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Photostability and Hydrolytic Forced Degradation Study of Albendazole: Validation of a Stability Indicating UV Spectroscopic Method Using Methanol

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KEYWORDS

Albendazole, Analytical method validation, Analytical Method development, Forced degradation

ABSTRACT:

Aim/Background: Albendazole (ABZ), a potent anthelmintic, is used against parasitic infections like giardiasis, trichuriasis and ascariasis. Forced degradation (FD) study was used to analyse the stability of drugs under stressful conditions such as elevated temperature, humidity, light, pH changes, oxidation and hydrolytic conditions. These studies determine the degradation pathways of any drug and it increases an interest to develop methods to ensure the safety as well as the efficacy of drug. The goal of this work was to develop and validate a UV Spectroscopic technique for ABZ's FD analysis using methanol.

Materials and Methods: The instrument utilized for method development was UV-Visible Spectrophotometer (Shimadzu UV-1900i) and validated as per ICH guidelines, while Sodium Hydroxide (NaOH), Hydrochloric acid (HCl) and Hydrogen Peroxide (H_2O_2) induced FD studies were carried out under various conditions.

Result: The maximum absorbance of ABZ was at 295nm, with linearity (R^2) of 0.9994. Validation studies demonstrated accuracy and precision with a percentage relative standard deviation (RSD) of lower than 2%. ABZ underwent degradation under hydrolysis and oxidative conditions. Aqueous hydrolysis of the drug shows 59.73% degradation, while acid hydrolysis showed 28.35% degradation, 18.38% degradation in alkaline hydrolysis and 35.12% in oxidative condition.

Conclusion: This study demonstrates a reliable, sensitive and straight forward analytical method for evaluating ABZ's FD using UV Spectroscopy. The ABZ drug substance showed degradation at hydrolysis and oxidative conditions. Thermal and photolytic stress had no impact on stability of ABZ.

1. INTRODUCTION

ABZ is a white or off-white crystalline powder belongs to anthelmintics, anti-custodial agents and antiprotozoal agents. ABZ has a molecular weight of 265.33 g/mol, as well as its molecular formula is $C_{12}H_{15}N_3O_2S$. ABZ is sparingly soluble in methanol and ethanol, but dissolves well in dimethylformamide. It is administered orally in tablet form (400 mg) or as a suspension (200 mg/5 mL). The drug has a melting point of 208-210 °C and should be stored in an air-tight container at room temperature. ABZ functions by attaching to colchicine site of tubulin, preventing its polymerization and preventing the assembly of microtubules and cell division. This ultimately leads to the death of the parasite. ABZ has a plasma half-life of around 8.5 hours. When taken with fatty foods, ABZ has increased absorption[1].

Pharmaceutical stability studies were designed to analyse the effects of time, temperature, humidity and radiation on the efficacy of drug substances throughout their shelf life. These studies are categorized into long-term and short-term stability studies, including real-time testing, accelerated and FD testing and photo-stability testing. FD studies involve subjecting the drug under intense conditions to detect potential degradation mechanism and products. The objectives of these studies include solving stability related problems,

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generating more stable formulations, elucidating the structure of degradation products and establishing degradation pathways. ICH guidelines such as ICH Q1A, Q1B, Q1C and Q2B guidelines outline the conditions for degradation such as hydrolytic degradation, oxidative and thermal degradation, photolytic and humidity conditions. In hydrolytic conditions the drugs are mixed with acidic or alkaline solutions and heated; oxidative conditions, where drugs are exposed to oxidation; in thermal conditions the drugs are exposed to elevated temperatures whereas in photolytic conditions the drugs are exposed to UV and visible light; and in humidity conditions the drugs are exposed to high humidity. By conducting FD studies, researchers can gain valuable information about the stability of drugs and these studies also helps to develop strategies to improve the shelf life and efficacy of the drug[2].

In this study, a straight forward, precise, cost effective technique was developed and validated according to ICH guidelines for ABZ using UV-VIS Spectroscopy and FD studies such as aqueous, acid and alkali hydrolysis, oxidative, thermal and photolytic degradation studies were performed and reported.

2. MATERIALS AND METHODS

Instrument

UV-Visible Spectrophotometer (Shimadzu UV-1900i) connected with LabSolutions UV Vis software with quartz cuvettes were used to measure the absorbance of ABZ drug substance.

Reagents and Chemicals

All the chemicals and solvents utilized in the process were AR Grade such as methanol which was used as the solvent and the reagents include HCl, NaOH and H_2O_2 solution were used for stress testing.

Preparation of Stock and Standard Solution

Stock solution of ABZ was made by solubilizing 0.01gm of the standard ABZ using methanol, then by dilution to 100 mL with methanol followed by sonication for 5 minutes, producing the concentration of 100 µg/mL. This 100 µg/mL solution was utilized for further dilution and preparation of different concentrated solution for development and validation.

Selection of wavelength (λ_{max})[10-12]

The Ultraviolet spectrum of pure ABZ drug was measured between 400 and 200 nm using Shimadzu UV-1900i UV-VIS Spectrophotometer.

Method Development[10-12]

The absorption maxima (λ_{max}) was recorded by analysing a 20 µg/mL standard solution of ABZ between 400 and 200 nm. From the stock (100 µg/mL) aliquots of 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 µg/mL concentrated solutions was prepared using methanol by appropriate dilution. Absorbances of prepared samples were measured using the λ_{max} and the measurements were performed six times and average absorbance values were taken for calibration curve.

Validation[2,3]

Linearity and Range

The ABZ drug substance showed linearity between the concentration range of 2 and 20 µg/mL. Absorbance was measured at λ_{max}, and a calibration curve was derived by plotting concentration against absorbance. This calibration curve facilitated the analysis of absorbance at the chosen wavelength. Regression approach is used for calculating the linearity.

Accuracy

Recovery studies were conducted to assess the method's accuracy, reliability and suitability. Pure ABZ was added to a pre analysed samples at 80%, 100% and 120% levels. The final concentrations in 10 mL volumetric flask were 18 µg/mL for 80% recovery (10µg sample + 8µg standard), 20 µg/mL for 100% recovery (10µg sample + 10µg standard) and 22 µg/mL for 120% recovery (10µg sample + 12µg standard). Absorbance was recorded at the specified wavelength (λ_{max}).

Precision

Intraday precision: Intraday precision was analyzed through the ABZ sample of 30 µg/mL prepared from the primary solution at various times throughout the same day, with precision assessed based on % RSD calculations.

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Interday precision: Interday precision was measured by evaluating the samples of 30 µg/mL ABZ solution on each of three consecutive days with %RSD serving as the metric for variability.

Limit of detection (LOD) and Limit of quantification (LOQ)

LOD and LOQ of ABZ were computed using standard deviation and slope of calibration curve. The LOD was computed employing the formula (3.3 × σ) / S, while the LOQ was assessed by the formula (10 × σ) / S, with both values subsequently computed. Where, σ is standard deviation of response and S is slope of calibration curve.

Ruggedness

The ruggedness was assessed by analysing the drug substance using different instruments and different analysts. A 30 µg/mL ABZ solution was analysed and the %RSD was determined to evaluate consistency and reliability.

Robustness

Robustness was assessed by analyzing the drug substance with small intentional change in the method under different conditions, such as temperature, solvent ratio and wavelength. Here, 10 µg/mL ABZ solution was examined and the %RSD was calculated to ensure the method's reliability.

Assay[2,3,11,12]

After precisely weighing twenty 400 mg ABZ tablets, an amount of tablet powder equivalent to 20 mg was made up to 100 mL using methanol. After 15 minutes of sonication, the solution underwent filtering using whatman filter paper to get the primary stock solution, which had a quantity of 200µg/mL. From this, 20 µg/mL sample solution was prepared, and the absorbance of this solution was taken into account.

Forced degradation studies[4-6,8,9]

Aqueous hydrolysis

About 10 mL of 1000 µg/mL ABZ was taken into a round bottom flask and added 10mL of distilled water and refluxed at 80 ℃ for 2 hours. After the process, cooled to room temperature and finally made up 10 µg/mL of solution using methanol. Blank preparation was done by omitting the sample.

Acid hydrolysis

About 10 mL of 1000 µg/mL ABZ was taken into a round bottom flask and added 10mL of 0.1M hydrochloric acid and refluxed at 80℃ for 2 hours. After the process, cooled to room temperature, neutralized with 0.1M sodium hydroxide solution and finally made up to 10 µg/mL of solution using methanol. Blank preparation was done by omitting the sample.

Alkali hydrolysis

About 10 mL of 1000 µg/mL ABZ was taken into a round bottom flask and added 10mL of 0.1M sodium hydroxide and refluxed at 80℃ for 2 hours. After the process, cooled to room temperature, neutralized with 0.1M hydrochloric acid solution and finally made up to 10 µg/mL of solution using methanol. Blank preparation was done by omitting the sample.

Oxidative degradation

About 10 mL of 1000 µg/mL ABZ was taken into a round bottom flask and added 10mL 3% hydrogen peroxide solution and refluxed at 80℃ for 2 hours. After the process, cooled to room temperature and finally made up to 10 µg/mL of solution using methanol. Blank preparation was done by omitting the sample.

Thermal degradation

About 100 mg of ABZ was taken in a Petri dish and kept in a hot air oven at 85℃ for 7 days to study the effect on the drug. After the process the sample was dissolved in methanol and further dilution was made to prepare 10 µg/mL.

Photolytic degradation

ABZ was subjected to photolytic degradation under UV radiation at an intensity of 1.2 million lux-h for 7 days. After the process the sample was dissolved in methanol and further dilution was made to prepare 10 µg/mL[1,5,6].

3. RESULTS

The goal of the current work was to design a sensitive, easy, quick and well-validated scientific technique for

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determining how ABZ degrades under various atmospheric circumstances. In accordance with ICH criteria, an ultraviolet spectroscopic approach was devised for the estimation involving forced degradation in the medicinal compound ABZ. Methanol was chosen as the solvent in this UV spectroscopic technique to estimate the drug compound's degradation.

Selection of wavelength

As shown in Figure 1 the maximum absorbance of ABZ standard was detected at 295nm. Figure 2 displays the overlay spectrum of ABZ at different concentration which confirms the maximum absorbance was at 295nm and it also ensures that the ABZ obeys Beer-Lambert rule[2,3].

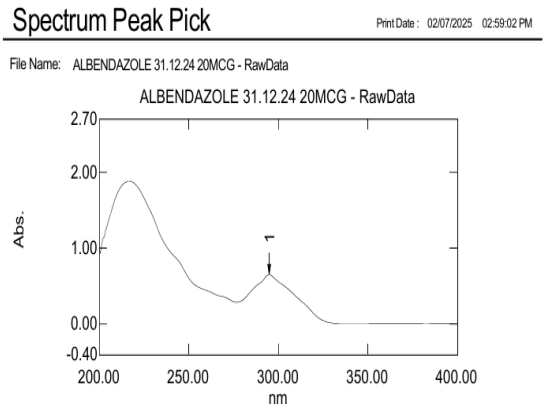

Figure 1: Wavelength maxima (λ_{max}) for the drug ABZ

Calibration curve, Linearity and Range

The ABZ was observed to show a linear response between the concentration range of 2 and 20 µg/mL. Absorbance measurements taken at 295nm and calibration curve was made, showing a high degree of linearity (R^2=0.9994) between concentration and absorbance. (Table 1 and Figure 3) [2,3].

A strong linear correlation was observed for ABZ over the concentration range between 2 and 20 µg/mL with R^2 value of 0.9994 at 295nm, as presented in Table 1 and illustrated in Figure 3.

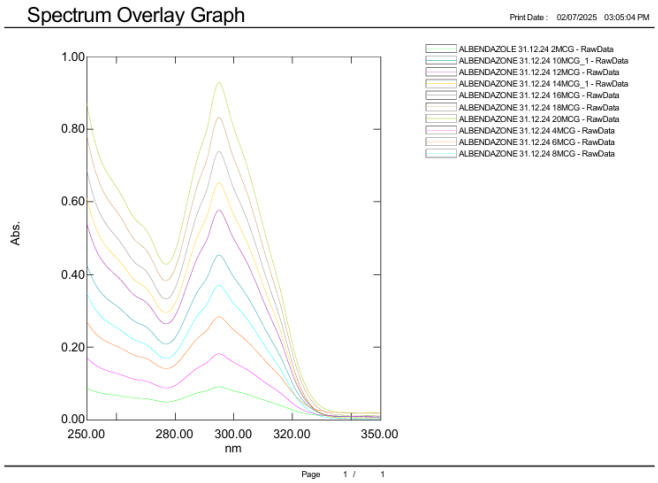

Figure 2: Spectrum overlay graph of ABZ

Table No. 1: Linearity study of ABZ

S.No	Concentration (µg/mL)	Absorbance
1.	0	0
2.	2	0.091
3.	4	0.181
4.	6	0.283
5.	8	0.370
6.	10	0.454
7.	12	0.577
8.	14	0.653
9.	16	0.739
10.	18	0.833
11.	20	0.929
Slope		0.0465
Intercept		-0.0006
Correlation Coefficient (R^2)		0.9994
LOD (µg/mL)		0.32
LOQ (µg/mL)		0.97

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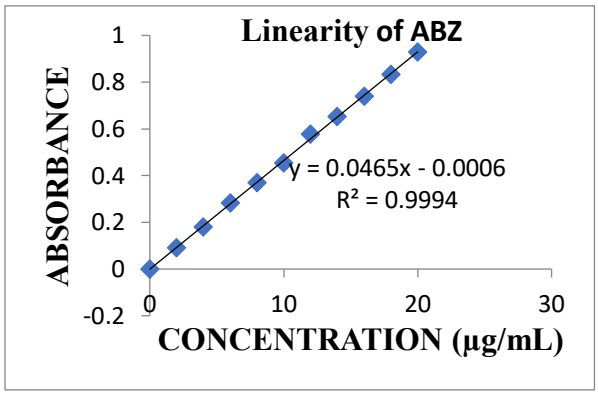

Figure 3: Linearity of ABZ at 295nm

Accuracy

The method's compatibility and accuracy were scrutinized through recovery experiments, which were carried out at distinct concentrations using the standard addition method. The recovery rates obtained were 100.29%, 100.77%, and 99.69%, indicating the method's specificity and accuracy. The % RSD was identified to be not exceeding 2%, confirming the method's accuracy which is displayed in Table 2.

Table No. 2: Accuracy studies of ABZ with statistical validation

Standard conc. (µg/mL)	Quantity added (µg/mL)	Recovery (µg/mL)	% recovery	Mean	SD	%RSD
10	8	8.01	100.12	100.29	0.0011	0.1379
10	8	8.01	100.12			
10	8	8.05	100.62			
10	10	10.01	100.01	100.77	0.003	0.3274
10	10	10.14	101.4			
10	10	10.09	100.9			
10	12	11.92	99.33	99.69	0.002	0.1958
10	12	11.96	99.66			
10	12	12.01	100.08			

Precision

A study of intraday and interday analysis was carried out and the % RSD was found to be less than 2% which was within the limit, as shown in table 3 and 4.

Table No. 3: ABZ Intraday precision

CONCENTRATION	ABSORBANCE		
	Morning	Afternoon	Evening
30µg/mL	1.461	1.44	1.494
	1.457	1.448	1.518
	1.476	1.457	1.531
MEAN	1.4644	1.4483	1.514
SD	0.0100	0.0085	0.0187
%RSD	0.6787	0.5762	1.2719

Table No. 4: ABZ interday precison

CONCENTRATION	ABSORBANCE		
	DAY1	DAY 2	DAY 3
30µg/mL	1.482	1.523	1.496
	1.493	1.538	1.523
	1.509	1.549	1.527
MEAN	1.4946	1.5366	1.5153
SD	0.0135	0.0130	0.0168
%RSD	0.8958	0.8611	1.1126

LOD and LOQ

The LOD of ABZ was 0.32 µg/mL and LOQ of ABZ was 0.97 µg/mL as presented in Table 1.

Ruggedness and Robustness

The ruggedness analysis was performed by several analysts and instruments at different times and validated and robustness method was also performed by slightly altering the method conditions such as wavelength, solvent ratio, temperature. Table 5 displays the percentage RSD of ruggedness and robustness which was found to be less than 2 %[2].

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Table No. 5: ABZ Ruggedness and Robustness study

Ruggedness			
Concentration	**Absorbance**		
	Analyst 1	**Analyst 2**	**Analyst 3**
30µg/mL	1.486	1.496	1.508
	1.496	1.499	1.508
	1.489	1.465	1.502
MEAN	1.4903	1.4866	1.506
SD	0.0051	0.0188	0.0034
%RSD	0.3443	1.2661	0.2300
Robustness			
Concentration	**Absorbance**		
	SAMPLE1	**SAMPLE2**	**SAMPLE3**
10µg/mL	0.621	0.609	0.616
	0.613	0.602	0.608
	0.617	0.606	0.612
MEAN	0.6153	0.6076	0.6116
SD	0.0060	0.0055	0.0055
%RSD	0.9795	0.9063	0.9004

Assay[2,3,11,12]

Twenty ABZ tablets were utilized for the quantification of the drug substance in the tablet formulation. The % RSD was identified to be less than 2% and the same was represented in Table 6.

Forced Degredation Studies

The FD study was performed with various conditions of acid (0.1M HCl), Base (0.1M NaOH), oxidation (3% H_2O_2), photolysis and thermal as per the requirement of ICH. The ABZ was found to be degraded at aqueous, acid, alkali and oxidative condition. The percentage degradation of ABZ was shown in table 7.

Table No. 6: Assay of ABZ tablets

Sample No.	**1**	**2**	**3**
Quantity taken (µg/mL)	20	20	20
Quantity found (µg/mL)	20.35	20.22	20.22
% Obtained	101.75	101.10	101.10
Average (%)	101.32		
SD	0.3753		
%RSD	0.3704		

Table No. 7: Forced degradation study of ABZ

Condition	**Temperature (°C)**	**Duration**	**Initial conc. (µg/ml)**	**Final conc. (µg/ml)**	**% degradation**
Aqueous hydrolysis	80	2 hours	10.23	4.12	59.73
Acid hydrolysis	80	2 hours	9.7	6.95	28.35
Alkaline hydrolysis	80	2 hours	10.34	8.44	18.38
Oxidation	80	2 hours	10.11	6.56	35.12
Thermal	85	7 days	10.16	10.13	_
Photolytic	_	7 days	_	10.11	_

4. DISCUSSION

The goal of the current work was to develop and validate a UV spectroscopy based FD method of study for ABZ. The solvent of choice was methanol and the wavelength maxima was identified to be 295 nm with a R^2 value of 0.9994. The calibration curve demonstrated a good linear connection across the concentration span of 2 to 20 µg/mL. This suggests that the absorbance and concentration have a strong relationship. Recovery rates of 100.29%, 100.77% and 99.69% were obtained from accuracy study by standard additional method at various levels i.e. 80%, 100% and 120%. The quantification

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approach developed was turned out to be straight forward, cost-efficient and time-efficient with acceptable limits in validation parameters. To get a concentration within the linearity range, the formulation was diluted after being extracted with methanol. Aqueous, acid and alkali hydrolysis, oxidation, thermal and photolytic degradation were among the parameters in FD investigations. The findings demonstrated that ABZ was stable in thermal and photolytic settings but degraded in hydrolytic and oxidative conditions.

5. CONCLUSION

A simple, cost effective, accurate and precise stability indicating UV-VIS spectroscopic technique for quantification of ABZ drug substance was developed and validated. In stability studies, ABZ drug substance showed degradation at hydrolytic and oxidative conditions. Thermal and photolytic stress had no discernible impact on the reliability of the drug. Validation results showed that the developed method was effective in estimating the content of ABZ in tablet formulation without the interference of excipients and related substances. The proposed approach might be applied for the everyday quality control test of ABZ drug substance. However, to get a comprehensive understanding of degradation, degradation products and metabolites further studies employing advanced analytical techniques are necessary.

CONFLICT OF INTEREST

No conflict of interest.

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