

Comparative In-Use Stability of Azithromycin Oral Suspension: Influence of Reconstitution Water, Storage Conditions, and Brand Formulation: A Foundation for Nanosuspension Development

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ABSTRACT

Background: The quality of the reconstitution water, storage temperature, and brand-specific formulation critically influence the in-use stability of reconstituted azithromycin oral suspensions. This study investigated these factors using two generic brands (Azimax® and Zomax®) reconstituted with tap water, deionized water, and sterile distilled water, stored at room temperature (27°C) and under refrigeration (2-8°C) for seven days.

Objective: This study investigates azithromycin suspension stability using three reconstitution water types and two storage conditions across two generic brands.

Method: A validated HPLC method with UV detection at 210 nm was employed for analysis. We determined degradation kinetics using zero-order, first-order, and second-order models.

Results: Azimax® demonstrated superior stability across all conditions, remaining within USP specification (2022) (95-105%) for up to six days with tap water at room temperature, compared to four days for Zomax®. Refrigeration did not consistently enhance stability, with Azimax® showing better stability at room temperature with tap water and deionized water. Degradation followed zero-order kinetics for both brands, with Zomax® exhibiting 2-5 times faster degradation rates ($k = 6.10-11.98\%$ /day) than Azimax® ($k = 1.16-2.80\%$ /day).

Conclusion: The observed degradation patterns highlight the critical importance of brand selection, reconstitution water quality, and appropriate storage recommendations while informing formulation development. This work provides critical baseline data that will guide the development of next-generation azithromycin nanosuspensions with enhanced stability profiles using nanoparticle engineering approaches.

Keywords

Azithromycin, Brand comparison, Degradation kinetics, Storage stability, Reconstitution, Generic formulations, HPLC, Zero-order kinetic.

Introduction

Stability is defined as the capacity of a drug substance or product to remain within established specifications, maintaining its

identity, strength, quality, and purity throughout its shelf life [1]. Oral liquid antibiotics typically have short stability periods, and in-use stability testing provides essential labeling information on preparation procedures, storage conditions, and use periods for multi-dose products after reconstitution [2,3]. Developing stable oral liquid formulations, particularly for pediatric use, remains a significant pharmaceutical challenge.

Azithromycin, a broad-spectrum macrolide antibiotic derived from erythromycin, exhibits improved acid stability due to its 15-membered ring structure with a methyl-substituted nitrogen [4]. However, degradation occurs primarily via acid-catalyzed hydrolysis of the ether bond, and temperature and pH influence stability [5,6]. Generic formulations may differ significantly in excipient composition and manufacturing processes, potentially leading to substantial variations in stability profiles.

In Sudan and other regions with challenging storage conditions, concerns about pharmaceutical stability are increasing due to frequent power outages and limited refrigeration access [7]. Patients often use alternative water sources for reconstitution, including tap water, when sterile water is unavailable.

Recent advances in nanoparticle engineering offer promising approaches to address stability and bioavailability challenges [8]. Nanosuspensions provide enhanced dissolution rates, improved stability through optimized stabilizer systems, and better patient compliance [9,10]. However, the rational design of such nanosuspensions requires a precise understanding of the degradation behaviour of the conventional suspension they are intended to replace. To date, no study has systematically compared the in-use stability of different generic azithromycin suspensions reconstituted with locally available water sources under storage conditions typical of resource-limited settings. This study therefore investigates azithromycin suspension stability using three reconstitution water types and two storage conditions across two generic brands, intending to establish a foundation for the rational design of more stable azithromycin nanosuspensions [11,12].

Materials and Methods

Materials

Azithromycin standard was obtained from Amipharm Laboratories (Sudan). HPLC-grade methanol and acetonitrile were purchased from Scharlau, Spain. Sodium 1-octanesulfonate was obtained from Central Drug House, India. Azimax® and Zomax® (azithromycin 200 mg/5 mL) were obtained from local pharmacies in Sudan. Deionized water, tap water, and sterile distilled water were used for reconstitution studies.

HPLC Method

Chromatographic Conditions:

- Column: ODS C18 (250 mm × 4.6 mm), 5 µm particle size
- Mobile phase: Acetonitrile: Octanesulfonate buffer: Methanol (40:40:20)
- Flow rate: 1.5 mL/min
- Detection wavelength: 210 nm
- Column temperature: 30°C
- Injection volume: 20 µL

Preparation of Mobile Phase: Octanesulfonate buffer (4.4 g dibasic potassium hydrogen orthophosphate and 500 mg sodium 1-octanesulfonate in 1000 mL water, pH adjusted to 8.2) was mixed with acetonitrile and methanol (40:40:20), filtered through

a 0.45 µm membrane, and degassed by sonication.

Standard Preparation: Accurately 0.04 g azithromycin standard was dissolved in 100 mL diluent (ammonium phosphate buffer pH 10:methanol: acetonitrile, 35:35:30) to obtain a 0.4 mg/mL concentration, filtered through a 0.45 µm nylon filter.

Sample Preparation: Azithromycin containers were reconstituted with the appropriate water volume (Azimax®: 9 mL labeled, 11 mL found; Zomax®: 8.5 mL labeled, 9 mL found), shaken for 15 minutes, allowed to stand for 30 minutes, and shaken again for 15 minutes. Before sampling, bottles were shaken for 20 seconds. 0.5 mL suspension was transferred to a 50 mL volumetric flask and completed with mobile phase, filtered, and injected.

Stability Study Design

Samples were stored at condition A (room temperature, 27°C) and condition B (refrigeration, 2-8°C) for seven days. Analysis was performed on days 1, 2, 3, 4, 5, 6, and 8. We determined degradation kinetics using zero-order (concentration vs. time), first-order (ln concentration vs. time), and second-order (1/concentration vs. time) models with linear regression analysis (R^2 values).

Statistical Analysis

Data were expressed as mean ± SD (n = 3). We defined the stability period as the number of days the product remained within 95–105% [13] of the label claim and treated it as a discrete, censored variable. Because the data were non-normally distributed and included censored values (<1), we used non-parametric methods. A p-value < 0.05 was considered statistically significant. All analyses were performed using SPSS version 27.0.

Results and Discussion

HPLC Method Validation

A sharp, symmetric peak was obtained for azithromycin at a retention time of approximately 10.5 minutes with no interference from degradation products or excipients (Table 1). System suitability parameters confirmed method suitability as stability-indicating.

Table 1: System suitability parameters for azithromycin standard:

Parameter	Mean ± SD	%RSD
Retention time (min)	10.760 ± 0.308	2.865
Area	526,785.5 ± 23,275.8	4.418
Tailing factor	1.085 ± 0.041	3.780
Theoretical plates	8,033.5 ± 904.4	11.257

Brand-Specific Stability

Azimax® demonstrated consistently superior stability compared to Zomax® across all storage conditions (Table 2). Due to the discrete nature of the stability data and the small sample size (n = 3), non-parametric analysis was applied; the difference between brands was statistically significant (p < 0.05). The stability periods are summarized in Table 2.

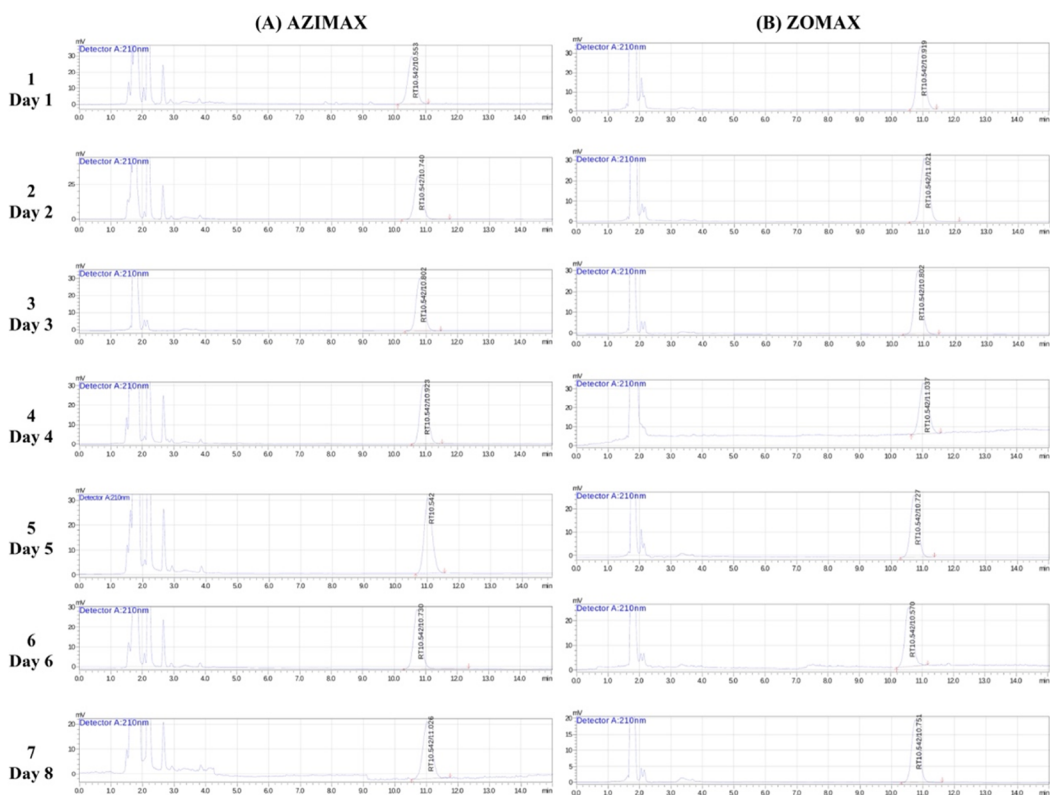


Figure 1: Comparative stability chromatogram profiles of A. Azimax® and B. Zomax® at room temperature using tap water for reconstitution - Day 1, Day 2, Day 3, Day 4, Day 5, Day 6, Day 8.

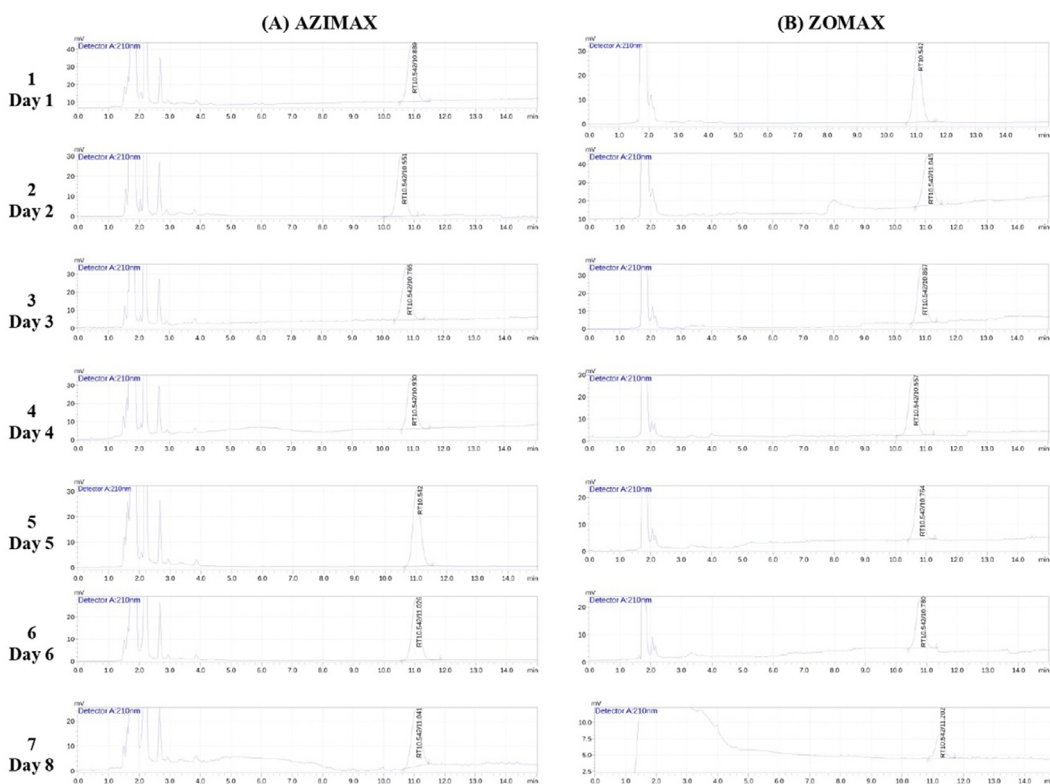


Figure 2: Comparative stability chromatogram profiles of A. Azimax® and B. Zomax® at Fridge using tap water for reconstitution - Day 1, Day 2, Day 3, Day 4, Day 5, Day 6, Day 8.

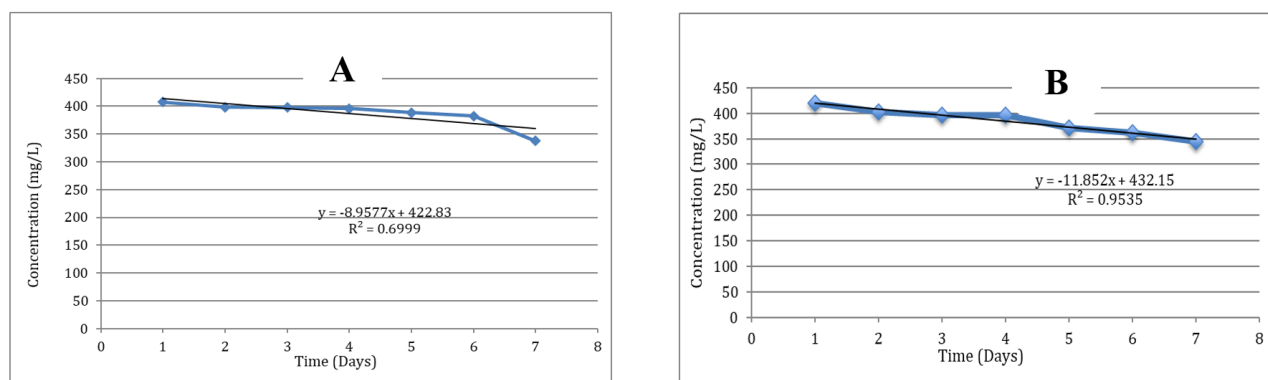


Figure 3: Degradation kinetics representing zero-order kinetics of Azimax® samples: A. at room temperature; B. in the fridge using tap water for reconstitution.

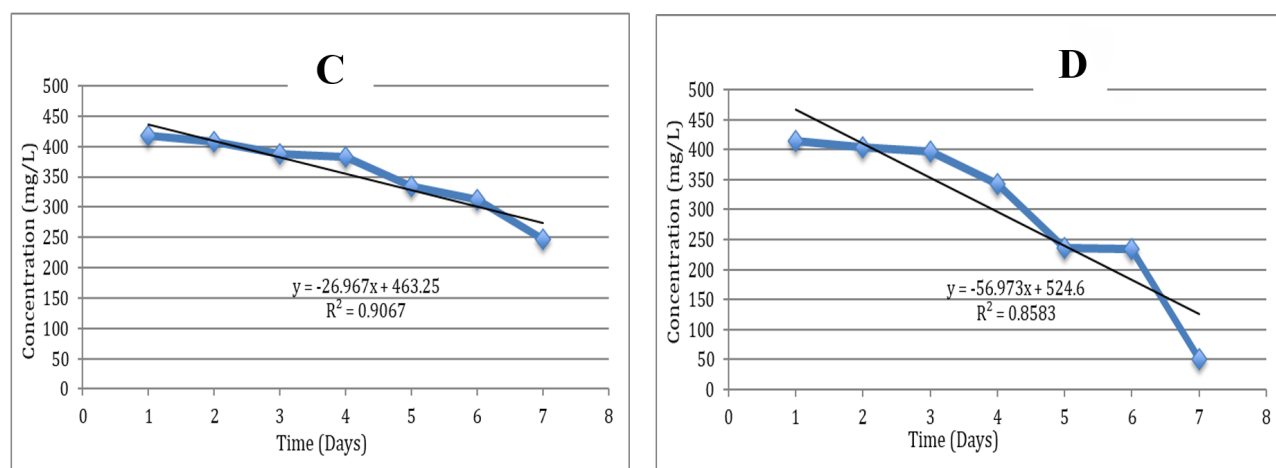


Figure 4: Degradation kinetics representing zero-order kinetics of Zomax® samples: C. at room temperature; D. in the fridge using tap water for reconstitution.

Table 2: Comparative stability periods (days) for both brands*.

Brand	Condition	Tap Water	Deionized Water	Sterile Water	Mean ± SD
Azimax®	Room Temp (27°C)	6	6	3	5.0 ± 1.73
Azimax®	Refrigeration (2-8°C)	5	4	6	5.0 ± 1.00
Zomax®	Room Temp (27°C)	4	<1	<1	1.7 ± 2.08
Zomax®	Refrigeration (2-8°C)	3	2	2	.3 ± 0.58

*The stability period is characterized as the duration of days during which the product remains compliant with United States Pharmacopeia specifications, specifically maintaining a potency range of 95 to 105% of the declared label claim.

Effect of Reconstitution Water Type

Tap water provided the longest stability for both brands under most conditions. At room temperature, both tap water and deionized water maintained stability for 6 days, whereas sterile water showed only 3 days of stability. Zomax® showed stability in tap water for 4 days at room temperature, but failed immediately in deionized or sterile water.

The finding that sterile water sometimes performed worse than tap

water suggests factors beyond microbial contamination influence stability, including pH, ionic strength, and trace impurities [14]. A recent study in Yemen similarly found that cooled distilled water provided acceptable stability, while heated water accelerated degradation [15].

Effect of Storage Temperature

Refrigeration did not consistently improve stability. Azimax® showed superior stability at room temperature with tap water (6 vs. 5 days) and deionized water (6 vs. 4 days), while refrigeration was superior with sterile water (6 vs. 3 days). Zomax® showed variable results with temperature.

This unexpected finding may be attributed to increased solubility of degradation products at lower temperatures, pH shifts accelerating acid-catalyzed degradation, and crystallization of excipients disrupting suspension structure [16]. This unexpected finding suggests that the relationship between storage temperature and stability is highly formulation-dependent, potentially due to excipient interactions or pH shifts at lower temperatures [17]. A recent study similarly reported temperature-dependent stability differences among azithromycin suspensions from different manufacturers [14].

Degradation Kinetics

Degradation followed zero-order kinetics for both brands ($R^2 > 0.90$), indicating concentration-independent degradation mechanisms (Table 3). Zomax® exhibited 2-5 times faster degradation rates than Azimax®.

Table 3: Kinetic parameters for both brands:

Brand	Condition	Water Type	Best Fit Model R^2	k (%/day)	T_{90} (days)*
Azimax®	Room Temp	Tap	Zero-order 0.9194	2.25	4.44
Azimax®	Fridge	Tap	Zero-order 0.9535	2.80	3.57
Azimax®	Fridge	Sterile	Zero-order 0.7356	1.16	8.62
Zomax®	Room Temp	Tap	Zero-order 0.9067	6.10	1.64
Zomax®	Fridge	Tap	Zero-order 0.8583	11.32	0.88
Zomax®	Fridge	Sterile	Zero-order 0.9380	6.60	1.52

* t_{90} = time to 90% remaining (10% degradation).

Implications for Nanosuspension Development

The stability challenges identified in this study provide important design criteria for developing azithromycin nanosuspensions. Several approaches have been reported for azithromycin nanoparticle preparation. Shariare et al. prepared azithromycin nanosuspensions (447.2 nm) using the evaporative precipitation into aqueous solution method [18]. Nanosuspensions with particle sizes of 189 nm have been achieved with 1-year physical stability at 4°C [19], while others have reported particles <100 nm using antisolvent precipitation with PVP as a stabilizer [20]. The fundamental understanding of azithromycin degradation gained from this study will guide the selection of appropriate stabilizers, pH conditions, and storage recommendations for future nanosuspension formulations.

Conclusion

This study demonstrates that the stability of reconstituted azithromycin varies significantly with brand formulation, water type, and storage conditions. Azimax® exhibited superior stability to Zomax® across all conditions, with tap water providing the best stability profile. Refrigeration did not consistently enhance stability, challenging common assumptions. Zero-order kinetics confirmed concentration-independent degradation mechanisms, providing essential parameters for formulation design.

The degradation kinetics data and identified stability challenges provide a critical baseline for azithromycin nanosuspension development. Polymeric encapsulation, nanocrystal formulations, and optimized stabilizer systems based on these kinetic parameters offer promising pathways to overcome current stability limitations and enable longer in-use shelf life with improved therapeutic outcomes.

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