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Dissolution Profiles of Innovator Azithromycin Tablets from Multiple Regulated Markets: Implications for Generic Product Development and WHO Prequalification

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ABSTRACT

Introduction: Generic medicines, including the World Health Organization (WHO) prequalified products, aim to ensure access to affordable products that are comparable to innovator products in terms of quality, safety, and efficacy. A comparative in vitro and in vivo evaluation of generic products against the reference listed drug (RLD) forms a basis of product development and regulatory approval. This study investigated the in vitro dissolution of azithromycin tablets (Zithromax, Pfizer), from different regulated markets. **Methods:** Four lots of Zithromax RLD tablets were procured from regulated markets in Switzerland, Germany, and Singapore at different time points. Dissolution testing was performed using a paddle apparatus operated at 75 rpm for 60 minutes with 900 mL of four different media: hydrochloric acid (HCl) pH 1.2, acetate buffer pH 4.5, phosphate buffer pH 6.8, and United States Pharmacopeial phosphate buffer (pH 6.0). Dissolution profiles were compared using the similarity factor (f_2) and one-way analysis of variance (ANOVA). **Results:** Dissolution profiles of all lots in the official medium (pH 6.0) were similar ($f_2 > 50$). However, at pH 4.5 and 6.8, pairwise comparisons among lots revealed dissimilarity for some lot pairs ($f_2 < 50$). In acidic conditions (0.1 N HCl, pH 1.2), all lots exhibited progressive degradation. One-way ANOVA of the dissolution profiles revealed no statistically significant differences ($p > 0.05$). **Conclusion:** Variability in dissolution profiles of Zithromax RLD tablets sourced from regulated markets highlights the challenges in selecting an appropriate comparator for generic product development. Such variability may complicate formulation development and delay the regulatory pathways, including the WHO prequalification, particularly for manufacturers in resource-limited settings.

Keywords: azithromycin, reference listed drug (RLD), dissolution variability, similarity factor (f_2), prequalification

INTRODUCTION

Generic medicines, including the World Health Organization (WHO) prequalified products, are therapeutically equivalent to innovator products in terms of quality, safety, and efficacy and serve as cost-effective alternatives (*1, 2*). Therapeutic equivalence is established by demonstrating pharmaceutical equivalence and bioequivalence with the innovator or reference listed drug (RLD). Generic medicines contain the same active ingredient(s) in the

same strength, dosage form, and route of administration as the innovator product (*3*). These products will have comparable risk-benefit profiles to their branded counterparts. Because the safety and efficacy of the active pharmaceutical ingredients have already been established for the innovator product, generic versions generally do not require animal and clinical studies. Instead, the regulatory authorities follow an abbreviated approval pathway based primarily on bioequivalence and comparative quality evaluation, thereby significantly reducing development time and product cost (*2*). Globally, generic medicines play a significant role in ensuring the accessibility of affordable alternatives, thereby improving medication adherence (*3*).

An innovator product is generally the first product approved and marketed following authorization by a stringent regulatory authority, with established quality, safety, and efficacy demonstrated through clinical studies (*4*). During generic product development, the RLD serves as the benchmark for formulation development, comparative dissolution testing, and bioequivalence studies, forming the foundation for regulatory approval. Both regulatory agencies and the WHO prequalification program require comparative in vitro and in vivo evaluation of generic products against an RLD procured from a well-regulated market, with proper documentation verifying shipment and storage conditions (*4*).

Azithromycin is a poorly soluble and moderately to highly permeable drug classified as Biopharmaceutics Classification System (BCS) class II/IV (*5*). It is a macrolide antibiotic used for treatment of bacterial infections in the respiratory tract, genitourinary tract, and ear, nose, and throat (*6*). WHO has included azithromycin 500-mg tablets for the treatment of yaws under its neglected tropical diseases prequalification program (*7*). Zithromax, manufactured by Pfizer, is recognized by WHO and regulatory agencies as the RLD for azithromycin (*8*). Despite the general assumption of consistency among RLD products sourced from regulated markets, limited published data are available on batch-to-batch variability in dissolution behavior. Variations in the dissolution profiles of RLDs can impact time and resources for generic product development and the WHO prequalification process.

The objective of the study was to investigate the in vitro dissolution behavior of Zithromax RLD tablets procured from different regulated markets at different time points. Dissolution profiles were evaluated using standard dissolution media and compared using the model-independent similarity factor (f_2) and one-way analysis of variance (ANOVA) (*9–11*).

METHODS

Four lots of Zithromax RLD tablets manufactured by Pfizer were procured from regulated markets in three countries: lot A from Switzerland (batch D00600), lot B from Germany (batch D11600), lot C from Singapore (batch 8153642), and lot D from Switzerland (batch E12204). Details are summarized in Table 1.

All lots were obtained with complete documentation in accordance with WHO requirements for comparator products–including labeling, invoice, shipping and storage conditions, and a signed authenticity statement confirming procurement from the specified regulated market (*8*).

Table 1. Product Information for Zithromax Tablets (Reference Listed Drug)

Lot	Batch Number	Expiry Date	Market Source
Lot A	D00600	Dec 2025	Pfizer AG, Zurich, Switzerland
Lot B	D11600	Feb 2026	Pfizer GmbH, Linkstr. Berlin, Germany
Lot C	8153642	Mar 2026	Pfizer Inc., Singapore
Lot D	E12204	Feb 2027	Pfizer AG, Zurich, Switzerland

Analytical grade chemicals and reagents including hydrochloric acid (HCl), sodium acetate, acetic acid, potassium dihydrogen phosphate, and sodium hydroxide were used without further purification. Ultrapure water used for analysis and preparation of reagents was prepared in-house (Milli Q, Merck). Suitable grade reagents were used for high-performance liquid chromatography (HPLC) analysis.

This study was conducted at Quest Pharmaceuticals Private Limited, Birgunj, Nepal, between February 2024 and February 2025. All dissolution experiments and analytical measurements were performed under controlled laboratory conditions following institutional standard operating procedures.

Dissolution Test

The dissolution test was performed using a 21 CFR Part 11-compliant paddle apparatus equipped with an autosampler (Sotax, SDT, India). The dissolution apparatus was routinely calibrated and qualified in accordance with institutional standard operating procedures complying with United States Pharmacopeia (USP) and applicable regulatory requirements. Performance verification of the dissolution system was conducted using Prednisone Tablets USP through an accredited third-party laboratory. The apparatus was maintained at 37 ± 0.5°C and 75 rpm. Four dissolution media with different pH levels were used: 900 mL of 0.1 N HCl (pH 1.2), acetate buffer (pH 4.5), phosphate buffer (pH 6.8), and the USP official medium (phosphate buffer, pH 6.0), without any surfactants (*9, 12, 13*). For each dissolution condition, 12 tablets from each RLD lot were tested. Samples (5 mL) were withdrawn at 5, 10, 15, 20, 30, 45, and 60 minutes using the autosampler with subsequent replenishment of an equal volume of fresh dissolution medium. Each sample was filtered through a 0.45-µm membrane filter.

The USP HPLC method used for quantification of azithromycin was verified for specificity, linearity, precision, accuracy, and system suitability in accordance with ICH Q2(R2) guidelines prior to the analysis of the dissolution samples (*14, 15*). The method was linear across the working concentration range with an R^2 value of 0.9998, it was accurate and precise with relative standard deviation values less than 0.8%, and it met the system suitability requirements.

Quantitative analysis was performed using an HPLC system (Prominence-I, Shimadzu, Japan) equipped with a UV detector. Chromatographic separation was achieved using an octadecylsilane (C18) column (150 x 4.6 mm, 5 µm) maintained at 50 °C, with a mobile phase consisting of acetonitrile, methanol, and buffer (pH 8.2) in a ratio of 9:3:8. The flow rate was 1.5 mL/min, the injection volume 50 µL, and the detection wavelength 210 nm (*12*). The procedure was repeated under all dissolution conditions for each RLD lot. Dissolution results were reported as the mean percentage of drug release from 12 tablets for each lot.

Data Analysis

Dissolution profiles in each medium for all Zithromax RLD lots were compared using the model-independent similarity factor (f_2) as recommended in regulatory guidelines (*9, 10*). An f_2 value between 50 and 100 was considered indicative of a similar dissolution profile. If both products achieved a drug release rate of 85% or higher within 15 min, then dissolution profiles were considered similar and calculation of f_2 was not necessary (*9, 10*). In addition, statistical comparison of dissolution data was performed using one-way analysis of variance (ANOVA), and a *p*-value less than 0.05 was considered statistically significant (*11*). All calculations were performed in Excel (Microsoft 365).

RESULTS

Dissolution Profiles

The mean dissolution profiles (average of 12 units) of four lots of Zithromax RLD tablets tested across multiple dissolution media are presented in Table 2.

Under acidic conditions (0.1 N HCl, pH 1.2), the drug release rate decreased gradually over time in all lots, indicating degradation of azithromycin under strong acidic conditions (Fig. 1A). In acetate buffer and phosphate buffers (pH 4.5, 6.0, and 6.8), all lots demonstrated progressive drug release, with more than 90% of the labeled amount released within 60 minutes (Fig. 1B–1D).

Minor differences in dissolution behavior among RLD lots were observed at early and intermediate time points, particularly within the first 20 minutes. However, the extent of drug release at later time points appeared comparable across all lots.

Similarity Factor Analysis

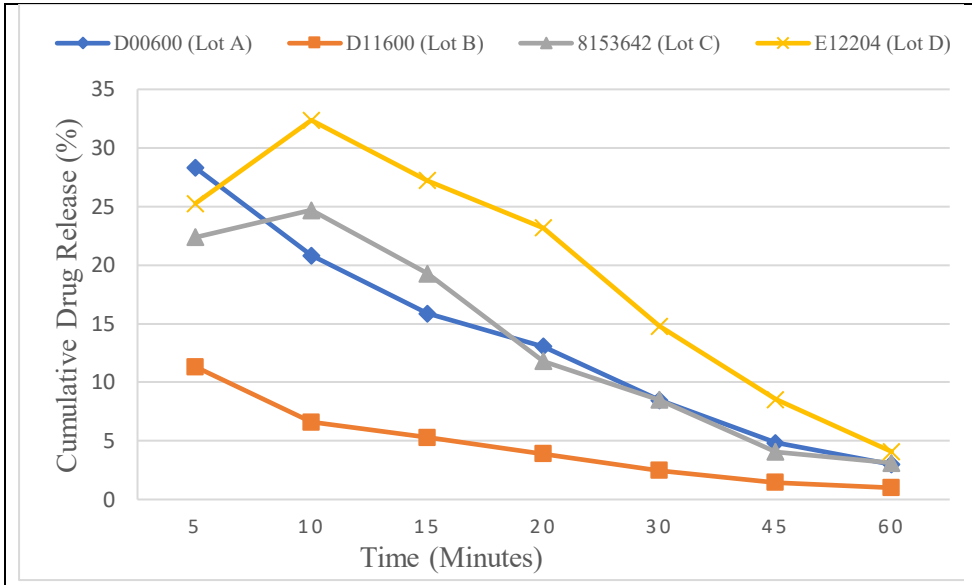
The dissolution test conducted under acidic conditions (0.1 N HCl, pH 1.2) showed degradation of azithromycin; therefore, f_2 factor was not calculated. Similarity factor analysis using the f_2 value in acetate buffer (pH 4.5) and phosphate buffers (pH 6.0 and 6.8) showed diverse results (Table 3).

In acetate buffer (pH 4.5), among six pairs of lots, three pairs (lot A vs C, lot A vs D, and lot C vs D) did not require f_2 calculation because more than 85% drug released was achieved within 15 minutes. The remaining three pairs had f_2 values less than 50, indicating dissimilar dissolution profiles (Table 3). Under the official dissolution condition (phosphate buffer, pH 6.0), all lots demonstrated similar dissolution profiles, with f_2 values greater than 50; the lowest f_2 value was observed for lot B vs D (51.01). In phosphate buffer (pH 6.8), two pairs (lot A vs C and lot B vs C) showed dissimilar profiles with f_2 values below 50. For lot C vs D, calculation of f_2 was not required due to rapid drug release (more than 85% within 15 minutes), and the remaining three pairs demonstrated similar drug dissolution profiles.

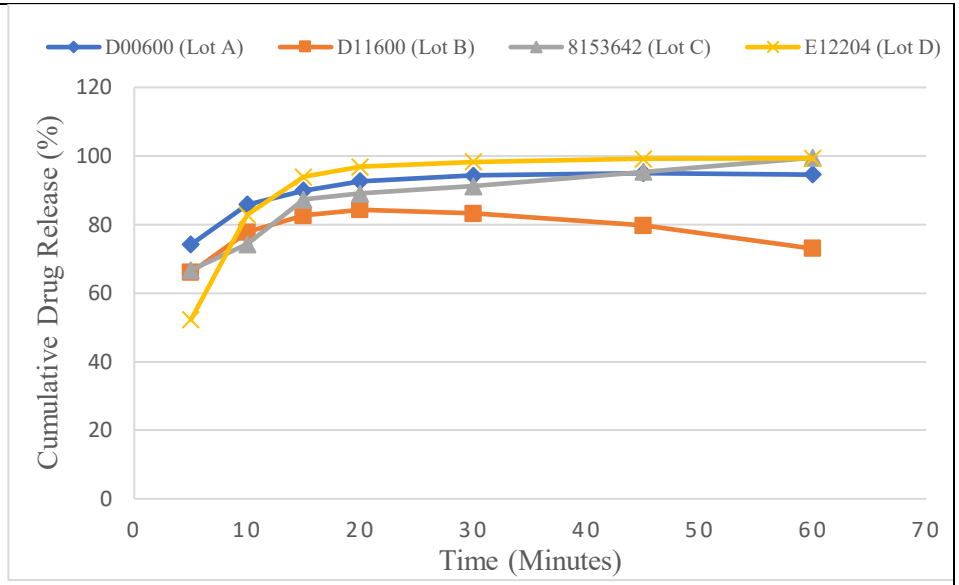
Table 2: Dissolution Test Results for Zithromax Tablets

	D00600 (Lot A)				D11600 (Lot B)				8153642 (Lot C)				E12204 (Lot D)			
pH	**1.2**	**4.5**	**6.0**	**6.8**	**1.2**	**4.5**	**6.0**	**6.8**	**1.2**	**4.5**	**6.0**	**6.8**	**1.2**	**4.5**	**6.0**	**6.8**
5 min	28.30	74.15	65.35	67.74	11.32	66.00	63.66	63.66	22.40	66.70	68.30	67.50	25.25	52.19	46.85	47.28
10 min	20.83	85.84	81.42	73.94	6.60	77.79	73.92	73.92	24.70	74.32	79.80	88.78	32.37	82.88	78.14	75.02
15 min	15.87	89.96	88.24	79.85	5.28	82.63	80.23	80.23	19.30	87.30	83.70	93.54	27.21	93.96	90.55	85.67
20 min	13.05	92.63	89.24	83.18	3.90	84.31	84.18	84.18	11.80	89.10	89.34	97.92	23.19	96.82	93.37	89.51
30 min	8.48	94.31	91.67	86.50	2.48	83.24	88.56	88.56	8.50	91.20	93.20	98.97	14.78	98.31	95.89	93.48
45 min	4.84	94.99	93.47	89.48	1.44	79.70	90.43	90.43	4.06	95.40	95.16	99.53	8.54	99.20	96.90	95.65
60 min	2.99	94.62	94.37	90.61	1.00	73.05	90.79	90.79	3.12	99.50	98.72	99.05	4.08	99.31	97.40	96.63

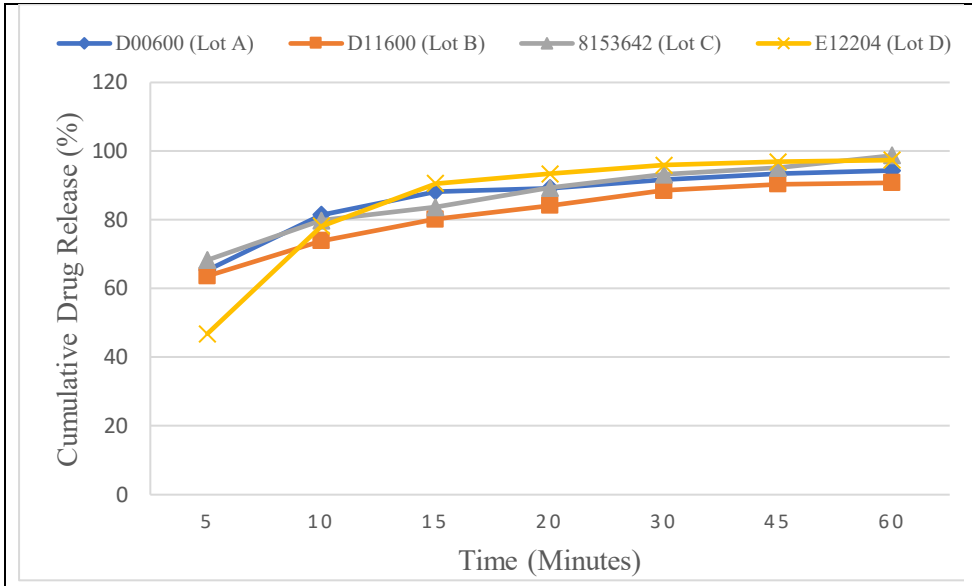
Values are cumulative mean drug release (%).



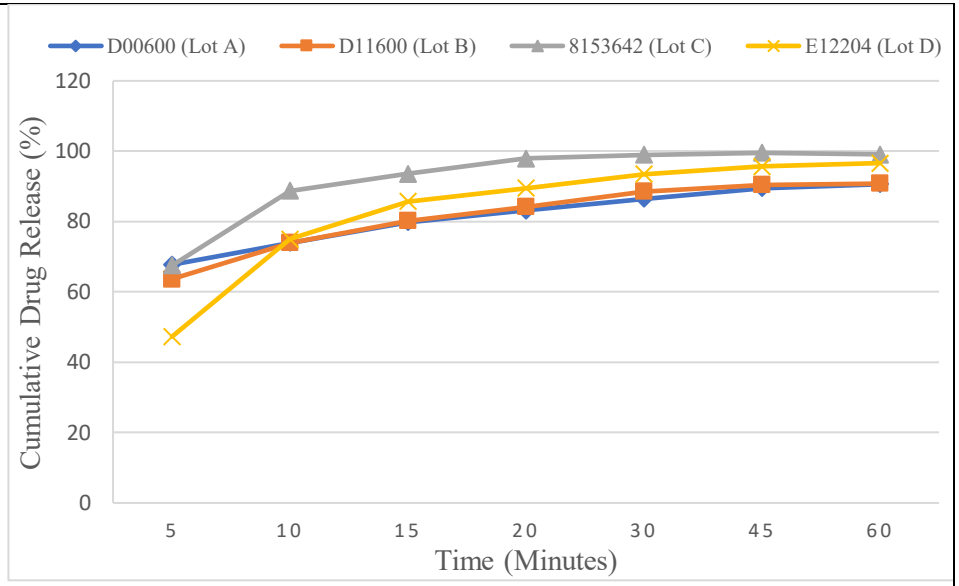
A. 0.1 M Hydrochloric acid, pH 1.2



B. Acetate buffer, pH 4.5



C. Phosphate buffer, pH 6.0



D. Phosphate buffer, pH 6.8

*Figure 1. Dissolution profiles of Zithromax tablets (reference listed drug) in different dissolution media. (**A**) Hydrochloric acid (pH 1.2); (**B**) acetate buffer (pH 4.5); (**C**) USP phosphate buffer (pH 6.0); (**D**) phosphate buffer (pH 6.8).*

Table 3: Similarity Factor (f2) Values for Zithromax Tablets

RLD lots	Acetate Buffer (pH 4.5)	Phosphate Buffer (pH 6.0)	Phosphate Buffer (pH 6.8)
Lot A vs Lot B	45.26	64.30	84.21
Lot A vs Lot C	NR	76.13	46.57
Lot A vs Lot D	NR	NR	51.25
Lot B vs Lot C	45.35	63.15	47.67
Lot B vs Lot D	39.71	51.01	55.54
Lot C vs Lot D	NR	52.72	NR

NR: not required.

Similar to the observations described above, drug release under acidic conditions (pH 1.2) was not included in the statistical analysis using ANOVA due to the degradation of azithromycin in all RLD lots. All *p*-values were greater than 0.05, ranging from 0.265 (lot A) to 0.837 (lot D), indicating that the variations were not statistically significant.

DISCUSSION

RLD products are generally assumed to provide a consistent benchmark for generic product development with reproducible dissolution profiles. Although the drug release profiles of all RLD lots in the official dissolution medium (pH 6.0) were similar, the results demonstrated previously unreported variability in dissolution behavior under identical conditions at pH 4.5 and 6.8. Furthermore, under the acidic condition (0.1 N HCl, pH 1.2), the dissolution profiles of all RLD lots showed a substantially lower release at the 60-minute timepoint compared with earlier sampling intervals, suggesting chemical instability and degradation of azithromycin. Although the degradation rate of azithromycin in acidic medium is slower than other macrolide antibiotics, it undergoes considerable degradation; previous studies reported 10% decay ($T_{1/10}$) of azithromycin at 37 °C in 20.1 minutes under acidic conditions (*16*).

Although the ANOVA test and f_2 values at pH 6.0 indicated similar dissolution profiles, a pairwise f_2 analysis revealed notable variability among the RLD lots at pH 4.5 and 6.8. These observations highlight an important challenge for generic manufacturers pursuing regulatory agency approval or WHO prequalification in the selection of appropriate RLD. The variability among RLD lots sourced from the regulated markets may complicate the selection of an appropriate target dissolution profiles and increase the number of formulations required to achieve similarity.

CONCLUSION

Unexpected variability in dissolution profiles of Zithromax RLD tablets sourced from regulated markets was observed under discriminatory dissolution conditions. Such variability may influence formulation development strategies for generic products intended for stringent regulatory approval or WHO prequalification. Careful evaluation of RLD batches is therefore necessary to ensure efficient and reliable development of generic medicines. This issue may be particularly relevant for manufacturers in resource-limited settings, where repeated reformulation and testing can significantly increase development costs and timelines. Early evaluation of multiple RLD batches during formulation development may provide a more representative understanding of innovator dissolution behavior and help mitigate the impact of potential lot-to-lot variability.

DISCLOSURES

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