

**CHEMICAL FORCE DEGRADATION ASSAY METHOD EVALUATION FOR
SIMULTANEOUS ESTIMATION OF ALBENDAZOLE AND IVERMECTIN IN BULK
VETERINARY DOSAGE FORM**Dr. Osman Ahmed*¹, Ashraf Unnisa¹, Meher Afrin¹, Mohammed Akthar Sulthana¹ and Dr. Anas Rasheed²¹Department of Pharmaceutical Analysis, Deccan School of Pharmacy, Hyderabad.²CSO, Gaelib Medications Private Limited, Hyderabad.***Corresponding Author: Dr. Osman Ahmed**

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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⁻¹ 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**

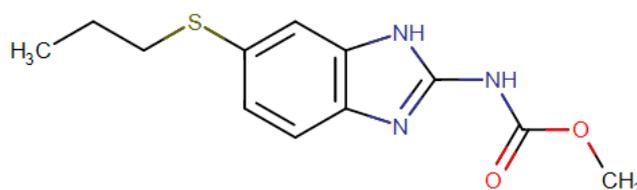
Veterinary pharmaceutical could be a field of pharmaceutical that's concerned with the avoidance, control, conclusion, and treatment of sickness, clutter, and harm in animals. It is additionally known as veterinary therapeutic hone. This can be in expansion to creature raising, cultivation, breeding, 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

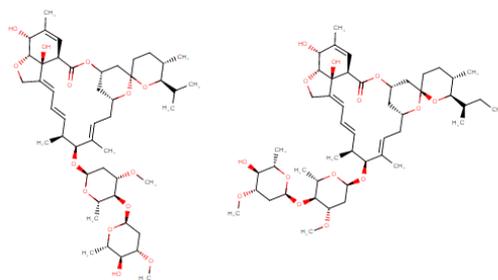


Fig. 2 Chemical Structure of Ivermectin.

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.

➤ 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.

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⁻¹ 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.

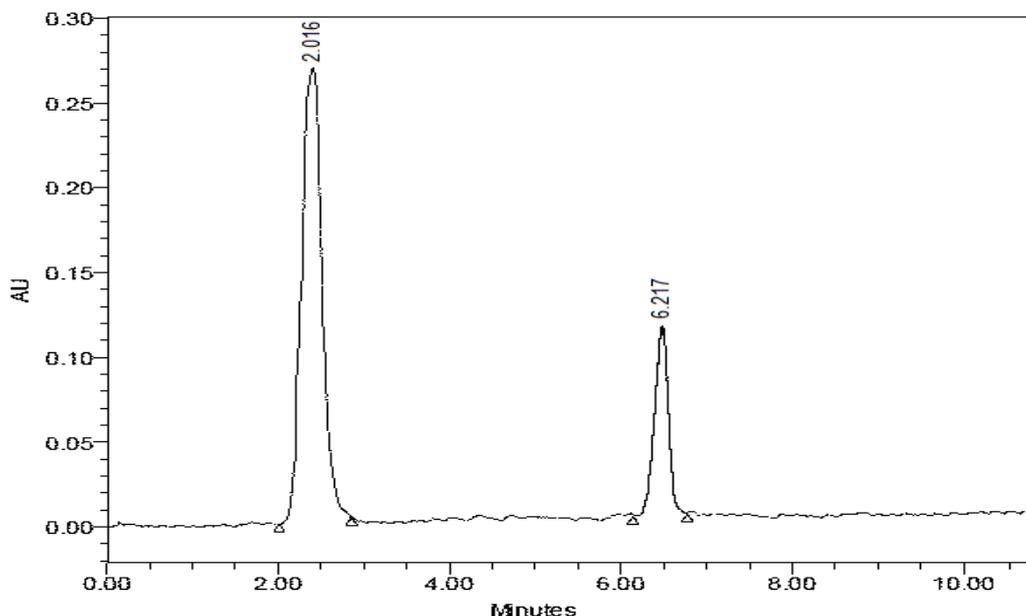
Preparation of ALBENDAZOLE and IVERMECTIN standard solution

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 μ g/ml of standard stock solution of working standard. Then it was ultrasonicated for 10 minutes and filtered through 0.20 μ membrane filter.

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

ALBENDAZOLE and IVERMECTIN in UPLC System



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

VALIDATION

ACCURACY

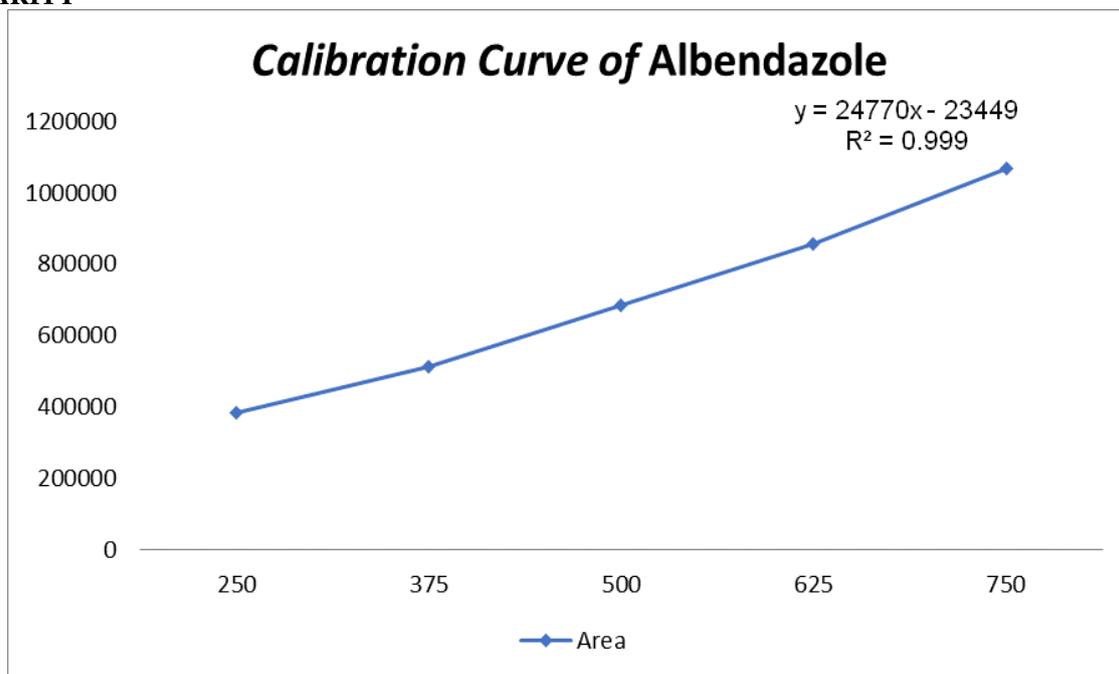
Results of accuracy study

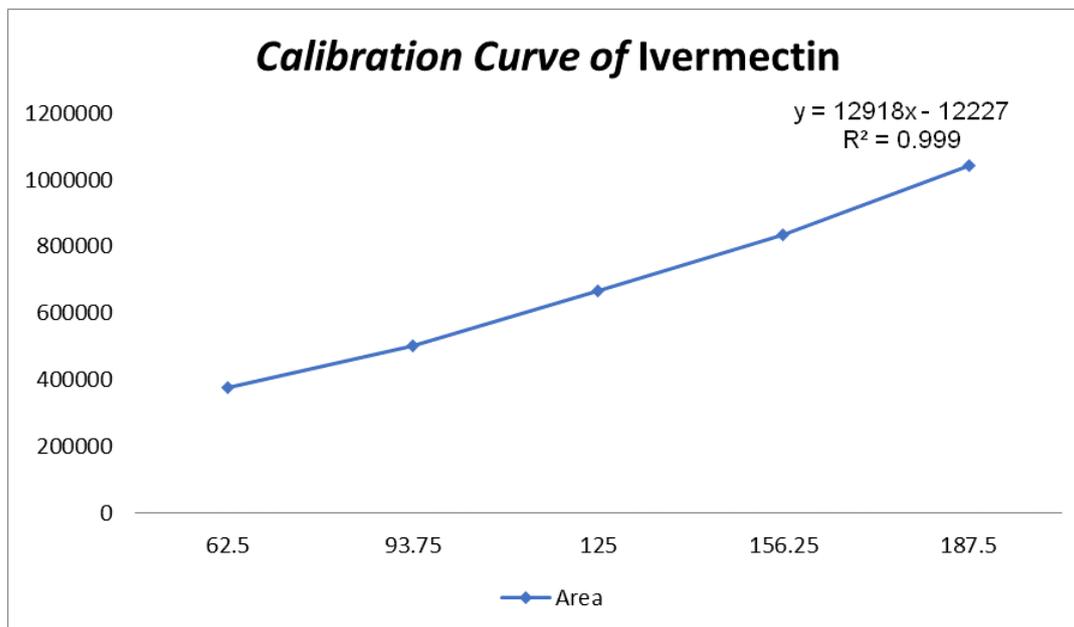
Drug	Level %	Amount added (μ g/ml)	Amount found (μ g/ml)	% Recovery	Mean recovery (%)	Std. Dev	% RSD
Albendazole	50	5.64	5.55	98.40	98.97%	0.306	0.78%
	100	11.88	11.72	98.65			
	150	16.43	16.41	99.87			
Ivermectin	50	1.25	1.24	99.20	98.98%	0.194	0.20%
	100	2.5	2.47	98.82			
	150	3.78	3.74	98.94			

PRECISION STUDY
METHOD PRECISION

Table No: 12. METHOD PRECISION.

<i>Replicate</i>	<i>ALBENDAZOLE</i>		<i>IVERMECTIN</i>
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%	

LINEARITY*Calibration Curve of Albendazole*



Calibration Curve of Ivermectin

Linearity level	ALBENDAZOLE		IVERMECTIN	
Level	Concentration (µg/ml)	Area	Concentration (µg/ml)	Area
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 coefficient	0.9992		0.9994	
Slope	247703		129188	
Intercept	234494		122276	

ROBUSTNESS

Parameter	ALBENDAZOLE		IVERMECTIN	
	Peak Area	% RSD	Peak Area	%RSD
Low	684654	0.03%	667849	0.04%
	684662		667793	
	684717		667786	
Actual	684756	0.02%	667741	0.02%
	684649		667851	
	684687		667845	
High	684758	0.02%	667734	0.03%
	684690		667814	
	684699		667703	

Robustness

RUGGEDNESS

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

Ruggedness

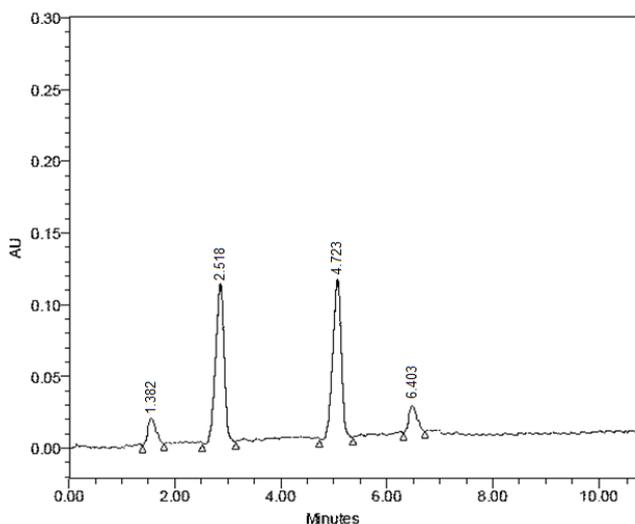
ANALYSIS OF FORMULATION

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

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.”

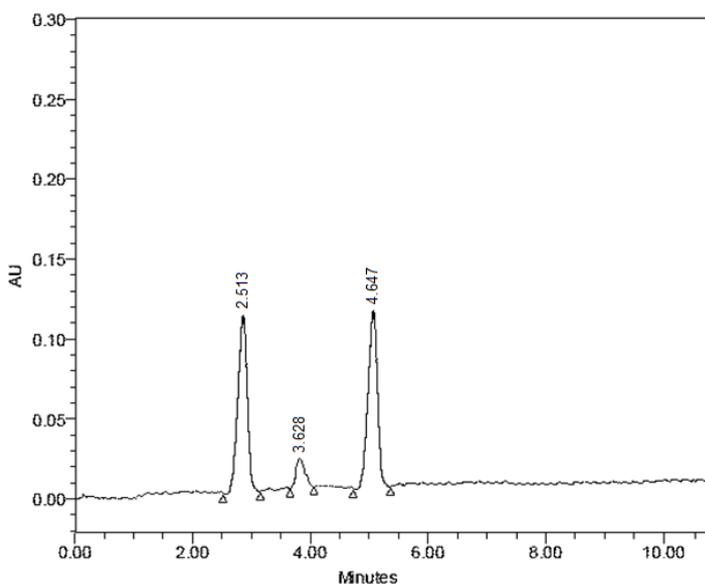


Acidic Degradation

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.”

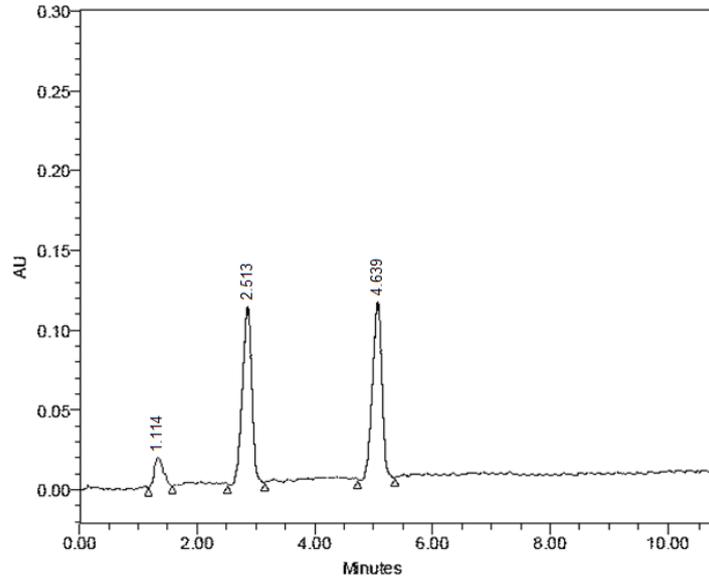


Basic Degradation

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.

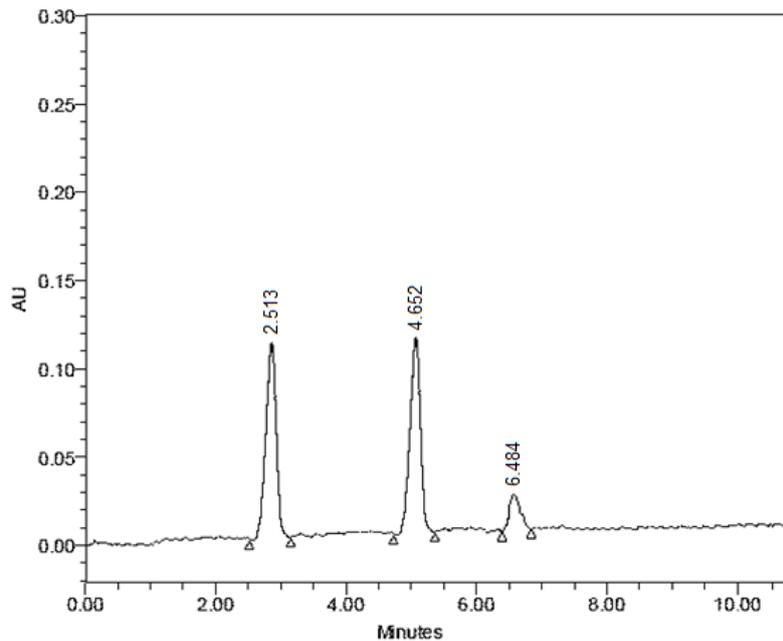


Oxidative Degradation

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.”

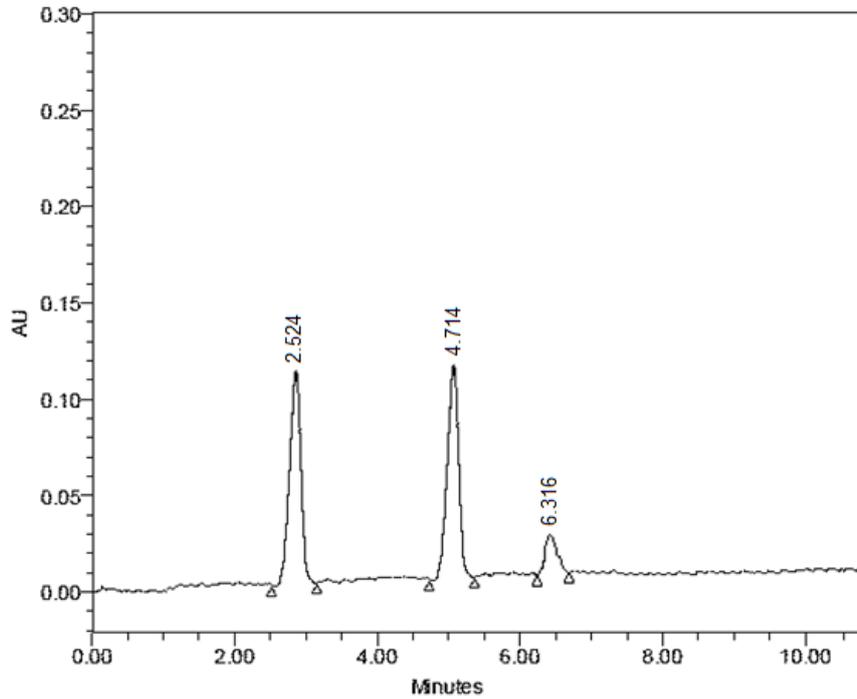


Wet Heat Degradation

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

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

Forced degradation Study

Acidic Degradation

$$\% \text{ Assay} = \frac{357338}{365461} \times \frac{04.05}{100} \times \frac{1}{25} \times \frac{100}{04.06} \times \frac{25}{1} \times \text{Error!} \times 98.60 = 96.26\%$$

Basic Degradation

$$\% \text{ Assay} = \frac{362849}{365461} \times \frac{04.05}{100} \times \frac{1}{25} \times \frac{100}{04.06} \times \frac{25}{1} \times \text{Error!} \times 98.60 = 97.75\%$$

Oxidative Degradation

$$\% \text{ Assay} = \frac{359276}{365461} \times \frac{04.05}{100} \times \frac{1}{25} \times \frac{100}{04.06} \times \frac{25}{1} \times \text{Error!} \times 98.60 = 96.78\%$$

Wet Heat

$$\% \text{ Assay} = \frac{362765}{365461} \times \frac{04.05}{100} \times \frac{1}{25} \times \frac{100}{04.06} \times \frac{25}{1} \times \text{Error!} \times 98.60 = 97.72\%$$

Photolytic Control

$$\% \text{ Assay} = \frac{363393}{365461} \times \frac{04.05}{100} \times \frac{1}{25} \times \frac{100}{04.06} \times \frac{25}{1} \times \text{Error!} \times 98.60 = 97.89\%$$

CONCLUSION

The RPUPLC techniques that were used in this study met all of the requirements. The approaches that have been proposed have the potential to generate more interest in the assessment of inadequate medical items and the scheduling of employment in the future. It was observed on all instances that unexpected recoveries had occurred, and it was revealed at the Convention that strategies may be employed for exams utilising the veterinarian dosage procedures.

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