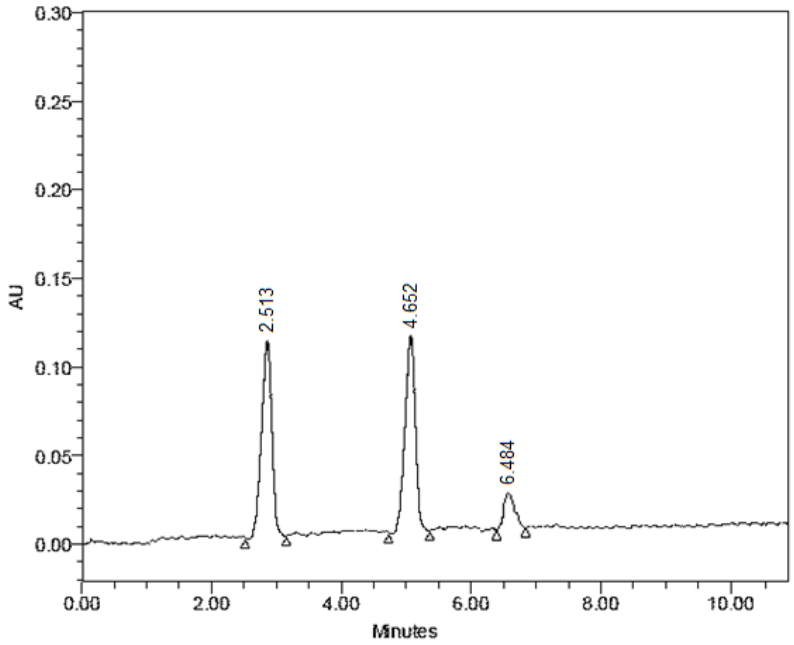
to prepare 100 ppm solution. The above sample was injected into the UPLC system. The chromatogram was recorded.



Oxidative Degradation

Wet Heat Degradation

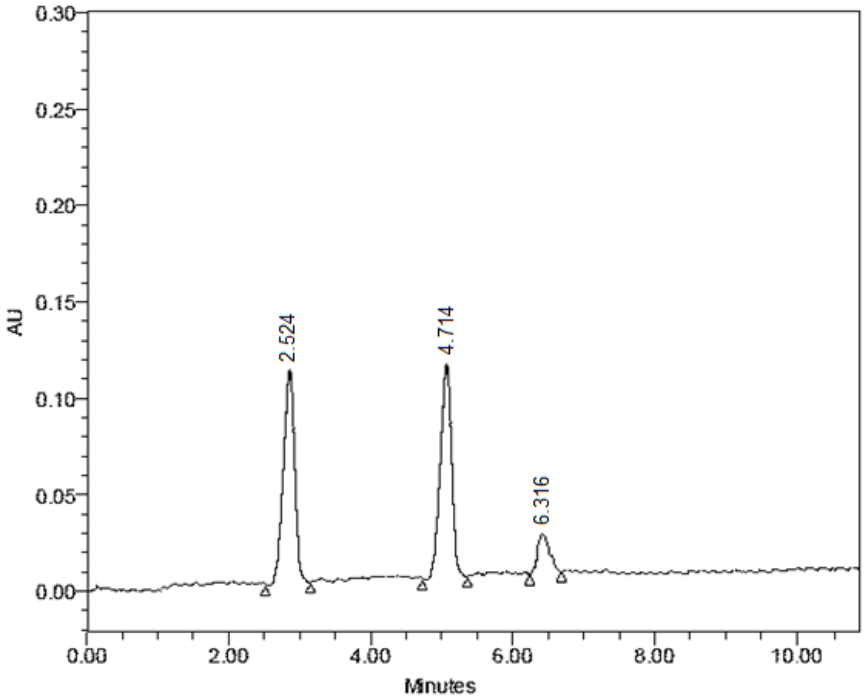
"Accurate 10 ml of pure drug sample was transferred to a clean and dry RBF. 30ml of HPLC grade water was added to it. Then, it was refluxed in a water bath at 60°C for 6 hours uninterruptedly. After the completion of reflux, the drug became soluble and the mixture of drug and water was allowed to cool at room temperature. Final volume was made up to 100 ml with HPLC grade water to prepare 100 ppm solution. It was injected into the UPLC system against a blank of mobile phase after optimizing the mobile phase composition, chromatogram was recorded."



Wet Heat Degradation

Photolytic

The photochemical stability of the drug was also studied by exposing the drug solution (4ml) to sunlight for 6 h. Twenty microlitres of the resultant solutions were injected onto column and the chromatograms were run as described.



Photolytic Degradation

Forced degradation Study

Nature of Stress	*Degradation condition*	*Time(h)*	*Number of degradation products*
Acidic	*60°C*	*6*	*2*
Basic	*60°C*	*6*	*1*
Oxidative	*RT*	*6*	*1*
Wet Heat	*105°C*	*6*	*1*
Photolytic	*AT*	*6*	*1*

Acidic Degradation

$$\% \text{ Assay} = \frac{357338}{365461} \times \frac{04.05}{100} \times \frac{1}{25} \times \frac{100}{04.06} \times \frac{25}{1} \times \textbf{Error!} \times 98.60 = 96.27\%$$

Basic Degradation

$$\% \text{ Assay} = \frac{362849}{365461} \times \frac{04.05}{100} \times \frac{1}{25} \times \frac{100}{04.06} \times \frac{25}{1} \times \textbf{Error!} \times 98.60 = 97.76\%$$

Oxidative Degradation

$$\% \text{ Assay} = \frac{359276}{365461} \times \frac{04.05}{100} \times \frac{1}{25} \times \frac{100}{04.06} \times \frac{25}{1} \times \textbf{Error!} \times 98.60 = 96.79\%$$

Wet Heat

$$\% \text{ Assay} = \frac{362765}{365461} \times \frac{04.05}{100} \times \frac{1}{25} \times \frac{100}{04.06} \times \frac{25}{1} \times \textbf{Error!} \times 98.60 = 97.73\%$$

Photolytic Control

$$\% \text{ Assay} = \frac{363393}{365461} \times \frac{04.05}{100} \times \frac{1}{25} \times \frac{100}{04.06} \times \frac{25}{1} \times \textbf{Error!} \times 98.60 = 97.87\%$$

CONCLUSION

The RPUPLC tactics used here meet all requirements. The techniques imply higher excitement for evaluating inadequate medicines and job schedule. The convention noted that techniques may be utilised for exams employing vet dosage methodologies.

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