European Journal of Chemistry

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pH and time effectiveness on azithromycin drug: A spectrophotometric approach

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RESEARCH ARTICLE

10.5155/eurjchem.15.2.120-127.2522

Received: 20 February 2024
Received in revised form: 7 April 2024
Accepted: 27 April 2024
Published online: 30 June 2024
Printed: 30 June 2024

KEYWORDS

Reduction
Drug stability
Acidity effect
Azithromycin
Spectrophotometry
Potassium permanganate

ABSTRACT

The present study describes a straightforward method to assess the quality control and diagnostic characteristics of three different brands of film-coated azithromycin tablets. The method is based on the reduction of potassium permanganate in a slightly alkaline solution using azithromycin. The effects of acidity and time were investigated to evaluate the reliability of the method. A spectroscopic technique was used to determine the concentration of azithromycin in a sample by measuring the decrease in potassium permanganate absorbance at a specific wavelength of 547 nm. Azithromycin causes decolorization of potassium permanganate with reduction. The method allowed the determination of azithromycin concentrations ranging from 3-15 µg/mL in the final solution. The usual components present in the azithromycin tablets were observed not to interfere with the method. The results obtained for the determination of azithromycin in tablets were in good agreement with the allowed limit.

Cite this: *Eur. J. Chem.* **2024**, *15(2)*, 120-127 **Journal website**: www.eurjchem.com

1. Introduction

Azithromycin has been widely used for the treatment of sexually transmitted infections and respiratory diseases since the mid-1980s [1]. Initially developed in 1980, its intended applications included the treatment of skin infections, uncomplicated chlamydial infections, and bacterial infections of the upper and lower respiratory tracts. Azithromycin is classified as a broad-spectrum antibiotic and is commonly prescribed for both adult and pediatric patients due to its increased efficacy against Gram-negative bacteria and its favorable safety profile [2]. Azithromycin is extensively used in clinical settings for the management of various bacterial infections, encompassing upper respiratory tract infections such as sinusitis and otitis media, lower respiratory tract infections, Helicobacter pylori infections, sexually transmitted infections, including gonorrhea, trachoma, and early syphilis, as well as urinary tract infections [3-7]. Azithromycin has been utilized as an additional treatment for chronic and aggressive periodontitis, providing various degrees of clinical and microbiological benefits. Although azithromycin is known for its antibacterial properties, it has also been demonstrated to possess supplementary anti-inflammatory and immunological effects [8].

The use of azithromycin as an antibiotic among hospitalized COVID-19 patients is alarmingly prevalent, with a high prescription rate [9]. The risk of bacterial superinfection, coupled with the challenge of distinguishing it from typical symptoms of COVID-19, serves as the main driving factor. In particular, early bacterial coinfection has been recognized as a significant contributor to morbidity and mortality in previous influenza pandemics [10]. Compared with erythromycin, azithromycin exhibits a broader spectrum of activity against Gram-negative bacteria and demonstrates considerable efficacy against Gram-positive species as well. Azithromycin, also known as an azalide antibiotic, possesses two basic amine groups on its 15-membered macrolide ring (Figure 1), which distinguishes it structurally from other macrolide antibiotics [11]. This unique ring configuration enhances its pharmacokinetics and antimicrobial coverage, while providing resistance against degradation in acidic environments [12].

https://dx.doi.org/10.5155/eurjchem.15.2.120-127.2522

Table 1. Weight variation results.

Brand	Weight, mg, mean±SD									
D1	W (mg)	742.4	721.1	722.6	729.6	707.4	745.5	720.4	713.1	730.4	730.2
	Mean±SD	726.3±2.2	726.3±0.7	726.3±0.5	726.3±0.45	726.3±2.60	726.3±2.64	726.3±0.81	726.3±1.81	726.3±0.56	726.3±0.53
Mean±SD = 726.3±12.5											
D2	W (mg)	862.3	871.7	866	866.5	872.8	870.7	867.5	868.4	871.1	867.0
	Mean±SD	868.4±0.7	868.4±0.38	868.4±0.27	868.4±0.27	868.4±0.5	868.4±0.2	868.4±0.1	868.4±0.0	868.4±0.2	868.4±0.16
Mean±SD = 868.4±3.203											
D3	W (mg)	961.2	980.0	969.7	964.5	957.7	965.1	963.2	985.5	998.4	980.4
	Mean±SD	972.6±1.17	972.6±0.76	972.6±0.29	972.6±0.83	972.6±1.5	972.6±0.77	972.6±0.96	972.6±1.32	972.6±2.65	972.6±0.8
Mean±SD = 972.6±12.98											

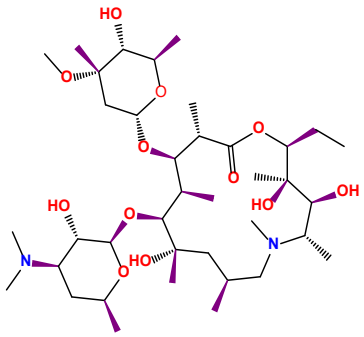


Figure 1. Structure of azithromycin.

This study aims to evaluate the quality of azithromycin tablets available in the market of Aden City, Yemen, using an ultraviolet/visible spectrophotometry instrument. This investigation uses a spectrophotometric method to assess the acidic and time-dependent efficacy of azithromycin. The main objective of this study is to understand how azithromycin behaves under acidic conditions and over time, as this knowledge is essential to enhance its therapeutic application. Unlike previous studies that have used different analytical methods or explored various aspects of azithromycin effectiveness while considering a wide range of factors or drugs, this research focuses solely on the acidic and time effectiveness of azithromycin. By shedding light on the understanding of azithromycin effectiveness, this study, along with the existing literature, contributes to scientific knowledge and offers valuable insights that can guide future investigations and clinical practice.

2. Experimental

Samples of azithromycin tablets were obtained from different sources, Azecen (500 mg/tablet, RFA Pharmaceutical Ind., Seiyun, Hadhramaut, Yemen), Zithrocin tablets (500 mg/tablet, Indi Pharma Private. Ltd., Ankleshwar, India), and Azicor tablets (500 mg/tablet, Bal Pharma Ltd., Rudrapura, India). Analytical grade potassium permanganate, potassium dihydrogen phosphate, and potassium hydrogen phosphate were procured from Glaxo Ltd., Mumbai, India. Absorbance measurements were carried out using an S-3100 Spectrophotometer equipped with a UV/Vis spectroscopy module and 10-matched quartz cells.

2.1. Weight variation test

To assess the quality of tablet manufacturing, a weight variation test was performed. This test is a standard procedure outlined in the Pharmacopeia for the evaluation and quality control of tablets. According to the United States Pharmacopeia (USP), British Pharmacopoeia (BP), and Indian Pharmacopoeia (IP), the acceptable limit for weight variation is ±5 [13,14]. For each batch, a random selection of 10 tablets was made and individually weighed to determine any variation in weight. The results of this test can be found in Table 1. To determine the upper and lower limits within the allowed percentage difference, further calculations are required (Equations 1 and 2):

$$\text{Average weight (\%)} = \frac{\text{Total weight}}{\text{No}} \times 100 \quad (1)$$

$$\text{Weight variation (\%)} = \frac{\text{Weight} - \text{Average weight}}{\text{Average weight}} \times 100 \quad (2)$$

2.2. Assay of commercial dosage form by the proposed method

Three commercially available azithromycin products, namely, Azicure (D1), Azicine (D2), and Zithrocin (D3), were obtained for analysis. For each formulation, ten tablets were carefully weighed and subsequently powdered [15]. The equivalent of 10 mg of azithromycin powder was then dissolved in a 1:1 solution of absolute ethanol and phosphate buffered saline (PBS) with a pH of 7.2, resulting in a solution of 2 mL. This solution was further diluted to a final volume of 100 mL using distilled water, followed by sonication for 15 minutes and filtration. The residues were thoroughly washed with distilled water. The resulting filtrate, along with the washing solution, was combined in a 100 mL volumetric flask and further diluted to the mark with distilled water, resulting in a final concentration of 0.10 mg/mL. To prepare the reaction mixture, 1 mL of the solution was taken and mixed with 1 mL of 0.01 M potassium permanganate solution and 1 mL of phosphate buffer with a pH of 7.2. The mixture was thoroughly mixed and further diluted to the mark with distilled water in a 10 mL volumetric flask. The reaction was allowed to proceed for 30 minutes. The absorbance of the resulting solutions was measured against a reagent blank in triplicate. The percentage content of azithromycin was then determined using Equation 3:

$$\text{\% Content} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 100 \quad (3)$$

2.3. Preparation of Mn (VII) stock solution

To prepare the Mn (VII) stock solution (0.01 mol/L), 0.16 g of potassium permanganate was dissolved in distilled water and then diluted to 100 mL [16]. Then 10 mL of the potassium permanganate solution (0.01 mol/L) was added to a 100 mL volumetric flask and diluted to a final volume of 100 mL.

Table 2. Optical characteristics of the proposed procedure.

Parameters *	Azithromycin
λ_{max}, nm	547
Beer's limit (µg/mL)	3-15
Molar absorptivity (L/mol/cm)	2.1994×10^4
Slope (b)	0.00346
Intercept (a)	0.128
Coefficient of variance (10 µg/mL of azithromycin)	0.03 (n = 3)
R^2	0.9787

* Optical characteristics for azithromycin such as Beer's law, molar absorptivity, and Sandell's sensitivity.

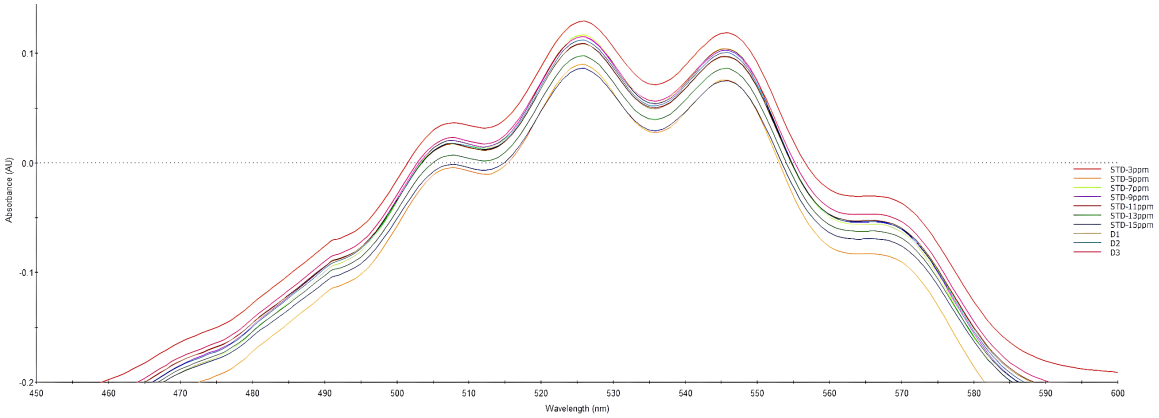


Figure 2. Absorption spectra of azithromycin+$KMnO_4$.

2.4. Preparation of the phosphate buffer solution (pH = 7.2)

In order to prepare a 100 mL solution of phosphate buffer (PBS), separate solutions of 1 M potassium hydrogen phosphate and 1 M potassium dihydrogen phosphate were first prepared. This was achieved by dissolving 34.8 g of potassium hydrogen phosphate and 21.5 g of potassium dihydrogen phosphate in 250 mL of distilled water for each solution. Subsequently, 71.7 mL of the potassium hydrogen phosphate solution was combined with 28.3 mL of potassium dihydrogen phosphate solution in a 100 mL volumetric flask. The pH of the resulting mixture was then accurately determined using a pH meter.

2.5. Preparation of standard azithromycin stock solution

The standard stock solution of azithromycin (100 µg/mL) was prepared by accurately weighing 10 mg/g and dissolving in a 1:1 solution of absolute ethanol: PBS (pH = 7.2) 3 mL and then diluted to 100 mL with distilled water [17].

2.6. Preparation of standard curve of azithromycin

In this work, aliquots of a standard azithromycin solution with concentrations ranging from 3-15 µg/mL were carefully transferred into a series of 10 mL volumetric flasks. To each flask, 10 mL of a potassium permanganate solution (0.01 M) was added, followed by the addition of 1.0 mL of phosphate buffer solution (PBS). To ensure consistency and homogeneity, the volume of the solution in each flask was adjusted to 10 mL by carefully adding water and thoroughly mixing the contents. After allowing the solutions to sit for 30 minutes, the absorbance of each solution was measured at a wavelength of 547 nm. To establish a baseline, a solution of absolute ethanol and PBS was used to zero the absorbance [18].

2.7. Preparation samples D1, D2, and D3

A total of 10 tablets of azithromycin were precisely weighed to determine the average weight per tablet. The tablets were then ground to a fine powder and an amount equivalent to 10 mg of powder was accurately weighed. To prepare the solution, the powdered sample was dissolved in a mixture of absolute ethanol and phosphate buffer solution (PBS) in a 1:1 ratio, with a pH of 7.2. The total volume of the solution was 3 mL, which was then further diluted with distilled water. To ensure the removal of any remaining solid particles, the solution was carefully filtered using Whatman Filter Paper No. 41 into a 100 mL volumetric flask. The residue left on the filter paper was thoroughly rinsed with distilled water and the washings were combined with the filtrate. Finally, the volume of the filtrate was adjusted to the mark on the volumetric flask by adding distilled water, bringing it to a total volume of 100 mL. The resulting solution was then subjected to analysis using the recommended procedure.

3. Results and discussions

3.1. Results of weight variation

In this experimental part, the objective was to assess the weight variation of the tablets. The results obtained were used to determine the weight ranges and variations for each tablet type. Table 1 presents the weight measurements for the tablets of D1 drug. The minimum and maximum weights observed ranged from 707.4 to 745.5 mg, with variations between ±2.60 and ±2.64. For tablets of D2 drug, the weight measurements ranged from 862.0 to 872.8 mg, with deviations ranging from ±0.70 to ±0.50. Lastly, the tablets of the D3 drug exhibited a minimum weight of 957.7 mg and a maximum weight of 998.4 mg, with deviations ranging from ±1.52 to ±2.65. On the basis of these results, it can be concluded that the tablets showed uniformity among themselves, meeting the criteria set by the experiment. The weight variation limit of ±5, which indicates that each tablet is identical, was not exceeded [19,20].

3.2. Stability against an oxidizing agent (potassium permanganate)

To establish the calibration curve for azithromycin, the recommended procedure was followed (Figure 2).

Table 3. Amount of investigated drugs.

Brands	Assay %, mean±SD, n = 3
D1	104.71±0.03
D2	100.96±0.06
D3	108.56±0.06

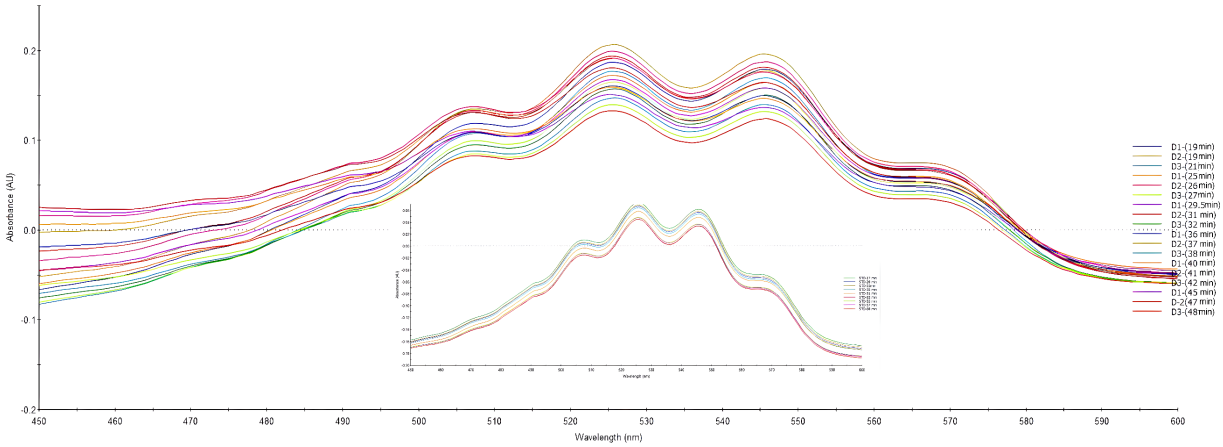


Figure 3. Stability oxidation reaction of azithromycin samples (D1, D2, D3) and standard solution (STD; 11 ppm) (inset) by potassium permanganate.

The procedure showed a linear relationship between absorbance and concentration. The concentration range used for the calibration curve was 2-20 µg/mL. Additional parameters related to the calibration curve are provided in Table 2 [21].

According to the recommended procedure [18], azithromycin was added to a solution containing 1.0 mL of potassium permanganate and 1.0 mL of PBS. To ensure homogeneity, the resulting mixture was diluted in 10 mL of deionized water with a thorough mixing. Then a calibration curve for azithromycin was constructed based on absorbance measurements. The linear relationship between absorbance and pure azithromycin concentration was observed within the range of 3-15 µg/mL.

To determine the percent content of azithromycin in market samples, the absorbance and peak area responses were measured using the proposed method. The percent content of azithromycin in commercial preparations was found to be 104.71% for D1, 100.96% for D2, and 108.56% for D3 (Table 3). It should be noted that the addition of potassium permanganate, a potent oxidizer, can cause a color change in the azithromycin standard solution, which in turn affects the absorbance of the solution. Therefore, it was essential to study the color stability of the chromogen to ensure accurate measurements. The color remained stable for approximately one hour, which allowed for reliable analysis. The proposed method for tablet formulation analysis demonstrated consistent and accurate results that were consistent with the claims on the drug label. The method was validated and proven to be straightforward, sensitive, accurate, and precise. The excipients present in the tablet dosage form did not interfere with the determination of azithromycin. Hence, this technology can be successfully employed for the routine analysis of azithromycin in tablet formulations [22].

3.3. Effect of time on azithromycin degradation

One milliliter of the stock solution was transferred to ten milliliter volumetric flasks. To each of these flasks, 1.0 mL of potassium permanganate solution (0.01 M) was added. The volume was then increased to 10 mL by adding PBS with a pH of 7.2, and the solution was thoroughly mixed. The absorbance of these solutions was measured at room temperature at various time intervals, as shown in Figures 3 and 4. Sivasubramanian *et al.* [23] reported that the visible spectrophotometric method for the determination of azithromycin in tablets lacks specificity. Furthermore, Sultana *et al.* [24] found that the rate of azithromycin degradation depends on the time interval. As the time interval increases, the absorbance gradually increases as well. The optimal time for each acid was determined as the time at which there was 100% recovery of the drug.

Based on the data depicted in Figure 4, it was observed that the reaction remained stable for a duration of 25 to 40 minutes. However, beyond this time frame, a decrease in absorbance was observed, accompanied by the appearance of a brown color. This brown color corresponds to the formation of manganese oxide (Mn^{2+}). These observations suggest that azithromycin is undergoing degradation in the presence of Mn ions, leading to a reduction in the oxidation state from +7 to +2 in the alkaline solution. In a related study conducted by Rodríguez-López *et al.* [25]. The degradation experiments conducted in water at pH = 4.0 revealed that azithromycin had a half-life of 216 hours. The degradation process was influenced by exposure to light or dark conditions during specific time intervals. The color remains stable for approximately an hour [22].

3.4. Effect of pH

In this experiment, 1 mL of azithromycin stock solution was transferred to multiple 10 mL volumetric flasks. To each flask, 1 mL of potassium permanganate (0.01 M) was added. The volume was then adjusted to 10 mL using a series of phosphate buffered saline (PBS) solutions with pH values ranging from 3.5 to 8. The solutions were carefully mixed and left at room temperature for 30 minutes. The absorbance of these solutions was measured at 525 nm (for Mn^{2+} produced and complexed with azithromycin) and 547 nm (for Mn^{7+} remaining) using Figures 5-7 as a reference for the maximum wavelength (λ_{max}). In their investigation, Rodrguez-López *et al.* [25] altered the pH level of the solution to 4.0 by employing 0.5 M NaOH and 0.5 M HCl. Furthermore, another investigation [24] examined the impact of acidity using various acids such as sulfuric, hydrochloric, phosphoric, and nitric acids.

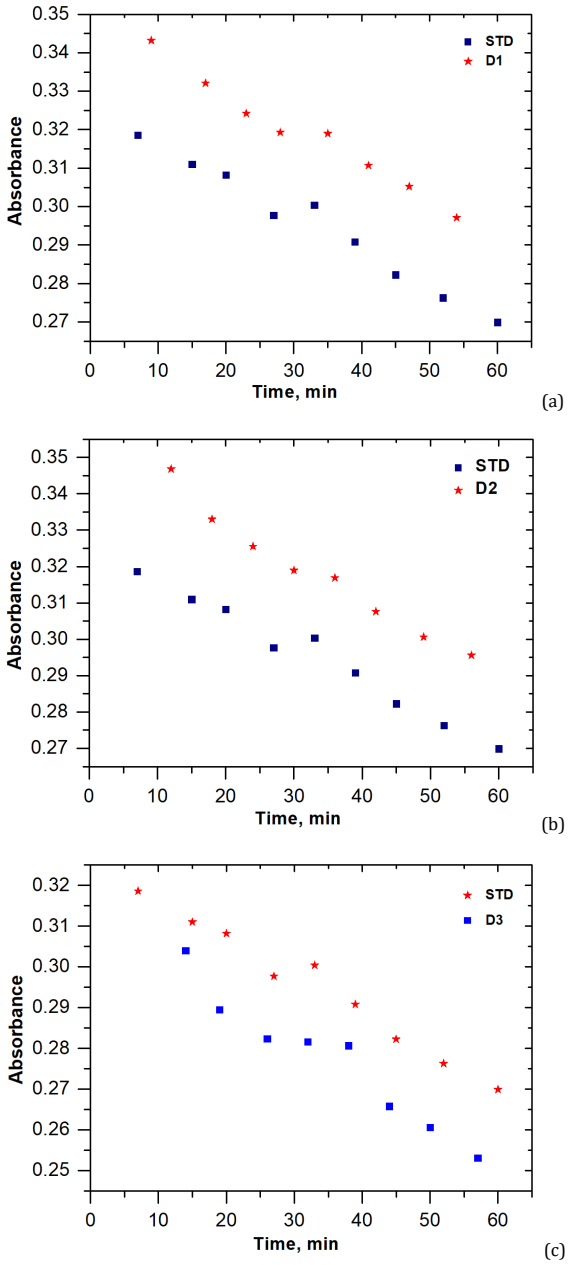


Figure 4. Relationship between absorbance (au) and time (min) for a standard solution (STD; 11 ppm) and samples; (a) D1, (b) D2, and (c) D3.

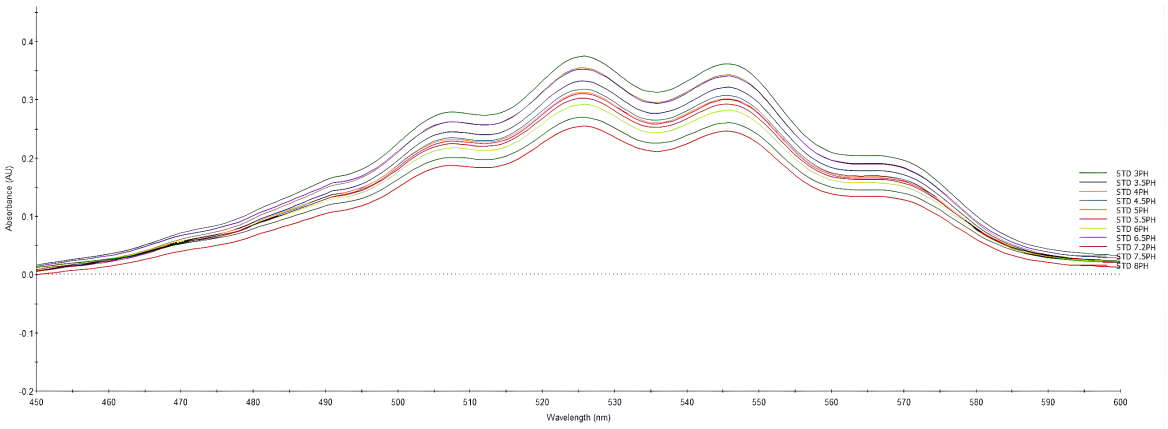


Figure 5. Absorption (au) *versus* wavelength (nm) for the effect of pH on STD.

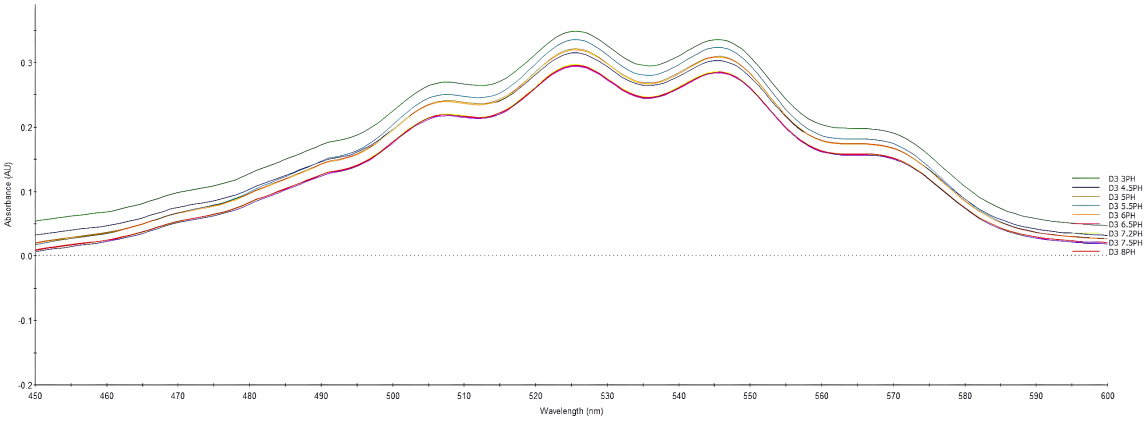
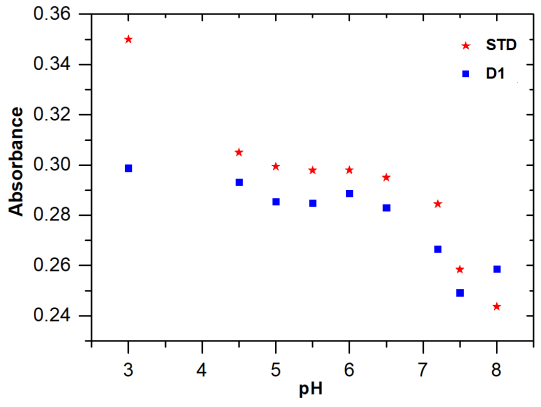
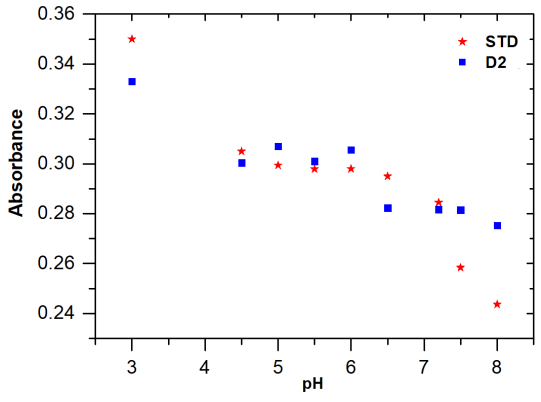


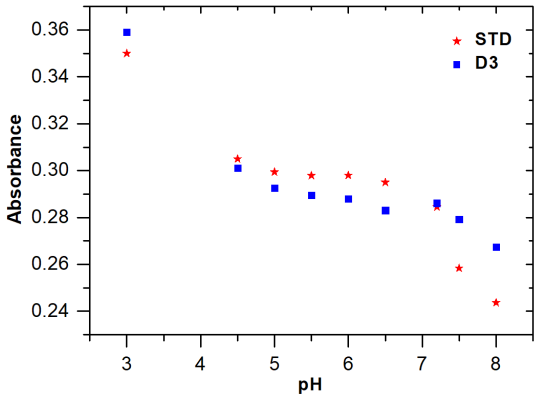
Figure 6. Absorption (au) *versus* wavelength (nm) spectra for the pH effect on D3.



(a)



(b)



(c)

Figure 7. Relationship between absorbance (au) and pH for standard solution (STD) and samples (D1, D2, and D3).

Interpreting the information in Figure 7, it can be inferred that the absorbance of potassium permanganate remains more consistent in an acidic medium compared to an alkaline one. However, the chemical interaction between potassium permanganate and azithromycin appears to be less favorable under acidic conditions. It is assumed that when the concentration of azithromycin decreases in an alkaline environment, naturally occurring media may be the optimal environment for its activity. Potassium permanganate exhibits accelerated degradation at elevated pH levels. The characteristic of maximum stability at pH between 5.0 and 6.5 for azithromycin has been previously reported by Farghaly and Mohamed [26], as well as Zhang *et al.* [27]. Furthermore, Čizmič *et al.* [28] found no significant effect on the degradation of azithromycin when varying the pH between 3.0 and 7.0. However, they did observe an increase in azithromycin degradation when the pH approached 10.

4. Conclusions

The weight variation uniformity test, as outlined in the Pharmacopeia, was successfully passed by all three brands of azithromycin tablets. This test stipulates that the average weight deviation for tablets should not exceed 5%. The observed differences in the mean weights across all brands could be attributed to the use of varying excipients in each brand. The spectrophotometric estimation of azithromycin demonstrated an interaction with potassium permanganate. It was found that the excipients present in the tablet formulation did not interfere with the determination of azithromycin. As a result, this method proves effective for routine analysis of azithromycin in tablet formulations. The quantity (%) and relative standard deviation (RSD) were determined to be 104.71, 0.3067 for brand D1, 100.96, 0.6623 for brand D2, and 108.56, 0.5491 for brand D3. This signifies that all brands were within their allowable limits.

Acknowledgements

We thank the Supreme Board of Drug and Medical Appliances, Aden, Yemen, for providing the UV-vis spectroscopy instrument to complete this research.

Disclosure statement

Conflict of interest: The authors declare that they have no conflict of interest.
Ethical approval: All ethical guidelines have been adhered to.
Sample availability: Samples of the compounds are available from the authors.

CRediT authorship contribution statement

Conceptualization: Adel Saeed; Methodology: Adel Saeed, Mokhtar Al-Sallmi, Amani Muthanna; Software: Ahmed Ahmed, Musab Hamood; Validation: Adel Saeed; Formal Analysis: Adel Saeed, Fadhel Qasam, Maysa Saleh; Investigation: Adel Saeed, Ahmed Ahmed, Fadhel Qasam; Resources: Ahmed Ahmed, Fadhel Qasam, Hadeel Alwan, Ibrahim Mohmed, Musab Hamood; Data Curation: Adel Saeed, Amani Muthanna; Writing - Original Draft: Ahmed Ahmed, Fadhel Qasam, Hadeel Alwan, Ibrahim Mohmed, Musab Hamood; Writing - Review and Editing: Adel Saeed, Mokhtar Al-Salimi, Ahmed Ahmed, Amani Muthanna; Visualization: Adel Saeed, Mokhtar Al-Salim; Supervision: Adel Saeed; Project Administration: Adel Saeed, Amani Mothana.

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