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Research Article

A Validated Stability-Indicating RP-HPLC Method for Albendazole in Pharmaceutical Finished Products

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Abstract

The study objective is to develop a rapid, isocratic, simple, and precise high-performance liquid chromatography (HPLC) method for quantifying albendazole in pharmaceutical dose form and bulk available in Bangladesh. A Shim-pack C18 column (250 × 4.6 mm, 5 µm) was used to separate albendazole at a detection wavelength of 235 nm. The mobile phase consisted of a 30:70% v/v mixture of methanol and a 0.2% orthophosphoric acid buffer, pumped at 1.0 ml/min. Acidic, basic, oxidative, photolytic, and thermal degradation at 50 ºC, 60 ºC, and 80 ºC for 1 to 3 hr for albendazole as per ICH Q1A (R2). The recovery level was 98.92 % - 101.68 %. Relative standard deviation (RSD) was below 5%. All the validation parameters were found to be within the limits according to International Conference on Harmonization (ICH) guidelines. Basic, Oxidative, and thermal degradation at 80 ºC vary from 5.08%- 13.89%, 5.84%-25.06%, and 7.96%-15.58%respectively for 1 to 3 hr. Mild impairment of the drug was observed for acidic, thermal at 50 ºC, 60 ºC, and photolytic degradation, and while moderate degradation occurred under basic, oxidative, and thermal conditions at 80 ºC. The batch-to-batch recovery variability of 98.9% to 100.4% for all the brands indicates excellent manufacturing consistency.

Keywords: Albendazole; Method validation; Stressed degradation study.

1. Introduction

Albendazole (ABZ) is chemically known as methyl-((5-propylthio)-1H-benzimidazol-2-yl) carbamate. It is recognized as a broad-spectrum anti-helminthic agent; it is effective against a wide variety of helminth parasites commonly found in both humans and animals. This effectiveness is the reason it is widely used for the treatment of such infections [1, 2].

Despite its widespread clinical use, accurately quantifying albendazole in pharmaceutical formulations remains a significant analytical challenge. Albendazole's poor solubility in water, its polymorphic nature, and its

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limited UV absorbance can negatively impact extraction efficiency, method sensitivity, and reproducibility. Additionally, albendazole is prone to degradation in acidic, alkaline, and oxidative environments, making it necessary to develop stability-indicating analytical methods. Many existing analytical techniques involve complex sample preparation, advanced instrumentation, or lack thorough validation, which limits their suitability for routine quality control analysis [3, 4]. From a regulatory standpoint, the quality assurance of albendazole formulations is crucial due to their inclusion in the World Health Organization's Model List of Essential Medicines and their extensive use in mass drug administration programs [5]. Regulatory authorities and pharmacopeial organizations require that analytical methods be validated according to international guidelines, such as ICH Q2(R1)/(R2), which emphasize key parameters including specificity, accuracy, precision, linearity, and robustness [6]. As a result, there is a continuing need for a simple, robust, and cost-effective analytical method that meets regulatory standards while being suitable for routine quality control. This study was conducted to address these analytical and regulatory needs by developing and validating an enhanced method for determining albendazole in pharmaceutical dosage forms.

A comprehensive literature survey reveals numerous analytical methods for the determination of albendazole (ABZ) in pharmaceutical and biological matrices, including capillary electrophoresis [7], HPLC with UV detection [8], mass spectrometry [9], fluorescence detection[10], thin-layer chromatography [11], UPLC [12], spectrophotometric [13], and spectrofluorimetric techniques [14]. Among these, high-performance liquid chromatography (HPLC) remains the preferred technique for routine quantification and stability assessment of albendazole owing to its superior accuracy, sensitivity, and reproducibility [15]. Although various HPLC-based methods exist, their routine use in quality control laboratories is often hindered by practical challenges. These include complex mobile phase compositions with buffer salts, long chromatographic run times, and the need for expensive organic solvents. For instance, methods using methanol-acetate buffer systems (pH 5.8; 70:30, v/v) take over 8 minutes per analysis [16, 17], while combination assays with albendazole and other active pharmaceutical ingredients typically approach 10 minutes [2]. These factors increase solvent consumption and maintenance demands, making high-throughput analyses difficult. Moreover, while some forced degradation studies of albendazole have been conducted, they often focus on isolated stress conditions and fail to demonstrate method specificity under varied stress scenarios [18]. This highlights the need for a validated analytical method that can consistently separate albendazole from its potential degradation products under different stress conditions, aligning with current regulatory standards.

The present study addresses these analytical and regulatory gaps by developing a rapid, cost-effective, and stability-indicating RP-HPLC method for the determination of albendazole in pharmaceutical formulations. The method is specifically optimized to reduce run time, simplify mobile phase composition, and minimize solvent and operational costs without compromising analytical performance. This approach is particularly relevant in resource-limited settings such as Bangladesh, where albendazole is extensively used in mass drug administration programs and where regulatory frameworks increasingly emphasize the availability of robust, validated analytical methods to ensure consistent product quality and patient safety [2]. A straightforward isocratic elution using 70% methanol and 30% 0.2% orthophosphoric acid achieved complete separation within 6 minutes. Employing methanol rather than acetonitrile offers a cost-effective and environmentally favorable alternative without compromising chromatographic efficiency. The objectives of this study are to develop a robust RP-HPLC method for the determination of albendazole in pharmaceutical formulations by first optimizing chromatographic conditions to achieve accurate, precise, and reproducible quantification. The method is then validated in accordance with ICH Q2(R1)/(R2) guidelines to ensure reliability for routine quality-control applications. Finally, albendazole stability is assessed under various forced degradation conditions, following ICH Q1A(R2) [19].

2. Material and Methods

2.1. Chemicals and reagents

Albendazole USP reference standard (Sigma-Aldrich, Germany), HPLC-grade methanol (Sigma-Aldrich,

Germany), Milli-Q water (Millipore, Bedford, MA, USA), Orthophosphoric acid (Sigma-Aldrich, Germany).

2.2. Sample preparation for pharmaceutical formulations

A precise aliquot of the pharmaceutical dosage form—either powdered tablets or liquid syrup—was weighed to achieve a final concentration within 80% to 120% of the established calibration curve range.

2.3. Stressed degradation studies

2.3.1. Acid hydrolysis

To evaluate the acid-induced degradation of albendazole (ABZ), three 50 mL Falcon tubes were each filled with 25 mL of a 10 µg/mL ABZ solution prepared in 0.1 N hydrochloric acid (HCl). The samples were refluxed at 30 °C for 1, 2, and 3 h. At each time point, one tube was removed and neutralized with 0.1 N sodium hydroxide to obtain neutral pH. The contents of each tube were then filtered and analyzed using HPLC to assess the extent of degradation under acidic conditions [20].

2.3.2. Basic hydrolysis

To study the effect of alkaline conditions on the degradation of albendazole (ABZ), three 50 mL Falcon tubes were each filled with 25 mL of a 10 µg/mL ABZ solution prepared in 0.1 N sodium hydroxide (NaOH). Samples were refluxed at 30 °C for 1–3 h and neutralized with 0.1 N hydrochloric acid (HCl) at each time point for necessary neutralization, filtered, and analyzed by HPLC to determine the extent of degradation under basic conditions [20].

2.3.3. Photolytic degradation

To study the effect of photolytic conditions on the degradation of albendazole (ABZ), three 50 mL Falcon tubes were each filled with 25 mL of a 10 µg/mL ABZ solution prepared using Milli-Q water. The samples were placed in a UV-light chamber and exposed to UVA radiation at ambient temperature for 1, 2, and 3 hours, respectively. At the end of each time point, one tube was removed from the chamber, filtered, and analyzed by HPLC to assess the extent of photodegradation [21].

2.3.4. Oxidative degradation

To investigate the effect of oxidative stress on the degradation of albendazole (ABZ), three 50 mL Falcon tubes were each filled with 25 mL of a 10 µg/mL ABZ solution prepared in 3% hydrogen peroxide (H_2O_2). The samples were maintained at ambient temperature in a water bath and exposed to oxidative conditions for 1, 2, and 3 hours, respectively. At the end of each time interval, one tube was withdrawn, and the contents were filtered and analyzed by HPLC to evaluate the extent of oxidative degradation [21].

2.3.5. Thermal degradation

To evaluate the thermal stability of albendazole (ABZ), three 50 mL Falcon tubes were each filled with 25 mL of a 10 µg/mL ABZ solution prepared in Milli-Q water. The samples were incubated in a temperature-controlled water bath set at 50 °C, 60 °C, and 80 °C for 1, 2, and 3 hours, respectively. After each time interval, one tube was removed from the bath, allowed to cool to room temperature, filtered if necessary, and analyzed by HPLC to assess the extent of thermal degradation [21].

Concentration deterioration rate, $C\ (\%) = \frac{(Xi - Xe) \times 100}{Xi}$

Where X_i = initial concentration of ABZ; X_e = final concentration of ABZ after each hour

2.4. Method validation

The method was validated using ICH analytical performance parameters [22].

2.4.1. Linearity

It was determined by preparing six different concentrations of the standard ABZ. Each sample was analyzed (n=3), and a calibration curve was obtained by plotting peak area versus concentration. Then, the regression analysis provided a correlation coefficient value (R^2) to determine the linearity [23].

2.4.2. Accuracy/ Recovery

Three concentrations of selected ABZ solutions were taken and subjected to HPLC (n=3), and each concentration was measured by linear regression equation. The result indicates the percent recovery or

accuracy. The following formula was used to determine the accuracy [24].

$$Recovery\ (\%) = \frac{\mathrm{Cm}}{\mathrm{Ct}} \times 100$$

Where Cm = mean concentration of three replicates; Ct = theoretical concentration

2.4.3. Precision

Calculation of standard deviation (SD) and relative standard deviation (RSD) for repeated measurements of various concentrations was used to assess precision. Interday and intraday precision were determined [25].

2.4.4. Specificity/ selectivity

It is the capacity to quantify the target analyte while accounting for other elements that can be anticipated to be present in the sample matrix. Analytes were shown to be free from influence from excipients or products of degradation in pharmaceutical formulations, guaranteeing that the components under examination are solely responsible for the peak response in the same retention durations [26].

2.4.5. Sensitivity

This is examined by testing the limit of detection (LOD) and limit of quantification (LOQ). LOQ is the lowest concentration at which the analyte can be quantified, while LOD is the lowest concentration at which the analyte can be detected but not necessarily quantified. LOD and LOQ were determined by regression statistics, including the ANOVA test [27].

2.4.6. Stability

To evaluate the stability of albendazole (ABZ), a 20 µg/mL solution was prepared in Milli-Q water and stored in tightly sealed amber glass vials at 4 °C in a laboratory refrigerator. Samples were withdrawn at weekly intervals over a predefined period (e.g., 1, 2, 3, and 4 weeks) and analyzed using the validated HPLC method [28].

2.4.7. Robustness

The robustness of the HPLC method for albendazole (ABZ) was assessed by varying two key parameters: flow rate and orthophosphoric acid concentration in the mobile phase. The flow rates tested were 0.90, 1.00 (standard), and 1.10 mL/min, while orthophosphoric acid concentrations were set at 0.1%, 0.2% (standard), and 0.3% v/v, with the methanol ratio maintained at 70% [29, 30].

2.5. System suitability

The following relations have been used to know system suitability parameters for a particular chromatogram: plate number, $N = 16\ (t_R/w)^2$, resolution, $Rs = 2\ (t_{R2}\text{-}t_{R1})/(W_1+W_2)$, retention factor, $k = t_R\text{-}t_0/t_0$, and tailing or asymmetry factor, $T_f = (a+b)/2a$. Where W1 and W_2 are the baseline peak widths of succeeding peaks, and t_0, t_{R1}, and t_{R2} are the retention times.
The acceptance criteria were monitored for acquired data [31].

3. Result and Discussion

3.1. Validation parameters

The procedure was verified according to the analytical performance parameters set by the International Council for Harmonization (ICH), including linearity, accuracy, precision, and stability.

3.2. Preparation of calibration curve

The calibration curve was prepared by taking 6 concentrations of standard ABZ, ranging from 5 ppm to 50 ppm. The calibration curve was prepared by plotting the peak area against theoretical concentration, and a linear regression equation was obtained:

$$Response\ (Y) = Slope\ (m) \times Concentration\ (x) + Intercept\ (c)$$

3.3. HPLC method development and optimization

The HPLC method for quantifying albendazole was optimized by evaluating eight mobile phase compositions of methanol and 0.2% orthophosphoric acid, with methanol content ranging from 50% to 85% (v/v) in 5% increments. Each composition was assessed based on key chromatographic performance parameters, including retention time (t_R), peak symmetry, tailing factor (T), resolution (Rs), and number of theoretical plates (N). Lower methanol concentrations resulted in

longer retention times and broader peaks, while higher concentrations decreased resolution and peak symmetry. The optimal performance was achieved with a 70:30 (v/v) ratio, where albendazole eluted at approximately 6 minutes, showing a sharp, symmetrical peak and efficient resolution from interferences. The absorption maximum was determined to be 235 nm through a UV spectral scan. Chromatographic separation was performed using a Shim-pack GIST C18 column (250 × 4.6 mm, 5 µm), with data processed via LabSolutions software and an injection volume of 20 µL.

3.4. Linearity

Working concentrations of 5, 10, 20, 30, 40, and 50 ppm of ABZ were used to create calibration curves based on the drug's peak area. The calibration equation for ABZ is y = 114136x + 53568, showing a linear relationship from 5 to 50 ppm, with a coefficient of determination (R^2) of 0.999. **Figure 1(a)** illustrates the calibration curve, demonstrating that concentration and response are directly proportional and meeting the criteria for method validation. Thus, the procedure is considered linear within this concentration range [31].

3.5. Sensitivity

The regression residuals were used to determine the limit of detection (LOD) and the limit of quantification (LOQ) using the formulas LOD = 3×Sxy/a and LOQ = 10×Sxy/a. It was revealed that the LOD and LOQ were, respectively, 0.71 and 2.39 ppm (**Table 1**). According to these findings, the technique is sufficiently sensitive for pharmaceutical assays [31].

Table 1. Validation performance of the proposed HPLC method

Validation Parameters		Albendazole
Linear range (ppm)		5-50
Slope (%RSD)		0.512
Correlation coefficient (R^2)		0.999
%RSD	Intra Day	0.043 - 0.259
Recovery (%)		93.07 - 100.66
%RSD	Inter Day	0.178 - 2.54
Recovery (%)		96.9 - 101.68
LOD		0.717
LOQ		2.39

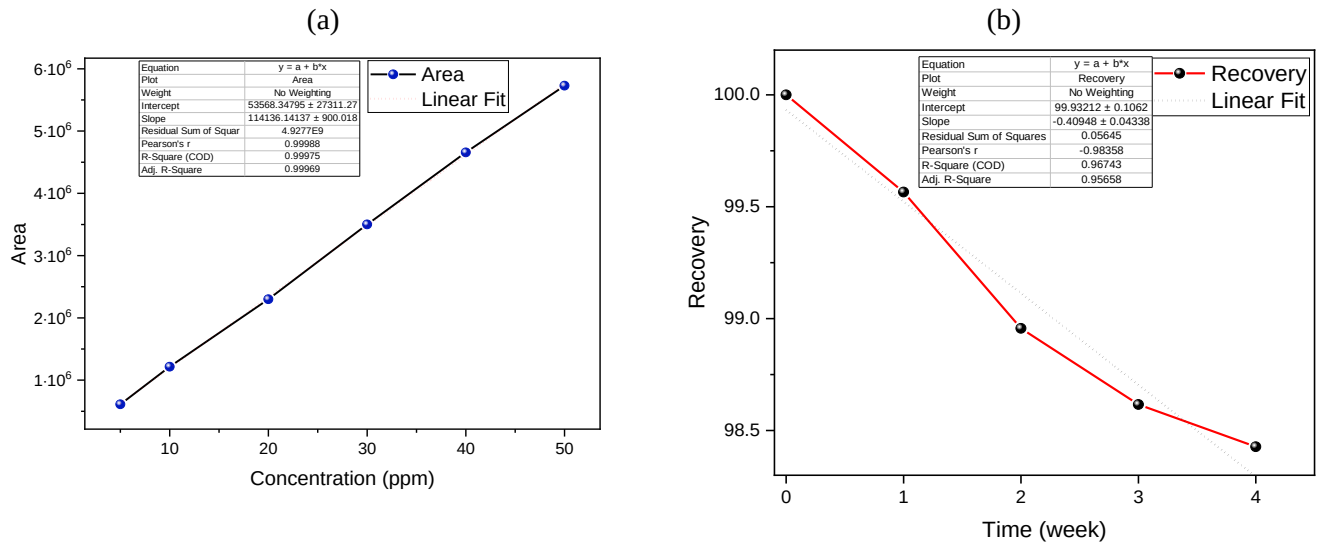


Figure1. (a) Calibration curve and (b) stability trend for ABZ.s

3.6. Recovery/accuracy

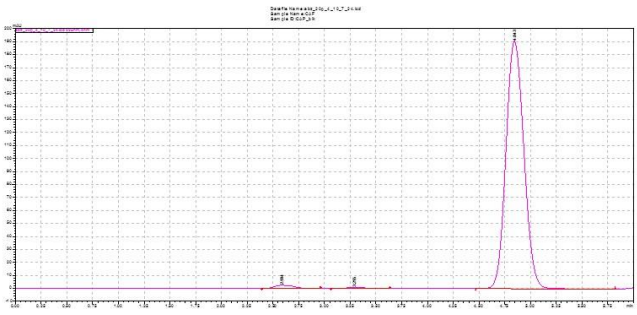
Recovery experiments demonstrated excellent drug accuracy. The intra-day assessment, conducted at six different concentrations (n=6), yielded results ranging from 98.67% to 100.66%. In the inter-day assessment, carried out over six separate days, the results ranged from 98.92% to 101.68%. **Table 2** presents the intra-day and inter-day recovery statistics for the proposed method. The percent recovery values fell within the range of 100 ± 2%, which confirms the accuracy of the developed approach beyond doubt [32].

3.7. Precision

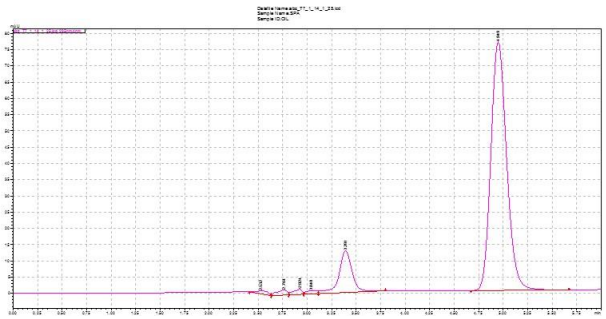
The precision is illustrated by the relative standard deviations (RSD) for ABZ at six different concentrations (n=6) during the intra-day assessment, which range from 0.043% to 0.259%. The inter-day assessment shows comparable RSD values ranging from 0.178% to 1.11%. **Table 2** presents the precision data for both intra-day and inter-day evaluations of the proposed method. The RSD values were all ≤ 2%, confirming the precision and repeatability of the developed method [33].

3.8. Specificity/selectivity

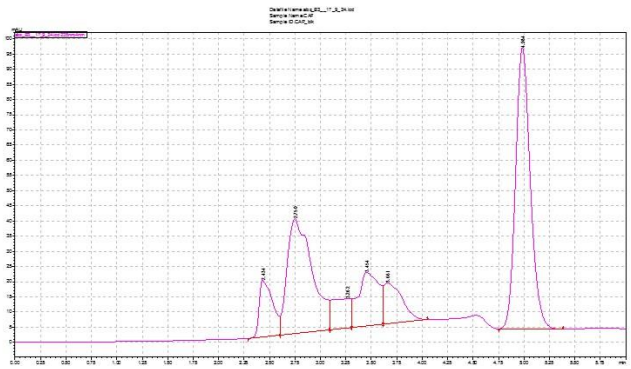
The absence of any peak in the same retention time revealed the specificity, which showed that the drug was identified free from interference from possible contaminants and decay products (Uddin et al., 2020b). Since there were no additional peaks on the retention periods of ABZ, it is clear from the chromatogram in **Figure 2** that the HPLC method was not interfered with by the formulation excipients under the selected chromatographic state. The method's great specificity is demonstrated by the results [34].



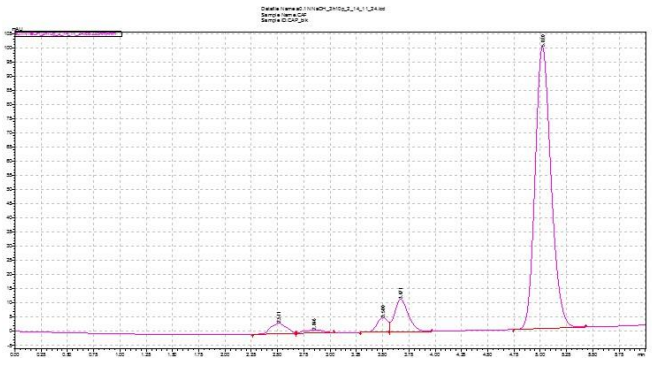
(a) Chromatogram of ABZ Standard (20 ppm)



(b) Chromatogram of ABZ after 3 hr in 3% H_2O_2



(c) Chromatogram of ABZ after 3 hr of thermal degradation at 80 ºC



(d) Chromatogram of ABZ after 3 hr in 0.1 N NaOH

Figure 2. Different Chromatograms of ABZ.

Table 2. Intraday and interday accuracy and precision data

	Actual Conc. (ppm)	Found Conc. (ppm) Mean±SD	RSD (%)	Recovery (%)
Inter Day	5	4.94 ± 0.05	1.11	98.92
	10	10.1 ± 0.14	1.36	101.0
	20	19.91 ± 0.04	0.178	99.59
	30	30.5 ± 0.24	0.794	101.7
	40	40.36 ± 0.3	0.741	100.9
	50	50.0 ± 0.42	0.831	100.0
Intra Day	5	4.93 ± 0.01	1.03	98.67
	10	10.05 ± 0.02	0.202	100.5
	20	19.99 ± 0.01	0.043	99.94
	30	30.20 ± 0.04	0.122	100.7
	40	40.07 ± 0.06	0.152	100.2
	50	49.51 ± 0.03	0.052	99.01

Each sample was analyzed three times (n=3)

3.9. Stability

Four tests were conducted over 30 days to determine how stable ABZ was in methanol that was kept in amber glass at 4 ºC in the refrigerator. The feedback from the freshly made standard solution and the aged solutions was compared. The findings demonstrated that there was no discernible degradation within the specified period, and the retention time and peak area of ABZ stayed nearly constant. As seen in **Figure 1(b)**, the compound's recovery was > 98 % for up to one month at 4 °C in the stored sample, but it gradually decreased [34].

3.10. Robustness

The experimental parameters, such as flow rate and orthophosphoric acid percent, did not have a significant effect on the method. However, the results in **Table 3** showed that these minor variations did not significantly impact the retention time, peak area, or resolution of albendazole, confirming the method's robustness [35].

3.11. System suitability

This test is necessary to guarantee a chromatographic system's high-quality performance (Das et al., 2024b). The calculated values; the number of theoretical plates, NTP = 3604.4±0.52 and tailing factor, T.F. = 1.097±0.38, resolution, Rs = 4.85±1.05, retention time, R.T. = 4.947 ± 0.30, and peak area = 1182422 ± 0.20 shown in **Table 4** demonstrated outstanding system suitability as all of the parameters were within the acceptance criteria. The acceptance criteria of the various parameters are Rs ≥ 2, T.F. < 2, NTP ≥ 2000**,** R.T.: % RSD ≤ 2%.

The analytical performance of the developed RP-HPLC method demonstrates acceptable linearity, accuracy, precision, robustness, and sensitivity for the quantification of albendazole, meeting ICH Q2(R1) requirements. The low variability observed in precision studies and consistent recovery values confirm that the method is reliable and free from significant systematic error. Compared with previously reported RP-HPLC methods for albendazole, which often employ longer run times, gradient elution, or complex mobile phase compositions [35], the present method provides comparable sensitivity and peak symmetry using a simpler and more economical chromatographic setup, enhancing its suitability for routine quality control applications. **Acceptance criteria for method validation** were as follows: Linearity: $r^2 \geq 0.999$, Accuracy: % Recovery 98–102%, %RSD < 2; Precision: %RSD < 2; Selectivity: no interference observed; Sensitivity: LOD, S/N ≥ 2–3; LOQ, S/N ≥ 10; Robustness: %RSD < 2; System suitability: Rs ≥ 1.5, Tailing Factor < 2, NTP ≥ 2000, and retention time %RSD ≤ 2 [36].

Table 3. Robustness data

Variations		Flow rate (mL/min)			Orthophosphoric Acid (%)		
		0.90	1.00	1.10	0.10	0.20	0.30
RT	Mean±SD	5.49±0.01	4.94±0.01	4.55±0.02	4.92±0.01	4.94±0.01	5.02±0.01
	RSD	0.12	0.30	0.41	0.12	0.30	0.20
Ar	Mean±SD	1313920±1313	1182422±2364	1078609±4638	1182282±1182	1182422±2364	1184280±118
	RSD	0.10	0.20	0.43	0.10	0.20	0.01
Rs	Mean±SD	4.33	4.85	4.19	4.72	4.85	5.09
	RSD	3.38±0.15	1.05±0.05	2.08±0.09	2.01±0.09	1.05±0.05	3.00±0.15

Each analysis was performed for three times (n=3); RT=Retention Time; Ar=Area; Rs=Resolution

Table 4. Validation parameters in terms of column efficiency and system suitability

	Column Efficiency			Suitability for Concentration 10 mg/L(n=5)	
	NTP	Tailing Factor (T_f)	Resolution Factor (R_s)	Retention Time	Area
Mean±SD	3604.4±18.7	1.097±0.05	4.85±0.05	4.947±0.01	1182422±3547
RSD	0.52	0.38	1.05	0.30	0.20

Each analysis was performed five times (n=5).

3.12. Pharmaceutical Product Assessment

The developed HPLC method was applied to determine the albendazole (ABZ) content in commercially available pharmaceutical formulations. A total of six different brands were selected, and four separate batches from each brand were analyzed. Each batch was tested in triplicate (n = 3) to assess consistency and accuracy. The results, summarized in **Table 5**, include the percentage recoveries and relative standard deviation (%RSD) values for each measurement. The percentage recoveries of ABZ from all tested products were found to be within the acceptable range of 100 ± 2%, and the %RSD values were all ≤ 2%, indicating excellent method precision and repeatability. The observed recoveries falling within this narrow range, with low RSD values, confirm that the method is both accurate and reliable. The low variability among replicate measurements suggests that the sample preparation process was consistent and that the method is robust. This further supports the suitability of the method for routine quality control analysis of ABZ in pharmaceutical formulations [6].

The observed batch-to-batch recovery variability, ranging from 98.9% to 100.4% across all tested brands, demonstrates excellent manufacturing consistency. This narrow variability indicates that each batch reliably delivers the intended albendazole concentration, reflecting precise formulation controls, accurate dosage filling, and stringent process validation. Regulatory standards, such as those in ICH Q2(R1), typically require assay recoveries within ±2% and relative standard deviation (%RSD) values ≤2% to confirm method and product consistency. Similarly, general pharmacopeial acceptance criteria for pharmaceutical assay results demand recoveries between 98–102% with %RSD ≤2%, ensuring both accuracy and precision [18]. By consistently meeting these tight recovery and precision limits, the batches clearly comply with regulatory expectations for content uniformity. Such compliance is essential to guarantee therapeutic efficacy, safeguard patient health, and uphold quality standards over the product lifecycle.

Table 5. Albendazole assay in the pharmaceutical sample by the developed HPLC method

Brand Name	Company Name	Dosage Form	Batch No.	Sample ID	Concentration (ppm)		Mean ± SD	RSD (%)
					Actual	Found		
Almex	SQUARE	Tablet	A	A.1	46.04	45.28	98.91 ± 0.98	0.99
				A.2	46.04	46.06		
				A.3	46.04	45.28		
			B	B.1	46.04	45.29	98.99 ± 1.09	1.10
				B.2	46.04	46.16		
				B.3	46.04	45.29		
			C	C.1	46.04	45.29	99.95 ± 1.68	1.68
				C.2	46.04	46.83		
				C.3	46.04	45.94		
			D	D.1	46.04	44.87	98.19 ± 1.50	1.53
				D.2	46.04	46.00		
				D.3	46.04	44.75		
Alben	SKF	Tablet	E	E.1	50.88	51.45	99.51 ± 1.43	1.43
				E.2	50.88	50.39		
				E.3	50.88	50.06		
			F	F.1	50.88	50.47	98.48 ± 0.68	0.69
				F.2	50.88	49.78		
				F.3	50.88	50.08		
			G	G.1	50.88	50.47	98.48 ± 0.68	0.69
				G.2	50.88	49.78		
				G.3	50.88	50.08		
			H	H.1	50.88	51.16	99.05 ± 1.36	1.37
				H.2	50.88	49.80		
				H.3	50.88	50.24		
Albendazole-DS	ALBION	Tablet	I	I.1	74.07	72.63	99.34 ± 1.20	1.21
				I.2	74.07	74.39		
				I.3	74.07	73.72		
			J	J.1	74.07	72.63	99.34 ± 1.20	1.21
				J.2	74.07	74.39		
				J.3	74.07	73.72		
			K	K.1	74.07	72.63	99.34 ± 1.20	1.21
				K.2	74.07	74.39		
				K.3	74.07	73.72		
			L	L.1	74.07	74.06	99.53 ± 1.92	1.92
				L.2	74.07	72.17		
				L.3	74.07	74.95		
Sintel	ACI	Tablet	M	M.1	36.89	36.81	100.41 ± 1.31	1.31
				M.2	36.89	36.72		
				M.3	36.89	37.60		
			N	N.1	36.89	36.02	99.64 ± 2.04	2.05
				N.2	36.89	36.73		
				N.3	36.89	37.53		
			O	O.1	36.89	36.87	99.89 ± 1.16	1.16
				O.2	36.89	36.42		
				O.3	36.89	37.27		
			P	P.1	36.89	36.41	98.71 ± 0.13	0.14
				P.2	36.89	36.47		
				P.3	36.89	36.37		

Almex	SQUARE	Suspension	Q	Q.1	80	81.43	99.77 ± 1.74	1.75
				Q.2	80	79.01		
				Q.3	80	79.02		
			R	R.1	80	81.43	100.17 ± 1.50	1.49
				R.2	80	79.94		
				R.3	80	79.06		
			S	S.1	80	79.69	99.80 ± 0.17	0.17
				S.2	80	79.91		
				S.3	80	79.93		
			T	T.1	80	81.12	100.11 ± 1.11	1.11
				T.2	80	79.48		
				T.3	80	79.68		
Alben	SKF	Suspension	U	U.1	80	79.88	99.77 ± 1.11	1.11
				U.2	80	80.68		
				U.3	80	78.91		
			V	V.1	80	80.82	99.82 ± 1.97	1.97
				V.2	80	80.72		
				V.3	80	78.04		
			W	W.1	80	80.04	99.99 ± 1.32	1.32
				W.2	80	78.91		
				W.3	80	81.02		
			X	X.1	80	80.11	100.07 ± 0.21	0.21
				X.2	80	79.87		
				X.3	80	80.19		

Each analysis was performed three times (n=3).

3.13. Forced degradation study

Forced degradation studies revealed that albendazole (ABZ) is relatively stable under acidic conditions, with degradation increasing modestly from 0.25% at 1 h to 4.83% at 3 h. In contrast, ABZ showed moderate susceptibility to alkaline hydrolysis, with degradation rising from 5.08% at 1 h to 13.89% at 3 h, likely due to cleavage of the benzimidazole ring. Thermal stability was generally good at lower temperatures, with degradation peaking at 3.47% after 3 h at 50 °C, increasing to 7.79% at 60 °C, and becoming more pronounced at 80 °C (15.58%), indicating temperature-dependent instability. Photostability testing demonstrated mild degradation, increasing from 0.34% to 4.74% over 3 h, suggesting that prolonged UVA exposure can induce some photolytic breakdown. Under oxidative stress, ABZ exhibited the highest susceptibility, with degradation rising sharply from 5.84% at 1 h to 25.06% at 3 h, likely due to oxidation of sulfur- or nitrogen-containing groups. These degradation patterns align with previous studies [34, 35], which reported similar products and pathways under hydrolytic, oxidative, thermal, and photolytic stress conditions **(Table 6)**.

Forced degradation studies indicate that albendazole is more susceptible to oxidative than to hydrolysis or thermal stress, consistent with degradation trends reported in earlier stability studies [4]. Albendazole primarily undergoes degradation through oxidative and hydrolytic pathways, yielding albendazole sulfoxide, albendazole sulfone, and carbamate [14]. Hydrolytic stress resulted in the formation of distinct degradation products that were chromatographically resolved from the albendazole peak, supporting the method's specificity. Although degradation was limited by the short stress duration, no co-elution at the analyte retention time was observed, and peak purity analysis confirmed the absence of interference from degradation products. These findings demonstrate that the method is capable of distinguishing albendazole from its degradation products under the applied stress conditions [16, 35].

Overall, while the degradation studies were not intended to replicate long-term stability testing, the observed

degradation behavior and chromatographic resolution support the claim that the method is stability-indicating within the scope of the applied stress conditions. The method's analytical performance and simplicity compare favorably with existing reports and justify its application in routine analysis and preliminary stability assessment [36].

4. Conclusion

A rapid, accurate, and robust HPLC method has been successfully developed and validated for the quantification of albendazole in pharmaceutical formulations, in accordance with ICH Q2 (R1) guidelines. This method demonstrated excellent linearity, precision, accuracy, and robustness. Forced degradation studies were conducted under acidic, basic, oxidative, photolytic, and thermal conditions, following ICH Q1A (R2) and Q1B guidelines. Albendazole is relatively stable under acidic, photolytic, and mild thermal conditions (≤ 60 °C), exhibiting less than 5% degradation. However, it shows moderate to high degradation under basic, oxidative, and elevated thermal conditions (at 80 °C), with oxidative degradation being the most severe. The method effectively separated albendazole from its degradation products, confirming its capability to indicate stability. The validated method was further applied to analyze six different commercial brands of albendazole available in Bangladesh. Analysis of multiple batches showed consistent drug content across all brands, with percentage recoveries ranging from 98.9% to 100.4% and % RSD values below 2%. This indicates high manufacturing quality and content uniformity. Overall, the proposed HPLC method is suitable for routine quality control, stability testing, and regulatory compliance of albendazole in pharmaceutical dosage forms.

Abbreviations

ABZ: Albendazole
HPLC: High-Performance Liquid Chromatography
LOD: Limit of Detection
LOQ: Limit of Quantification
ICH: International Conference on Harmonization
LC-MS: Liquid Chromatography-Mass Spectrometry
RP: Reversed Phase
SD: Standard Deviation
RSD: Relative Standard Deviation
NTP: Number of Theoretical Plates
T.F.: Tailing Factor
Rs.: Resolution
R.T.: Retention Time
MeOH: Methanol
OPA: Orthophosphoric Acid

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Conflict of interest

All authors unequivocally affirm that they have no conflicts of interest to declare.

Data availability

Data will be provided upon request.

Table 6. Relative degradation (%) of Albendazole under various stress conditions

Stress Conditions \ Duration (hr)		1st hr (Mean±SD)	2nd hr (Mean±SD)	3rd hr (Mean±SD)
Acid hydrolysis (1N HCl)		0.25±0.072	3.47±0.149	4.83±0.140
Basic hydrolysis (1N NaOH)		5.08±0.106	11.77±0.368	13.89±0.036
Thermal hydrolysis	50◦C	0.34±0.087	1.10±0.098	3.47±0.180
	60◦C	2.03±0.095	3.13±0.095	7.79±0.085
	80◦C	7.96±0.128	8.98±0.062	15.58±0.062
Photo hydrolysis (UV-A)		0.34±0.052	1.44±0.053	4.74±0.050
Oxidative hydrolysis (H_2O_2 -3%)		5.84±0.128	15.75±0.030	25.06±0.286

Each analysis was performed three times (n=3).

Authors Contributions

Maliha Momtaj: Formal analysis, Investigation, Validation, Resources, Writing – original draft. **Suman Das:** Conceptualization, Methodology, Writing – original, Writing – review & editing, Investigation, Validation, Supervision. **Md. Samrat Mohay Menul Islam:** Data curation**,** Writing – review & editing. **Md. Miraz Hossain:** Formal analysis, Validation, Investigation. **Nusrat Jahan Mouri:** Formal analysis, Writing – review & editing. **Md. Afzal Hossain:** Writing – review & editing. **Sujan Kanti Das:** Formal analysis, Investigation. **Sreebash Chandra Bhattacherjee:** Investigation, Writing – review & editing. **Rasheda Akter:** Investigation, Writing – review & editing

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