RP-HPLC method development and validation of Albendazole and its impurity

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Abstract: Oxibendazole is a type of benzimidazole that is commonly used as an antiparasitic medication for both humans and animals. However, it is also a significant impurity found in albendazole, and it is crucial to follow the ICH Q3B criteria when analysing oxibendazole impurities. Therefore, it is recommended to use a simple, fast, sensitive, and precise RP-HPLC approach to identify oxibendazole impurities in bulk and pharmaceutical formulations of albendazole.To separate the oxibendazole impurity, acetonitrile and 10 nM potassium phosphate were used as a mobile phase. Orthophosphoric acid was used to accurately adjust the pH of the mobile phase to 2.03, ensuring optimal conditions. A nucleosil C18 column (250 x 4.6 mm, 5 µm) was used for the separation process, and it effectively provided the necessary separation. The gradient elution was set at a wavelength of 235 nm and a flow rate of 1 mL/min. The analytical technique was successfully designed and validated. The AQbD technique was used to optimize the analytical conditions for the suggested methodology, and the Design Expert 13® trial version was used for the central composite design optimization of analytical conditions. The procedure's linearity was verified using a regression coefficient of 0.999 within a working range of 0.5 to 3 µg/ml. Accuracy research showed results of 99.94–100.10% at 50, 100, and 150% levels of the working concentration. The oxibendazole impurity's average retention time was found to be 6.40 minutes, with a relative standard deviation of less than 2%, indicating high accuracy. The limits of detection (LOD) and quantification (LOQ) were found to be 0.073 and 0.091 µg/ml, respectively. Following the ICH Q2(R1) criteria, other validation criteria, such as robustness, were also evaluated. In conclusion, the proposed approach is suitable for analysing albendazole and oxibendazole in bulk and pharmaceutical formulations, making it ideal for detecting oxibendazole impurities.

1 INTRODUCTION

Thorough testing and analysis are necessary parts of the verification process to ensure that albendazole formulations are free from contaminants that could endanger user health. It is important to continuously evaluate the stability of these formulations over time to further verify their safety and effectiveness and to ensure that no new contaminants arise during storage or ageing. The quality of these formulations is influenced by the materials and procedures used during the production process. These products may contain various contaminants such as synthetic precursors, side reaction products, unreacted raw materials, intermediates, and degradation products [1]. However, the benefits of delivering these contaminants are offset by the toxicological adverse effects they typically induce. It is therefore crucial to keep a constant watch on these contaminants to maintain a high-quality industrial environment. Oxibendazole (Albendazole Impurity I EP) has a chemical makeup of methyl ester of 5-propoxy-1H-benzo[d]imidazole-2-yl carbamate; (5-Propoxy-1H-benzimidazol-2yl) carbamic acid. [Fig 1.]. [2]

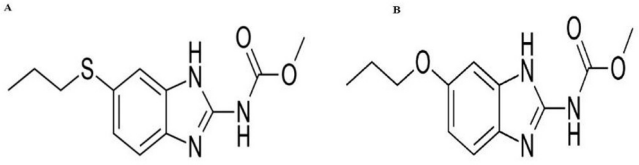

Fig. 1 (A) Chemical structure of Albendazole (B) Oxibendazole

The anthelmintic drug oxibendazole is used to treat parasitic infestations such as roundworms, strongyles, threadworms, pinworms, and lungworms in horses and other domestic animals. Different analytical methods have been developed for detecting the residues of this drug in bulk medications, milk, and human plasma as reported in literature studies [3].

The current study aimed to identify the oxibendazole impurity in albendazole solid formulation using RP-HPLC technology. Quality by Design (QbD) methodology was used to optimize the analytical conditions, which focuses on the quality expected during the planning phase. AQbD is an instrument that reports quality from the planning stage by relying on predetermined goals, risk evaluations, and risk management [Figure 2]. By using QbD, fewer experiments are needed to establish a technique. Additionally, the analytical technique provides a better understanding of how variables can affect significant metrics [4-5].

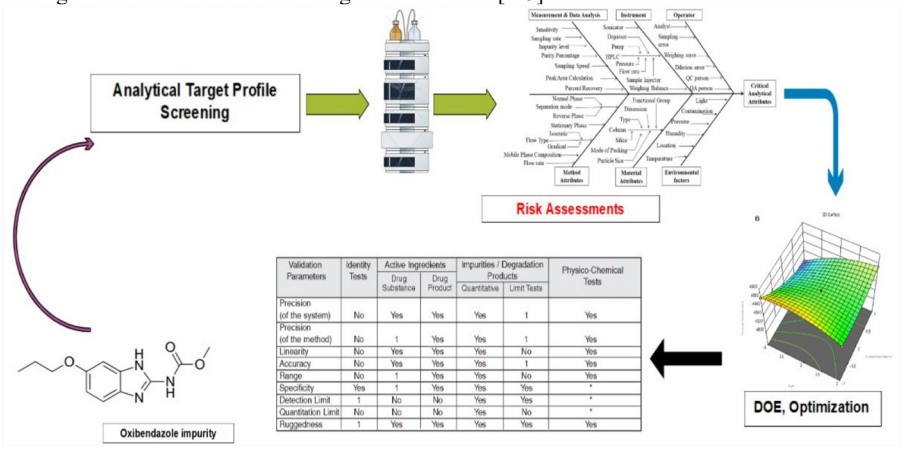

Fig. 2 The schematic representation of the analytical method QbD approach

2 Material and methods

For the analysis of the impurity and albendazole the Ashirwad Analytical from Panchkula, India provided a free sample of albendazole and the impurity was provided by the Pharma affilaites, Panchkula, India. Only HPLC-grade chemicals were used, which were all procured from Merk India. The solvents and solutions were sonicated and filtered through a membrane filter before being employed. The Jasco HPLC System and a PDA detector were used for the chromatographic process. The sample was injected into the analytical column (C18 Columns 250 x 4.6 mm, 5µm) through a rheodyne sample injection port. The output response was recorded using the Borwin-PDA program (Version 1.5).

2.1 Initial characterization of impurity and API

The solubility of both oxibendazole impurity and albendazole was determined in various solvents, including water, methanol, and ethyl acetate. Fourier-transform infrared spectroscopy (FTIR) was used to analyze the functional groups present in the compounds and confirm their identity [Fig 3].

2.2 Determination of λ max

2.2.1 Mobile phase preparation

To prepare the mobile phase, a mixture of acetonitrile, 10 nM potassium dihydrogen phosphate, and orthophosphoric acid was made in a 40:60 v/v ratio until the pH reached 3.5. The mobile phase was then passed through a 0.45 µm membrane paper filter and ultrasonically sonicated for ten minutes [6].

2.2.2 Preparation of the stock solution

To prepare a 1000 µg/ml concentration of the drug, 10mg of the albendazole was mixed with 10 ml of 1% methanolic sulfuric acid to form the standard drug stock solution. From this stock solution, we produced additional doses ranging from 5 to 25 µg/ml. A similar procedure was followed to prepare the dilutions of the impurities[7].

2.2.3 Detection of wavelength

Additional dilutions of the standard stock solution were made with the mobile phase and the UV spectrum was scanned to acquire spectra. It was found that both albendazole and its primary impurity, Oxibendazole, exhibited significant absorption at 235 nm [8].

2.3 Optimization of Analytical Conditions

During the screening process, critical parameters such as the column temperature, mobile phase ratio, buffer pH, flow rate, and injection volume were identified. The central composite design (CCD) was then utilized to maximize the separation of oxibendazole impurities [9]. The independent variables, which included the mobile phase's pH (X2) and composition (X1), were examined for their impact on the dependent variable. Table 1 shows the independent factors used in the experiment [10].

Table. 1 Conversion of factor levels into units **for experimental design of impurities**

Levels	Mobile phase ratio (Methanol: Phosphate buffer:)	pH
-1	30:70	2
0	40:60	4
+1	50:50	6

The experimental design was developed for the separation process, the independent variables were the type of column used, the flow rate of the mobile phase, and the system's temperature. The number of theoretical plates (Y2) indicates how effective the separation process is, while the retention time (Y1) is the amount of time taken for a component to elute from the column. The recovery (Y3) of a compound from the sample post-column passage is used to measure its effectiveness [11]. To get the best composition predictions, an overlay plot and desirability function are used. The anticipated analytical conditions were determined using Design-Expert Software and numerical optimization techniques [12].

The Design-Expert-13® program was used to statistically analyze the experimental design's outcomes. Statistical validation involved assessing the distribution of the correlation coefficient F-value (R2), adjusted R-squared (R2 Adj), predicted R-squared (R2 Pred), PRESS statistics, and adequate precision (AP) produced by the ANOVA provision to determine the model's appropriateness and reliability. A model was considered significant if the F-value was $p < 0.05$. It was considered acceptable if the difference between R2 Adj and R2 Pred was less than 0.2. The PRESS statistics were used to determine the measure of fit, where a smaller PRESS value was preferred [13-14].

Design Expert software-13® was used to generate diagnostic graphs to compare externally studentized residuals with planned and actual plots for the impurity experimental design. The plots were examined to determine if the points fell within the designated limits [15-16].

2.4 HPLC method development

2.4.1 Selection of mobile phase and chromatographic conditions [17-18]

To conduct chromatographic separation, albendazole and oxibendazole were used as working standard solutions. The experiments were carried out by changing the ratios of acetonitrile to buffer, and by using a buffer with different pH values, to obtain the required system suitability characteristics. The optimal mobile phase was determined to be acetonitrile: 10 nM potassium dihydrogen phosphate with a pH of 3.5, adjusted with orthophosphoric acid in a 40:60 v/v ratio. After 11 trials, it was found that this mobile phase showed high resolution and adequate peak characteristics at a flow rate of 1 ml/min [19].

Chromatograms and system suitability parameters
The mobile phase was carefully applied to the column, ensuring full saturation and resulting in continuous back pressure at the optimal flow rate. Following this, a precisely measured working standard solution containing 1 µg/ml oxibendazole and 200 µg/ml albendazole was injected into the system [20-21].

2.5 Method validation [22]

2.5.1 Linearity

Six replicates of each concentration were extracted and introduced into the system from the standard albendazole stock solution. The linearity of the correlation between the drug's peak area and concentration was established over the concentration range of 100-600 µg/ml [23].
Similarly, from the oxibendazole standard stock solution, six duplicates of each concentration were extracted and introduced into the apparatus. It was observed that the relationship between the drug's peak area and concentration was linear over the concentration range of 0.5-3 µg/ml [24].

2.5.2 Precision

The precision of the method was determined by evaluating intra-day and inter-day variability. For intra-day variability, three concentrations of albendazole (200, 400, and 600 µg/ml) were assessed with three replicates each, and the percent relative standard deviation was calculated. Inter-day variability was evaluated over three consecutive days with the same concentrations and replicates, and the percent relative standard deviations were calculated accordingly [25].
Similarly, for oxibendazole, three duplicates of three distinct concentrations (1, 2, and 3 µg/ml) were examined in one day, and the percent relative standard deviation was calculated. Inter-day variability was also investigated over three consecutive days with three different concentrations and three replicates, and the percent relative standard deviations were calculated [26].

2.5.3 Accuracy

To establish the accuracy of the method, a recovery study was conducted for both drug and impurity. Pure albendazole was added to sample solutions at selected levels (50%, 100%, and 150%), with a basic concentration of 200 µg/ml. Additionally, standard oxibendazole impurity (0.3, 0.6 & 0.9 µg/ml) was spiked, and chromatograms were obtained after injecting in triplicates. The concentration of drug and impurity was calculated using the linearity equation of albendazole and oxibendazole [27-28].

2.5.4 Limit of Detection (LOD)

To determine the sensitivity of the proposed method, the lowest concentrations in a sample that can be detected but not always measured - LOD and LOQ - were analysed. LOD represents the lowest concentration in a sample that can be accurately measured, while LOQ represents the lowest amount of analyte in a sample that can be accurately measured. The drug and impurity concentrations in the lower part of the calibration curve were used to calculate LOD and LOQ using the equation $3.3 \times \sigma/S$ and $10 \times \sigma/S$, respectively [29].

2.5.6 Robustness

The robustness of the method was tested by varying chromatographic conditions, such as flow rate, pH, and wavelength, and observing their effects.

3 Results

3.1 FTIR study

To characterize the oxibendazole, the first step was to record the FTIR (Fourier Transform Infrared Spectroscopy) spectrum of the impurity. This was done by making a pellet using a small quantity of impurity powder and KBr, which was then placed between two discs. Once this was done, the prepared KBr disc was placed in the sample holder and analysed to obtain the FTIR spectra of the impurity (oxibendazole).

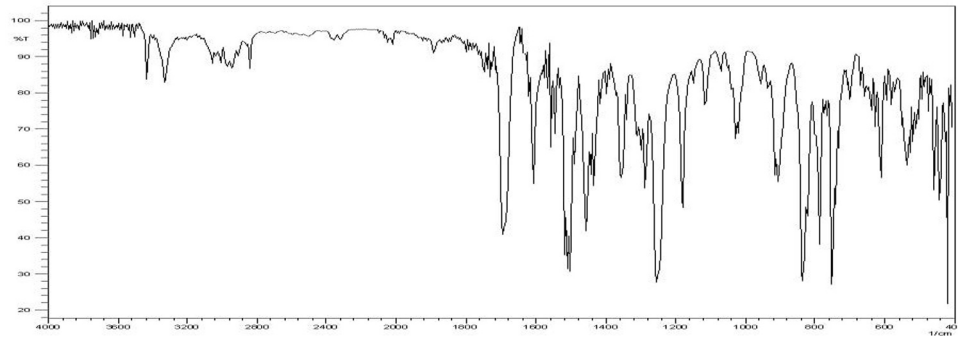

Fig. 3 IR spectra of oxibendazole depicted the principal peak 3328 n-h stretching, 2980 CH3/CH2 C-H (aromatic) stretching 1712 amide stretching 1650 C=C stretching 1450 C-H bending methyl group

3.2 Determination of λmax

To measure the wavelength of albendazole and oxibendazole impurity, an ideal ratio of 40:60 v/v in mobile phase acetonitrile and 10 nM potassium dihydrogen phosphate was used. The solution was filtered through 0.45 M membranes and subjected to 10 minutes of ultrasonication in an ultrasonicater. For the albendazole standard stock solution, 10 milligrams of albendazole were dissolved in one percent methanolic sulfuric acid to create a solution of 1000 µg/ml. Additional dilutions were made using the original stock solution. Similarly, for the impurity standard stock solution of oxibendazole, 10 mg of the drug was dissolved in 10 ml of 1% methanolic H2SO4 to make a 1000 µg/ml concentration. Additional dilutions were made using the original stock solution. [Fig 4 A and B]

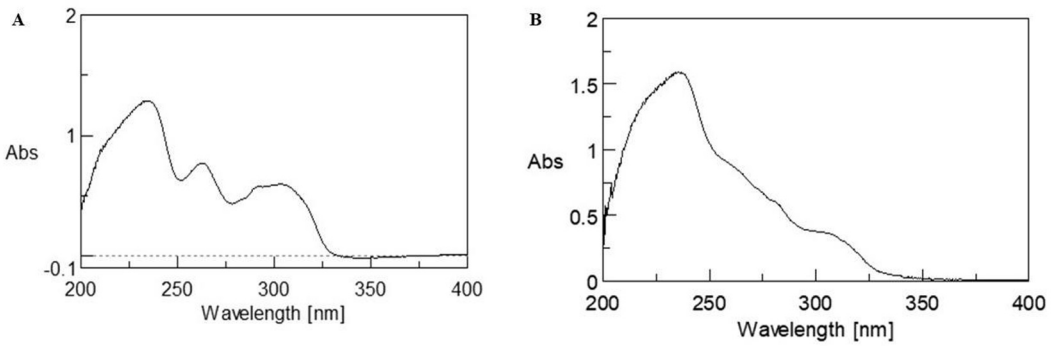

Fig. 4 UV spectrum of A. albendazole and B. oxibendazole in mobile phase acetonitrile and 10 nm potassium dihydrogen phosphate in an optimized ratio of 40:60 v/v. (10 µg/ml)

3.3 Experimental design for optimization of analytical method conditions

Throughout the optimization investigation, a range of measurements and analyses were conducted to assess the recoveries, theoretical plates, and retention time. The software algorithm recommended a total of eleven trial runs, which were conducted across three separate levels of the design matrix. The outcome of these experimental runs enabled us to create a robust mathematical model, which accurately describes the results obtained from the trials. [Table 2].

Table. 2 Experimental runs and response variables oxibendazole impurity

		Factor 1	Factor 2	Response 1	Response 2	Response 3
Std	**Run**	**A: Mobile Phase Ratio**	**B: pH**	**Retention Time**	**Theoretical Plates**	**Recovery**
		%		Min	Numbers	%
10	1	0	3	6.39	4858.14	98.73

11	2	0	3	6.38	4858.13	98.75
8	3	0	4.414214	6.87	4824.14	98.91
2	4	1	2	6.18	4872.84	97.99
7	5	0	1.585786	6.21	4847.72	98.56
1	6	-1	2	6.25	4858.21	99.23
4	7	1	4	6.86	4816.98	99.27
9	8	0	3	6.37	4857.97	98.79
3	9	-1	4	6.89	4881.14	98.51
6	10	1.414214	3	6.31	4863.05	98.49
5	11	-1.41421	3	6.28	4898.21	98.56

The study assigned independent variables, such as pH and mobile phase composition, to levels +1, 0, and -1, and examined their effects with response variables. Highly significant models were constructed for each response variable ($p < 0.05$).

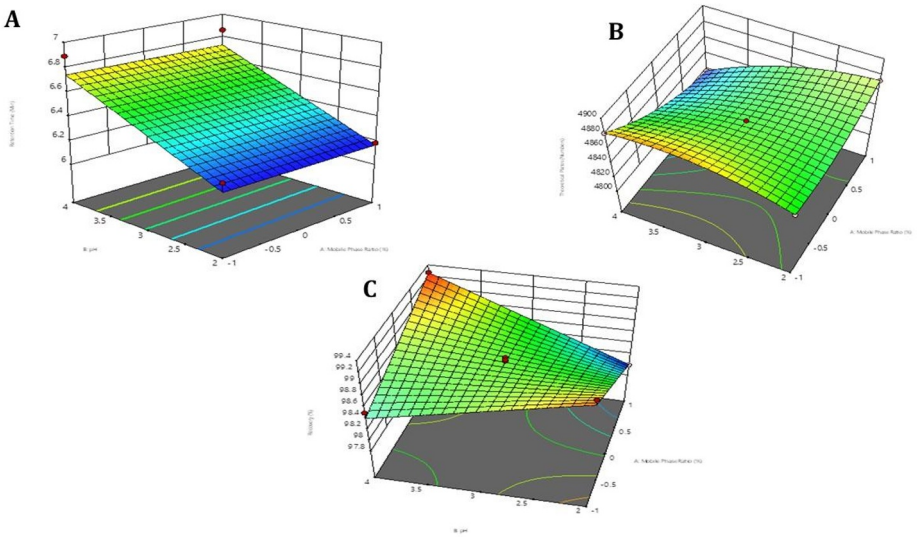

Fig. 5 Effect on (a) retention time (b) theoretical plates (c) and (d) percent recovery as a function of mobile phase ratio and mobile phase pH

The model's residual mean square and mean square were both greater than F, indicating an excellent fit to the data. Using DOE software, values compared the anticipated retention, theoretical plate, and recovery values and found that the model predicted them with good accuracy, with only a 5% error rate. [Table 3]

Table. 4 Predicted values for response variables and data from experiments Recovery, Theoretical Disks, and retention time

Run	**Retention Time**			**Theoretical Plates**			**Recovery**		
	Actual Value	Predicted Value	% Error*	Actual Value	Predicted Value	% Error*	Actual Value	Predicted Value	% Error*
1	6.39	6.45	-0.93023	4858.14	4858.08	0.001235	98.73	98.71	98.73
2	6.38	6.45	-1.08527	4858.13	4858.08	0.001029	98.75	98.71	98.75
3	6.87	6.85	0.291971	4824.14	4823.72	0.008707	98.91	98.89	98.91
4	6.18	6.16	0.324675	4872.84	4873.36	-0.01067	97.99	98.00	97.99
5	6.21	6.06	2.475248	4847.72	4847.15	0.011759	98.56	98.52	98.56

6	6.25	6.18	1.132686	4858.21	4858.78	-0.01173	99.23	99.15	99.23
7	6.86	6.73	1.931649	4816.98	4817.4	-0.00872	99.27	99.27	99.27
8	6.37	6.45	-1.24031	4857.97	4858.08	-0.00226	98.79	98.71	98.79
9	6.89	6.74	2.225519	4881.14	4881.61	-0.00963	98.51	98.41	98.51
10	6.31	6.44	-2.01863	4863.05	4862.59	0.00946	98.49	98.61	98.49
11	6.28	6.46	-2.78638	4898.21	4897.68	0.010821	98.56	98.81	98.56

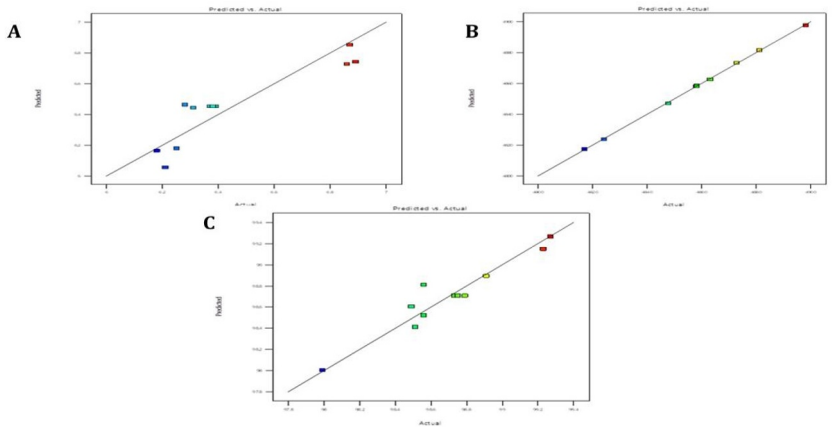

Fig. 6 The actual vs predicted values plots of (a) retention time (b) theoretical plates (c) recovery

Optimization of analytical conditions

The program is designed to improve the performance of chromatography by adjusting the mobile phase ratio and pH level. This process helps to maximize the retention time, theoretical plates, and recovery of target molecules. The program selects the most appropriate combination based on the specific requirements. For instance, after testing various combinations, it was found that using a mobile phase ratio of 0.499 and a pH level of 2.030 resulted in 6.17 minutes of retention time, 4861.570 theoretical plates, and 98.30% recovery. To achieve these optimal values, the values were converted to the translation factor coded level and lowered the pH to 2.03. Based on these calculations, it was recommended to use a mobile phase ratio of 40:60 (acetonitrile and 10 nM potassium dihydrogen phosphate) to achieve the best results. [Fig 7]

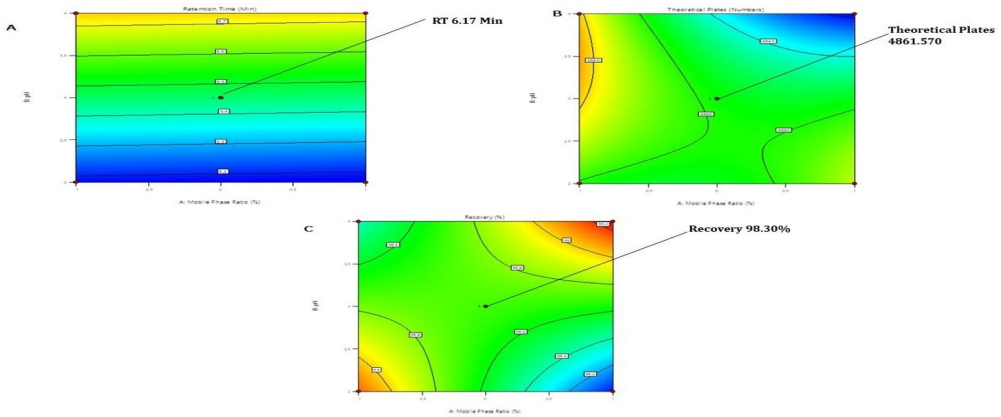

Fig. 7 Mobile phase ratio (0.499) pH 2.030 which provided the retention time of 6.17 min, theoretical plates 4861.570, and the % recovery of 98.30% at a 95% confidence interval with a desirability value of 1

3.4 Validation of analytical method

3.4.1 Chromatogram and system suitability parameters

The column was saturated with the mobile phase and carefully monitored the pressure to ensure the appropriate flow rate. Then the working standard solution, containing oxibendazole at a concentration of 1 µg/ml and albendazole at 200 µg/ml was introduced into the system. The results of the system's applicability were then recorded and are presented in detail in Table 4 and Fig. 8.

Table. 6 System suitability study

Name	RT (Min) ± RSD	Concentration (µg/ml)	Area (µV. Sec)	Plates	Asymmetry
Oxibendazole	6.399 ± 0.114	1	6537	4748	1.07
Albendazole	11.095 ± 0.087	200	1709397	11863	0.98

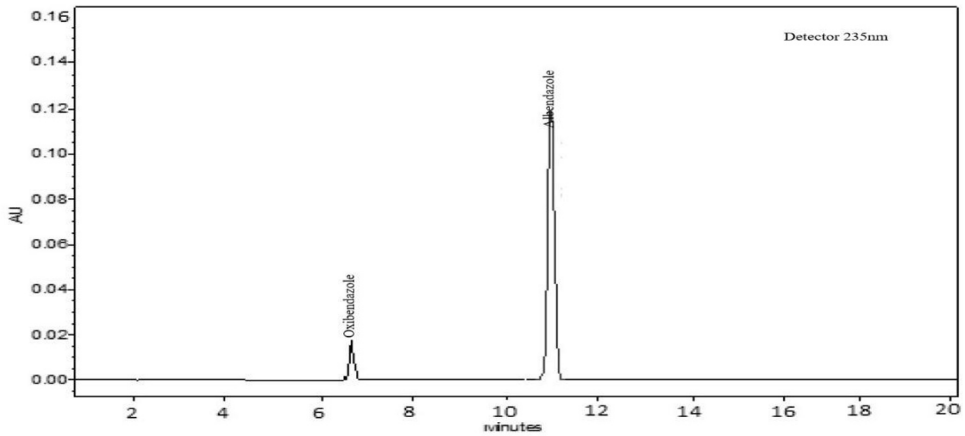

Fig. 8 HPLC chromatogram of a standard mixture of Albendazole (200 µg/ml) and oxibendazole (1 µg/ml)

3.4.2 Linearity

Six replicates were taken from the standard stock solution of albendazole (100-600 µg/mL) and oxibendazole (0.5-3 µg/mL) at each concentration and injected into the system. Calibration curves were drawn to establish the linear relationship between the concentrations and peak areas of the drug and impurity. [Fig 9 and 10]

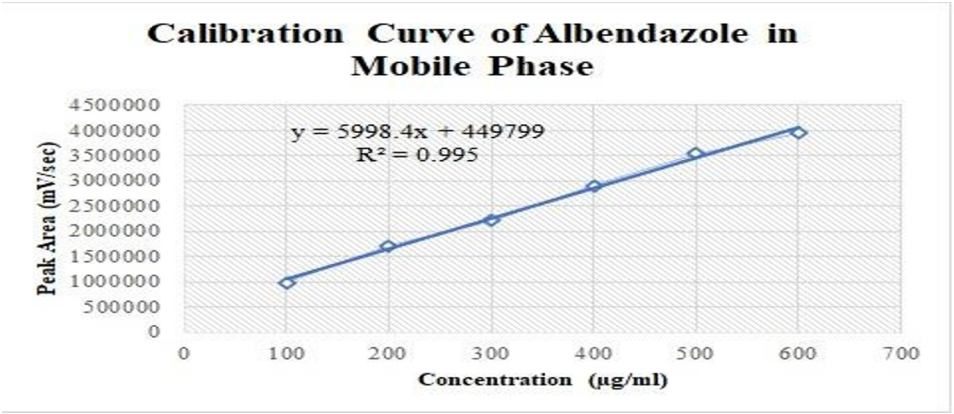

Fig. 9 Albendazole calibration curve in the mobile phase (Acetonitrile: Potassium dihydrogen phosphate at pH 2.3)

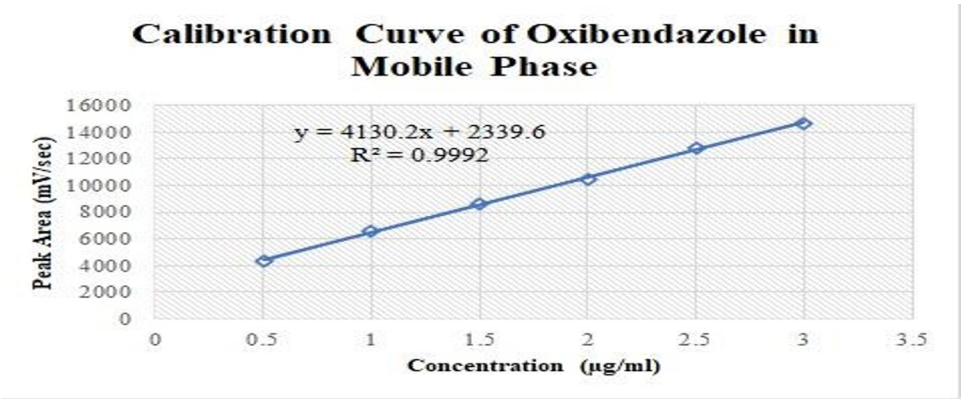

Fig. 10 Oxibendazole calibration curve in the mobile phase (Acetonitrile: Potassium dihydrogen phosphate at pH 2.3)

3.4.3 Precision

The method's precision was evaluated by testing intra-day and inter-day variability using three concentrations and three replicates of albendazole (200, 400, and 600 µg/mL) and oxibendazole (1, 2, and 3 µg/mL). The percent relative standard deviation was calculated.

Table. 5 Inter-day precision results of albendazole and impurity

Albendazole			**Oxibendazole**		
Concentration (µg/ml)	**Avg peak Area (µV. Sec)**	**Mean % Recovery ± SD**	**Concentration (µg/ml)**	**Avg peak Area (µV. Sec)**	**Mean % Recovery ± SD**
200	1657053	101.142 ± 0.44	1	6514	100.76 ± 0.44
200	1666067		1	6480	
200	1666430		1	6509	
400	2827255	100.310 ± 1.34	2	10730	99.54 ± 1.77
400	2851304		2	10465	

400	2891211		2	10490	
600	4113717	101.443 ± 0.56	3	14611	100.51 ± 1.36
600	4077274		3	14822	
600	4111365		3	14945	
% RSD*		0.91	**% RSD***		1.265

*Average of three determinations

Table. 6 Inter-day precision results of albendazole and impurity

Albendazole			**Oxibendazole**		
Concentration (µg/ml)	**Avg peak Area (µV. Sec)**	**Mean % Recovery ± SD**	**Concentration (µg/ml)**	**Avg peak Area (µV. Sec)**	**Mean % Recovery ± SD**
200	1646652	100.68 ± 1.56	1	6561	101.23 ± 0.87
200	1679351		1	6510	
200	1647035		1	6491	
400	2889510	101.33 ± 0.42	2	10604	100.20 ± 1.16
400	2899173		2	10718	
400	2879256		2	10528	
600	4071092	100.88 ± 1.25	3	14696	100.37 ± 0.85
600	4041046		3	14895	
600	4129189		3	14738	
% RSD*		1.11	**% RSD***		0.961

*mean value of three determinations

3.4.4 Accuracy

The method's accuracy was determined by conducting a recovery study of a drug and its impurity. Pure albendazole was added to sample solutions at different levels (50, 100, and 150%) with a basic concentration of 200 µg/ml. Standard oxibendazole impurity was also added, and chromatograms were obtained after injecting three replicates. The linearity equation of albendazole and oxibendazole was used to calculate the concentrations of the drug and impurity. [Table 7-8]

Table. 7 Accuracy study of albendazole

Level	**Conc. (µg/ml)**		**Area**	**% Recovery**	**Mean**	**% RSD**
	Sample	**Std**				
50 %	200	100	2234308	99.166	99.239	1.271
			2213617	98.016		
			2258967	100.536		
100 %	200	200	2906199	102.377	101.463	0.788
			2870526	100.891		
			2876086	101.122		
150 %	200	300	3530462	102.716	101.987	0.713
			3486862	101.262		
			3508483	101.983		

Table 8. Accuracy study of oxibendazole

Level	Conc. (µg/ml)		Area	% Recovery	Mean	% RSD
	Sample	Std				
50 %	0.592	0.3	5998	99.525	100.105	0.514
			6026	100.286		
			6034	100.504		
100 %	0.592	0.6	7285	100.620	101.081	0.419
			7312	101.169		
			7326	101.454		
150 %	0.592	0.9	8498	100.072	99.947	0.428
			8461	99.470		
			8512	100.299		

3.4.5 LOD and LOQ

The limit of detection (LOD) and limit of quantification were calculated using the equations 3.3 x σ/S and 10 x σ/S, respectively. The method was able to detect the lowest amounts of albendazole and oxibendazole, which were found to be 111.25 µg/ml and 0.223 µg/ml, respectively. Additionally, the limit of quantification for the drug and impurity were determined to be 337.12 µg/ml and 0.667 µg/ml, respectively.

3.4.6 Robustness

The robustness of the method was tested by varying chromatographic conditions, such as flow rate, pH, and wavelength, and observing their effects.

Table. 9 Robustness Study

Drug	% RSD Found for Robustness Study								
	pH			Flow Rate (1 ml/min)			Wavelength (nm)		
	3.4	3.5	3.6	0.9	1.0	1.1	234	235	236
Albendazole	0.316	0.347	0.219	0.736	0.370	0.451	0.397	0.197	0.214
Oxibendazole	0.475	0.592	0.768	0.885	1.453	0.699	0.199	0.473	0.171

*Average of three determinations

4 Discussion

In this investigation, a quality-by-design enabled approach was used to analyse the oxibendazole impurity in bulk and albendazole formulation. The optimization process involved evaluating the theoretical plates, recovery reactions, and retention time. Based on the observed data, the DOE program made predictions using statistical analysis. The accuracy and reliability of the model were evaluated by comparing the projected values with the actual observed values. This allowed for additional experimental conditions to be adjusted and refined to enhance retention time, theoretical plate count, and recovery percentage in subsequent experiments.

With an error percentage of less than 5%, the model exhibited strong predictability. The retention period of the oxibendazole impurity increased with decreasing mobile phase pH, possibly because oxibendazole is a basic impurity (pKa 4.56), and partial ionization occurs at pH values below 3.0. The retention period of oxibendazole is significantly decreased at high pH levels because of non-ionization, particularly when the mobile phase's pH value is two units over its pKa value. Changing the mobile phase ratios led to a considerable variation in the impurity's region time. Raising the mobile phase ratio or the ratio of 10 nM potassium dihydrogen phosphate also extended the impurity's retention period.

The theoretical plates decreased as the pH values increased, resulting in a broad peak on the chromatograms. On the other hand, oxibendazole had a strong peak with a good number of theoretical plates at low pH. It is suggested that the impurity (Oxibendazole) becomes partially or completely ionizable when the concentration of phosphate

buffers is increased. The non-ionizable forms become more hydrophobic, resulting in substantial retention on the non-polar stationary phase.
The mobile phase's pH shift had the least impact on the percentage recovery. At 235 nm, it was discovered that the retention times of albendazole and oxibendazole in the current solvent, as well as the composition of the mobile phase, were respectively 6.399 ± 0.114 and 11.095 ± 0.087. The technique exhibited high linearity between the concentration range of 100-600µg/ml and 0.5-3µg/ml for albendazole and oxibendazole, respectively. Good recovery was demonstrated by the approach at concentrations of 50, 100, and 150%
In summary, a validated RP-HPLC technique has been devised for the analysis of oxibendazole impurity in albendazole formulation and bulk. The created approach is fast, accurate, and precise since it is based on quality by design. Based on the statistical assessment of the method's good linearity and its validation for many parameters, we concluded that the suggested methodology may be used for the rapid and accurate assessment of oxibendazole impurity in pharmaceutical formulations.

Table. 10 Summary of analytical method validation parameters

S. N	Parameters		Albendazole	Oxibendazole
1	Linearity and Range		100-600µg/ml	0.5-3.0 µg/ml
2	Regression equation		Y=5998x+44979	4130x+2339
3	Regression coefficient		0.995	0.999
4	Specificity		Specific. PDA analysis: peak purity 99.7	Specific. PDA analysis: peak purity 99.5
5	Precision	Intra Day	100.96 ± 0.92	100.265 ± 1.269
		Inter Day	101.08 ± 1.12	100.60 ± 0.967
6.	Accuracy	50%	99.239	100.105
		100%	101.463	101.081
		150%	101.987	99.947
7	Robustness		The system suitability parameters were found well within acceptance criteria as per system suitability	
8	LOD		3.074 µg/ml	0.073 µg/ml
9	LOQ		9.316 µg/ml	0.091 µg/ml

5 Conclusion

The experimental methods and analytical techniques used in this study were successfully validated in accordance with ICH recommendations. To validate the proposed method, recovery studies and preliminary analysis of a standard sample were conducted. The study developed a validated RP-HPLC method for the measurement of oxibendazole impurity in bulk and pharmaceutical dosage forms. The suggested method was found to be rapid, accurate precise, and easy to use. Furthermore, the method demonstrated excellent precision and reproducibility, making it suitable for routine quality control analysis. The method's sensitivity was also satisfactory, as it could detect even trace amounts of oxibendazole impurity in pharmaceutical formulations.

6 Reference

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