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ANALYTICAL METHOD DEVELOPMENT AND ITS VALIDATION OF FENBENDAZOLE IN BULK AND MARKETED FORMULATION

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ABSTRACT

The present study reveals that novel, simple Analytical Method Ultraviolet Spectrophotometer & High Performance liquid chromatography was developed of for the analysis of Fenbendazole. In the ultraviolet Spectrophotometer of Fenbendazole is detected at 221 nm. The proposed method is fully validated & found to be linear over a workable drug concentration & R^2 was found to be 0.998, accurate, Precise & robust and suitable for routine analysis. HPLC method development was done. HPLC developed method Chromatographic Separation was achieved on the C18 ODS (250- X 4.6MM,5µm) column using Mobile Phase Acetonitrile: Methanol in different ratio (85:15) as mobile phase at a flow rate 0.8ml/minute, 30 min run time & 20µl injection volume with 30°C column temperature is employed for the analysis. The Ultraviolet spectrophotometric method was developed using Schimadzu 1800. The method was validated as per ICH Q2B guideline. This is fast & inexpensive method. It is suitable for research laboratories as well as for quality control analysis in pharmaceutical industries.

Keywords: Fenbendazole, UV, HPLC and Validation.

INTRODUCTION

1.1 ANALYTICAL METHOD DEVELOPMENT AND METHOD VALIDATION

Analytical chemistry is a branch of chemistry which deals with identification of components (qualitative) and determination of quantity of components (quantitative) of substances or samples or mixture. There are two types of analysis, one is qualitative analysis and another one is quantitative analysis. In qualitative analysis, there is identification of components or analyte of mixture or sample is carried out. In quantitative analysis, there is determination of amount of components or analyte of mixture or sample is carried out (Kenkel J, 2003). Analytical data is required not only in chemistry but also in other sciences like biology, zoology, arts such as painting and sculpture, archaeology, space exploration and clinical diagnosis. Important areas of application of analytical chemistry are quality control in manufacturing industries, monitoring and control of pollutants, clinical and biological studies, geological assays, fundamental and applied research (Kissinger PT, 2002).

ANALYTICAL METHOD

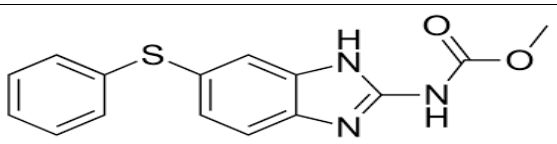
Analytical method includes use of a specified technique and detailed-stepwise instructions which are used in qualitative, quantitative or structural analysis of a sample for one or more analytes (Kissinger PT, 2002). Analytical methods are mainly classified into two types: Clas- - od in which the signal is proportional to the absolute amount of analyte is called classical method. A method in which the signal is proportional to the analytes concentration is called instrumental method (Harvey D, 2000). Classical methods are divided into 3 main types are: a) Separation of analyte, b) Qualitative analysis and c) Quantitative analysis. Separation of analyte includes extraction, distillation, precipitation and filtration. Qualitative analysis includes boiling point, freezing point, colour, odour, density, reactivity and refractive index. Quantitative analysis includes gravimetric analysis and volumetric analysis. Instrumental methods are divided into four main types are: a) spectroscopic methods, b) electrochemical methods, c) chromatographic methods and d) other

techniques. Spectroscopic methods include ultraviolet-visible spectroscopy, infrared spectroscopy, Raman spectroscopy, atomic absorption spectroscopy and atomic emission spectroscopy, x-ray spectroscopy and nuclear magnetic spectroscopy. Electrochemical methods include Potentiometry, Coulometry and Voltametry. Chromatographic methods include column chromatography, paper chromatography, thin layer chromatography, high performance liquid chromatography, gas chromatography and modern methods (LC-MS, GC-MS, LC-MS-MS, GC-MS-MS, LC-NMR and GC-NMR). Other techniques include x-ray methods, radioactivity, mass spectrometry, optical methods (Refractometer, optical rotation) and thermal methods (Thermogravimetry, differential thermal analysis and differential scanning calorimetry) (Ravisankar P, et al., 2015; Jeffery GH, 1989).[1]

FENBENDAZOLE

Fenbendazole is a broad spectrum benzimidazole anthelmintic used against gastrointestinal parasites including : giardia, roundworms, hookworms, whipworms, the tapeworms genus Taenia(but not effective against Dipylidium caninum, a common dog tapeworm), pinworms, aelurostrongylus, paragonimiasis, strongyles, and strongyloides that can be administered to shep, cattle, horse, fish, dogs, cats, rabbits, most reptiles, Dwarf fresh water shrimp tanks Fenbendazole (FEN) is chemically methyl 5-(phenyl thio)-2-benzimidazolecarbamate is a as Planaria and Hydra treatments as well as seals. It is soluble in methanolic HCl. It is official in Indian Pharmacopoeia (IP), British Pharmacopoeia (BP) and United States Pharmacopoeia (USP).[1-5] Fenbendazole acts on helminthes primarily by binding to tubulin and disrupting the tubulin microtubule equilibrium; its utility as an antiparasitic drug results from differences in the structures of tubulin in mammalian cells and in lower organisms, which lead to its greater binding to tubulin, and therefore greater inhibition of polymerization, in the parasites[6-8]. In addition, the limited absorption of fenbendazole from the intestine results in low levels of the drug and its active metabolites in tissue relative to the levels within the gut, to which the targeted parasites are exposed[5,9,10] . Several widely used anticancer drugs produce their antineoplastic effects by disrupting either microtubule formation (vincristine; vinblastine) or microtubule depolymerization (paclitaxel; docetaxel)[8-11], suggesting that fenbendazole could have antitumor effects. Some data in the literature support this hypothesis[8]. A Fenbendazole containing diet combined with supplemental high dose of vitamins was reported by [Gao *et al].* to inhibit growth of a human lymphoma xenografted into scid mice[12] ; it was unclear whether this reflected a direct effect of the drug on tumor cells or stimulation of host immune responses.[Bai *et al].* reported that fenbendazole reduced the engraftment of brain tumors in nude mice[13]. [Chung *et al].* reported in a meeting presentation[14] that high doses of fenbendazole, albendazole and mebendazole inhibited the growth of paclitaxel-resistant tumors.[16]

Table 1: Monograph of Fenbendazole

Parameter	Description
Name	Fenbendazole
Chemical Structure	
Molecular formula	$C_{15}H_{13}N_3O_2S$
Molecular weight	299.349 g/mol
Description	White to dirty white coloured crystalline powder
Melting Point	233 ℃
Refractive index	1.6740
Solubility	Sparingly soluble in Dimethylformamide , very slightly soluble in methanol, phosphate Buffer (pH 7.4). Insoluble in water
Chemical class	Benzimidazole

Elimination Half life

The half-life for plasma elimination for fenbendazole in rats is 6 hours, rabbits 13 hours, dogs 15 hours and sheep 2 to 3 days. Elimination of fenbendazole is predominantly by the faecal route. The liver appears to be the main target tissue in all species tested.[26]

Metabolism

Fenbendazole is metabolized in the liver to oxfendazole partially gets reduced back to fenbendazole in the liver and rumen. also, fenbendazole itself is an active metabolite of another anthelmintic drugs, febantle.[19-24]

MECHANISM OF ACTION FENBENDAZOLE

Mechanism action of Fenbendazole is a benzimidazole that acts by binding to tubulin, an essential structural protein of microtubules. By blocking the microtubules in worms the uptake of glucose is blocked which eventually depletes glycogen reserve.[25]

The molecular mode of action of all benzimidazole, including fenbendazole, consists in binding to tubulin, a structural protein of microtubules. These microtubules are important organelles involved in the motility, the division and the secretion processes of cells in all living organisms. In the worms the blocking of microtubules perturbs the uptake of glucose, which eventually empties the glycogen reserves. This blocks the whole energy management mechanism of the worms that are paralyzed and die or are expelled.

Since cell division is also disturbed, worm egg production and development is also blocked by benzimidazoles, i.e. most of them also have an ovicidal effect.2[28]

SIDE EFFECT

Fenbendazole is of low acute toxicity after oral exposure. No acute exposure limit is available. Based on limited human data it appears that doses up to 500 mg per person did not result in adverse effects. Moreover, single doses up to 2,000 mg per person were reported to cause no adverse effects.[26]

MATERIALS AND METHODS

1. Material

Analytical method development of UV and HPLC of Fenbendazole

A. Equipment and materials

List of Instrument used

1. High Performance Liquid Chromatography (HPLC)- SHIMADZU
Model : Lc -2030c 3D CAT. NO.: 228-45202-48 SERIAL NO.: L21455401683AE 100-240~ 50-60Hz 600VA
2. UV spectrometry- SHIMADZU Model : UV -1800 CAT. NO.: 206-25400-58 SERIAL NO. :A11635580531 ML 220-240~ 50-60Hz 140VA
3. Electronic balance - SHIMADZU
4. Ultrasonicator- Modern Science India

List of Reagents/Chemicals

Fenbendazole, Phosphate buffer, Acetonitrile, water and Methanol

Method Development

Steps for Method development of Fenbendazole

Selection of solvent to be used as diluent and mobile phase

a. Choosing the suitable solvent in which Drug (Fenbendazole) is soluble and stable
b. It must be easily available, economical and of the HPLC grade.

Selection of column. In order to select the wavelength for carrying out the analysis, critical examination of the Ultraviolet absorbance spectra of the drug should be done. Selection of the mobile phase: For the mobile phase, suitable solvent system selects for better separation of the analyte, the first variable to be decided is whether an organic or aqueous eluent should be used. With the HPLC analysis, either an aqueous eluent

A. Reagents, chemicals and formulations used

Pure drug sample of Fenbendazole was used without further purification. HPLC grade sample water, Acetonitrile, Methanol was used.

B. Selection of solvent

The ideal property of a solvent be that the drug should be completely soluble in the solvent used. Drug should be stable in the solvent used and should be economical. Various solvents were studied for the solubility of Fenbendazole. The Fenbendazole Sparingly soluble in Dimethylformamide, very slightly

soluble in methanol, phosphate Buffer (pH 7.4), Insoluble in water Therefore mobile phase was selected as a solvent of choice for Fenbendazole.

Buffer Preparation
Dissolve 2.38 g of disodium hydrogen Phosphate, 0.19 gm of potassium dihydrogen phosphate and 8.0 g of sodium chloride in sufficient water to produce 1000 ml.

Preparation Standard solution
Standard solutions were prepared by dissolving the drugs in the diluents and diluting them to the desired concentration. Buffer and diluents used for the standard preparation and sample preparation were prepared as follow Buffer Dissolve 2.38 g of disodium hydrogen Phosphate, 8.0g of sodium chloride in sufficient water to produce 1000ml. Adjust the pH composed of the Fenbendazole standard was accurately weighed and transfer.

Preparation of Mobile phase
The selection of mobile phase was according to polarity and non- polarity of solvent. The Mobile phase containing Acetonitrile: Methanol in different ratios (85:15) sonicate to degassed and mix well. change in column

Preparation of working standard solution
From the standard stock solution of Fenbendazole 1ml from each solution taken and added in 10 ml volumetric flask and diluted up to the mark with Phosphate Buffer solution.

Assay preparation
Ten tablets were weighed and finely powdered. A quantity of powder equivalent to one tablet containing 150 mg of fenbendazole was transferred in a 100 mL volumetric flask, accurately weigh 10 mg tablet drug dissolve the 100ml of buffer solution (PH 7.4) make up the volume. The solution was filtered through Whatman filter paper 41.

Selection of diluent and mobile phase for HPLC Method development and validation
Primary thinking about the selection of diluent and mobile phase was important for good chromatographic conditions if the same solvent concentration ratio of diluent and mobile phase was used. So, for the selection of diluent, solubility of Fenbendazole
was taken as reference. Because of the free soluble nature of drug in solvent acetonitrile, Methanol it was selected. But the issue of the Methanol is very slightly soluble so it was decided to add 15 percent of methanol in it after some trial took. So the final ratio of diluent is Acetonitrile: Methanol (85:15). The selection of Methanol with chromatographic parameters was done in the HPLC system by trial and error method and by referring the literature survey about it. Finalized Diluent and Mobile phase solvents ratio was **Acetonitrile: Methanol** with ratio (85) :(15) respectively.

Selection of Wavelength for Fenbendazole
In the literature survey, it was found that many HPLC with UV detector having low detection sensitivity due to lack of strong chromophores in the drug molecule. So it was found that the maximum wavelength of the Fenbendazole is within the in range.

RESULT AND DISCUSSION

Method Validation of UV Spectroscopy

1) Linearity

Table 1: Observation for linearity study of Fenbendazole

Sr.No	Concentration (ppm)	Absorbance at 221 nm
1	4	0.492
2	6	0.698
3	8	0.858
4	10	1.058
5	12	1.267

Y = 0.955 x +0.1106

R^2= 0.9982

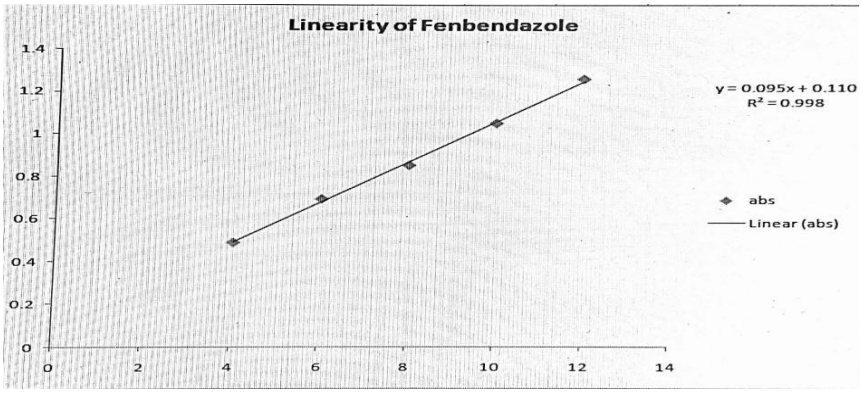


Fig. 1: Graph of Linearity in Phosphate Buffer solution PH 7.4 by UV

Calibration curve data was constructed in the range of 2 to 10 µg/ml. Beer's law was observed over this concentration range. The correlation coefficient (was found to be 0.9982. The regression equation Y=0.955x+0.1106 was found to be linear.

2) PRECISION

a) INTRADAY PRECISION

Table 2: Result of Intraday precision study of Fenbendazole

Sr. No	Concentration (ppm)	Absorbance at 221 nm			Mean (x)
		Morning	Afternoon	Evening	
1	8	0.799	0.815	0.818	0.810
2	8	0.813	0.817	0.816	0.815
3	8	0.815	0.817	0.827	0.819
4	8	0.818	0.827	0.819	0.821
5	8	0.820	0.830	0.829	0.826
6	8	0.826	0.832	0.830	0.829

S.D= 0.0013
%RSD= 0.15%

b) Interday Precision

Table 3: Result of Interday precision study of Fenbendazole

Sr.No	Concentration (ppm)	Absorbance at 221 nm			Mean (x)
		Day 1	Day 2	Day 3	
1	8	0.813	0.820	0.818	0.817
2	8	0.809	0.815	0.816	0.813
3	8	0.796	0.823	0.816	0.811
4	8	0.818	0.797	0.810	0.808
5	8	0.822	0.825	0.815	0.820
6	8	0.795	0.819	0.810	0.808

Robustness

1. Intraday Robustness

Table 4: Result of Intraday Robustness study of Fenbendazole

Sr.No	Concentration (ppm)	Absorbance at 221 nm			Mean (x)
		Morning	Afternoon	Evening	
1	8	0.830	0.832	0.839	0.833
2	8	0.828	0.840	0.845	0.837
3	8	0.813	0.823	0.835	0.823
4	8	0.829	0.829	0.831	0.829
5	8	0.839	0.825	0.835	0.833
6	8	0.815	0.842	0.840	0.832

S.D= 0.0055
%RSD= 0.66%

2. Interday Robustness

Table 5: Result of Interday Robustness study of Fenbendazole

Sr.No	Concentration	Absorbance at 221 nm			Mean (x)
		Day 1	Day 2	Day 3	
1	8	0.832	0.831	0.835	0.832
2	8	0.815	0.833	0.838	0.828
3	8	0.835	0.828	0.820	0.827
4	8	0.830	0.825	0.834	0.829
5	8	0.825	0.830	0.831	0.828
6	8	0.830	0.826	0.833	0.829

S.D= 0.00087
%RSD= 0.10%

3. Ruggedness

a) Intraday Ruggedness

Table 6: Result of Intraday by change in analyst 24 hour

Sr.No	Concentration	Absorbance at 221 nm			Mean (x)
		Morning	Afternoon	Evening	
1	8	0.839	0.842	0.875	0.852
2	8	0.845	0.849	0.851	0.848
3	8	0.855	0.859	0.862	0.858
4	8	0.901	0.870	0.873	0.881
5	8	0.870	0.902	0.912	0.894
6	8	0.865	0.873	0.863	0.867

S.D= 0.0079
%RSD= 0.91%

b) Interday Ruggedness

Table 7: Result of Interday Ruggedness by change in analyst 24 hour

Sr.No	Concentration (ppm)	Absorbance at 221 nm			Mean (x)
		Day 1	Day 2	Day 3	
1	8	0.841	0.845	0.849	0.845
2	8	0.845	0.850	0.855	0.850
3	8	0.850	0.871	0.875	0.860
4	8	0.912	0.869	0.830	0.870
5	8	0.879	0.903	0.825	0.869
6	8	0.880	0.891	0.860	0.877

S.D= 0.0055
%RSD= 0.63%

The Ruggedness was carried out by change in analyst and the difference of % RSD is negligible indicates that the method is rugged.

4. Summary

Table 8: Summary of Precision

Sr.No.	Parameter	SD	%RSD
1	**Precision**		
	1. Intraday precision	0.0089	0.15%
	2. Interday precision	00.0089	0.10%
2	**Robustness**		
	1. Intraday precision	0.0055	0.66%
	2. Interday precision	0.00087	0.10%
3	**Ruggedness**		
	1. Intraday precision	0.0079	0.91%
	2. Interday precision	0.0055	0.63%

The summary of the precision is given in above table and % RSD was found to be≤ 2.

5. Accuracy

Table 9: Result of recovery study by UV

Sr. No	Drug	Percentage Recovery			Mean	SD	%RSD
		50%	100%	150%			
1	Bulk	98.5%	100%	99.96%	99.48%	0.234	0.2340
2	Tablet	99.9%	101%	101.4%	100.4%	0.57	0.5677

6. Limit of detection

$$LOD = \frac{3.3 \times Standard\ deviation}{Slope}$$

$$LOD = \frac{3.3 \times 0.0013}{0.955}$$

LOD= 0.0044

7. Limit of quantitation

$$LOQ = \frac{10 \times Standard\ deviation}{Slope}$$

$$LOQ = \frac{100.0013}{0.955}$$

LOQ= 0.013

The LOD and LOQ were found to be 0.0044µg and 0.013 µg respectively, which indicates the sensitivity of method.

Summary

Table 10: Summary of Method validation

Sr. No	Parameter	Observation
1	Linearity range	1-10 µg/ml
2	Slope	0.955
3	Intercept	0.1106
4	Correlation coefficient	0.9982
5	LOD	0.0044
6	LOQ	0.013

The developed UV method was validated as per ICH guidelines and the method is simple, cost effective to determine the Fenbendazole.

HPLC Method Validation

The method development aimed to develop HPLC method for drug Fenbendazole According to a plan of work firstly find the physical properties of the working standard of Fenbendazole.

Solubility of Fenbendazole working standard

For solubility of drug is checked with different solvent, the solubility checked adding the 10 mg Fenbendazole standard into the to the 10 ml respective organic solvents and check the solubility by Visual method.

Table 11: Solubility of working standard of Fenbendazole

Sr. No	Solvent	Remark
1	Phosphate Buffer (pH 7.4)	Freely soluble less need sonicate
2	Methanol	Very slightly soluble
3	Acetonitrile	Freely soluble less need sonicate
4	DMSO(Dimethyl Sulfoxide)	Sparingly soluble

Selection of diluent and mobile phase for HPLC Method development and validation

Primary thinking about the selection of diluent and mobile phase was important for good chromatographic conditions if the same solvent concentration ratio of diluent and mobile phase was used. So, for the selection of diluent, solubility of Fenbendazole was taken as reference. Because of the free soluble nature of drug in solvent acetonitrile, Methanol it was selected. But the issue of the Methanol is very slightly soluble so it was decided to add 15 percent of methanol in it after some trial took. So the final ratio of diluent is Acetonitrile: Methanol(85:15). The selection of Methanol with chromatographic parameters was done in the HPLC system by trial and error method and by referring the literature survey about it. Finalized Diluent and Mobile phase solvents ratio was **Acetonitrile: Methanol** with ratio (85) :(15) respectively.

Some trials were mentioned as following and find an optimized HPLC method for drug Fenbendazole

Trial 1

Referencing study the solubility of the drug was useful for selecting the diluent and mobile phase only concentration of ratio of Acetonitrile and Water(90:10) respectively was decided. Firstly, the used with acetonitrile used with Methanol with the same ratio.

Diluent: Acetonitrile : water (90:10)
Mobile Phase: Same as diluent.
Column: C18 ODS (250- X 4.6MM,5µm)
Flow rate: 0.8ml/min
Wavelength: 221nm
Pump mode: Isocratic
Injection volume: 10µl
Column temperature: 30 °C
Sample Temperature: 10 °C
Run Time: 30 minutes

Remark

In this trial, the Fenbendazole RT at 15 min with low plate count and peak shape also not proper.

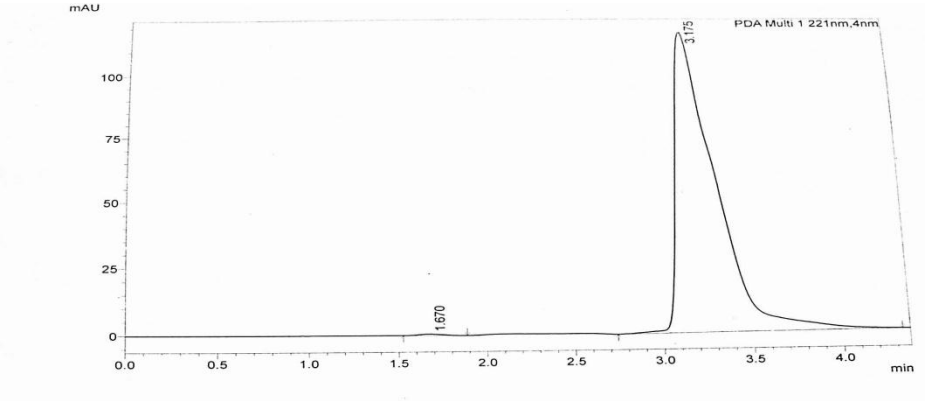


Fig. 1: Representative chromatogram of trail 01

Trial 2

Preparation of mobile phase

Mobile phase containing Acetonitrile : Water in different ratios (85:15) sonicate to degassed and mix well. Change in column.

Chromatographic Conditions

Diluent: Acetonitrile : Water (85:15)
Mobile Phase: Same as diluent.
Column: C18 ODS (250- X 4.6MM,5µm)
Flow rate: 0.8 ml/min
Wavelength: 221nm
Pump mode: Isocratic
Injection volume: 10µl
Column temperature: 30 °C
Sample Temperature: 10 °C

Remark

In this trial, the Fenbendazole RT at 20 min with low plate count and peak Shape also not proper.

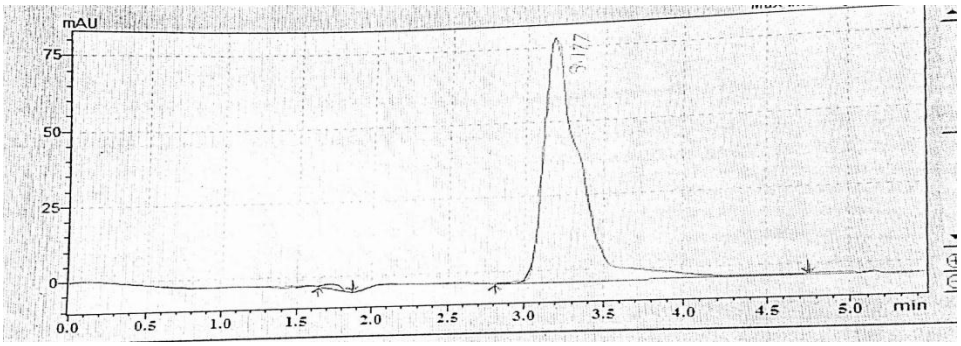


Fig. 2: Representative chromatogram of trail 02

Trial 3

Preparation of mobile phase

Mobile phase containing Phosphate Buffer: Acetonitrile different ratios (80:20) sonicate to degassed and mix well. Change in column.

Chromatographic Conditions

Diluent: Phosphate Buffer: Acetonitrile (80:20)
Mobile Phase: Same as diluent.
Column: C18 ODS (250- X 4.6MM,5µm)
Flow rate:0.8 ml/min
Wavelength :221nm
Pump mode: Isocratic
Injection volume: 10µl
Column temperature: 30 °C
Sample Temperature: 10 °C

Remark

In this trial the Fenbendazole RT was increased to with low plate count and peak shape also Not good.

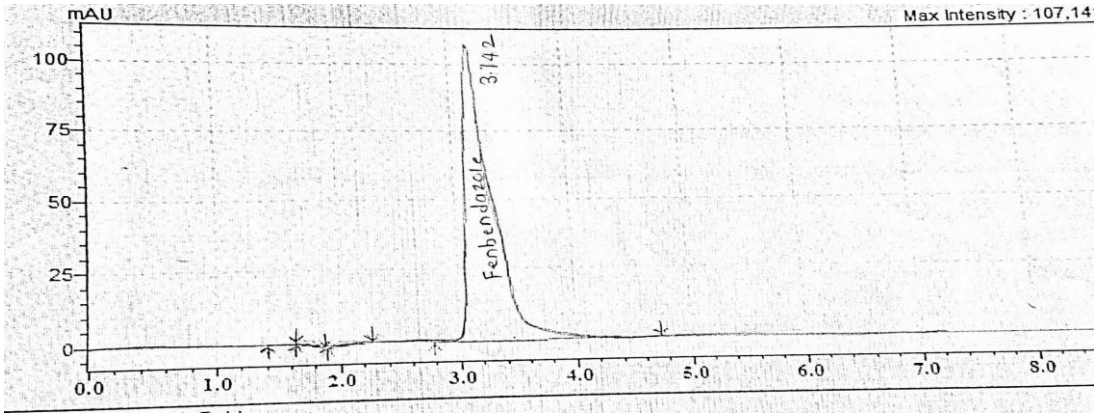


Fig. 3: Representative chromatogram of trail 03

Trial 4

Preparation of mobile phase

Mobile phase containing Phosphate Buffer: Acetonitrile different ratios (90:10) sonicate to degassed and mix well. Change in column.

Chromatographic Conditions

Diluent: Phosphate Buffer: Acetonitrile (90:10)
Mobile Phase: Same as diluent.
Column: C18 ODS (250- X 4.6MM,5µm)

Wavelength :221nm
Flow rate: 0.8ml/min
Pump mode: Isocratic
Injection volume: 10µl
Column temperature: 30 °C
Sample Temperature: 10 °C

Remark
In this trial, the Fenbendazole RI was 12 min with good plate count and peak shape also not good.

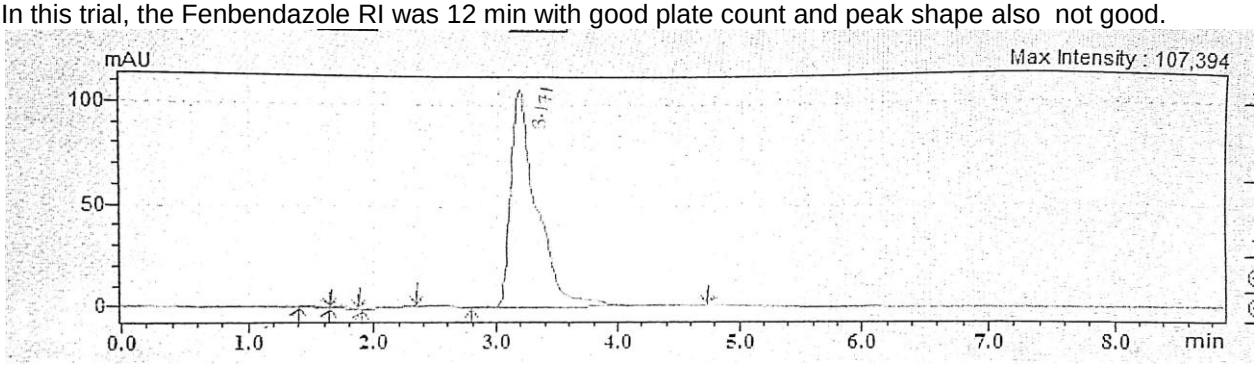


Fig. 4: Representative chromatogram of trail 04

Trial 5
Preparation of mobile phase
Mobile phase containing Acetonitrile: Methanol different ratios (70:30) sonicate to degassed and mix well. Change in column.

Chromatographic Conditions
Diluent: Acetonitrle: Methanol (70:30)
Mobile Phase: Same as diluent
Column: C18 ODS (250- X 4.6MM,5µm)
Wavelength :221nm
Flow rate: 0.8 ml/min
Pump mode: Isocratic
Injection volume: 10µl
Column temperature: 30 °C
Sample Temperature: 10 °C

Remark
In this trial, the Fenbendazole RT was 3.1 min with good plate count, and peak shape.

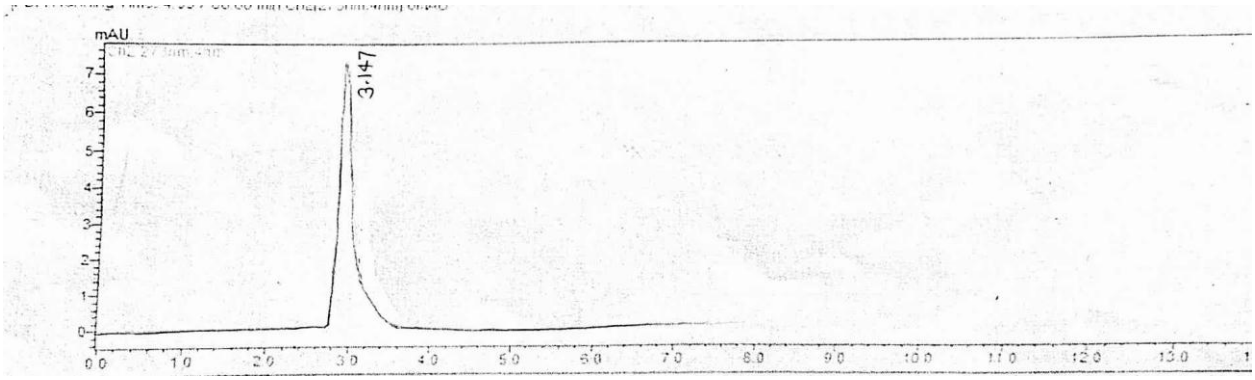


Fig. 5: Representative chromatogram of trail 05

Trial 6

Preparation of mobile phase

Mobile phase containing Acetonitrile: Methanol different ratios (85:15) sonicate to degassed and mix well. Change in column.

Chromatographic Conditions

Diluent : Acetonitrile : Methanol (85:15)
Column: C18 ODS (250- X 4.6MM,5µm)
Wavelength :221nm
Flow rate: 0.8 ml/min
Pump mode: Isocratic
Injection volume: 10µl
Column temperature: 30 °C
Sample Temperature: 10 °C

Remark

In this trial, the Fenbendazole RT was 3.2 min with good plate count and peak shape also good.

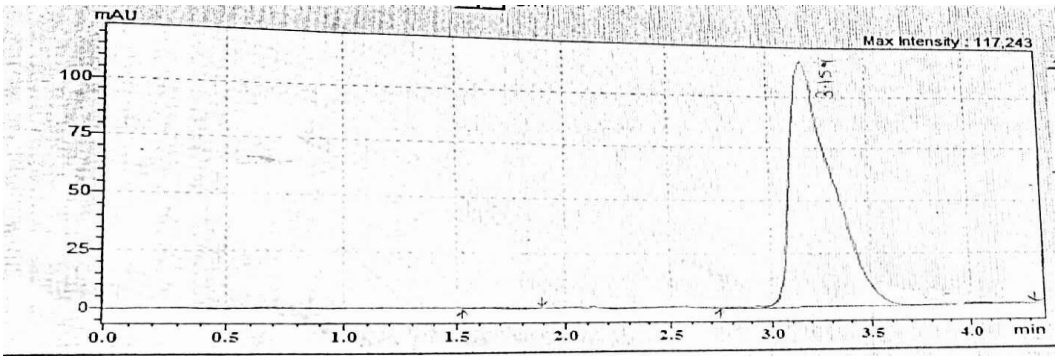


Fig. 6: Representative chromatogram of trail 06

Representative chromatogram for Blank

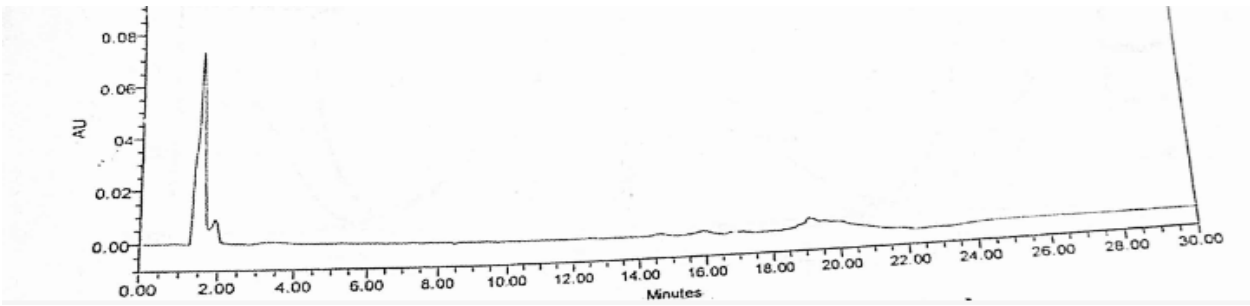


Fig. 7: Optimization development of Chromatogram

Representative chromatogram of Fenbendazole

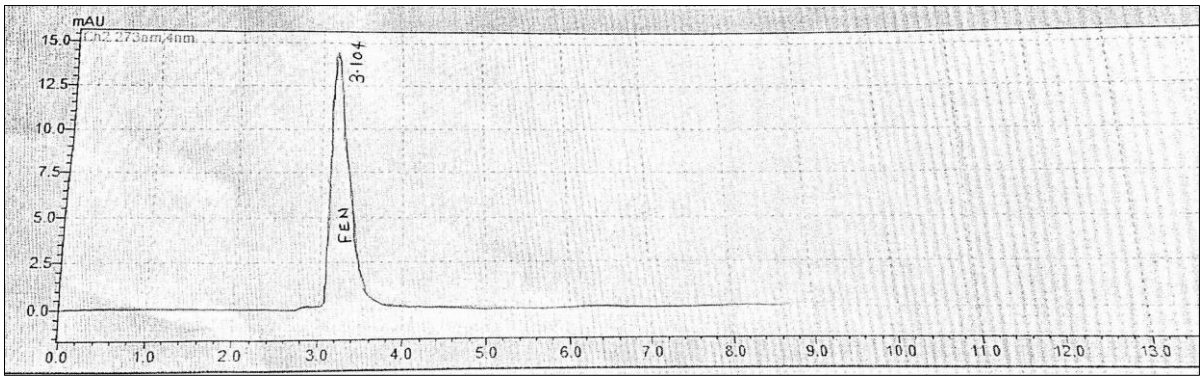


Fig. 8: Optimization development of Chromatogram

Representative chromatogram of Fenbendazole

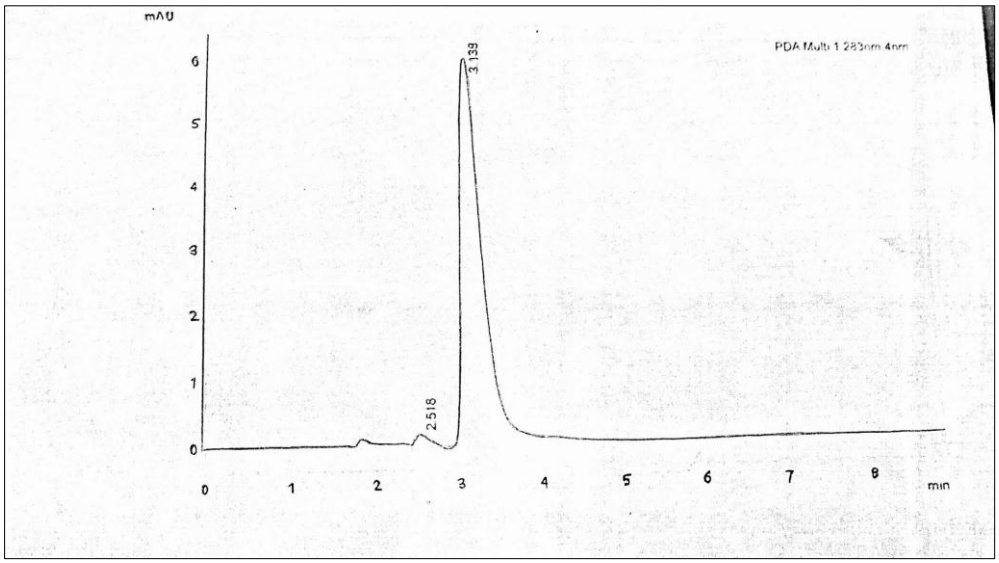


Fig. 9: Optimization development of Chromatogram

Representative chromatogram of Fenbendazole

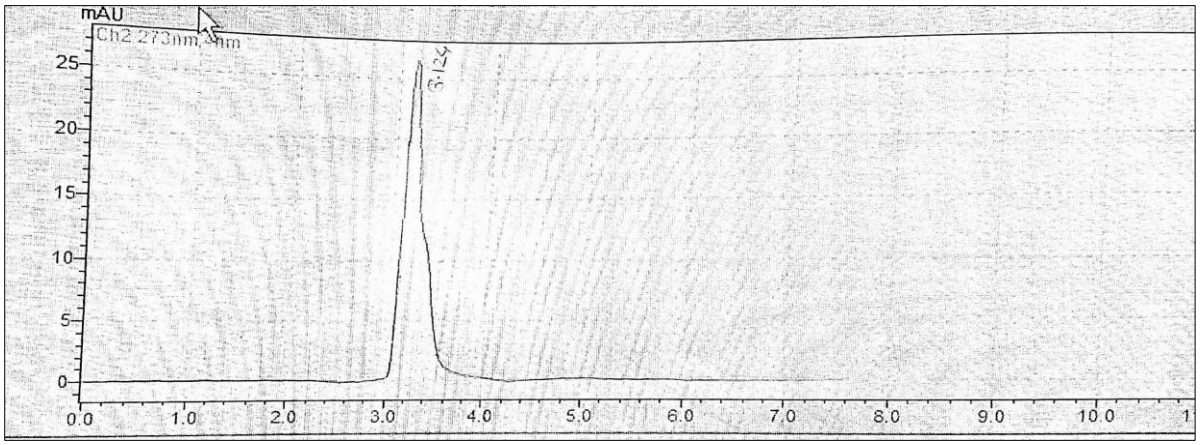


Fig. 10: Optimization development of Chromatogram

Representative chromatogram of Fenbendazole

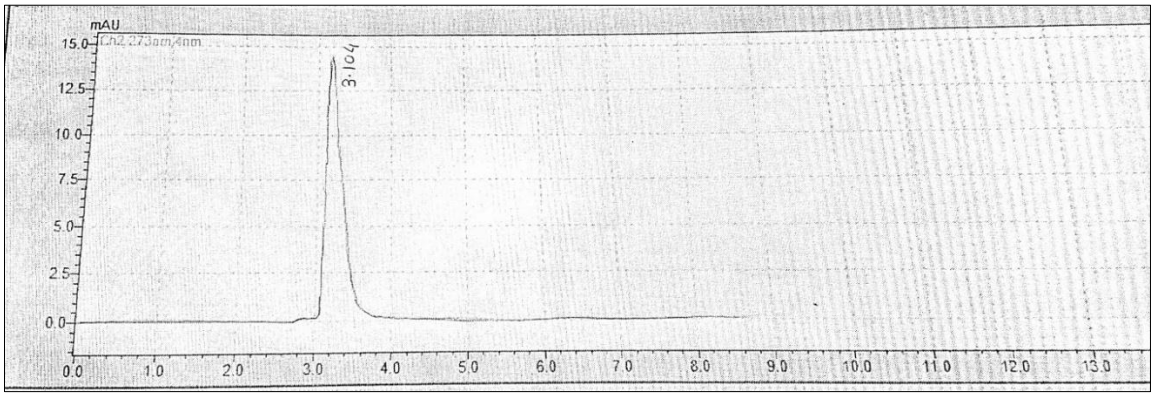


Fig. 11: Optimization development of Chromatogram

Validation of Developed HPLC Method

Preparation of standard stock solution

10 mg of Fenbendazole weighed accurately and Dissolve 100 ml of Phosphate buffer solution transferred into 100 ml of volumetric flask containing mobile phase (Acetonitrile: Methanol) (85:15).

1. **Specificity**

   Specificity of developed HPLC method was done to find out none of the peaks in the blank and any impurity regarding Fenbendazole was not observed at the retention time of working standard Fenbendazole.

2. **Linearity**

   Linearity of stock solution – 3-18 µg/ml

Table 12: Linearity of Fenbendazole

Sr No	Concentration	Volume of stock solution Taken	Concentration of diluent	Average area
1	3 ppm	0.3ml	10ml	21977
2	6 ppm	0.6 ml	10ml	40185
3	9 ppm	0.9ml	10ml	51302
4	12 ppm	1.2ml	10ml	68889
5	15 ppm	1.5ml	10ml	84519
6	18 ppm	1.8ml	10ml	103421

Y= 5312.5x +5934.6

R^2=0.9962

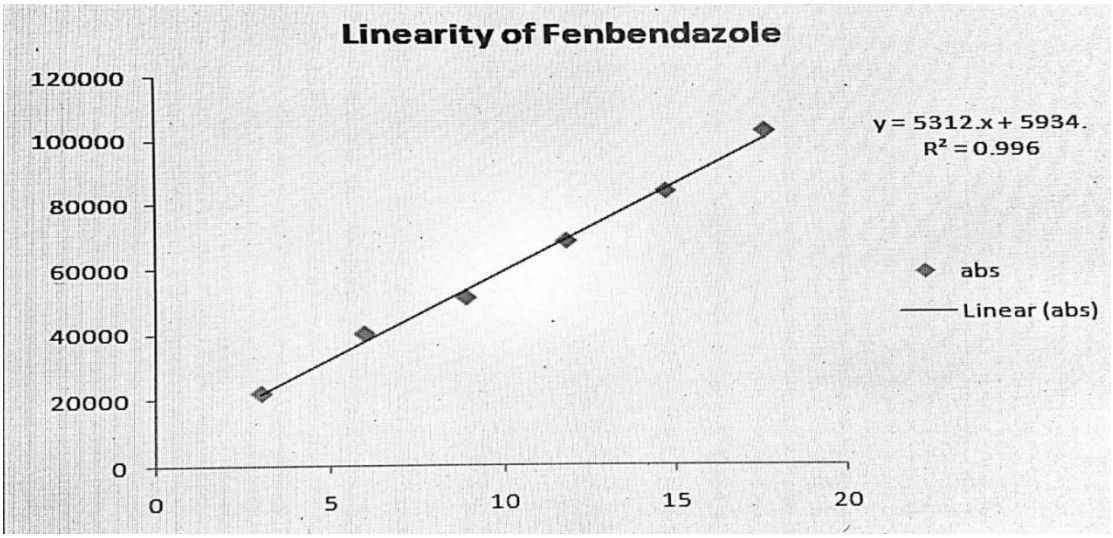


Fig. 14: Linearity graph of standard drug Fenbendazole

Conclusion

The linearity for Fenbendazole stock solution was found between 3-18 µg/ml. The correlation factor (R^2) was found to be 0.996.

1) **Precision**

   i) **Intraday precision**

Table 13: Result for Intraday precision of Fenbendazole

Sr. No	Concentration (ppm)	Area at 221 nm			Mean (x)
		Morning	Afternoon	Evening	
1	3ppm	21870	21975	22018	21954
2	9ppm	51298	51310	51318	51308
3	18ppm	103422	103398	103444	103421

SD = 519.8990

% RSD = 0.8

a. Limit of detection

$$LOD = \frac{3.3 \times Standard\ deviation}{Slope}$$

$$LOD = \frac{3.3 \times 519.8990}{5312.5}$$

LOD = 0.3229

b. Limit of quantitation

$$LOQ = \frac{10 \times Standard\ deviation}{Slope}$$

$$LOQ = \frac{10 \times 519.8990}{5312.5}$$

LOQ = 0.9786

ii) Interday precision

Table 14: Result for Interday precision of Fenbendazole

Sr. No	Concentration (ppm)	Area at 221 nm			Mean (x)
		Day 1	Day 2	Day3	
1	3ppm	21875	21960	22112	21982
2	9ppm	51292	51310	51378	51326
3	18ppm	103321	103428	104410	103719

SD = 763.35
%RSD =1.4 %

a. Limit of detection

$$LOD = \frac{3.3 \times Standard\ deviation}{Slope}$$

$$LOD = \frac{3.3 \times 763.35}{5312.5}$$

LOD= 0.4741

b. Limit of quantitation

$$LOQ = \frac{10 \times Standard\ deviation}{Slope}$$

$$LOQ = \frac{10 \times 763.35}{5312.5}$$

LOQ=1.4368

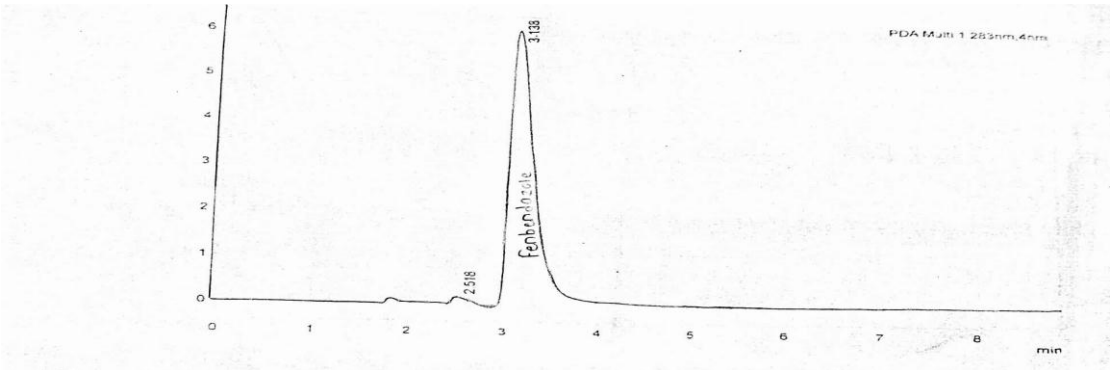


Fig. 15: Chromatogram of Precision for method development

4) Robustness

Standard flow rate 1ml/min

Table 15: Result for Standard of Fenbendazole

Sr No	Injection No	Concentration of diluents(ppm)	Average area
1	Inj 1	6 ppm	40185
2	Inj 2	6 ppm	40168
3	Inj 3	6 ppm	40189
4	Inj 4	6 ppm	40178
5	Inj 5	6 ppm	40192
6	Inj 6	6 ppm	40179

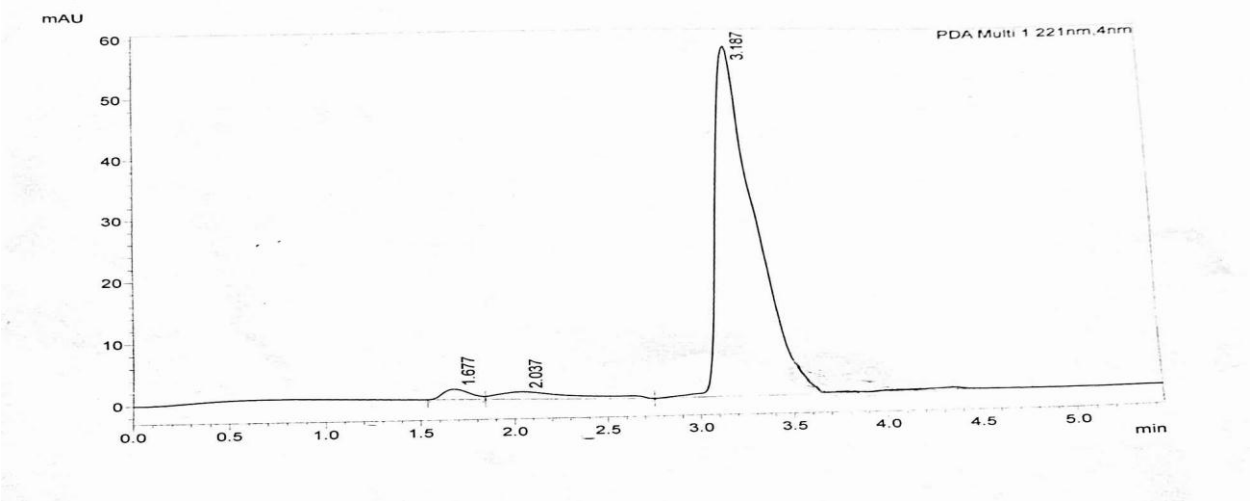


Fig. 16: Chromatogram of Robustness for method development

A) Change in flow rate

1) Minus flow (0.9ml/min)

Table 16: Result of robustness study (Minus Flow)

Sr. No	Injection No	Concentration of diluent	Average area (x)
1	Inj 1	6 ppm	40395
2	Inj 2	6 ppm	40310
3	Inj 3	6 ppm	40326
4	Inj 4	6 ppm	40354
5	Inj 5	6 ppm	40368
6	Inj 6	6 ppm	40371

SD = 31.26

%RSD=0.07 %

2) Plus flow (1.1ml/min)

Table 17: Result of robustness study (plus Flow)

Sr. No	Injection No	Concentration of diluent	Average area(x)
1	Inj 1	6 ppm	40095
2	Inj 2	6 ppm	40110
3	Inj 3	6 ppm	40078
4	Inj 4	6 ppm	40089
5	Inj 5	6 ppm	40096
6	Inj 6	6 ppm	40106

SD = 11.56

%RSD = 0.02 %

B) Change in ratio of mobile phase

a) Minus ratio (80:20)

Table 18: Result of robustness study (Minus ratio)

Sr. No	Concentration of diluents(ppm)	Injection set	Average area(x)
1	6 ppm	Inj 1	40382
2	6 ppm	Inj 2	40389
3	6 ppm	Inj 3	40377
4	6 ppm	Inj 4	40398
5	6 ppm	Inj 5	40386
6	6 ppm	Inj 6	40396

SD = 8.07
%RSD = 0.019 %

b)Plus ratio (90:10)

Table 19: Result of robustness study (plus ratio)

Sr. No	Concentration of diluent	Injection set	Average area(x)
1	6 ppm	Inj 1	40092
2	6 ppm	Inj 2	40118
3	6 ppm	Inj 3	40072
4	6 ppm	Inj 4	40094
5	6 ppm	Inj 5	40088
6	6 ppm	Inj 6	40102

SD = 15.25
% RSD = 0.038 %

4) Recovery Study

Table 20: Sample preparation table for recovery studies

Sr. No	Concentration Level	Amount added concentration (ppm)		(100ppm) (ml)	
		Fenbendazole	Test	Fenbendazole	Test
1	80%	3.2	6	0.32	0.6
2	100%	4	6	0.4	0.6
3	120%	4.8	6	0.48	0.6

Result:

Table 21: Result of Fenbendazole

Sr.No	Average area	%	Amount Recovery (ppm)	Amount Recovery %Recovery
1	44106	80%	7.185	99.79
2	44096		7.183	99.76
3	44101		7.184	99.78
1	49354	100%	8.129	102.86
2	49648		8.128	109.15%
3	49659		8.17	101.25%
1	5206	120%	8.80	100%
2	52211		8.712	99.98%
3	52202		8.701	99.96%

Summary and conclusion

Summary

The objective of the present work study was to develop and validate a method for Fenbendazole by UV spectroscopy and HPLC. It is a one of the most accurate methods widely used for the qualitative and quantitative analysis of drug product. Analytical method development and validation play as an important role in discovery, development and manufacture of pharmaceuticals. The use of HPLC for routine analysis as a part of quality control is rapidly increasing owing to the numerous advantages of the technique such as cost, time for analysis, and sensitivity. Study was conducted firstly developed UV Spectroscopy method for solubility of drug is checked with different solvent, select the wavelength drug. In HPLC method development tried a number of chromatographic parameter have been evaluated in order to optimize the method in the analysis of method development in HPLC. An appropriate mobile phase, column temperature, wavelength and isocratic method are developed. Optimum separation was

observed in mobile phase ratio Acetonitrile: methanol (85:15) at retention time for Fenbendazole 3.1 minutes. Validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose After HPLC method development, validation of the HPLC method done as per ICH guidelines and include accuracy, precision, specificity, limit of detection, limit of quantification, linearity, range and robustness. The main objective of an analytical method validation is form development stage of the Fenbendazole in Bulk and Marketed Formulation.

CONCLUSION

Analytical method development and its validation of Fenbendazole in Bulk and marketed formulation has been developed and validated for the fenbendazole in bulk and marketed formulation. From the results is of method validation experiments, it is concluded that the proposed developed method it was linear, sensitive, selective, specific, precise, accurate, robust and having stability indicating characteristics. Method development and validation play an important role in the pharmaceutical industry. The method was validated as per ICH guidelines. These analytical methods are critical elements of pharmaceutical development so it is very important to develop efficient and accurately validated analytical methods to develop safe and effective drug formulation. Validating a developed method is important as it is meaningless it the method cannot be reproduced or rugged. All the analytical performed parameters are checked as per the approved validation Protocol and found to be well within specified acceptance criteria. Hence it is concluded that this method capable to produce the accurate and precise and reproducible result during routine analysis and can be use for formulation containing same a active pharmaceutical ingredient. The present method can be used for routine quality control of Fenbendazole by the quality control laboratories.

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REFERENCES

1. Analytical Method Development and Validation Ramole Rina, Mohini Baile and Ashish Jain. Department of Quality Assurance, Shri. D. D. Vispute College of Pharmacy and Research Center, Mumbai, India.
2. http://www.drugbank.com (accessed on 2/12/2014).
3. http://www.druginfosys.com (accessed on 2/12/2014).
4. Indian Pharmacopoeia, Indian Pharmacopoeia Commission India. Government of India Ministry of Health and Family Welfare. The Indian Pharmacopoeia Commission, Ghaziabad, India. 2007;2:1336-7.
5. The United States Pharmacopeia-36, National Formulary 31. The United States Pharmacopoeia Convention, Inc. Rockville, Md, USA, 18th edition. 2007;1:2116.
6. British Pharmacopoeia. British Pharmacopoeia Commission. The Medicine and Healthcare Products Regulatory Agency, London. 2011;74.
7. Dawson PJ, Gutteridge WE and Gull K. A comparison of the interaction of antihelminth benzimidazoles with tubulin isolated from mammalian tissue and the parasitic nematode Ascaridia galli. Biochem Pharm. 1984;33:1069-1074.
8. Lacey E. The role of the cytoskeletal protein, tubulin, in the mode of action and mechanism of drug resistance to benzimidazoles. Int J Paracytol. 1988;7:885-936.
9. Spagnuolo PA, Hu J, Hurren R, Wang X, Gronda M,Sukhai MA, DiMeo A, Boss J, Ashall I, Za vareh R, Fine N, Simpson CD, Sharmeen S, Rottapel R and Schimmer AD. The antihelmintic flubendazole inhibits microtubule function through a mechanism distinct from Vinca alkaloids and displays preclinical activity in leukemia and myeloma. Blood. 2009;115:4824-4833.
10. Düwel D. Fenbendazole II. Biological properties and activity. Pestic Sci. 1977;8:550-555.
11. Pritchard RK, Kelly JD, Bolin TD, Duncombe VM and Fagan MR. The effect of iron and protein deficiency on plasma levels and parasite uptake of [^{14}C] fenbendazole in rats infected with Nippostrongylus brasiliensis. Aust J Exper Biol Med Sci. 1981;59:567-573.
12. Tannock I, Hill R, Bristow R and Harrington L. The Basic Science of Oncology. 4th edition, McGraw-Hill, New York, NY. 2004.
13. Bai R-Y, Staedtke V, Aprhys CM, Gallia GL and Riggins GJ. Antiparasitic mebendazole shows survival benefit in two preclinical models of glioblastoma multiforme. Neuro Oncol. 2011;13:974-892.

14. Gao P, Dang CV and Watson J. Unexpected antitumorigenic effect of fenbendazole when combined with supplementary vitamins. J Am Assoc Lab Anim Sci. 2008;47:37-40.
15. Chung I, Barrows C, Wilson A, Rummel N, Badaruddin S, Mizokami A, Banyard J and Zetter B. Benzimidazole as novel therapeutic agent for metastatic prostate cancer. Poster session presented at the Annual Meeting of the American Association for Cancer Research, Washington DC, April 17-21. 2010.
16. Keller WC. Fenbendazole. (WHO Food Additive Series 29) available at http://www. inchem.org/documents/jecfa/jecmono/v29je04.htm. Last accessed January 2, 2013.
17. Overgaard J. Hypoxic radiosensitization: Adored and ignored. J Clin Oncol. 2007;25:4066-4074.
18. en.wikipedia.org
19. Indian Pharmacopoeia, Indian Pharmacopoeia Commission India. Government of India Ministry of Health & Family Welfare, The Indian Pharmacopoeia Commission, Ghaziabad, Ind 2018;2:2038.
20. en.m.wikipedia.org.
21. vcahospital.com.
22. Plumb's Veterinary Drug Handbook, Fifth Edition, 2005.
23. Junquera P. Fenbendazole, anthelmintic for veterinary use on cattle, sheep, goats, pig, poultry, horses, dogs and cats against roundworms and tapeworms. Parasitipedia. Retrieved 2015-09-08.
24. Junquera P. Oxfebendazole, anthelmintic for veterinary use on cattle, sheep, goats, horses, dogs and cats against roundworms and tapeworms. Parasitipedia. Retrieved 2015-09-08.
25. Junquera P. Febantel for veterinary use on dogs, cats, cattle, sheep, goats, pig and poultry against roundworms and tapeworms. Parasitipedia. Retrieved 2015-09-08.
26. Toku-e.com.
27. Patents.google.com.
28. http://www.inchem.org/documents/jecfa/jecmono/v041je03.htm.
29. Shabir G. A HPLC method development for pharmaceutical analysis. 2004 Pharmaceutical technology Europe.
30. Mandapati Varaprasad Reddy. HPLC determination of fenbendazole and ivermectin simultaneously in bulk and pharmaceutical dosage forms. Indo Am J P Sci. 2017;4(05).