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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING HPTLC METHOD FOR ALBENDAZOLE

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ABSTRACT

Albendazole belongs to the benzimidazole class of Anthelmintic. The present study describes degradation of Albendazole under ICH [Q1A (R2)] prescribed stress conditions (hydrolysis, oxidation, dry heat, wet heat and photolysis) and establishment of a stability-indicating HPTLC method. Different degradation products were observed for Albendazole when each was exposed to different stress conditions. For HPTLC, aluminium plate precoated with Silica Gel 60 F254 and mobile phase consisting of toluene: methanol: ammonia 8: 2: 0.5 v/v/v was used to achieve separation. Quantitation was done at 244 nm. The retention factor for albendazole is 0.41. The method exhibited good linearity (r2>0.998) over the studied range of 200-700 ng/band. The method was validated as per ICH Q2 R1 guidelines and results were in limit. This method was found to be simple, specific, precise and stability indicating.

KEYWORDS: Albendazole, HPTLC, Stability Indicating Method, Validation.

INTRODUCTION

Albendazole is chemically named as Methyl [5-(propylthio)-1H-benzoimidazole-2-yl]carbamate with molecular formula $C_{12}H_{15}N_3O_2S$.[13] It retains the broad spectrum activity and excellent tolerability of its predecessor. Albendazole causes degenerative alteration in the tegument and intestinal cells of the worm by binding to the colchicines-sensitive site of tubulin, thus inhibiting its polymerization or assembly into microtubules.[1]

Various UV and HPLC assay methods are also reported in the literature for the estimation of Albendazole individually and in-combination with other drugs Stability indicating RP-HPLC

method,[2,3] Development and validation UV method,[4-7] Rapid quantitative assay by UHPLC,[8] HPLC method.[9,10] According to literature survey there is no official method for the Forced Degradation Studies of Albendazole by HPTLC in tablet dosage form. Hence, an attempt has been made to develop new method for Forced Degradation study of Albendazole in accordance with the ICH guidelines.

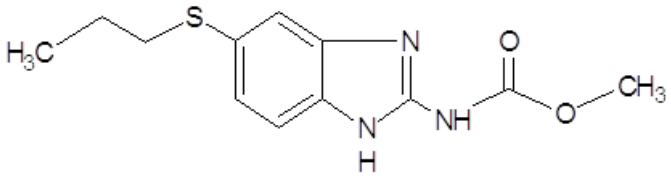

Fig. 1: Structure of Albendazole.[13]

The drug stability test guidelines Q1A (R2) issued by International Conference on Harmonization (ICH) requires that analytical test procedures for stability samples should be fully validated and the assay should be stability indicating. The aim of the present study accordingly was to establish inherent stability of the albendazole through stress studies under a variety of ICH recommended test conditions[11] and its validation.[12]

MATERIALS AND METHODS

For stability indicating HPTLC method development, Camag HPTLC system consisting of Linomat-5 applicator, Camag TLC Scanner 3 and Win CATS software V 1.4.2.8121 were used. For photo-degradation studies, UV Chamber was used Thermal degradation studies, Hot air oven was used. All the weighing was done on Shimadzu balance (Model AUW-120D).

Working standard of Albendazole was provided by Glaxo smithklin pharmaceutical Ltd and used as such without further purification. Methanol HPLC grade, Toluene, ammonia, Conc. HCl, Na OH and H_2O_2, H_2O used were of analytical reagent grade. Marketed formulation of albendazole was purchased from local market.

Preparation of Standard Solutions

Standard stock solution of albendazole was prepared by dissolving 10 mg of drug in 10 ml of methanolic Glacial acetic acid to get concentration of 1000 µg/mL. From the standard stock solution working standard solution was prepared to contain 100µg/ml of albendazole.

Stress Degradation Studies

Stress degradation studies were carried under condition of acid/ base/ neutral hydrolysis, oxidation, dry heat and photolysis. For each study, two samples were prepared: the blank subjected to stress in the same manner as the drug solution and working standard solution subjected to stress conditions. Dry heat and photolytic degradation was carried out in solid state.

Degradation under Alkali Catalysed Hydrolytic Condition

Working standard solution, 1ml was mixed with 1ml of 0.01N Na OH and reflux at 60^0C for 15 min. Cool at RT and neutralized. The solution was spotted on TLC plate using applicator.

Degradation under Acid Catalysed Hydrolytic Condition

Working standard solution, 1ml was mixed with 1ml of 0.01N HCL and reflux at 60^0C for 15 min. Cool at RT and neutralized. The solution was spotted on TLC plate using applicator.

Degradation under Neutral Hydrolytic Condition

Working standard solution, 1ml was mixed with 1ml water. The solution was refluxed at 60^0C for 15 min. The solution was cooled to room temperature. Volume was made to 10ml. The solution was spotted on TLC plate using applicator.

Degradation under Oxidative Condition

Working standard solution, 1ml was mixed with 1ml 3% solution of H_2O_2 and kept for 15 min at RT then the solution was spotted on TLC plate using applicator.

Degradation under Dry Heat

Dry heat studies were performed by keeping drug sample in oven (80^0C) for a period of 2 hrs. Samples was withdrawn, dissolved in methanolic glacial acetic acid and diluted to get 100µg/ml as final conc. of the solution was spotted.

Photo-Degradation Studies

Photolytic studies were also carried out by exposure of drug to UV light for 24 hrs. Sample was weighed, dissolved and diluted get 100µg/ml. The solution was spotted.

Analytical Method Validation

1. Linearity and Range

Linearity was found for ABZ by using standard stock solution 100 µg /ml. To establish linearity, the stock solution was applied on the plate using 100 µl syringe with the help of Linomat V applicator, to give spots of concentrations 200-700 ng/spot for Albendazole. Application volume 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 was applied on plate using Linomat 5 to obtain concentrations 200, 300, 400, 500, 600,700ng/spot. Plate was developed according to proposed method. After the development, plate was scanned at 244 nm and results obtained are shown in Table No.1 and calibration plot obtained was shown in Fig. No.3

2. Precision

Precision of the method was studied as intra – day and inter – day variations. Intra -day variation was determined by analyzing three different concentrations for three times within a day and Inter- day precision was assessed by three different concentrations for three different days, over a period of week. The Intra-day and Inter-day variation was measured at three different concentrations 200, 400, 600, ng/ band. Intraday and interday precision assures the repeatability of test results. The % RSD found was below 2. Result of intraday and interday precision was shown in Table No. 4 and Table No. 5 respectively.

3. Accuracy

To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed tablet sample solution at three different levels, 80%, 100% and 120%. At each levels of the amount, three determinations were performed. Results of recovery study are shown in Table No. 6 and statistical validation is shown in Table No. 7.

Assay (Preparation of Sample Solution)

Twenty tablets of Hizole were weighed accurately and finely powdered. Powder equivalent to 10 mg of ABZ was transferred to 10 ml volumetric flask. This was sonicated with Methanolic GAA for 5mins and the volume was made up to 10 ml with the same solvent. Resulting solution (1ml) was further diluted to 10 ml with the same solvent to give 100µg/ml. Line equation obtained from calibration plot was used to calculate label claim of marketed formulation of ABZ. Results of Tablet analysis are shown in Table No. 2 and Table No.3

4. Limit of Detection

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantitated as an exact value. Based on the Standard Deviation of the response and the slope, detection limit (DL) may be expressed as

$DL = 3.3 \sigma S$.

5. Limit of Quantitation

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy. Based on the Standard Deviation of the response and the slope, the quantitation limit (QL) may be expressed as

$QL = 10 \sigma S$ Where,

σ = the standard deviation of the response for the lowest conc. in the range

S = the slope of the calibration curve

6. Specificity

For the determination of interference of excipients, Magnesium stearate were added in concentration 300 ng to each standard stock solution of ABZ and then assayed by proposed method and it was found that the assay results were unaffected by the presence of such excipients. Results of specificity are shown in Table No. 9

7. Robustness

In the robustness study, the influence of small, variations in the analytical parameters on peak area were examined. Robustness was studied by different deliberate variations. In the chromatographic conditions like saturation time does not make any significant change. Results are shown in Table No.8

RESULTS AND DISCUSSION

Optimization of Mobile Phase

Chromatographic separation studies were carried out on the working standard solution of albendazole (100µg/ml). Initially, trials were carried out using various solvents. After several trials, toluene: methanol: ammonia 8: 2: 0.5 v/v/v was chosen as the mobile phase, for HPTLC, which resulted in good resolution and acceptable peak parameters. The retention factor was found to be: Albendazole = 0.41 (Fig.No.2).

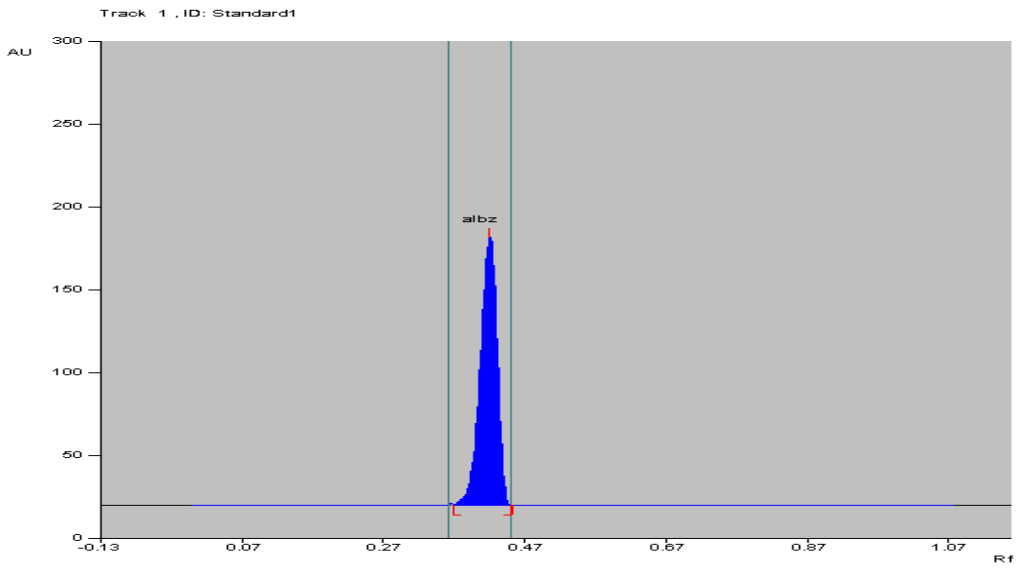

Fig. 2: Typical Densitogram of Albendazole (Rf =0.41).

Table 1: Data for Linearity of Albendazole.

Sr. No.	Conc. (µg/mL)	Area
1	200	2792.63
2	300	3725.48
3	400	4553.2
4	500	5365.92
5	600	6137.88
6	700	6921.4

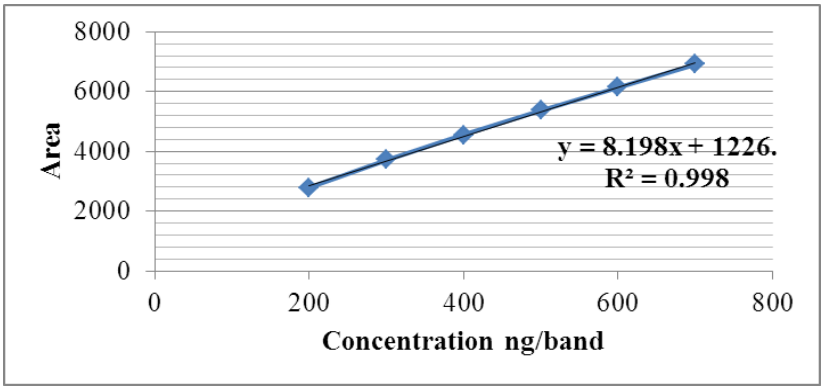

Fig. 3: Calibration Curve of Albendazole.

Table 2: Data for Tablet Analysis (Assay) of ABZ.

Sr. no.	Brand name	Conc. (ng/band)	Label claim (mg)	Amount found (mg)	% amount recovered
1	Hizole	400	400	403.6	100
2		400	400	401	100.2
3		400	400	411	102.8

Table 3: Statistical Validation of Tablet Analysis.

Mean*	SD	%RSD	SE
101%	42.85945	0.941982	24.74564

***Average of three determinations

Table 4: Data for Intraday Precision of ABZ.

Conc. (ng/band)	Mean*	SD	% RSD	SE
200	2752.62	26.91	0.997	15.54
400	4635.87	71.63	1.54	41.35
600	6410.07	79.99	1.24	46.18

Table 5: Data for Interday Precision of ABZ.

Conc. (ng/band)	Mean*	SD	% RSD	SE
200	2788.63	35.79	1.28	3.58
400	4608.45	21.09	0.45	2.16
600	6329.45	8.80	0.13	1.57

Table 6: Data for Recovery (Accuracy) Study of ABZ.

Level of addition	Tablet drug conc. (ng/band)	Standard drug added (ng/band)	Total conc.(ng/band)	Drug recovered (ng/band)	% Recovery
80%	300	240	540	540.92	100
	300	240	540	537.27	99
	300	240	540	536.33	99
100%	300	300	600	595.78	99
	300	300	600	598.28	99.71
	300	300	600	617.17	102.8
120%	300	360	660	682.89	103
	300	360	660	679.37	102.93
	300	360	660	682.35	103

Table 7: Statistical Validation of ABZ.

Level of addition	% Mean recovery	SD	% RSD	SE
80%	99.33	19.87368	0.35244	0.1147441
100%	100.50	95.85837	1.552013	55.34548
120%	102.97	15.54357	0.208106	8.974349

***Average of three determinations

Table 8: Data for Robustness Study of ABZ.

Conc. (ng/band)	Time (minutes)	Mean*	SD	% RSD	SE
200	30	2996.71	31.15	1.03	17.98
	35	2834.25	14.27	0.50	8.24
400	30	4969.60	15.74	0.31	9.08
	35	4829.91	23.24	0.48	13.42
600	30	6726.03	87.06	1.29	50.26
	35	6418.56	65.96	1.02	38.08

***Average of three determinations

Table 9: Data for Specificity Study of ABZ.

Level of addition	Standard (ng/band)	Magnesium stearate (ng/band)	Mean Area*	SD	% RSD	SE
80%	300	240	3743.56	49.15	1.31	28.38
	300	240				
	300	240				
100%	300	300	3644.93	3.589	0.098	2.07
	300	300				
	300	300				
120%	300	360	3651.73	44.95	1.23	25.95
	300	360				
	300	360				

Stress Degradation Studies

Hydrolysis at Basic pH

Albendazole 89.13% was recovered with peak of degradation (Fig. No. 3)

Hydrolysis at Acidic pH

Albendazole 90.71% was recovered with peak of degradation (Fig. No. 4)

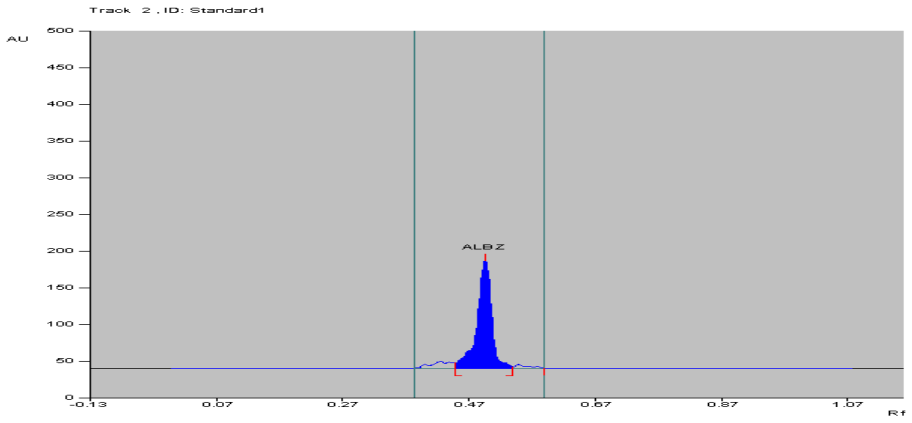

Fig. 4: Degradation Peak of Albendazole in Alkali.

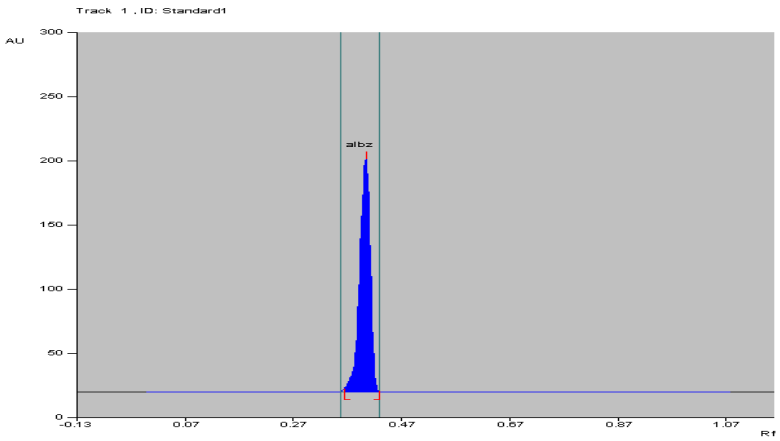

Fig. 5: Degradation Peak of Albendazole in Acid.

Under Neutral Hydrolysis

Albenadzole 87.97% was recovered with peak of degradation (Fig.No.5).

After Oxidative Condition

Albendazole 90.49% was recovered with peak of degradation (Fig.No.6).

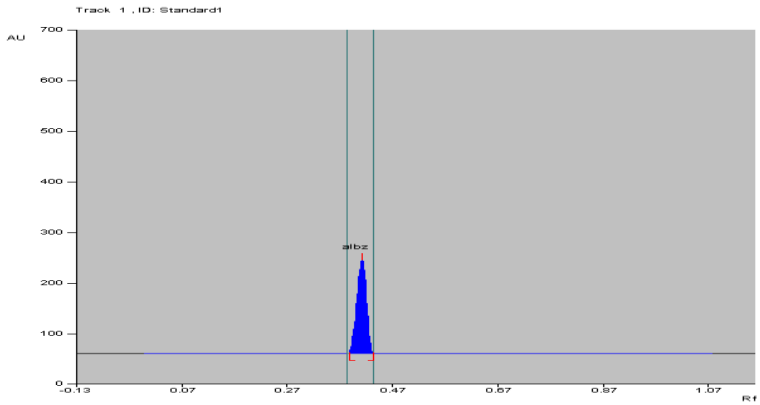

Fig. 6: Degradation Peak of Albendazole under H_2O.

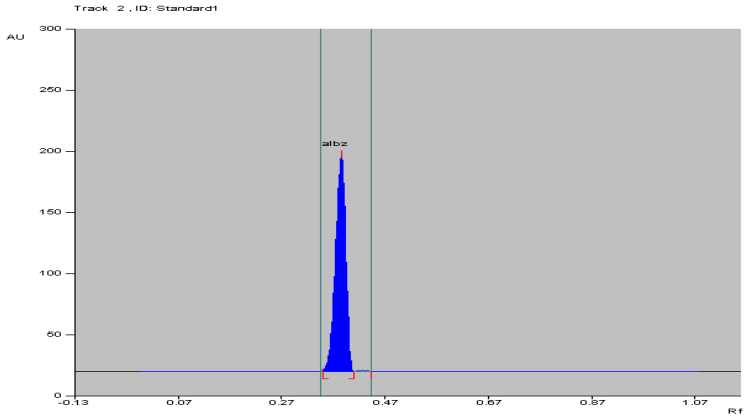

Fig. 7: Degradation Peak of Albendazole under H_2O_2.

After the Dry Heat

Degradation was observed for albendazole with 94.99% recovery

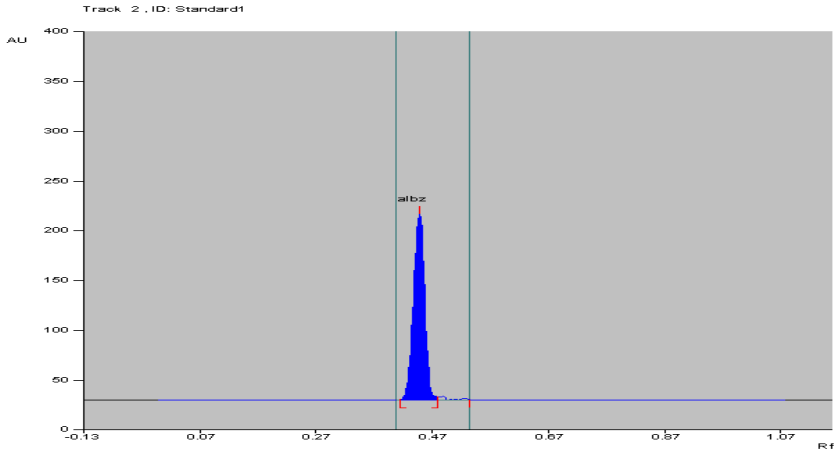

Fig. 8: Degradation Peak of Albendazole under Heat.

After the Photo Degradation

Degradation was observed for Albendazole with 94.05% recovery

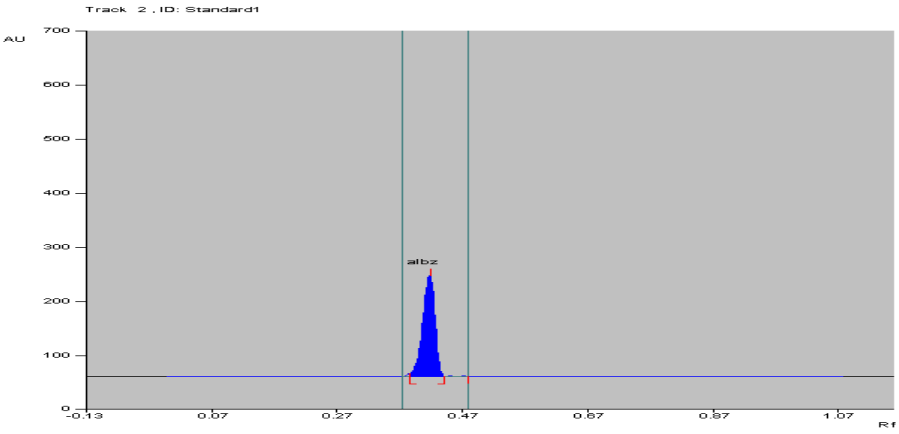

Fig. 9: Degardation Peak of Albendazole under UV Light.

Literature survey revealed that no stability indicating HTPLC method has been reported for the determination of albendazole. Stress degradation studies were carried out under condition of acid/ base/ neutral hydrolysis, oxidation, dry heat and photolysis. For acidic and basic hydrolysis various normalities was tried. The exposure to 0.01 N HCL at 60^0C for 15 min and 0.01N NaOH at 60^0C for 15 min was optimized condition for degradation. Since it resulted in % degradation not more than 20%.For neutral hydrolysis, oxidation, dry heat and photolysis not more than 20% degradation was achieved. The goal is to obtain about not more than 20%

degradation of active compound.Stress degradation results are summarized in Table No.10 & results of method validation are given in Table No.11

Table 10: Summary of Stress Degradation of Albendazole.

Sr. No.	Type of Degradadation	Degradation Conditions	Standard Peak Area	Degraded Peak Area	(%) Degraded	(%) Recovery of Drug
1	Acid	0.01N HCL at 60^0C 15min	2735.24	2481.30	10%	90.17%
2	Base	0.01N NaOH at 60^0C 15min	2751.34	2452.50	11%	89.13%
3	Oxidation	H_2O2 3%, 15min	2740.45	2479.86	10%	90.49%
4	Hydrolytic	H_2O 60^0C 15min	2741.24	2411.31	12%	87.97%
5	Photolytic	UV light 24hr	2794.00	2628.14	6%	94.07%
6	Thermal	Hot Air Oven 2hr	2705.24	2569.78	5%	94.99%

Table 11: Summary of Validation Study for Albendazole.

Sr. No.	Parameters	High performance thin layer chromatography method
1	λ_{max} (nm)	244
2	Beer's law limit (ng/band)	200-700
3	Regression equation [y]	Y=8.198x + 1226.
4	Slope [m]	8.198
5	Intercept [c]	1226.
6	Correlation coefficient [r^2]	0.998
7	Limit of detection (LOD) (ng/band)	48.54
8	Limit of quantitation (LOQ) (ng/band)	147.10

***RSD:** Relative standard deviation, † **LOD:** Limit of detection, ‡ **LOQ:** Limit of Quantitation.

CONCLUSION

The degradation conditions mentioned above were arrived at, after number of initial trials for optimization of extent of degradation. Overall study comprised of stability indicating method development for Albendazole. This method can be used for stability testing of this drug in dosage forms. The developed method was found to be simple, sensitive and selective, accurate, precise, and repeatable for analysis of albendazole in market formulation without any interference from the excipients. Finally it was concluded that the method is sensitive, simple, economical and has the ability to separate the drug from degradation products.

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