

**EVALUATION AND VALIDATION OF A UPLC METHOD FOR ESTIMATION OF ALBENDAZOLE 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<sup>-1</sup> shows good resolution. The PDA detector response of Albendazole 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 Albendazole. The column was maintained at ambient temperature.

**KEYWORDS:** Veterinary dosage form, Albendazole and Hypersil BDS C18.

**INTRODUCTION**

**Veterinary Pharmaceutical**

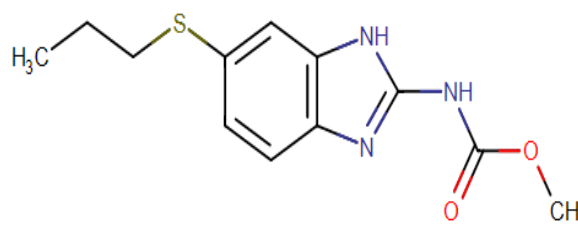
Veterinary research about incorporate the anticipation, control, conclusion, and treatment of animal sicknesses, as well as the ponder of animal science, welfare, and care, among other things. Perspectives of veterinary investigate that rise above species boundaries incorporate the ponder of actually 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**



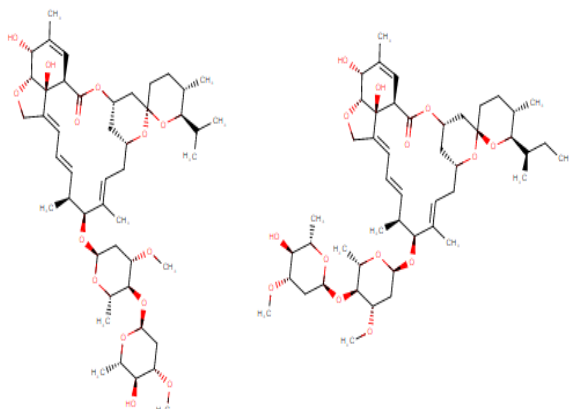
**Fig. 1: Chemical Structure of Albendazole.**

**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 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

### ALBENDAZOLE in UPLC System

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

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<sup>-1</sup> shows good resolution. The PDA detector response of ALBENDAZOLE was studied and the best wavelength was found to be 225 nm showing highest sensitivity.

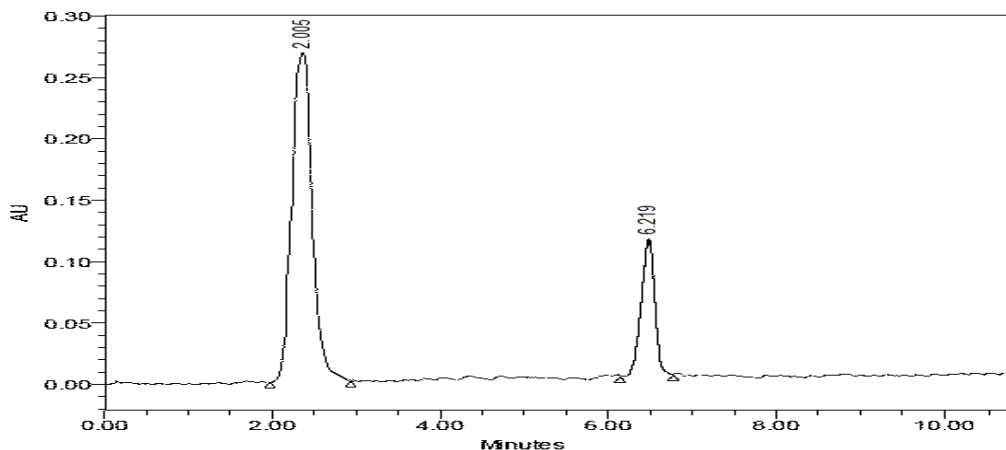
The mixture of two solutions Water -acetonitrile in the ratio of 70:30% v/v”. Finally, the pH was adjusted to 7.65 by sodium hydroxide. with gradient programming was used as mobile phase at 1.5mL/min was found to be an appropriate mobile phase for separation of ALBENDAZOLE. The column was maintained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

#### Preparation of internal standard solution

Weighed accurately about 10 mg of ALBENDAZOLE 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 standard solution

Weighed accurately about 10 mg of ALBENDAZOLE 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 (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<br>S. No. | ALBENDAZOLE           |        | IVERMECTIN |
|---------------------|-----------------------|--------|------------|
|                     | 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<br>Level | ALBENDAZOLE           |         | IVERMECTIN            |         |
|--------------------------|-----------------------|---------|-----------------------|---------|
|                          | 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**

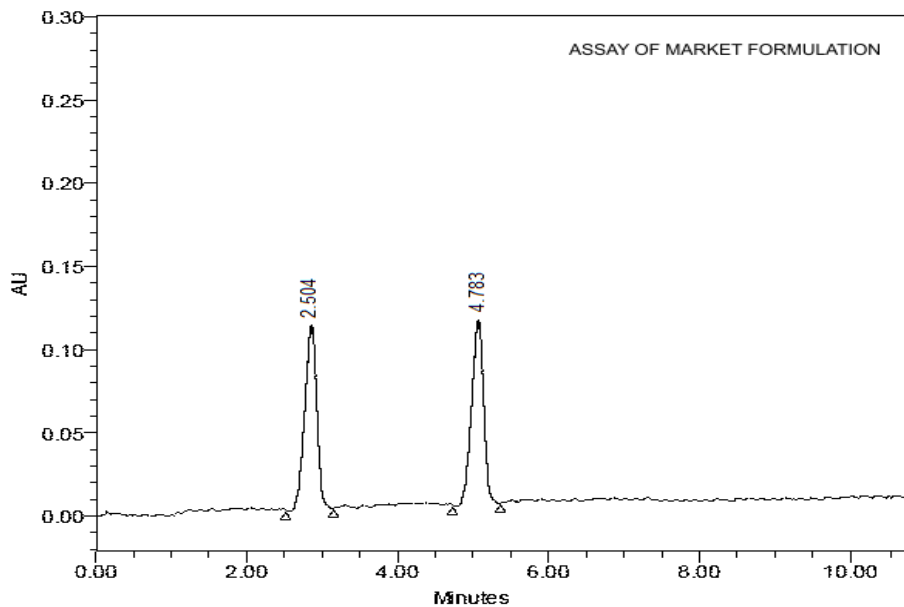
| Parameter | ALBENDAZOLE |       | IVERMECTIN |       |
|-----------|-------------|-------|------------|-------|
|           | Peak Area   | % RSD | Peak Area  | %RSD  |
| Low       | 684655      | 0.03% | 667844     | 0.04% |
|           | 684663      |       |            |       |
|           | 684714      |       |            |       |
| Actual    | 684755      | 0.02% | 667743     | 0.02% |
|           | 684645      |       |            |       |
|           | 684686      |       |            |       |
| High      | 684757      | 0.02% | 667732     | 0.03% |
|           | 684698      |       |            |       |
|           | 684694      |       |            |       |

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



**Assay of Market Formulation**

**Calculation formula for Market sample**

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

**Market sample**

$$\% \text{ Assay} = \frac{1332097}{1344160} \times \frac{12.5}{100} \times \frac{1}{25} \times \frac{100}{12.5} \times \frac{25}{1} \times \frac{625.65}{625} \times 99.98 = 99.18\%$$

**CONCLUSION**

The RPUPLC tactics used here meet all of the metrics needed to be implemented. The approaches proposed for future development give improved excitement for the assessment of inadequate medicines and the scheduling of employment. All of the unexpected recoveries were detected, and it was revealed at the Convention that tactics may be employed for tests using veterinarian dosing procedures.

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