CODEN (USA): IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.583278

Available online at: http://www.iajps.com *Research Article*

HPLC DETERMINATION OF FENBENDAZOLE AND IVERMECTIN SIMULTANEOUSLY IN BULK AND PHARMACEUTICAL DOSAGE FORMS

Battula Sreenivasa Rao[1], Mandapati Varaprasad Reddy[1*], Bhatraju Sreenivasa Rao[2]

[1]Department of Chemistry, GITAM Institute Technology, GITAM University, Visakhapatnam, Andhrapradesh, India-530045.

[2]Wockhardt Research Centre, Chikalthana, Aurangabad, Maharastra, India-431006.

Abstract:

In the present study, a simple, precise and accurate high performance liquid chromatography with photodiode array detector was developed for the simultaneous estimation of ivermectin & fenbendazole in bulk and tablet dosage forms. A Zorbax C8 column (250 cm × 4.6 mm × 5 µm) with mobile phase consisting of 0.1 M potassium dihydrogen orthophosphate and methanol (60:40 v/v) having pH 4.5 (adjusted with orthophosphoric acid) was used. The flow rate was 1.2 ml/min and the effluents were detected at 268 nm. The retention times of ivermectin and fenbendazole were found to be at 3.296 min and 7.257 min, respectively. The linearity of the selected drugs was obtained in the range of 2-6 µg/ml (ivermectin) and 60-80 µg/ml (fenbendazole). The results of analysis have been validated according to ICH guidelines. The results for all validation parameters were found to be within the acceptance limit. Ivermectin and fenbendazole were degraded under acidic, alkaline, oxidative, thermal and photolytic degradation conditions. All the peaks of degraded products were well resolved from the peaks of ivermectin and fenbendazole. The developed and validated method can be employed as a stability-indicating, as it could efficiently separate ivermectin and fenbendazole from their degradation products.

Key words:*Ivermectin, fenbendazole, HPLC, Stability indicating, Assay*

Corresponding author:

Mandapati Varaprasad Reddy,
Department of Chemistry,
GITAM Institute Technology, GITAM University,
Visakhapatnam, Andhrapradesh,
India-530045.
Email: mpr_ac@yahoo.co.in
Phone: +91-9730925446

QR code

Please cite this article in press as Mandapati Varaprasad Reddy et al, ***HPLC determination of fenbendazole and ivermectin simultaneously in bulk and pharmaceutical dosage forms****, Indo Am. J. P. Sci, 2017; 4(05).*

INTRODUCTION:

Ivermectin is a semisynthetic and anthelmintic agent active against numerous types of parasites[1]. It is derived from avermectins (active broad-spectrum, anti-parasitic agent), which is obtained as the fermentation product of *Streptomyces avermitilis*[2]. In humans, as an antiparasitic agent, ivermectin is used in the treatment of onchocerciasis, strongyloidiasis, enterobiasis, ascariasis, filariasis, trichuriasis and scabies[3]. Recently, Ivermectin is being studied as an antiviral agent against the viruses that are responsible for chikungunya and yellow fever [4]. Ivermectin is also widely used as an antiparasitic agent against endoparasites and ectoparasites of domestic animals [5].

Fenbendazole is an antinematodal drug used in veterinary medicine and belongs to the benzimidazole class of compounds[6]. Fenbendazole is an active metabolite of anthelmintic agent, febantel[7]. As an anthelmintic agent, fenbendazole is used in treating gastrointestinal parasites in cattle, fish, sheep, dogs, cats, rabbits and horses [8].

The chemical structures of ivermectin and fenbendazole are given in Figure 1. The combination of ivermectin and fenbendazole is available in the market as Fentin Bolus (strength: Fenbendazole 3000 mg and Ivermectin 100 mg) [9]. This combination is commonly used as dewormer in animals. It is used for treatment of lungworms, roundworms and is active against ova, larva and adult worms. It is also used for treatment of ticks, lice, mites and scabies.

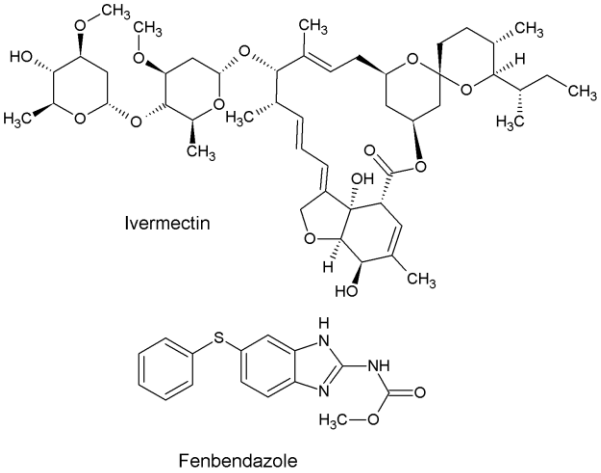

Fig 1: Chemical structure of drugs

Literature review reveals that only one HPLC method was reported for the estimation of ivermectin and fenbendazole in combination [10]. The method was carried out by using Qualisil BDS C18 (250 mm ×4.6 mm, 5µm) column. The mobile phase consisted of acetonitrile, methanol and phosphate buffer (pH 4.5) in the ratio of 50:20:30 (*v/v*). The flow rate, injection volume and detection were 1.0 ml/min, 20 µl and 254 nm, respectively. The retention times of ivermectin and fenbendazole were 4.0 min and 6.3 min, respectively.

However no stability indicating HPLC method has been reported for simultaneous estimation ivermectin and fenbendazole in bulk and in pharmaceutical formulations. So, the aim of the present work was to develop an accurate, precise and sensitive stability indicating HPLC method for the simultaneous estimation of ivermectin and fenbendazole in bulk and pharmaceutical dosage form.

MATERIALS AND METHODS:

Chemicals, solvents, reference standards and tablet dosage forms:

All chemicals were of analytical grade. Potassium dihydrogen phosphate, hydrogen peroxide, hydrochloric acid, sodium hydroxide and orthophosphoric acid were acquired from Sd. Fine Chemicals Ltd., Mumbai, India. HPLC grade methanol was obtained from Merck India Ltd., Mumbai, India. Milli-Q water (Millipore, USA) was used throughout the experiments.

Reference standards of ivermectin and fenbendazole were from Lara Drugs Private Limited, Telangana, India as gift. The marketed formulation of ivermectin and fenbendazole (Fentin bolus, strength: Fenbendazole 3000 mg and Ivermectin 100 mg, Zenley animal health, Haryana, India**)** were obtained from local pharmacy market.

HPLC instrument and conditions:

The HPLC equipment used in the present investigation was a Waters 2695 alliance system coupled with Waters 2998 photodiode array detector and Waters empower2 software. The chromatographic analysis of ivermectin and fenbendazole was performed at 30˚C using Zorbax C8 analytical column (250 mm × 4.6 mm, 5 µm). The column was eluted with the mobile phase (0.1M potassium dihydrogen phosphate and methanol in the ratio of 60:40 (*v/v*); pH of the mobile phase was adjusted to 4.5 with dilute orthophosphoric acid) in isocratic mode at a flow rate of 1.2 ml/min. Mobile phase was degassed using ultrasonic bath and filtered through 0.45 µm pore size membrane filter former to analysis. Ivermectin and fenbendazole was detected using photodiode array detector set at 268 nm. The total run time of the method was 8 min and the injection volume was 20 µl.

Standard solutions:
Ivermectin (100 mg) and fenbendazole (3000 mg) was accurately weighed and transferred to 250 ml calibrated volumetric flask. It was dissolved in mobile phase (50 ml) with the aid of sonication. The final volume was made up to the mark with mobile phase to produce stock solution (ivermectin - 0.4 mg/ml and fenbendazole - 12 mg/ml). Working solutions of ivermectin (2, 3, 4, 5 and 6 µg/ml) and fenbendazole (60, 90, 120, 150 and 180 µg/ml) were prepared using suitable aliquots of stock solution. All the solutions were stored in refrigerator until use.

Calibration curves:
Appropriate aliquots of ivermectin and fenbendazole stock solution were taken in different 10 ml volumetric flasks and diluted up to the mark with mobile phase to obtain final concentrations of 2-6 µg/ml and 60-180 µg/ml of ivermectin and fenbendazole, respectively. The solutions were injected into the HPLC system using a 20 µl loop and chromatograms were recorded. Calibration curves were constructed by plotting peak areas of drug *versus* drug concentrations. The regression equations were calculated for ivermectin and fenbendazole.

Assay of selected drug combination in tablet dosage form:
Average weight of ten tablets content (each containing 3000 mg of fenbendazole and 100 mg of ivermectin) was determined. A quantity of powder equivalent to 3000 mg of fenbendazole and 100 mg of ivermectin was weighed and transferred to 250 ml volumetric flask. Mobile phase (50 ml) was added to the same flask and sonicated for 20 min. The volume was made up to 250 ml with mobile phase. The theoretical concentration of the stock solution was 0.4 mg/ml and 12 mg/ml of ivermectin and fenbendazole, respectively. The solution was filtered using 0.45 µm pore size membrane filter. Appropriate dilution (4 µg/ml and 120 µg/ml of ivermectin and fenbendazole, respectively) was prepared with mobile phase for analysis. The solution was analyzed using the above described chromatographic conditions.

Method validation [11]:
System suitability:
System suitability parameters were established with five replicate injections of the working standards (4 µg/ml and 120 µg/ml of ivermectin and fenbendazole, respectively). The system suitability parameters were calculated as per United States Pharmacopoeia [12]. The parameters were relative standard deviation of retention time of selected drugs, relative standard deviation of peak areas, tailing factor, plate count and resolution.

Selectivity:
The probable interference from components of mobile phase and common tablet excipients was evaluated by comparing the chromatograms of mobile phase blank, placebo blank and tablet sample solution (ivermectin- 4 µg/ml and fenbendazole- 120 µg/ml) with standard solution (ivermectin- 4 µg/ml and fenbendazole- 120 µg/ml).

Specificity:
The specificity of the method was assessed by using different International Conference on Harmonization prescribed stress conditions (acidic, basic, oxidative, thermal and photolytic) [13]. Tablet powder equivalent to 100 mg of ivermectin and 3000 mg of fenbendazole was used for degradation studies.
Acid degradation was carried out using 0.1 N HCl and sonication for 30 min at ambient temperature. The tablet powder was taken in a 100 ml volumetric flask. 10 ml of 0.1 N HCl was added to the flask and sonicated for 30 min. After completion of the stress, the solution was neutralized by using sufficient volume of 0.1 N NaOH and completed up to the mark with mobile phase.
Alkaline degradation was carried out using 0.1 N NaOH and sonication for 30 min at ambient temperature. The tablet powder was taken in a 100 ml volumetric flask. 10 ml of 0.1 N NaOH was added to the flask and sonicated for 30 min. After completion of the stress, the solution was neutralized by using sufficient volume of 0.1 N HCl and completed up to the mark with mobile phase.
Oxidative degradation was performed at ambient temperature using 30% H_2O_2 and sonication for 30 min. Degradation with H_2O_2 was done by taking tablet powder in 100 ml volumetric flask followed by adding 10 ml of 30% H_2O_2. The mixture was sonicated for 30 min. The flask was completed up to the mark with mobile phase.
Thermal degradation was performed at 105°C for 30 min in oven. Tablet powder was kept in petri dish in oven at 105°C for 30 min. Thereafter, the tablet powder was cooled and transferred to 100 ml volumetric flask. 30 ml of mobile phase was added, mixed well and diluted up to the mark with mobile phase.
The photo degradation was studied by exposing the tablet powder kept in petri dish to direct sunlight in for 24 h. The photo degraded sample was cooled and transferred to a 100 ml volumetric flask containing 30 ml of mobile phase and mixed well. The volume of the flask was completed up to mark with mobile phase.

For analysis, all the degraded sample solutions were diluted with mobile phase to obtain final concentration of 4 µg/ml and 120 µg/ml of ivermectin and fenbendazole, respectively. The samples were filtered through 0.45 µm pore size membrane filter. 20 µl solution of the degraded samples were injected into the HPLC system and analyzed using the chromatographic conditions described earlier.

Limit of detection (LOD) and limit of quantitation (LOQ):
The signal-to-noise ratio approach was used to calculate the LOD and LOQ values. The concentration level that yields a signal-to-noise ratio of about 10:1, at which ivermectin and fenbendazole can be readily quantified, was reported as the limit of quantitation. The concentration level that yields a signal-to-noise ratio of about 3:1, at which ivermectin and fenbendazole can be readily detected, was reported as the limit of detection.

Precision:
Precision was studied on six injections of the working standard solution (4 µg/ml and 120 µg/ml of ivermectin and fenbendazole, respectively). The mean peak area and percent relative standard deviation (%RSD) was calculated.

Accuracy:
Accuracy was studied on six injections of the working standard solution (4 µg/ml and 120 µg/ml of ivermectin and fenbendazole, respectively). The percent assay of drug was calculated.

Recovery studies:
Accuracy was further evaluated by carrying the recovery studies at three concentration levels. Recovery studies were performed by adding three different amounts of standard selected drugs (i.e, 50%, 100%, and 150% of the drug) to the preanalyzed tablet solution. The resultant was reanalyzed three times.

Robustness:
By introducing small changes in mobile phase flow rate and column temperature, the effects on the system suitability parameters were examined. Robustness of the method was determined at a concentration level of 4 µg/ml and 120 µg/ml ivermectin and fenbendazole, respectively.

RESULTS AND DISCUSSION:

Analytical method development:
The HPLC method was developed as a stability indicating method to quantify ivermectin and fenbendazole simultaneously in the presence of the possible degradation products of the drugs. A Zorbax C8 analytical column (250 mm × 4.6 mm, 5 µm) with temperature set at 30˚C was used. During development step, 0.1 M KH_2PO_4: methanol in different ratios, flow rates & pH were tried and the responses were recorded. On the basis of responses and chromatographic parameters studied a mobile phase of 0.1 M KH_2PO_4: methanol in the ratio of 60:40 (*v/v*) with flow rate of 1.2 ml/min and final pH adjusted to 4.5 with orthophosphoric acid was selected as suitable mobile phase conditions. Under these conditions, ivermectin and fenbendazole were eluted at 3.296 min and 7.257 min, respectively. 268 nm was selected for detection as both the selected drugs showed good response at this wavelength. Figure 2 depicts the chromatogram obtained with the optimized chromatographic conditions.

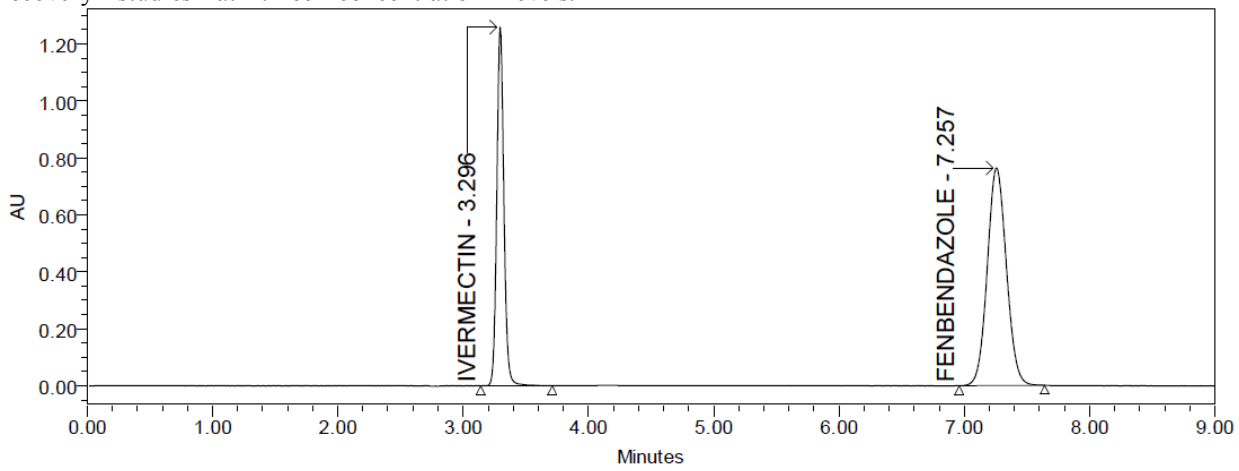

Fig 2: Chromatogram of ivermectin and fenbendazole obtained with optimized chromatographic conditions

Method validation:

System suitability:
The results of system suitability are given in Table 1. The values are within the acceptance criteria, indicating the good performance of the system.

Selectivity:
A typical HPLC chromatogram of mobile phase blank, placebo blank and tablet sample solution and standard solution are shown in Figures 3, 4, 5 and 6, respectively. The HPLC chromatograms recorded for the mobile phase blank and placebo blank revealed no peaks at retention time of ivermectin and fenbendazole. The reproducibility of retention times of ivermectin & fenbendazole between standard solution and tablet sample solution proved good selectivity of the method.

Table 1: Chromatographic characteristics of system suitability

Parameters	Ivermectin		Fenbendazole		Recommended limit
	Value*	RSD (%)	Value*	RSD (%)	
Retention time	3.304	0.087	7.363	0.641	RSD ≤2
Peak area	5540620	0.393	9258149	0.218	RSD ≤2
Resolution	-	-	20.336	0.804	> 1.5
Plate count	15874	0.530	10533	0.336	> 2000
Tailing factor	1.102	0.994	1.072	0.780	≤ 2

*mean of five values

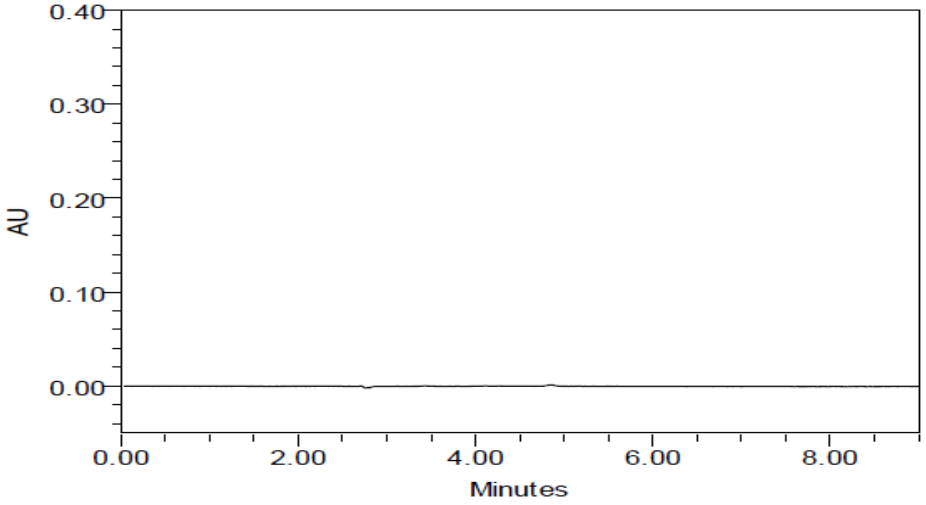

Fig 3: Chromatogram of mobile phase blank

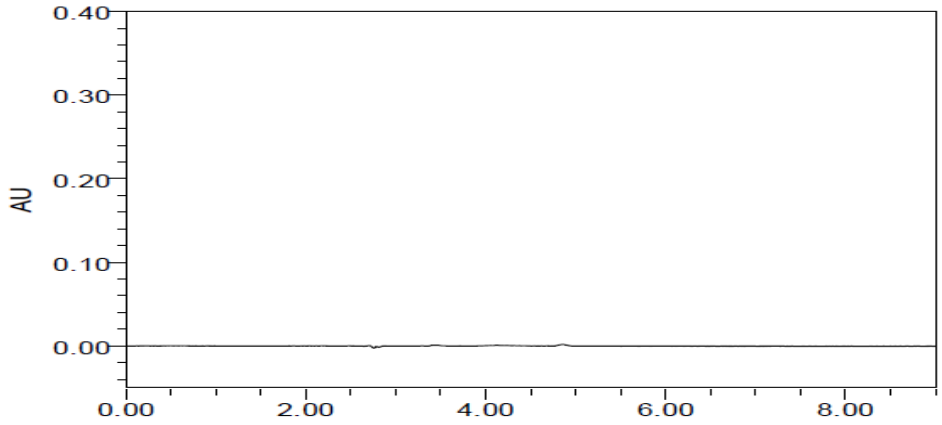

Fig 4: Chromatogram of placebo blank

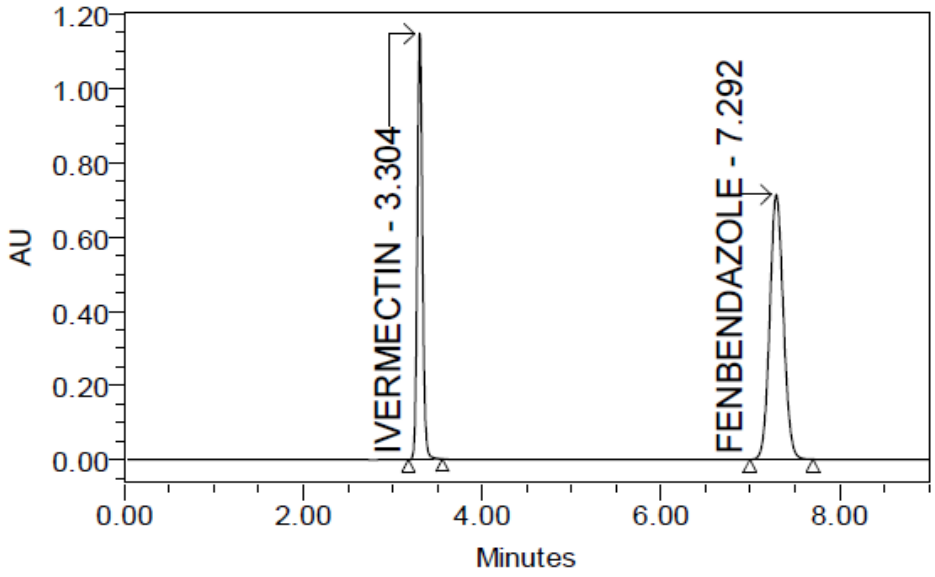

Fig 5: Chromatogram of tablet sample solution (ivermectin- 4 µg/ml and fenbendazole- 120 µg/ml)

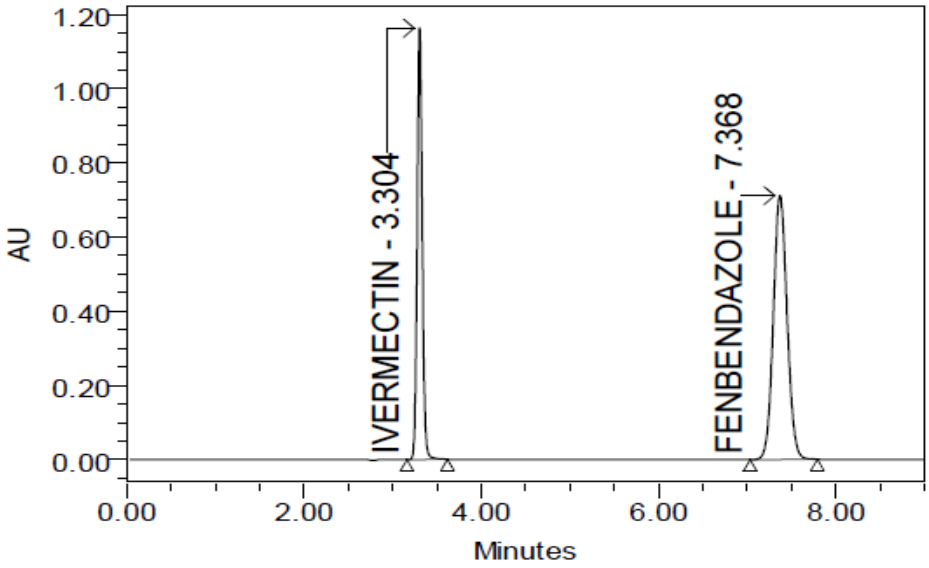

Fig 6: Chromatogram of standard solution (ivermectin- 4 µg/ml and fenbendazole- 120 µg/ml)

Linearity:
The regression equation for ivermectin was y = 85509 *x* + 18.36 by plotting peak area (y) versus the concentration (*x*) studied from 2-6 µg/ml and the regression coefficient (R^2) was 0.9998. The regression equation for fenbendazole was y = 77834 *x* – 37158 by plotting peak area (y) versus the concentration (*x*) studied from 60-180 µg/ml and the regression coefficient (R^2) was 0.9990. According to results obtained, there is linear regression and there is no deviation from linearity for ivermectin and fenbendazole.

Sensitivity:
The LOD and LOQ of ivermectin by the proposed method were found to be 0.004 µg/ml and 0.013 µg/ml, respectively. The LOD and LOQ of the proposed method for fenbendazole were 0.198 µg/ml and 0.661 µg/ml, respectively. The results show the good sensitivity of the present method.

Specificity and stability indicating nature of the method:
Drugs, ivermectin and fenbendazole, got degraded in all the stress conditions applied (Table 2). The

percent degradation of fenbendazole is less when compared with the ivermectin degradation, indicating that the fenbendazole is more stable than ivermectin in all the stress conditions applied. Four peaks, in addition to ivermectin and fenbendazole peaks, were observed in acid hydrolysis, alkali hydrolysis, oxidative hydrolysis, thermal degradation and photolysis conditions. The number of degradants produced and their retention times are given in Table 2. The peaks of degradation products were resolved well from the peaks of ivermectin and fenbendazole (Figure 7-11). The results obtained from the peak purity tool revealed that the peak response of ivermectin and fenbendazole was pure in all cases and thus proved the absence of degradants at the same retention time. The results proved the specificity and stability indicating nature of the proposed method.

Table 2: Stability of ivermectin and fenbendazole in stress conditions

Drug	Parameter	Type of stress condition				
		Acid	Alkali	Hydrogen peroxide	Thermal	Photo
Ivermectin	Peak area	5237936	5213712	5251900	5267017	5288351
	% Recovery	94.388	93.952	94.640	94.912	95.297
	% degradation	5.612	6.048	5.360	5.088	4.703
	Purity angle	0.149	0.147	0.131	0.125	0.145
	Purity threshold	0.441	0.414	0.407	0.407	0.417
Fenbendazole	Peak area	9045862	9035793	9012737	9016345	9002857
	% Recovery	97.728	97.620	97.371	97.410	97.264
	% degradation	2.272	2.380	2.629	2.590	2.736
	Purity angle	0.231	0.187	0.192	0.195	0.197
	Purity threshold	0.64	0.598	0.57	0.558	0.554
Retention time of degradants (min)		2.979, 3.784, 4.162 & 4.360	2.799, 3.810, 4.199 & 4.410	2.807, 3.838, 4.228 & 4.444	2.813, 3.853, 4.250 & 4.471	2.812, 3.855, 4.257 & 4.480

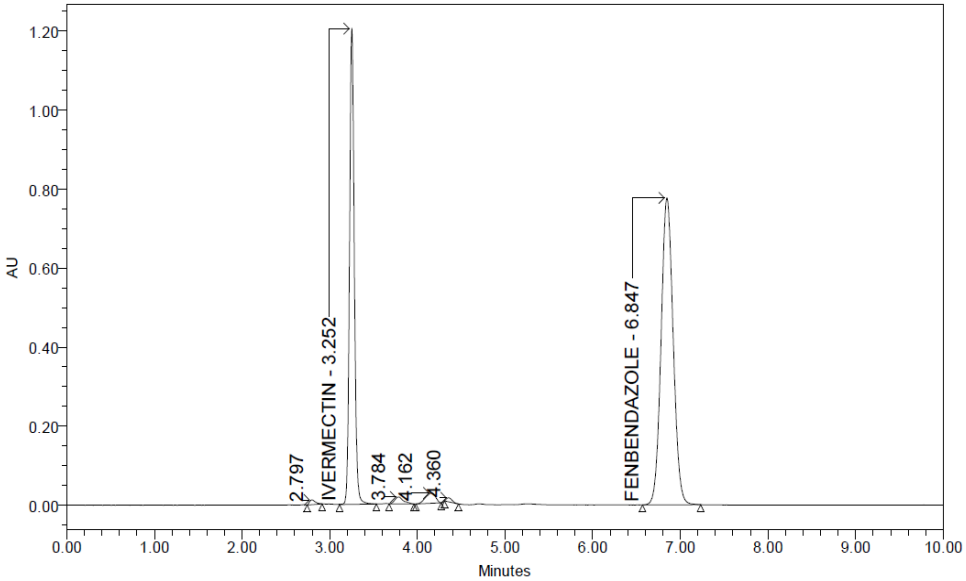

Fig 7: Chromatogram of acid induced degradation

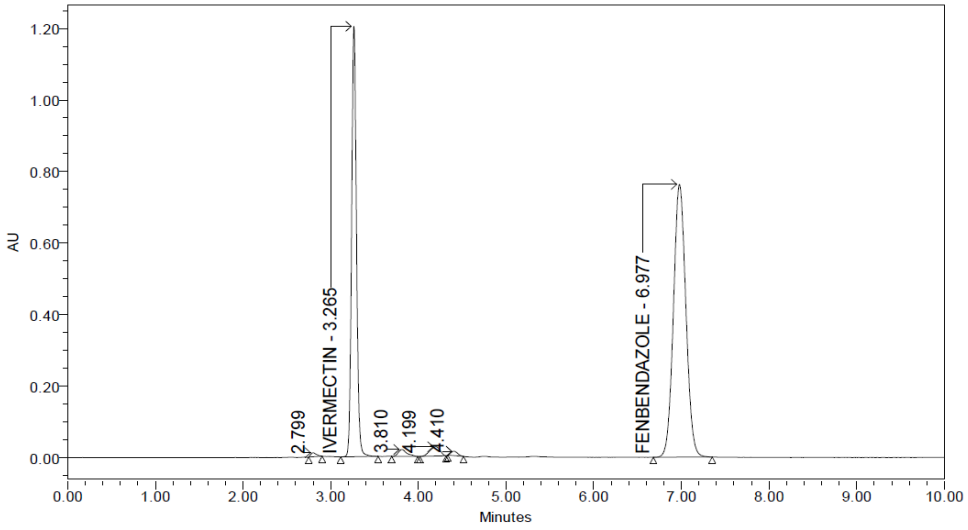

Fig 8: Chromatogram of alkali induced degradation

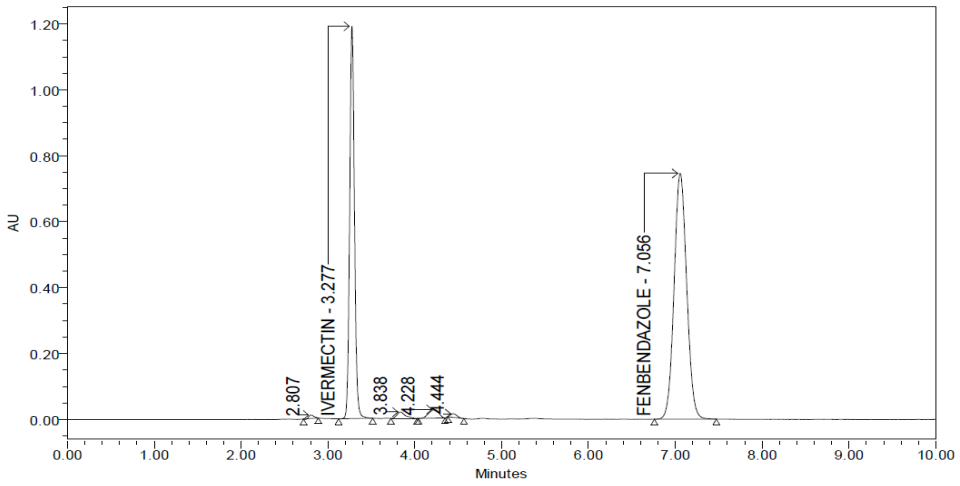

Fig 9: Chromatogram of hydrogen peroxide induced degradation

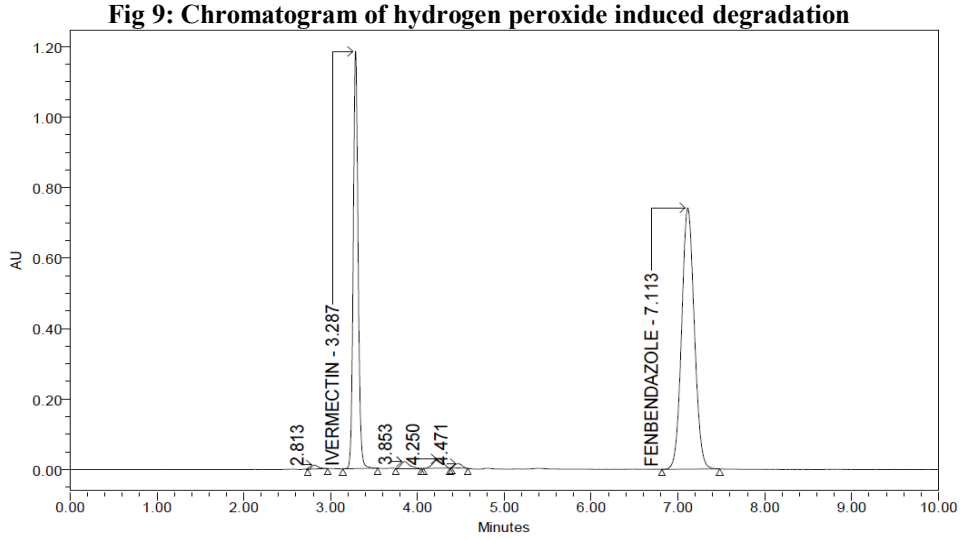

Fig 10: Chromatogram of thermal degradation

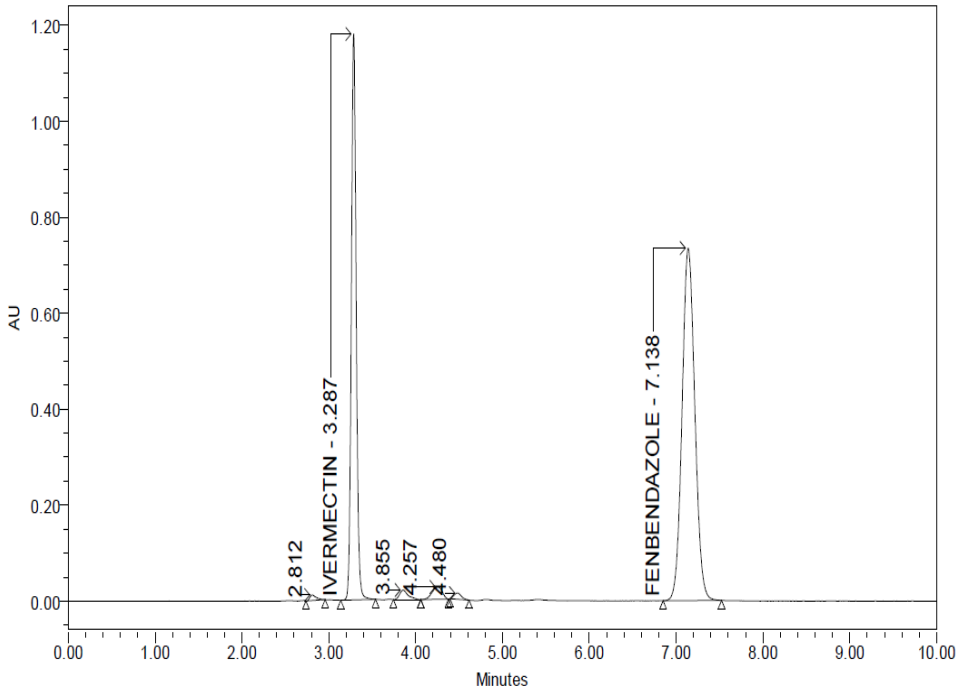

Fig 11: Chromatogram of photo degradation

Precision and accuracy:

The precision and accuracy was expressed as %RSD of drug peak area response and % assay of drug, respectively. The %RSD value measured during evaluation of precision was in the range of 0.0426-0.0442% for ivermectin and 0.0302-0.306% for fenbendazol, confirming the method is precise (Table 3). The % assay calculated during the evaluation of accuracy was 99.56% and 99.27% for ivermectin and fenbedazole, respectively (Table 3). The good percent assay proved the accuracy of the method.

.

Table 3: Results of precision and accuracy of the method

Sample No.	Ivermectin (4 µg/ml)		Fenbendazole (120 µg/ml)	
	Peak area response	Assay (%)	Peak area response	Assay (%)
1	5543095	99.540	9255194	99.270
2	5546153	99.600	9255805	99.270
3	5541103	99.510	9259760	99.320
4	5547536	99.620	9255258	99.270
5	5542078	99.530	9251212	99.230
6	5544146	99.560	9253477	99.250
Average	5544019	99.560	9255117	99.268
RSD	0.0442	0.0426	0.0306	0.0302

Recovery study:
The mean recovery of ivermectin and fenbendazole using standard addition method was found to be in the range of 99.545-99.668% and 99.803-100.368% (Table 4), respectively which shows no significant interferences from tablet excipients.

Robustness:
The results of the robustness tests are shown in Table 5. The results of robustness study confirmed that the small and deliberate changes in the flow rate of mobile phase and column temperature did not present significant effect on the system suitability parameters of the proposed method, indicating the robustness of the method.

Table 4: Recovery study of ivermectin and fenbendazole

Spiked Level (%)	Concentration of drug (µg/ml)		Recovery (%)	Mean (%)
	Added	Found		
Ivermectin				
50	2.00	1.993	99.644	99.668
	2.00	1.996	99.795	
	2.00	1.991	99.566	
100	4.00	3.980	99.508	99.545
	4.00	3.982	99.552	
	4.00	3.983	99.576	
150	6.00	5.972	99.532	99.550
	6.00	5.972	99.533	
	6.00	5.975	99.586	
Fenbendazole				
50	59.40	59.597	100.331	100.368
	59.40	59.629	100.385	
	59.40	59.631	100.389	
100	118.80	119.057	100.216	100.224
	118.80	119.084	100.239	
	118.80	119.058	100.217	
150	178.20	177.768	99.758	99.803
	178.20	177.890	99.826	
	178.20	177.888	99.825	

Table 5: Results of robustness test

Parameter varied	Retention time	Peak area	Plate count	Tailing factor	Resolution
Ivermectin					
Flow rate – 1.1 ml/min	4.127	5754924	18122	1.11	-
Flow rate – 1.3 ml/min	2.741	3793612	15668	1.10	-
Column temperature-29°C	3.288	4613813	16806	1.09	-
Column temperature-31°C	3.266	4587200	17351	1.07	-
Fenbendazole					
Flow rate – 1.1 ml/min	9.065	9493804	12648	1.07	21.80
Flow rate – 1.3 ml/min	6.003	6245188	9833	1.07	19.45
Column temperature-29°C	7.212	7539682	10953	1.07	20.46
Column temperature-31°C	6.845	7528375	12003	1.05	20.14

CONCLUSION:

A HPLC method with photodiode array detector has been developed and validated for the simultaneous estimation of ivermectin and fenbendazole in bulk and tablet dosage forms. From the results of method validation experiments, it is concluded that the proposed method was linear, sensitive, selective, specific, precise, accurate, robust and having stability indicating characteristics. The present method can be used for routine quality control of ivermectin and fenbendazole by the quality control laboratories.

Acknowledgements:

The author, M.V. Reddy, expresses his deep sense of gratitude to the Department of Chemistry, GITAM Institute Technology, GITAM University, Visakhapatnam for their encouragement and facilities provided throughout the project.

REFERENCES:

1.Saunders handbook of veterinary drugs: Small and large animal (4 ed.). Elsevier health sciences, 2015, 420. ISBN 978-0-323-24486-2.

2.Fisher MH, Mrozik H. The chemistry and pharmacology of avermectins. Annu Rev Pharmacol Toxicol, 1992, 32:537–53.

3."Ivermectin". The American Society of Health-System Pharmacists. Retrieved 16 January 2016.

4.Varghese FS, Pasi K, Sabine G, Maxim B,Leena H, Krister W, Beate MK,Tero A. Discovery of berberine, abamectin and ivermectin as antivirals against chikungunya and other alphaviruses. Antiviral Res, 2016, 126: 117–24.

5.Thomas BB. A review of the pharmacology and clinical uses of ivermectin. Can Vet J, 1987, 28: 512–517.

6.Griffith R, Yaeger M, Hostetter S, Tell LA, Wetzlich S, Vickroy T, Lillie B, MacFarlane W, Laudenslager T, Whitley E, Dzikamunhenga R, Larson W. Safety of fenbendazole in Chinese ring-necked pheasants (Phasianuscolchicus). Avian Dis, 2014, 58: 8-15.

7. Junquera, P. (2015-07-26). Febantel for veterinary use on dogs, cats, cattle, sheep, goats, pig and poultry against roundworms and tapeworms. Parasitipedia. Retrieved 2015-09-08.

8.Junquera, P. (2015-07-26). Fenbendazole, anthelmintic for veterinary use on cattle, sheep, goats, pig, poultry, horses, dogs and cats against roundworms and tapeworms. Parasitipedia. Retrieved 2015-09-08.

9.http://www.pcdfranchiseindia.com/fenbendazole-ivermectin-bolus-3314563.html

10. Pradeep S, Chandrashekar J. Analytical method development and validation for simultaneous estimation of ivermectin and fenbendazole in bulk dosage form by RP-HPLC. World J Pharm Pharma Sci, 2016, 5: 1614-24.

11. International Conference on Harmonization, ICH Guidelines, Validation of analytical procedures technical requirements for registration of pharmaceuticals for human use: Text and Methodology Q 2 (R1), International Conference on Harmonization, Geneva, Switzerland, November 2005.

12.United States Pharmacopoeia, “United states pharmacopeial convention,” Tech. Rep. USP 27, NF 22, United States Pharmacopoeia, Rockville, Md, USA, 2004.

13.Geneva: Proceeding of the International Conference on Harmonization; 1996. ICH, Q1(B), Harmonized Tripartite Guideline, Stability testing: Photostability testing of new drug substances and products, in.