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STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF LEVAMISOLE HCl AND ALBENDAZOLE IN TABLET DOSAGE FORM

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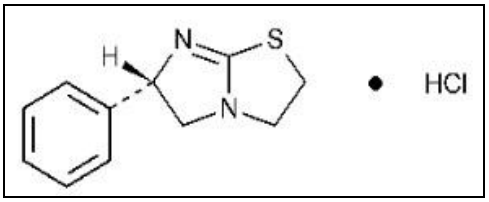
ABSTRACT
The purpose of the investigation was to develop a new RP-HPLC Method for simultaneous estimation of Albendazole and Levamisole HCl in pharmaceutical dosage forms. Chromatography was carried out on an Shiseido C_{18} column (4.6 x 250mm, 5µ particle size) with a isocratic mobile phase Phosphate buffer : Acetonitrile 30:70 (v/v) (adjusted to pH 5 with 10 M potassium hydroxide), at a flow rate of 1.0 mL/min and the detection was carried out using a UV detector at 217 nm. Validation parameters such as system suitability, linearity, precision, accuracy, specificity, limit of detection (LOD), limit of quantification (LOQ), Stability of sample & standard stock solutions, robustness and degradation studies were determined as reported in the International Conference on Harmonization guidelines. The retention times for Albendazole and Levamisole HCl were 3.177 min and 5.370min respectively. The percentage recoveries of Albendazole and Levamisole HCl were 100.60% and 98.40% respectively. The relative standard deviation for assay of tablets was found to be less than 2%. The Method was fast, accurate, precise and sensitive hence it can be employed for routine quality control of tablets containing both drugs in quality control laboratories and pharmaceutical industries.

KEYWORDS: Albendazole, Levamisole HCl, RP-HPLC Method, Stability indicating Method, Validation.

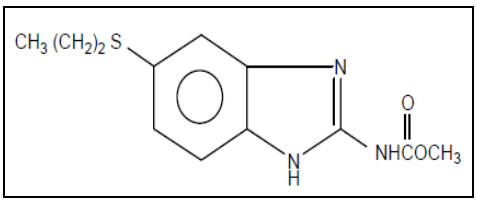
MATERIALS AND METHODS

Albendazole Methyl 5 – propylthio – 1H – Benzimidazol- 2-yl- carbomate.

It is official in Indian Pharmacopeia 2014, British Pharmacopeia 2008 and United States Pharmacopoeia 29NF30. It is freely soluble in Formic acid, slightly soluble in methanol, Ethanol and dilute acids. Molecular weight of Albendazole 265.33 gm/mol and Molecular formula is C_{12} $H_{15}N_3O_2S$.



Levamisole HCl



Albendazole

Levamisole HCl (5) 2, 3, 5, 6-tetrahydro-6-phenylimidazo [2, 1-6] 6-thiazole hydrochloride is act as Anthelmintic. It is official in Indian Pharmacopeia 2014, British Pharmacopeia 2008 and United States Pharmacopoeia 29NF30. It is freely soluble in water, soluble in ethanol, slightly soluble in Dichloromethane. Molecular weight of Levamisole HCl is 240.8 g/mol and Molecular formula is C_{11} H_{12} N_2 S, HCl.

Albendazole is obtained from Umedica Laboratories Vapi, Gujarat, India.
Levamisole HCl obtained from NuCare Laboratories, Mehsana, Gujarat, India.

Instrumentation and Chromatographic method

The analysis of drug was carried on RP- HPLC, by using Shimadzu LC 2010CHT using Shiseido C_{18} (250 mm x 4.6 mm, 5 µm) column and with mobile phase composition of Phosphate Buffer: Acetonitrile (30:70 % v/v) pH 5, at a flow rate of 1ml/min was used. Detection

was carried out at 217 nm. Retention time of Albendazole and Levamisole HCl was found to be 3.177 min and 5.370 min.

Determination of maximum absorbance
The standard solution of Albendazole (20 µg/ml) and Levamisole HCl (7.5 µg/ml) in methanol was individually scanned over the range of 200nm-400nm.

Its overlay graph showed that the drugs absorb at 217nm. So, the wavelength selected for the determination of Albendazole and Levamisole HCl was 217nm.

Standard Stock Solution I of Albendazole (1000µg/mL)
100mg of Albendazole was accurately weighed and transferred to 100mL volumetric flask and dissolved in Methanol and sonicated for about 10min. Volume was made up to the mark with Methanol to give a solution containing 1000µg/mL Albendazole solution.

Standard Stock Solution I of Levamisole HCl (1000µg/mL)
100mg of Levamisole HCl was accurately weighed and transferred to 100mL volumetric flask and dissolved in Methanol and sonicated for about 10min. Volume was made up to the mark with Methanol to give a solution containing 1000µg/mL Levamisole HCl solution.

Standard Stock Solution II of Albendazole (200µg/mL)
20mL of Standard stock Solution I was transferred in 100mL volumetric flask and Volume was made up to the mark with Methanol to give a solution containing 200µg/mL Albendazole solution.

Standard Stock Solution II of Levamisole HCl (75µg/mL)
7.5mL of Standard stock Solution I was transferred in 100mL volumetric flask and volume was made up to the mark with methanol to give a solution containing 75µg/mL Levamisole HCl solution.

Preparation of Binary Mixture
0.5mL, 0.75mL, 1mL, 1.25mL, 1.5mL of Standard Stock Solution II was transferred with 0.5mL, 0.75mL, 1mL, 1.25mL, 1.5mL of Levamisole HCl Standard Stock Solution II respectively in five different 10mL volumetric flask. Volume was made up to the mark with Mobile Phase to give a solution containing 10µg/mL, 15µg/mL, 20µg/mL, 25µg/mL, 30µg/mL Albendazole and 3.75µg/mL, 5.62µg/mL, 7.5µg/mL, 9.37µg/mL, 11.25µg/mL of Levamisole HCl solution respectively in a binary mixture were injected to the system with stated chromatographic conditions.

Method validation
The Proposed method was validated according to ICH guidelines. The parameters assessed were linearity, precision, accuracy, LOD and LOQ.

System Suitability
System suitability tests are an integral part of liquid chromatography. They are used to verify that resolution and reproducibility of chromatography system are adequate for the analysis to be done. System Suitability was performed on standard solution and system suitability parameters were calculated at the start of study for each parameter.

Linearity and Range
The linearity was determined at three levels over the range of 10-30 µg/ml of Albendazole and 3.75-11.25 µg/ml of Levamisole HCl. Peak area of above linearity solution preparations were taken at each concentration three times.

Accuracy
Recovery studies were carried out by addition of standard drug to the sample at 3 different concentration levels (80%, 100% and 120%) taking into consideration percentage purity of added bulk drug samples. These solutions were subjected to re-analysis by the proposed method and Results are calculated.

Precision
Repeatability Study
Standard solutions of 10,20,30 µg/ml Albendazole and 3.75, 7.5, 11.25 µg/ml Levamisole HCl were prepared and chromatograms were recorded. Area was measured of the same concentration solution three times and %RSD was calculated.

Intra-day precision
Mixed solutions containing 10, 20, 30 µg/ml Albendazole and 3.75, 7.5, 11.25 µg/ml Levamisole HCl were analysed three times on the same day % R.S.D was calculated.

Inter-day precision
Mixed solutions containing 10, 20, 30 µg/ml Albendazole and 3.75, 7.5, 11.25 µg/ml Levamisole HCl were analysed on three different days and % R.S.D was calculated.

Limit of Detection and Limits of Quantitation Limit of Detection (LOD)
From the linearity curve equation, the standard deviation (SD) of the intercepts (response) was calculated. The limit of detection (LOD) of the drug was calculated by using the following equation designated by International Conference on Harmonization (ICH) guideline.

$$LOD = 3.3\times \text{Intercept / Slope}$$

Limit of Quantitation (LOQ)
The limit of quantitation (LOQ) of the drug was calculated by using the following equation designated by International Conference on Harmonization (ICH) guideline.

$$LOQ = 10 \times \text{Intercept / Slope}$$

Robustness
The robustness of the method was established by making deliberate minor variations in the following method parameters.
a) pH of mobile phase: ± 0.2
b) Flow rate: ± 0.2 ml/min.
c) Change in the ratio of component in the mobile phase: ± 2%.

Stability studies
Stability Studies was carried out on the drug in order to check the stability of the drug by providing various stress conditions like acid, base, oxidation and thermal degradation compared with normal conditions. The purpose of force degradation method is to provide evidence that the analytical method is efficient in determination of drug substances in commercial drug product in the presence of its degradation products.

Acid hydrolysis: 1mL of Standard Stock Solution II of Albendazole, Levamisole HCl and Sample Stock Solution were transferred in three different 10mL volumetric flask, to it 2mL of 0.1NHCl was added and kept for 4hrs at 40ºC and then 2mL of 0.1N NaOH was added for neutralization and diluted up to the mark with Mobile Phase.

Alkaline hydrolysis: 1mL of Standard Stock Solution II of Albendazole ,Levamisole HCl and Sample Stock Solution were transferred in three different 10mL volumetric flask; to it 2mL of 0.1N NaOH was added and kept for 4hrs at 40ºC and then 2mL of 0.1N HCl was added for neutralization and diluted up to the mark with Mobile Phase.

Oxidative degradation: 1mL of Standard Stock Solution II of Albendazole, Levamisole HCl and Sample Solution were transferred in three different 10mL volumetric flask; to it 2mL of 3% H_2O_2 was added and kept for 3hrs at 60ºC and then diluted up to the mark with Mobile Phase.

Thermal degradation: Weighed 50mg of both drugs and 50mg equivalent weight of tablet powder was taken in a clean and dry Petri dish and covered with Aluminium foil. Petri dish was kept in oven at 80 ºC for 8 hr. An Accurately weighed 10mg of dry heated drug was transferd to 100mL volumetric flask. It was dissolved using mobile phase and final volume was made up to 100mL. Solution of 7.5µg/mL of Levamisole HCl and 20µg/mL of Albendazole was prepared from above solution.

Photo degradation: 1mL of Standard Stock Solution II of Albendazole ,Levamisole HCl and Sample Solution were transferred in three different 10mL volumetric flask was kept in UV chamber for 6 hrs and then diluted up to the mark with Mobile Phase.

RESULT AND DISCUSSION

Method development

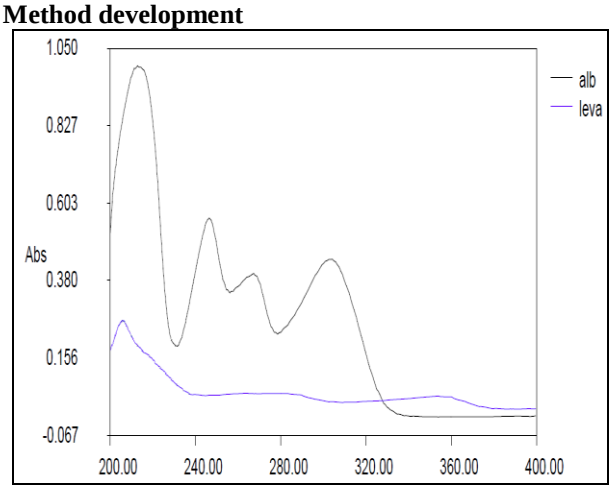


Figure No: 1 Determination of detection wavelength.

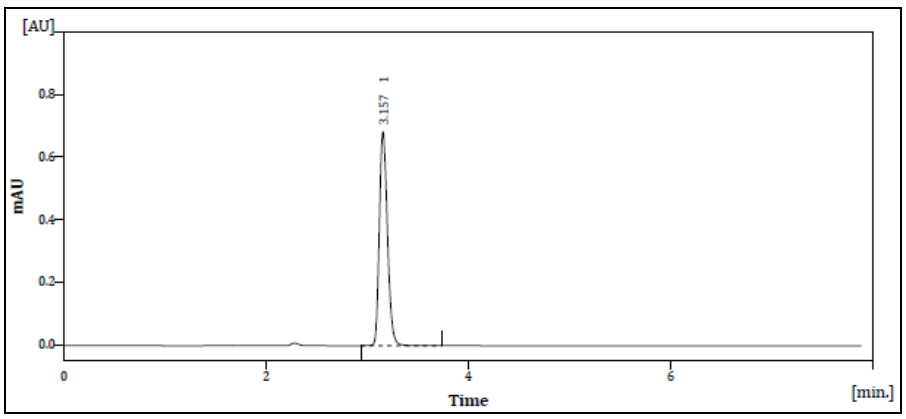


Figure No: 2 Chromatogram for Albendazole.

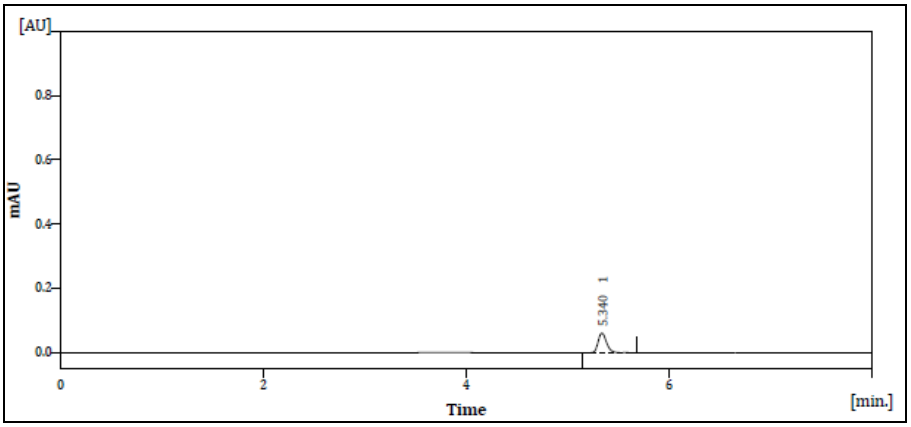


Figure No: 3 Chromatogram for Levamisole HCl.

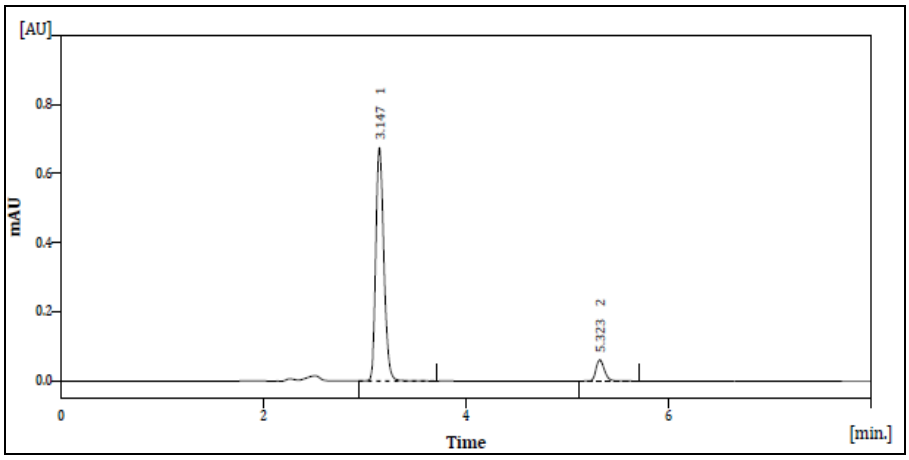


Figure No: 4 Chromatogram for Formulation.

Linearity

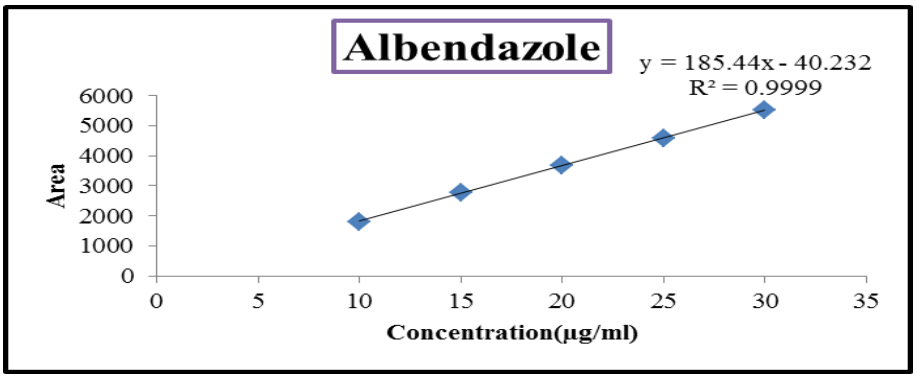


Figure No: 5 Calibration curve of Albendazole.

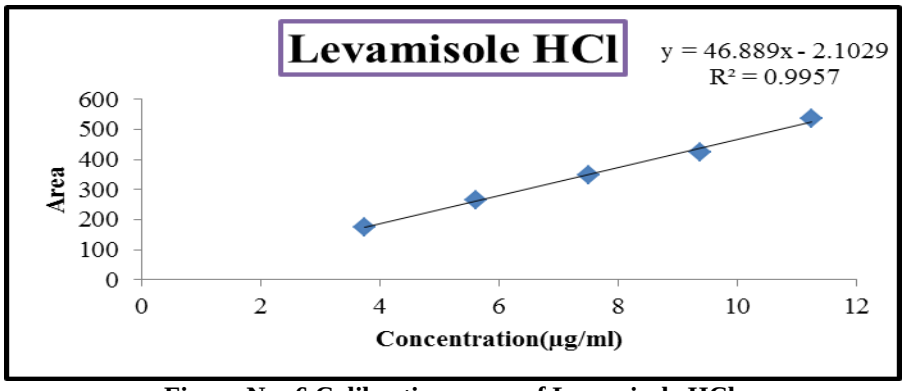


Figure No: 6 Calibration curve of Levamisole HCl.

Table 1: System suitability parameters.

Factor	Albendazole	Levamisole HCl	Specification as per IP and USP 34 NF 29
Conc (µg/ml)	20 µg/ml	7.5 µg/ml	-
R_t (min)	3.1416	5.3515	-
Resolution	14.57		Greater than 1.5
Theoretical plate number	7280	18672.8	Not less than 2000
Tailing factor	1.3441	1.382	Not greater than 2

Accuracy

Table 2: Recovery study of Albendazole.

Level	Amount of sample taken (µg/ml)	Amount of standard spiked (µg/ml)	Total amount (µg/ml)	Std. Amount recovered (µg/ml)	% Recovery	Mean %Recovery ± SD (n=3)	% RSD
80%	10	8	18	7.897	98.716	99.779±0.932	0.934
	10	8	18	8.036	100.459		
	10	8	18	8.013	100.164		
100%	10	10	20	9.901	99.017	99.542±0.591	0.593
	10	10	20	10.018	100.182		
	10	10	20	9.942	99.428		
120%	10	12	22	11.990	99.923	99.537±0.388	0.389
	10	12	22	11.897	99.147		
	10	12	22	11.944	99.541		

Table 3: Recovery study of Levamisole HCl.

Level	Amount of Sample taken (µg/ml)	Amount of Standard spiked (µg/ml)	Total amount (µg/ml)	Std. Amount recovered (µg/ml)	% Recovery	Mean % Recovery ± SD (n=3)	% RSD
80%	3.75	3	6.75	2.966	98.872	100.08±1.124	1.123
	3.75	3	6.75	3.032	101.093		
	3.75	3	6.75	3.008	100.289		
100%	3.75	3.75	7.5	3.719	99.199	99.79±0.629	0.630
	3.75	3.75	7.5	3.766	100.452		
	3.75	3.75	7.5	3.739	99.728		
120%	3.75	4.5	8.25	4.513	100.292	99.78±0.507	0.508
	3.75	4.5	8.25	4.467	99.278		
	3.75	4.5	8.25	4.489	99.770		

Precision

Table 4: A Repeatability study of the drugs.

	Albendazole	Levamisole HCl
Sr. No.	Area	Area
1	3687.377	356.038
2	3694.823	356.719
3	3695.404	357.436
4	3691.122	356.361
5	3698.559	357.076
6	3698.190	357.805
Avg	3694.245	356.906
SD	4.3075	0.6639
%RSD	0.11660	0.18604

Table 5: B. Interday Precision.

	Albendazoel		Levamisole HCl		
Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% RSD	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% RSD
10	1797.567±1.866	0.1038	3.75	173.866±0.355	0.2043
20	3677.635±4.323	0.1175	7.5	355.310±0.719	0.2025
30	5505.797±6.423	0.1167	11.25	531.614±1.071	0.2014

Table 6: C. Intraday Precision.

	Albendazoel		Levamisole HCl		
Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% RSD	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% RSD
10	1796.411±1.939	0.1079	3.75	173.714±0.343	0.1974
20	3673.606±4.078	0.1110	7.5	354.963±0.686	0.1934
30	5496.229±6.447	0.1173	11.25	531.086±1.048	0.1973

Limit of Detection and Limits of Quantitation Limit of Detection (LOD)

Table 7: LOD and LOQ of the drugs.

Drugs	LOD (µg/mL)	LOQ (µg/mL)
Albendazole	0.2186	0.6625
Levamisole HCl	0.7417	2.2447

Robustness

Table 8: Robustness for Albendazole.

Sr. No.	Albndazole (20µg/ml)					
	pH:5		Flow Rate		Mobile Phase	
	pH 5.2 (+ 0.2 units)	pH 4.8 (−0.2 units)	(+ 0.2 units)	(− 0.2units)	(+ 0.2 %)	(− 0.2%)
1	3513.658	3772.517	3594.812	3817.179	3587.596	3776.312
2	3535.684	3798.695	3617.037	3839.552	3613.415	3798.695
3	3550.775	3806.582	3631.156	3953.371	3628.053	3809.831
Avg	3533.372	3792.598	3614.335	3836.701	3609.688	3794.946
SD	18.666	17.832	18.322	18.263	20.484	17.071
%RSD	0.5282	0.4701	0.5069	0.4760	0.5674	0.4498

Table 9: Robustness for Levamisole HCl.

Sr. No.	Levamisole HCl (7.5 µg/ml)					
	Ph:5		Flow Rate		Mobile Phase	
	pH 5.2 (+0.2 units)	pH 4.8 (-0.2 units)	(+ 0.2 units)	(− 0.2units)	(+ 0.2 %)	(− 0.2%)
1	339.282	364.215	347.083	368.515	346.390	364.581
2	341.399	368.729	349.235	370.691	348.885	366.622
3	343.529	366.884	352.187	372.849	351.347	368.916
Avg	341.403	366.609	349.221	370.685	348.874	366.731
SD	2.123	2.336	2.132	2.167	2.478	2.151
%RSD	0.6219	0.6374	0.6105	00.5845	0.7104	0.5866

Force Degradation studies

Table no 10: Force Degradation studies of drugs.

Stress Condition	% Degradation of API		% Degradation of pharmaceutical dosage form	
	Albendazole	Levamisole HCl	Albendazole	Levamisole HCl
Acid Hydrolysis	7.12	6.56	8.85	7.14
Alkaline Hydrolysis	9.39	8.05	8.74	6.95
Oxidation	8.97	8.37	9.65	9.07
Thermal	6.97	7.18	6.14	8.29
Photo	6.27	7.08	6.09	7.95

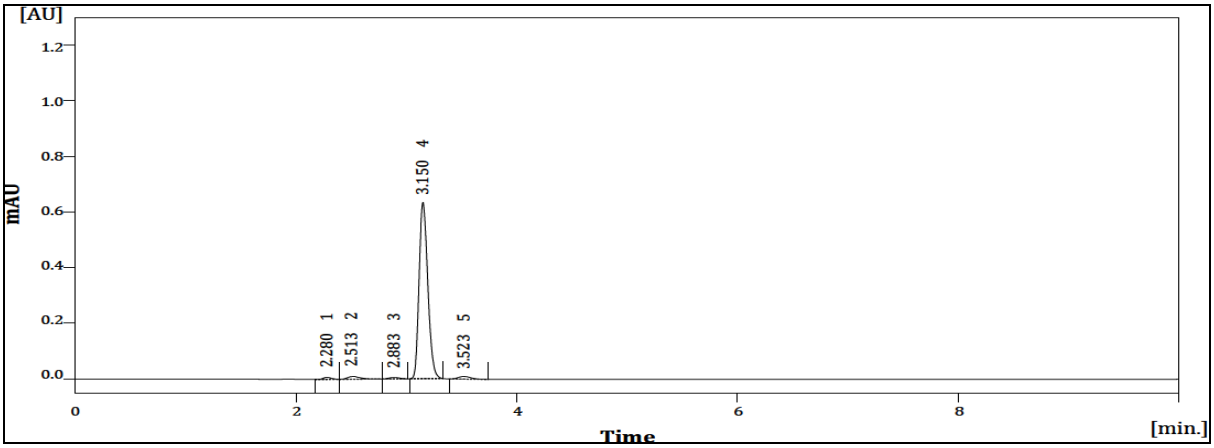


Figure no 7: Acid Hydrolysis of Albendazole.

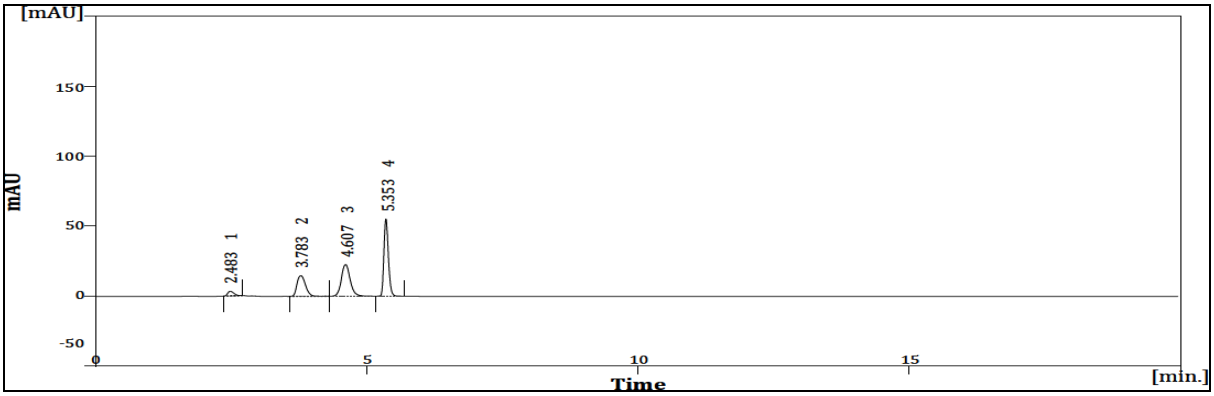


Figure no 8: Acid Hydrolysis of Levamisole HCl.

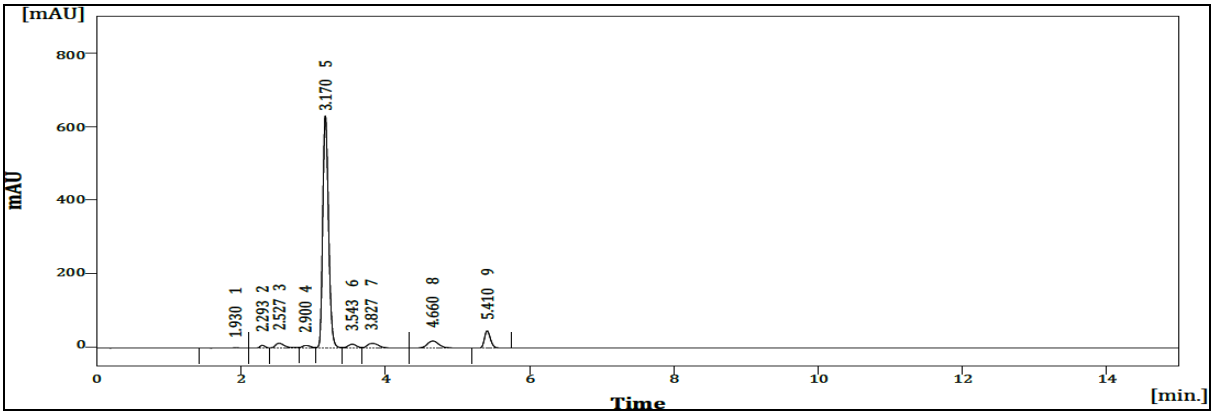


Figure no 9: Acid Hydrolysis of Formulation.

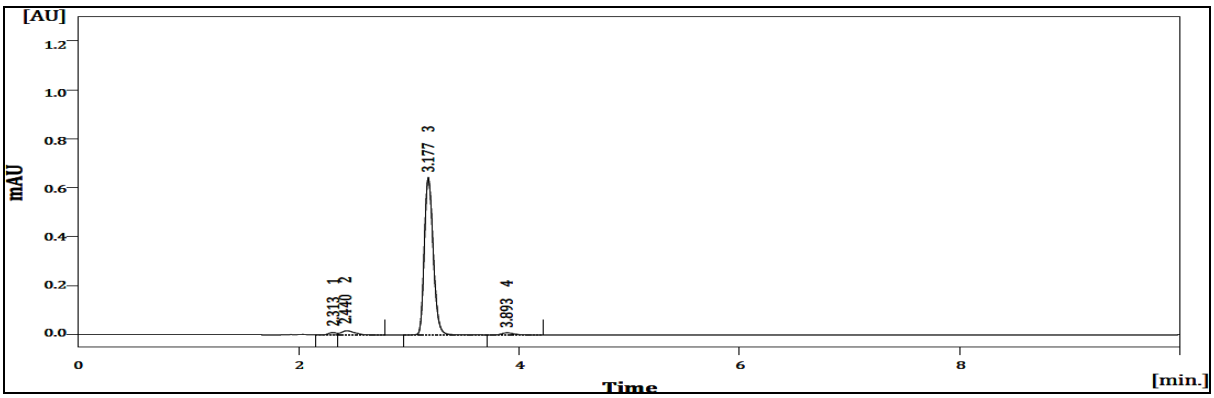


Figure no:-10 Alkali Hydrolysis of Albendazole.

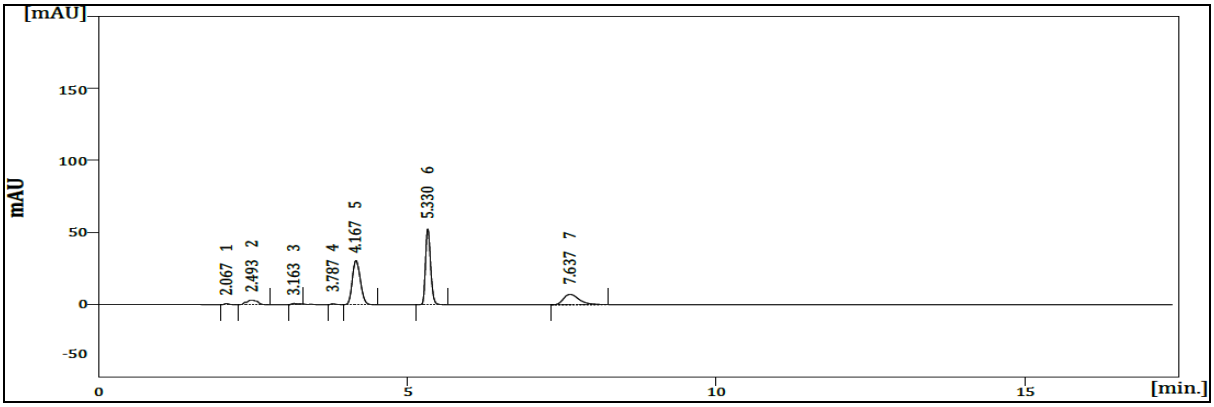


Figure no:-11 Alkali Hydrolysis of Levamisole HCl.

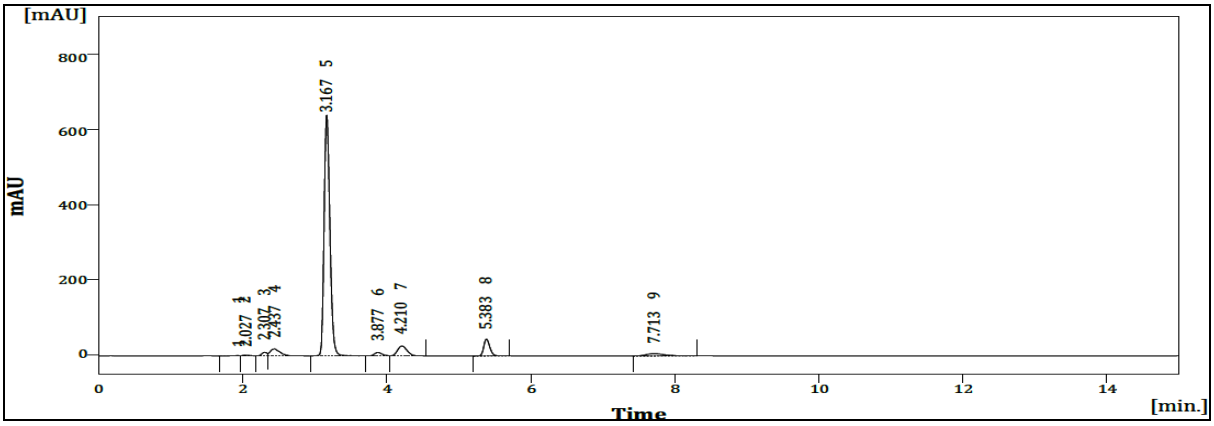


Figure no:-12 Alkali Hydrolysis of Formulation.

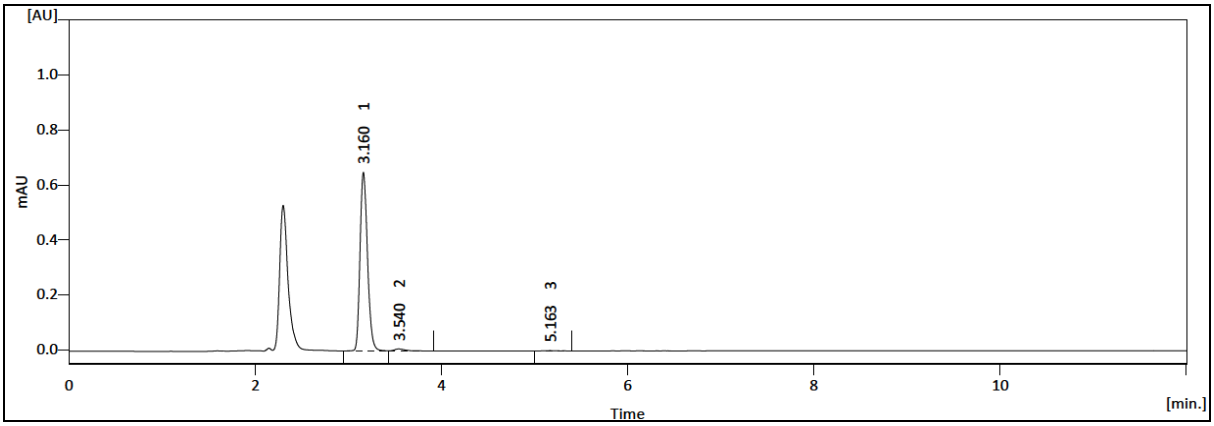


Figure no:-13 Oxidative Hydrolysis of Albendazole.

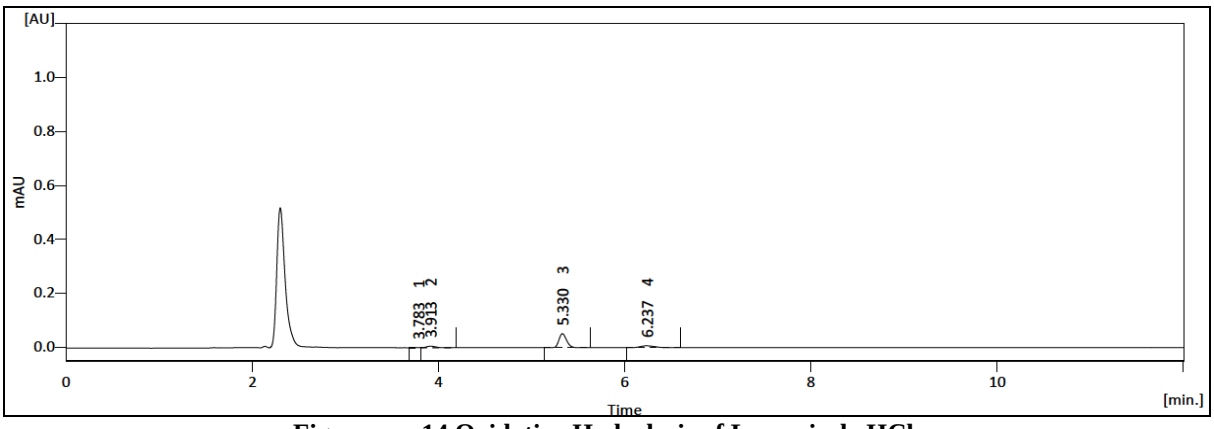


Figure no:-14 Oxidative Hydrolysis of Levamisole HCl.

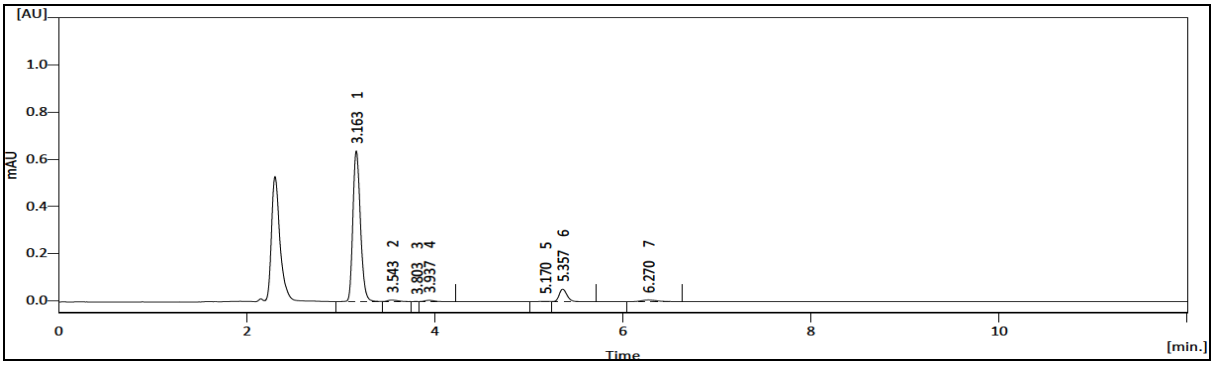


Figure no:-15 Oxidative Hydrolysis of Formulation.

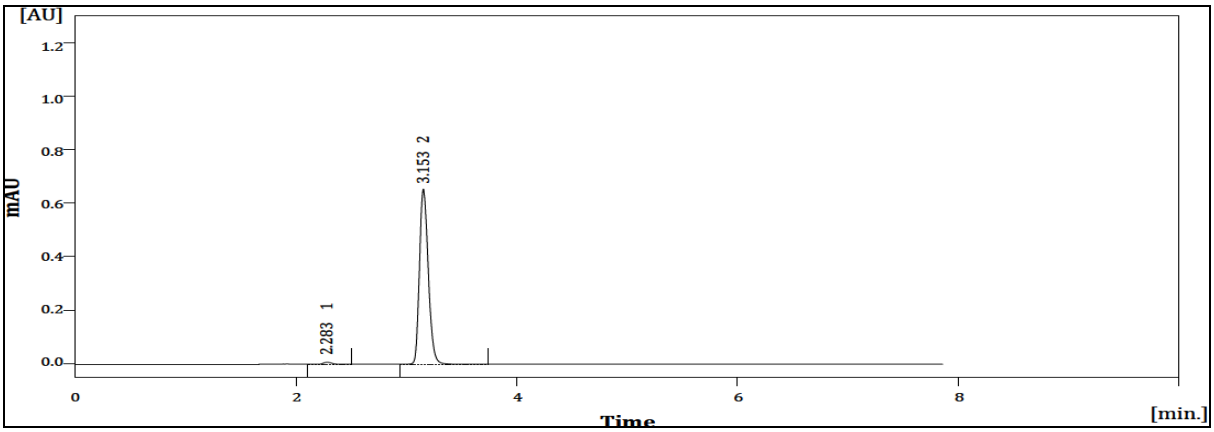


Figure no:-16 Thermal Hydrolyisis of Albendazole.

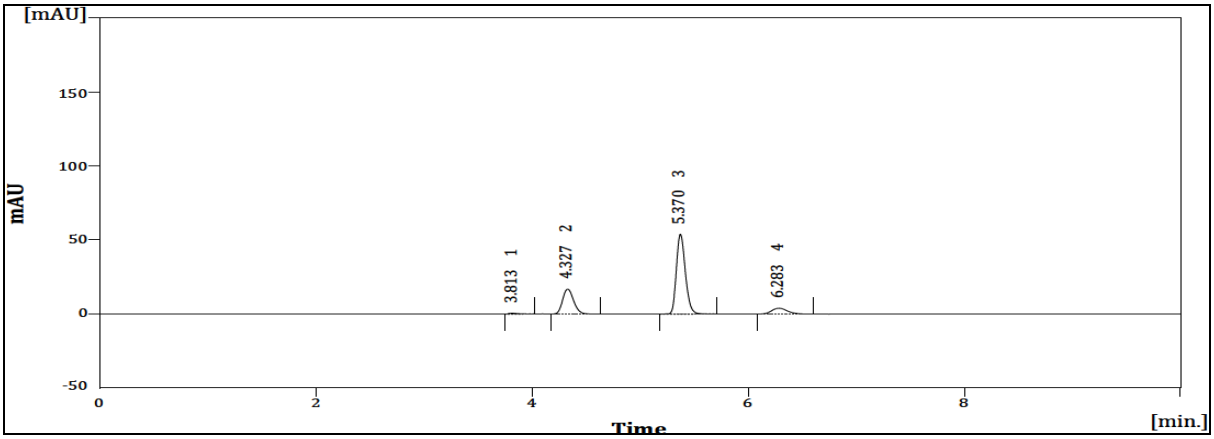


Figure no:-17 Thermal Hydrolyisis of Levamisole HCl.

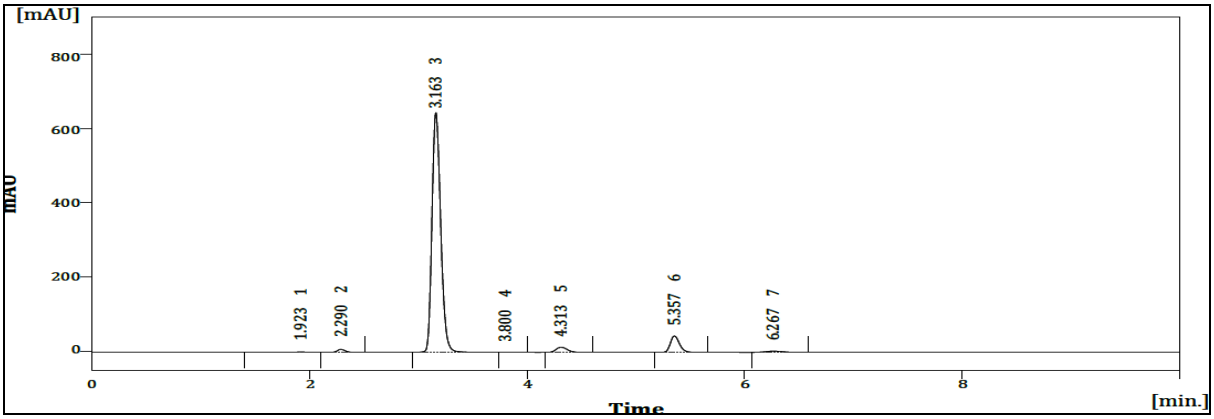


Figure no:-18 Thermal Hydrolyisis of Formulation.

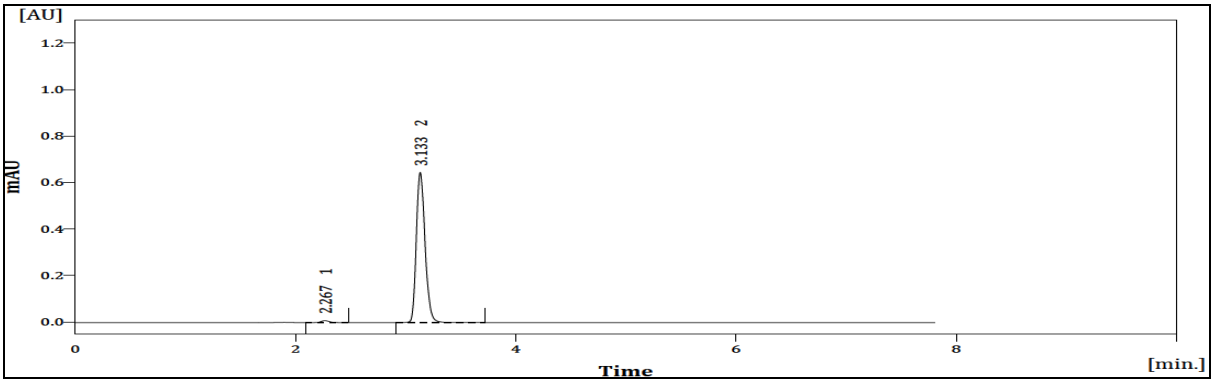


Figure no:-19 Photo Degradation of Albendazole.

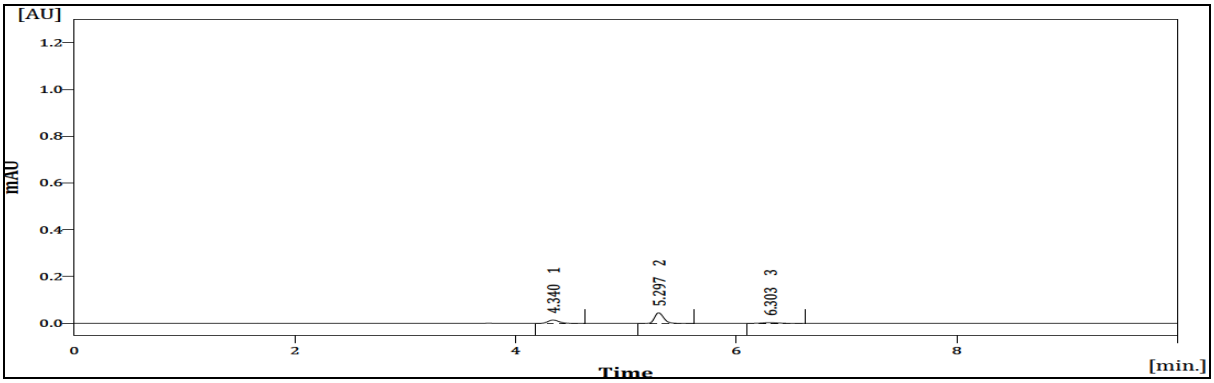


Figure no:-20 Photo Degradation of Levamisole HCl.

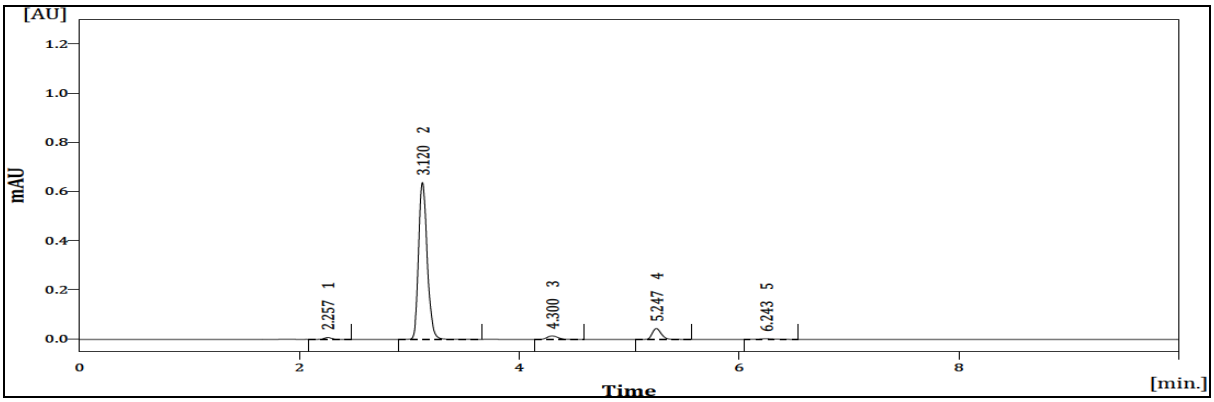


Figure no:-21 Photo Degradation of Formulation.

CONCLUSION

- Development HPLC Method can resolve all Degradants peak of drug. No chromatographic interference from tablet excipients was found.
- It is concluded that the developed method is specific. The test parameters were also performed and were found to be within acceptable criteria. The method can be successfully employed for the simultaneous determination of Albendazole and Levamisole HCl in pharmaceutical formulation.

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