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Research Article

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR ESTIMATION OF IVERMECTIN AND ALBENDAZOLE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, specific, accurate and stability-indicating reversed phase high performance liquid chromatographic method was developed for simultaneous estimation of Ivermectin and Albendazole, Supel cosilTM (150 x 4.6 mm i.d.,5 µ particle size) column used and a mobile phase composed of Acetonitrile: Methanol: Buffer (pH 7.0) (51:25:24 v/v/v). The retention time of Ivermectin and Albendazole were found to be 7.733 and 2.173 min respectively. Linearity was established for Ivermectin and Albendazole in the range of 6-36 µg/ml and 200-1200 µg/ml respectively. The percentage recovery of Ivermectin and Albendazole were found to be in the range of 98.77-99.63% and 98.67-100.09% respectively. The drug was subjected to acid and alkali hydrolysis, oxidation, dry heat and photolytic degradation. The degradation studies indicated, Ivermectin showed degradation in acid, alkali, Oxidation and dry heat while it was found stable in photolytic condition and Albendazole showed degradation in Acid, Alkali, Oxidation and Dry heat while it was found stable in Photolytic condition . This method can be successfully employed for the quantitative analysis of Ivermectin and Albendazole in bulk drugs and formulations.

KEY WORDS: Albendazole (ALB), Ivermectin (IVE), RP-HPLC method, Stability indicating RP-HPLC method, Validation.

1. INTRODUCTION

Ivermectin is broad-spectrum Antiparasitic agent Chemically Known as 5-O-demethyl-22,23-dihydroavermectin-A1a5-O-Demethyl-25-de(1-methylpropyl)-22,23-dihydro-25-(1-methylethyl)avermectin-A1a:22,23 Dihydroavermectin B1b. It is mainly used in the human in the treatment of Onchocerciasis (River blindness) and also effective against other worm infestation[1, 3]. Albendazole is Broad-spectrum Antiparasitic agent developed as a veterinary Anthelmintic chemically Known as a Methyl [5-(propylthio)-1H-benzoimidazol-2-yl] carbamate. It is effective against pin worm infection, ascariasis, trichuriasis, infection with both hook worm species[1, 4]. Combined Albendazole and Ivermectin are a more efficacious treatment for intestinal helminthes and W. bancrofti infections in children[2]. The chemical structures of Ivermectin and Albendazole are shown in Fig.1 & 2. Commercially fixed combination of IVE (12 mg) and ALB (400 mg) is available in the market as tablet and Suspension formulation. The ratio in marketed formulation is 1:33 respective for IVE and ALB.

Ivermectin is official in IP, BP where as Albendazole is official in IP, BP[3, 4]. Several analytical methods like HPLC[5, 6], HPTLC[7, 8], UPLC[9], and UV[10] have been reported for estimation of Ivermectin and Albendazole by single drug or also by combining with other drugs. On literature survey RP-HPLC method reported for combined dosage form of IVE and ALB[11]. The literature survey indicated that no stability indicating RP-HPLC method was proposed for IVE and ALB.

The International Conference on Harmonization (ICH) guideline entitled "Stability testing of new drug substances and products" requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance[12]. An ideal stability-indicating method is one that resolves the drug and its degradation products efficiently. Consequently, the implementation of an analytical methodology to determine Ivermectin and Albendazole in presence of its degradation products is rather a challenge for pharmaceutical analyst. Therefore, it was thought necessary to study the stability of IVE and ALB under acidic, alkaline, oxidative, photolytic and dry heat conditions. This paper reports validated stability-indicating HPLC method for simultaneous determination of IVE and ALB in presence of their degradation products. The proposed method is simple, accurate, reproducible, stability-indicating and suitable for routine determination of IVE and ALB in its dosage form. The method was validated in compliance with ICH guidelines[13].

2. MATERIALS AND METHODS

2.1 Materials Used

- Albendazole (Jay Pharmaceutical pvt, Ltd.)
- Ivermectin (Jay Pharmaceutical pvt. Ltd.)
- Membrane Filter-0.45 µm pore size, 47mm diameter (Sartorius India Pvt. Ltd., Mumbai, India).
- Millipore Millex-HV Hydrophilic PVDF Syringe filter-0.45 µm Pore size, 33mm diameter (Millipore India Pvt. Ltd., Mumbai, India).
- Whatman filter paper no. 41 (Whatman International Ltd., England).

2.2 Chemical and Reagents Used

- HPLC grade methanol, acetonitrile, water (Merck Chemicals Ltd. India)
- Potassium Dihydrogen ortho phosphate, Ortho Phosphoric acid, Hydrochloric acid, Sodium hydroxide, and Hydrogen peroxide (AR- grade, Merck Chemicals Ltd., Ahmadabad, India)

2.3 Instruments and Apparatus Used

- Agilent Technologies 1260 infinity Series HPLC instrument equipped with Auto-sampler and DAD or UV detector.(Software EZ chrome)
- An analytical balance (Sartorius)
- pH meter (Thermo electron Crop., Pune, India)
- Ultra sonicator (Electrolab, India)
- Hot air oven (TO-90S, Thermolab)
- Calibrated Volumetric flasks- 10, 50, 100 ml
- Graduated Pipettes- 1, 2, 5, 10 ml
- Measuring cylinder- 100 ml (Borosil)
- Beaker- 100, 250, 500 ml (Borosil)

2.4 Chromatographic System

A Liquid Chromatography-model Agilent Technologies 1260 Infinitely Series Equipped with LC 20 AD Quaternary Pump, SIL-20AD auto sampler, CTO-20A column oven, SPD-M20A photodiode array detector, Software EZ Chrome.

2.5 Preparation of Buffer Solution (KH_2PO_4) pH 7.0

Accurately weigh and transfer 0.68gm of potassium Dihydrogen orthophosphate in 500 ml of glass beaker previously dried. Dissolve it in about 450 ml of HPLC grade water and also adjust to pH7.0 ±

0.05 with dilute NaOH make up 500 ml volume with HPLC grade water. Mix well and Filter through 0.45µ membrane filter.

2.6 Preparation of Mobile Phase

Acetonitrile, methanol and filtered buffer solution were mixed in the proportion of 51:25:24 v/v/v and sonicated for 15 minutes.

2.7 Preparation of Standard Solutions

A. Preparation of Standard Stock Solution of Ivermectin: (S_1)

Accurately weighed quantity of Ivermectin 50 mg was transferred into 50 ml volumetric flask, dissolved and diluted up to mark with mobile phase. This will give stock solution having strength of 1000 µg/ml.

B. Preparation of Working Standard Solution of Ivermectin: (W_1)

100 µg/ml of Ivermectin was prepared by diluting 5 ml of Stock Solution in 50 ml with Mobile phase.

C. Preparation of Standard Stock Solution of Albendazole: (S_2)

Accurately weighed quantity of Albendazole 250 mg was transferred into 100 ml volumetric flask, dissolved in DMSO and diluted up to mark with mobile phase. This will give stock solution having strength of 2500 µg/ml.

2.8 Test Sample Preparation

Take equivalent weight of 583.75 mg of tablet powder into 100 ml volumetric flask and 10 ml diluents was added into it. The content of the flask were sonicated for 10 min to dissolve the active ingredient completely and the volume was made with the mobile phase. 1 ml from this solution was transferred into a 10 ml volumetric flask and the volume was made with the mobile phase. This sample solution containing working concentrations of 12 µg/ml of Ivermectin and 400 µg/ml of Albendazole was then analyzed for assay determination.

2.9 Assay Determination of IVE and ALB marketed formulation (Evimectin-A, Tablet)

The Prepared test sample was chromatographed for 10 min under the optimized chromatographic conditions. From the peak areas obtained in the chromatogram, the concentrations of IVE and ALB in the test sample were individually calculated using the related linear regression equations of their respective calibration curves. The amounts of IVE and ALB per each mg Tablet were then found out from their respective concentrations in the test sample and their assay was determined.

2.10 Method Validation

A. System Suitability test Parameters

System suitability testing is an internal part of a liquid chromatographic method, and it is used to verify that the chromatographic method is able to produce good resolution between the peaks of interest with high reproducibility. The system suitability was determined by making six replicate injections from a freshly prepared standard solution of 12 µg/ml of IVE and 400µg/ml of ALB and analyzing each solute for its retention time (Rt), Number of theoretical plates (N), resolution (RS) and tailing factor (T). The system suitability method acceptance criteria set in each validation run were- a %RSD of peak areas and retention times less than 1.0, Capacity factor > 2.0, tailing factor ≤ 2.0, and theoretical plates > 2000.

B. Selectivity:

It is ability of the method to measure specifically the analyte of interest, in the presence of other components, such as impurities, degradation products, excipients that be expected to be present in the sample preparation.

C. Linearity and Range:

From working std. Solution of IVE (W_1) (0.6, 1.2, 1.8, 2.4, 3.0, 3.6 ml.) were transferred into series of 10 ml volumetric flask and diluted up to mark with mobile phase. This yielded solution of 6, 12, 18,

24, 30, 36 µg/ml of IVE and from working std. solution of ALB (S_2) (0.8, 1.6, 2.4, 3.2, 4.0, 4.8 ml) were transferred into series of 10 ml volumetric flask and dilute up to mark with mobile phase. This yielded solution of 200, 400, 600, 800, 1000, 1200 µg/ml. An aliquot of 20µl of each solution was injected under operating chromatographic condition. Plot the calibration curve of area versus respective concentration and find out correlation co-efficient and regression line equation for IVE and ALB. Each response was an average of five determinations.

The linearity data are presented in Table 2 & 3. Calibration curve of IVE and ALB is presented in Figure 9 & 10.

D. Precision

i. Repeatability:

Repeatability was determined by analyzing IVE and ALB test solution having the concentration 12µg/ml of IVE and 400µg/ml of ALB. Measure six times. Calculate %RSD for IVE and ALB.

The data for repeatability for IVE and ALB is shown in Table 4.

ii. Intraday precision

Intraday precision was determined by analyzing of IVE and ALB standard solution in the range 12, 18, 24 µg/ml of IVE and 400, 600, 800 µg/ml of ALB in triplicate. Calculate %RSD for IVE and ALB.

The data for Intraday precision for IVE and ALB is shown in Table 5.

iii. Interday precision:

Interday precision was determined by analyzing of IVE and ALB standard solutions in the range 12, 18, 24 µg/ml of IVE and 400, 600, 800 µg/ml of ALB in different days. Calculate %RSD for IVE and ALB.

The data for Interday precision for IVE and ALB is shown in Table 6.

E. Accuracy

Accuracy was determined by calculating recovery of IVE and ALB by the standard addition method. The known amounts (0.6, 1.2, 1.8 ml) of working standard solution of IVE was added to 1 ml sample solution of IVE (12 µg/ml) and the known amount (0.8, 1.6, 2.4 ml) of working standard solution of ALB was added to 1 ml sample solution of ALB (400 µg/ml) in 10 ml of volumetric flask and diluted up to mark with mobile phase Each solution was injected in triplicate and recovery was calculated for regression equation of calibration curve by measuring peak areas.

The data of accuracy for IVE and ALB are shown in Table 7.

F. Limit of detection and Limit of Quantitation

LOD and LOQ of the drug were calculated using following equations according to ICH guideline. LOD = 3.3 σ/s and LOQ = 10 σ/s Where σ is the SD of the response and S is the slope of the calibration curve.

The data of LOD and LOQ for IVE and ALB are shown in Table 8.

G. Robustness:

The robustness study was performed to evaluate the influence of small but deliberate variation in the chromatographic condition. The robustness was checked by changing three small changes.

1. Mobile phase flow rate (1±0.1ml/min)
2. Mobile phase composition (ACN + methanol +buffer
3. pH (±0.2 units)
4. Column temperature (25±2 ˚C)

After each changes sample solution was injected and % assay with system suitability parameters were checked.

The data for Robustness for IVE and ALB is shown in Table 9.

2.11 Forced degradation Study

A. Preparation of Sample Solution

Accurately weigh and transferred about 583.75 mg of tablet powder in 100 ml volumetric flask. Add about 5 ml of DMSO and 20 ml of methanol and sonicate till it dissolve completely by maintaining temperature around 25°C and make up the volume up to mark with mobile phase. Take 1 ml of above solution transfer in 10 ml volumetric flask make up volume up to 10 ml with mobile phase.

B. Chromatographic Conditions

The chromatographic separations were performed using supel cosil^{TM} LC-ABZ (150 cm× 4.6mm, 5µm particle size) column. The optimum mobile phase consisted of Acetonitrile, methanol and KH_2PO_4 buffer (pH 7.0) in ratio of 51:25:24 %v/v. Auto sampler 20 µl was used and kept at 30°C temperature. Analysis was done with flow rate of 1.0 ml/min at 245 nm wavelength by using diode array detector (DOD).

C. Acid Degradation

Transfer an accurately weighed quantity about 1 ml of sample solution in to 10 ml of 4 different volumetric flasks. Add each flask 1.0 ml 0.1 N hydrochloric acid. Store flask at 60°C for 1, 2, 4 and 6 hr. After the stipulated time period remove the flask from water bath & cool the content. Add each flask 1.0 ml 0.1N sodium hydroxide. Dilute to volume with mobile phase mix evenly.

D. Alkali Degradation

Transfer an accurately weighed quantity about 1 ml of sample solution in to 10 ml of 4 different volumetric Flask. Add each Flask 1.0 ml 0.1 N Sodium hydroxide. Store Flask at 60°C for 1, 2, 4 and 6 hr. After the stipulated time period remove the flask from water bath & cool the content. Add each flask 1.0 ml 0.1N Hydrochloric acid. Dilute to volume with mobile phase mix evenly.

E. Oxidation

Transfer an accurately weighed quantity about 1 ml of sample solution in to 10 ml of 4 different volumetric Flask. Add each flask 1.0 ml 3% Hydrogen peroxide. Store Flask at 60°C for 1, 2, 4 and 6 hr. After the stipulated time period remove the flask from water bath & cool the content. Add each flask 1.0 ml 0.1N sodium hydroxide. Dilute to volume with mobile phase mix evenly. The small peak observed at retention time of 2.313 Rt is compared with blank chromatogram of 3% H_2O_2. Shows that peak was of H_2O_2 not of Albendazole and Ivermectin.

F. Dry heat

Accurately weigh and transfer about 1 ml of sample solution exposed under heat at 80°C for 1, 2, 4 and 6 hr in 10 ml of 4 different volumetric Flask. Add about 5 ml of mobile phase and sonicate to dissolve it completely and make volume up to the mark with mobile phase.

G. Photolytic degradation

Sample of tablet exposed under sun light and UV light for 24 hr. Take 5 mg of powder in 10 ml of volumetric flask. Add 5 ml of mobile phase than sonicate it dissolve completely. Than make volume up to 10 ml with mobile phase.

3. RESULT

The mobile phase consisting of ACN: Methanol: Buffer (51: 25: 24 %v/v/v), 1.0ml/min flow rate was optimized which gave sharp peak with minimum tailing factor for IVE and ALB (fig. 2). The retention time for IVE and ALB were 7.733 and 2.173 min respectively. UV spectra of IVE and ALB showed the λ_{max} at common wavelength 245 nm, so this wavelength was selected as the detection wavelength. Shown Fig. 1

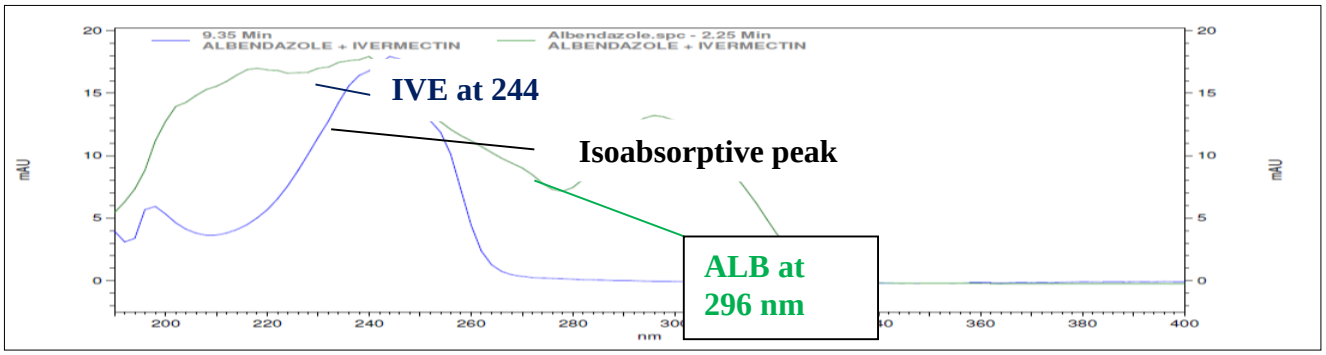

Figure 1: Overlain spectra of IVE and ALB

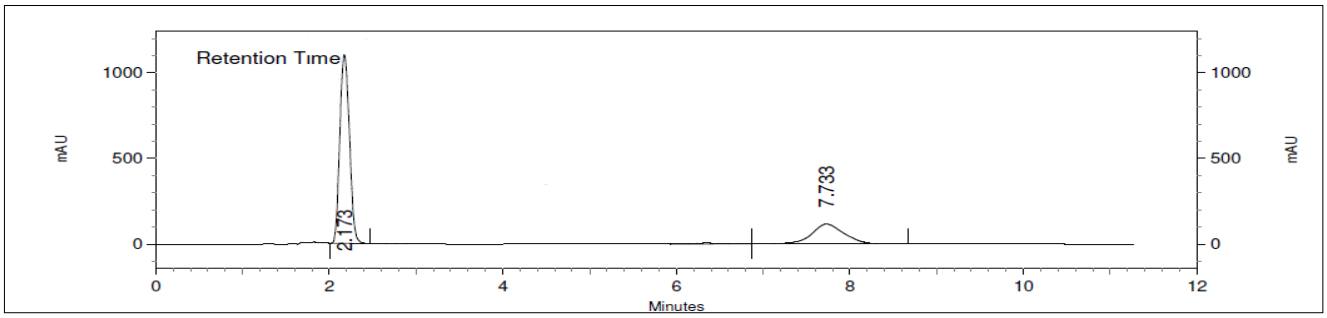

Figure 2: Chromatogram of STD. IVE and ALB

3.1 Selectivity

The analytical method was found to be selective as no interferences of excipients were found in separation. Representative chromatogram of Ivermectin and Albendazole diluents, Standard, and sample were shown in figure 3, 4, 5, and 6.

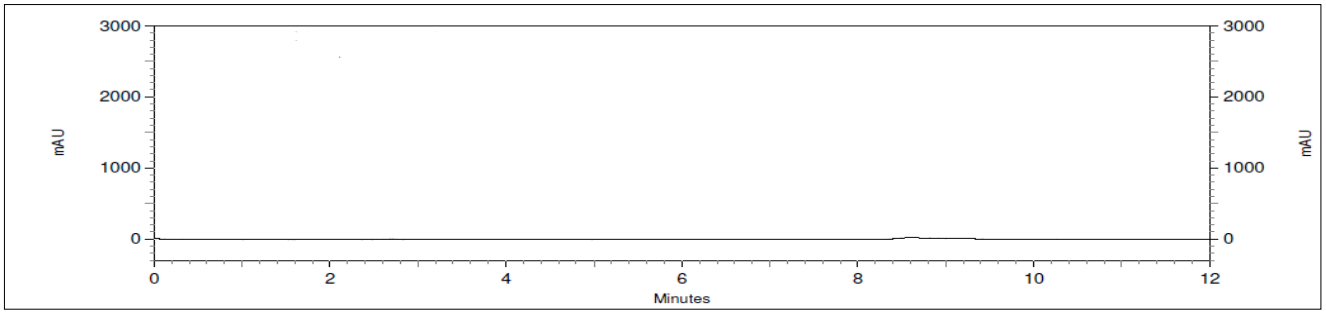

Figure 3: Chromatogram of Diluents

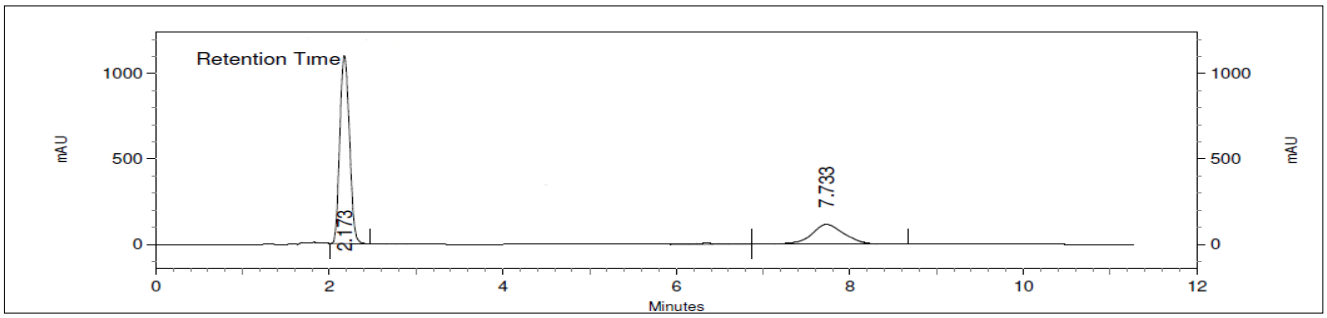

Figure 4: Chromatogram of IVE and ALB

Table 1: System suitability parameter for IVE and ALB

Drug	Retention time	Area	Theoretical Plate	Tailing Factor	Resolution
ALB	2.173	17862177	6808.98	1.130	0.00000
IVE	7.733	6325711	8745.01	1.026	2.23680

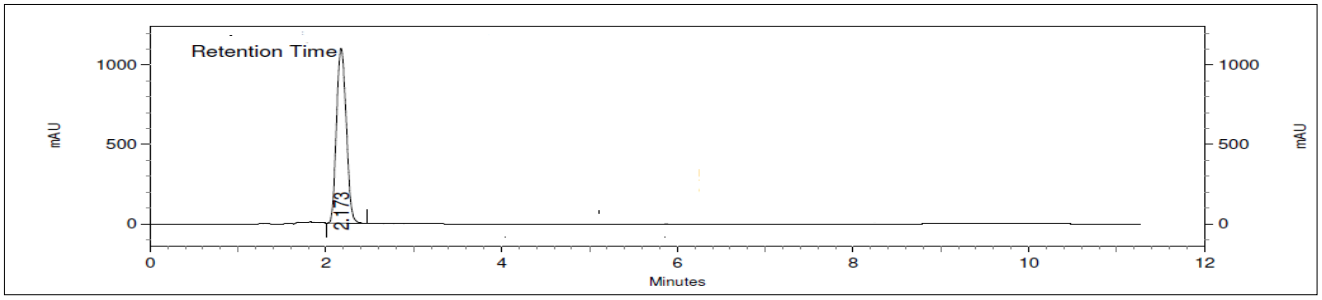

Figure 5: Chromatogram of Std. ALB (Rt: 2.173 min)

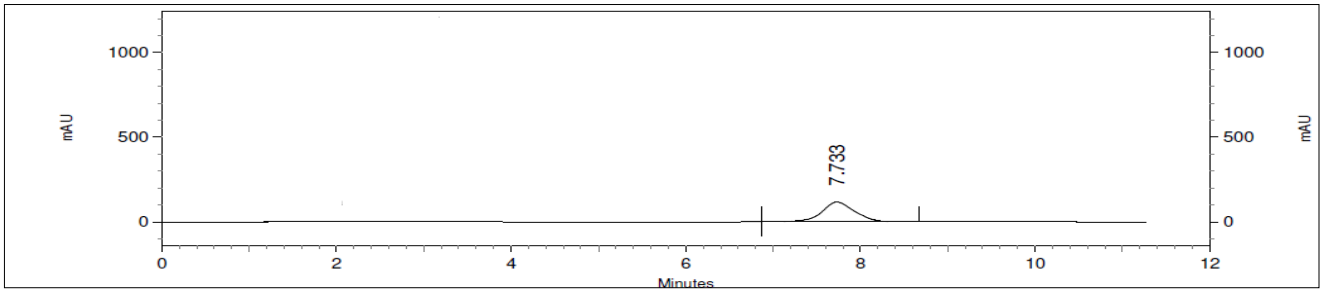

Figure 6: Chromatogram of Std. IVE (Rt: 7.733 min)

3.2 Linearity and Range

The linearity for IVE and ALB was found in the range of 6-36 µg/ml and 200-1200 µg/ml. The overlain chromatogram of IVE and ALB are presented in Figure 7 & 8. The linearity data are presented in table 2. Calibration curve of IVE and ALB is presented in Figure 9 & 10 respectively. Correlation coefficient, slope and intercept are listed in table 3.

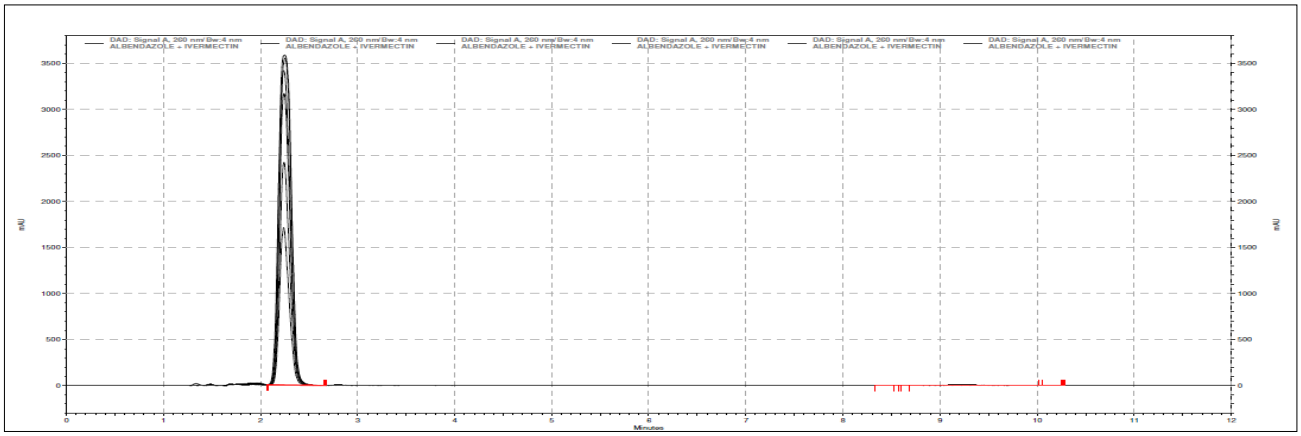

Figure 7: Overlain Chromatogram of ALB and IVE.

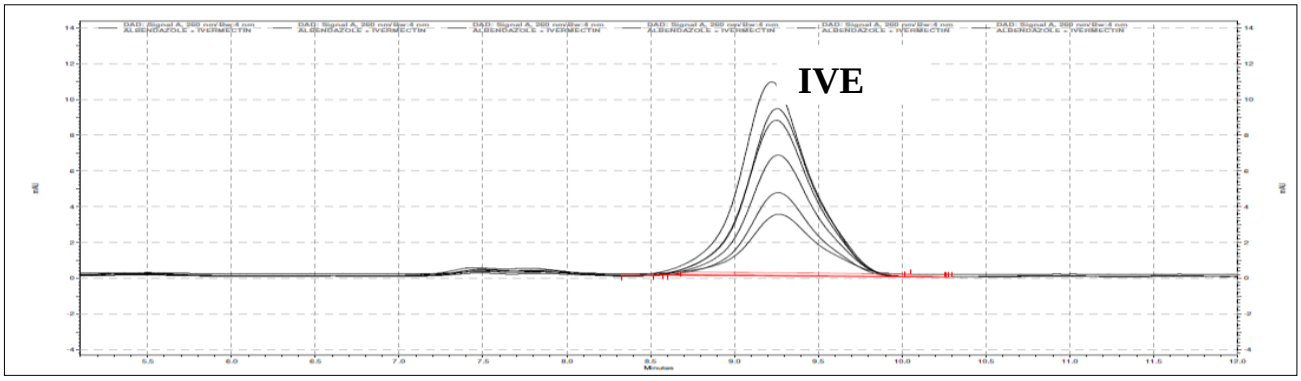

Figure 8: Zoom Overlain spectra of Ivermectin

Table 2: Linearity Data for ALB and IVE

Sr. no.	ALB (µg/ml)	Peak Area*± S.D.	IVE (µg/ml)	Peak Area*± S.D.
1	330	24968560.4±36259.71	10	194809.4±51.964
2	495	37481231.4±12733.68	15	282688.6±847.102
3	660	49977017.4±12985.71	20	411708.8±1009.831
4	825	61479350±3992.621	25	487201±56.04908563
5	990	74977773±6057.833	30	572101.8±2110.994
6	1155	85483925±3314.151	35	674249.8±159.6690325

*Average of five determinations.

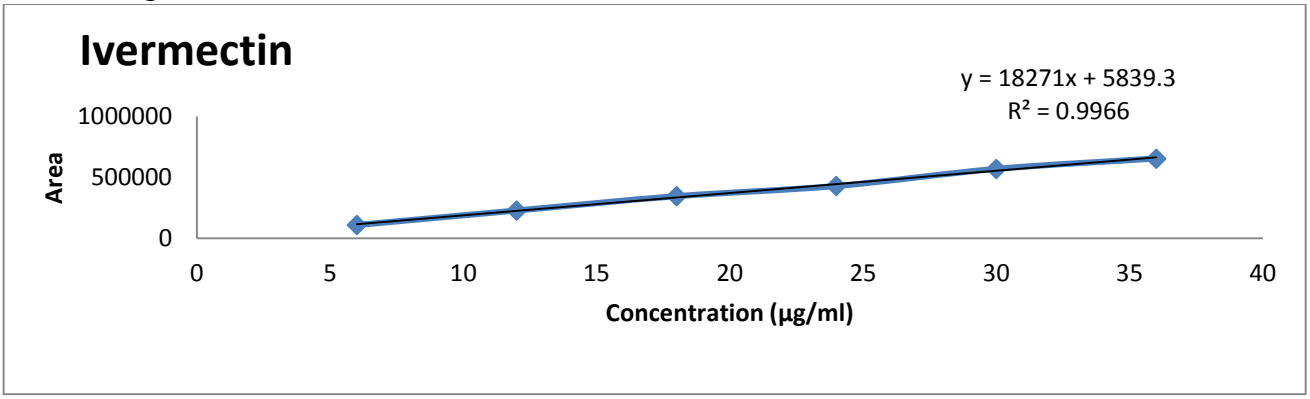

Figure 9: Calibration curve of Ivermectin

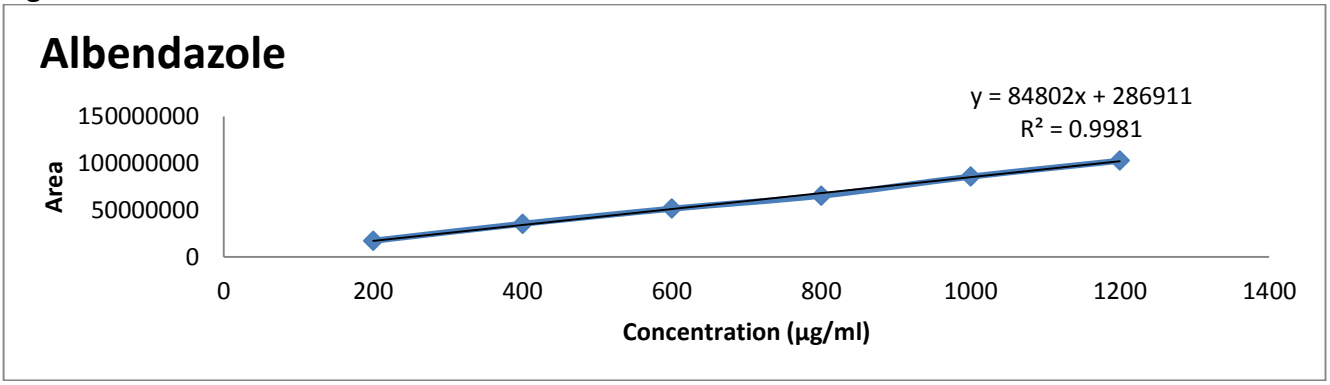

Figure 10: Calibration curve of Albendazole

Table 3: Regression Analysis data for ALB and IVE

Regression Analysis	ALB	IVE
Regression equation	Y= 84802x+28691	Y=18271x+5839
Correlation co-efficient (R^2)	0.998	0.996
Slope(S)	84802	18271
Intercept(σ)	28691	5839

3.3 Precision

A. Repeatability

The data of repeatability for ALB and IVE is shown in table 4. The % RSD for Repeatability data was found to be 0.1086 % for ALB and 1.033% for IVE.

Table 4: Repeatability data for estimation of ALB and IVE

Sr. no.	Drug	Conc. (µg/ml)	Mean Peak Area*± S.D.	Mean %Assay*± S.D.	%RSD
1	ALB	400	35080108±38129.93	99.36±0.108	0.1086
2	IVE	12	226042±2336.875	98.97±1.023	1.033

*Average of six determination

B. Intra-day precision

The data for intraday precision for ALB and IVE is shown in table 5. The %RSD for Intraday precision was found to be 0.065-0.144% for ALB and 0.928-1.168% for IVE.

Table 5: Intra-day precision data for estimation of ALB and IVE

Conc.(µg/ml)		Mean Peak Area*± S.D.		Mean % Assay* ± S.D.		%RSD	
ALB	IVE	ALB	IVE	ALB	IVE	ALB	IVE
400	12	35121710±50607.04	225201±2663.92	99.48±0.143	98.16±1.116	0.144	1.168
600	18	51042203±58163.42	345140±3848.01	99.17±0.113	99.84±1.113	0.113	1.114
800	24	65055001±42300.5	424141±3938.77	99.45±0.064	98.76±0.9172	0.065	0.9286

*Average of three determination

C. Interday precision

The data for inter-day precision for ALB and IVE is shown in table 6. The %RSD for inter-day precision was found to be 0.0249-0.0907% for ALB and 0.088-0.311% for IVE.

Table 6: Inter-day precision data for estimation of ALB and IVE

Conc.(µg/ml)		Mean Peak Area*± S.D.		Mean % Assay* ± S.D.		%RSD	
ALB	IVE	ALB	IVE	ALB	IVE	ALB	IVE
400	12	34822699 ±25661.74	225171± 624.23	98.65±0.0624	98.59±0.273	0.0633	0.277
600	18	51053388 ±45618.05	340119.3 ±1059.57	99.19±0.0900	98.39±0.306	0.0907	0.311
800	24	64108594 ±15977.88	428137.3 ±376.96	98.011±0.0244	99.69±0.087	0.0249	0.088

*Average of three determination

3.4 Accuracy

Accuracy of the method was confirmed by recovery study from marketed formulation at three level of standard addition. Percentage recovery for 1ALB it was % and for IVE it was %. The Result are shown in Table 7. Recovery greater than 98 % with low S.D. justifies the accuracy of the method.

Table 7: Recovery data for ALB and IVE from tablet formulation

Level of Accuracy	Amt. of sample (µg/ml)		Amt. of drug added (µg/ml)		Total conc. In µg/ml		Amt. Recovered Mean ±S.D.(µg/ml)		% Recovery*	
	ALB	IVE	ALB	IVE	ALB	IVE	ALB	IVE	ALB	IVE
50%	400	12	200	6	600	18	598.45±0.647	17.78±0.023	99.74	98.77
100%	400	12	400	12	800	24	789.40±0.541	23.83±0.05	98.67	99.29
150%	400	12	600	18	1000	30	1000.96±0.02	29.89±0.051	100.09	99.63

*Average of three determination

3.5 Limit of Detection and Limit of Quantitation

Table 8: Limit of Detection and Limit of Quantitation

ALB		IVE	
LOD	LOQ	LOD	LOQ
0.0789 µg/ml	0.239 µg/ml	0.0116 µg/ml	0.0353 µg/ml

3.6 Robustness

The typical variation studied under this parameter are flow rate, mobile phase composition, change in pH, temperature. The results are shown in the table 9.

Variation seen was within the acceptable range respect to peak asymmetry and theoretical plates, so the method was found to be robust.

Table 9: Data of Robustness

Condition	Variation	ALB			IVE		
		% Assay	S.D.	% RSD	% Assay	S.D.	%RSD
Temp. (30 ± 2°C)	28°C	99.95	0.059	0.059	98.75	0.933	0.945
	32°C	98.65	1.042	1.056	98.03	0.432	0.441
Flow Rate (1 ± 0.1 ml/min)	1.1 ml/min	99.45	0.890	0.895	98.96	1.61	1.62
	0.9 ml/min	98.54	0.106	0.107	99.08	0.933	0.941
Organic phase Modifier ACN (51±2%): Methanol (25): buffer (24±2%) (v/v/v)	49:25:26(v/v/v)	98.13	1.74	1.06	98.69	0.46	0.917
	53:25:22(v/v/v)	99.82	0.188	0.188	98.54	1.38	1.40
pH (7.0 ± 0.2)	pH 6.8	99.91	0.073	0.68	97.74	0.47	0.64
	pH 7.2	98.14	1.775	1.80	98.87	0.95	0.97

3.7 Applicability of the Method

Analysis of marketed formulation

Application of the proposed method was tested by analyzing the commercially available tablet formulation. The results are shown in table 10.

Table 10: Analysis of market formulation

Tablet	Label Claim (in mg/tablet)		Amount Found*±SD		%Assay*±S.D	
Evimectin-A	ALB	IVE	ALB	IVE	ALB	IVE
	400	12	399.15±0.23	11.95±0.0152	99.78±0.015	99.58±0.023

*Average of six determination

3.8 Summary

Table 11: Summary of Validation parameter for developed chromatographic method.

Sr No.	Parameter	Albendazole	Ivermectin
1	Linearity Range	400-1200 µg/ml	6-36 µg/ml
2	Co-relation coefficient	0.998	0.996
3	Precision(%RSD)		
	Repeatability	0.108%	1.033%
	Intra-day precision	0.065-0.144%	0.928-1.168%
	Inter-day precision	0.0249-0.0907%	0.088-0.311%
4	Accuracy (% Recovery)	98.67-100.09%	98.77-99.63%
5	Limit of detection(LOD)	0.0789 µg/ml	0..239 µg/ml
6	Limit if quantification(LOQ)	0.0116 µg/ml	0.0353 µg/ml
7	Selectivity	Selective	Selective
8	Assay (%)	99.58±0.023	99.78±0.015

3.9 Force degradation

3.9.1 Acid Degradation

It was observed that one degradation impurity peak on refluxing it in methanolic 0.1N HCl for 6 hr and chromatogram of drug in formulation 0.1N HCl.

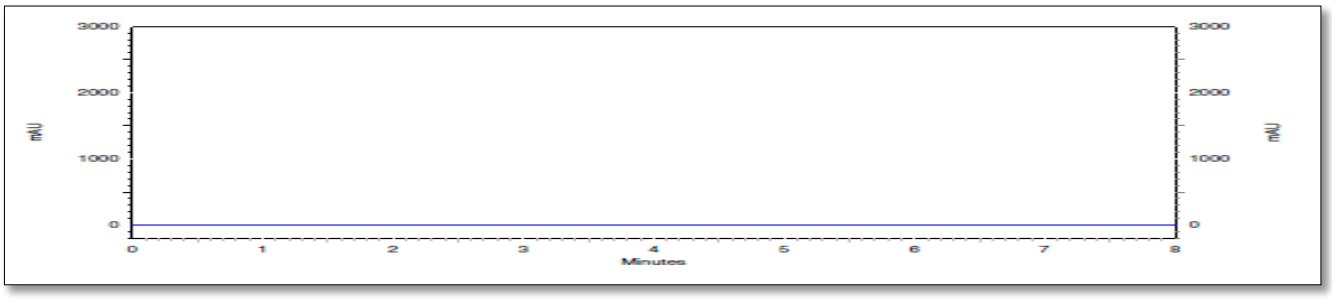

Figure 11: Chromatogram of acid degradation of Blank

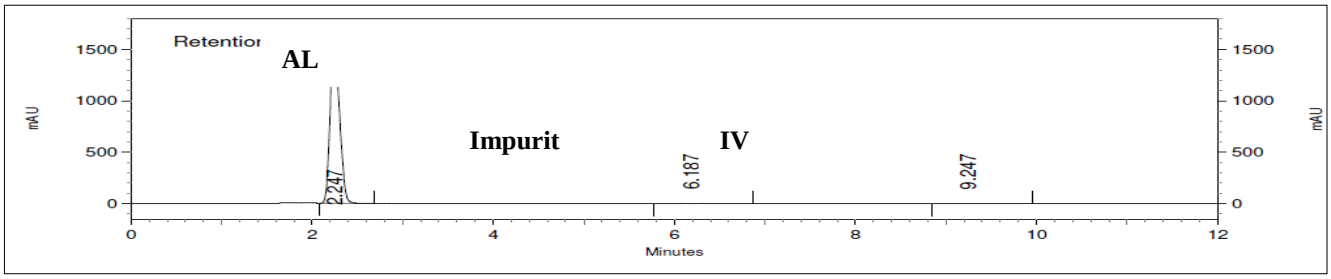

Figure 12: Chromatogram of acid degradation of ALB and IVE (6 hr)

3.9.2 Alkali Degradation

It was observed that Two degradation impurity peak on refluxing it in methanolic 0.1N NaOH for 8 hr and chromatogram of drug in formulation 0.1N NaOH.

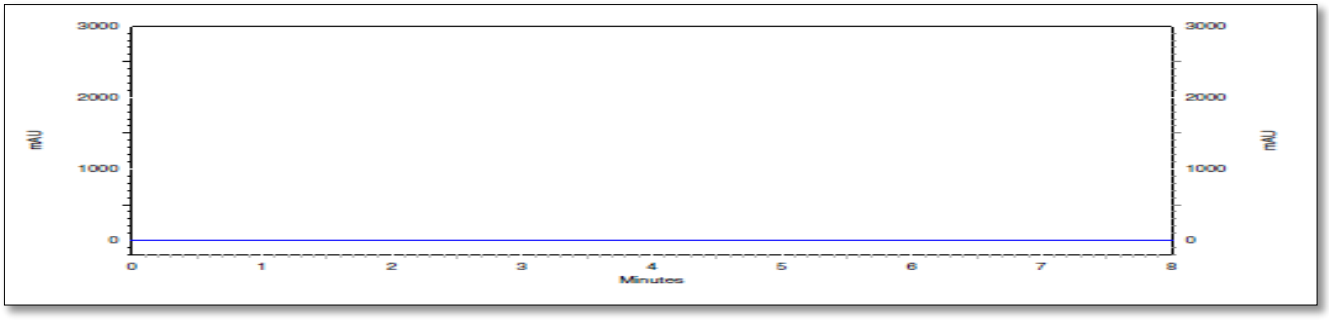

Figure 13: Chromatogram of alkali degradation of Blank

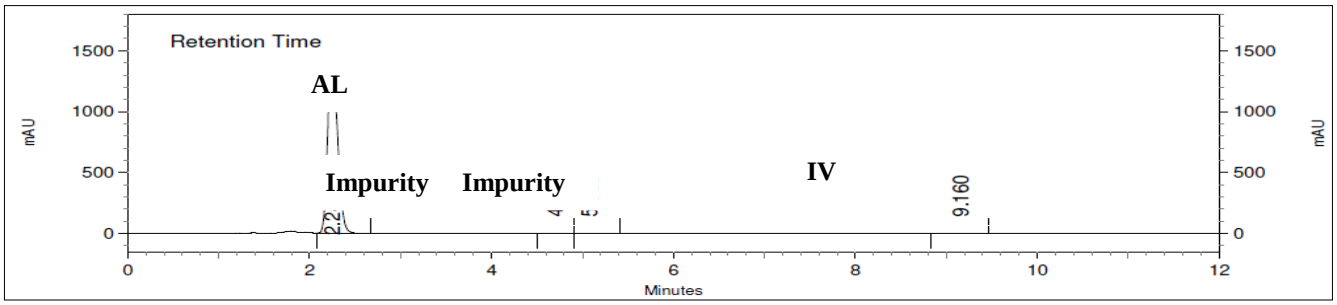

Figure 14: Chromatogram of alkali degradation of ALB and IVE (6 hr)

3.9.3 Oxidation

The small peak observed at retention time of 2.313 Rt is compared with blank chromatogram of 3% H_2O_2. Shows that peak was of H_2O_2 not of Albendazole and Ivermectin.

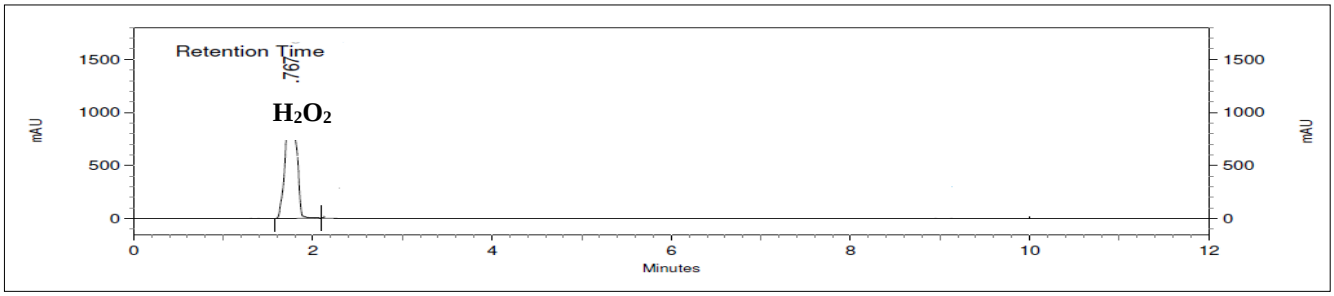

Figure 15: Chromatogram of 3% H_2O_2 degradation of Blank

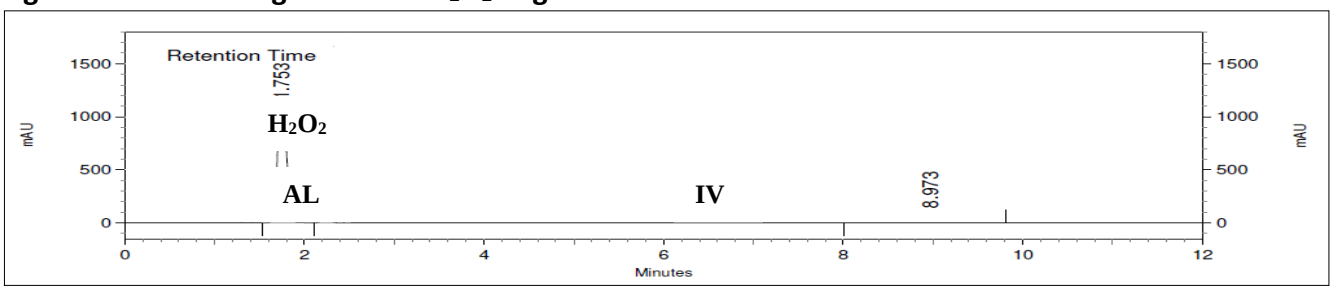

Figure 16: Chromatogram of dry heat degradation of ALB and IVE (6 hr)

3.9.4 Dry heat

It was not observed any Impurity peaks but change minor retention time so given the same degradation.

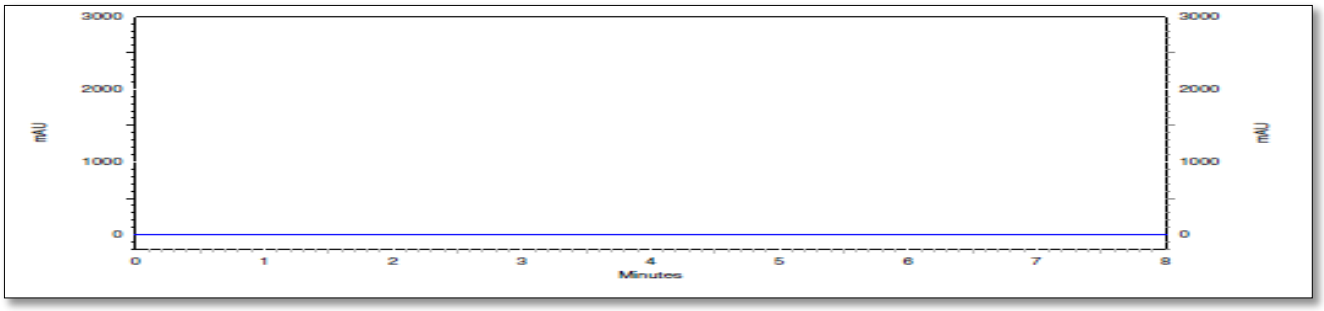

Figure 17: Chromatogram of dry heat degradation blank

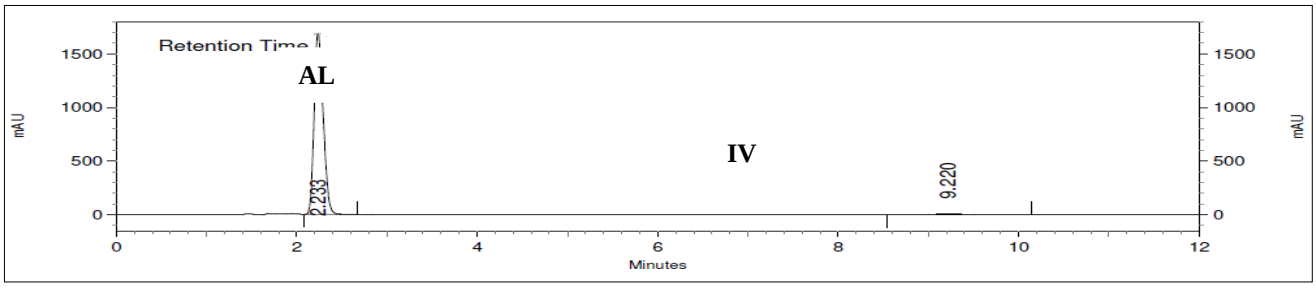

Figure 18: Chromatogram of dry heat degradation ALB and IVE (6 hr)

3.9.4 Photolytic degradation

It was stable to photolytic degradation condition.

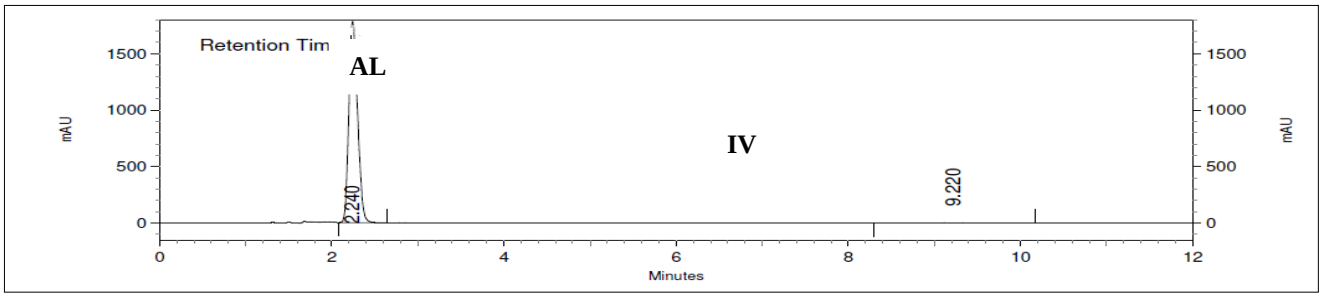

Figure 19: Chromatogram of photolytic degradation of ALB and IVE

Table 12: Result of Force degradation study of Albendazole and Ivermectin

Stress condition	Time (hr)	Albendazole		Ivermectin		%degradation		No. of extra peak	Relative retention time of extra peak
		R.T.	Peak purity	R.T.	Peak purity	ALB	IVE		
As such	-	2.173	-	7.733	-	-	-	-	-
Acidic 0.1N HCl	6	2.247	0.99978	9.247	0.99991	21%	18.95%	1	6.187
Alkali 0.1N NaOH	6	2.247	0.99962	9.160	0.99992	23.76%	17.76%	2	4.707, 5.080
3% H_2O_2	6	2.227	1.00000	8.973	0.99998	15.33%	14.72%	-	-
Thermal	6	2.233	1.00000	9.220	1.00000	9.78%	13.64%	-	-
UV Light	24 hr	2.240	0.999	9.220	0.99998	-	-	-	-

4. DISCUSSION

In the present Work Simple, Accurate, Sensitive, Rapid & Precise analytical methods had been developed and validated. In RP-HPLC method was developed for the simultaneous determination of ALB and IVE, using a Supel cosil^{TM} LC-ABZ C_{18} , 5µ (150×4.6 mm) column and a mobile phase composed of ACN: Methanol: Phosphate buffer (KH_2PO_4) (51:25:24, %v/v), pH 7. The retention times of ALB and IVE were found to be 2.173min and 7.733 min, respectively. Linearity was established for ALB and IVE in the range of 400-1200 µg/ml and 6-36 µg/ml with correlation coefficient is 0.998 and 0.996 respectively. The Intraday precision % RSD value are found 0.065-0.144% for ALB and 0.928-1.168% for IVE and Interday precision % RSD values found 0.0249-0.0907% for ALB and 0.088-0.311% for IVE. The % Recovery found that 98.67-100.09% for ALB and 98.77-99.63% for IVE. The method is also found robust for various changed conditions. The proposed method passes all the validation requirements of ICH Q2R1. 1 In Force degradation study degradation of both drug at 6 hr in Acidic, Alkali, oxidation and thermal condition were 21%, 23.76%, 15.33% and 9.78% respectively for Albendazole and 18.95%, 17.76%, 14.72% and 13.64% respectively for Ivermectin. There was no degradation in Photolytic condition.

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