

Research Article

Comparative *in Vitro* Dissolution Profile of Commercial Azithromycin Dihydrate 500 mg Tablet Preparations in the Philippines

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Abstract

This study seeks to compare the *in vitro* multi-point dissolution profiles between 3 off-patent products azithromycin dihydrate 500 mg and the innovator product. The paddle-type dissolution apparatus was used. This was rotated at a speed of 50 rpm using 0.1-N HCl as media. Random samples were withdrawn after certain time points and assayed for azithromycin dihydrate. Comparison between test samples and the innovator product was done by computing their similarity (f_1) and dissimilarity (f_2) factors and by fitting them to various kinetics of drug release during dissolution. Test samples 1 and 3 were comparable to the innovator product because they complied with f_1 and f_2 specifications. Test sample 2, however, gave higher f_2 values making it non-equivalent with the innovator product. The Higuchi and Korsmeyer-Peppas kinetics of drug release characterized most of the dissolution profiles. This study showed that test samples 1 and 3 are equivalent with the innovator products in terms of comparative *in vitro* dissolution profiles where extra-Fickian release behaviors were exhibited by all the preparations.

1. Introduction

Among the macrolide antibiotics, azithromycin has the longest biological half-life because of its high volume of distribution, where tissue concentrations are much higher than blood levels. High concentrations are taken up into white blood cells and, therefore, plasma concentrations are of little value as a guide to efficacy [1]. A 500 mg daily dose for 3 days is optimum for patient compliance in the eradication of mostly respiratory tract infections without the inconvenience of multiple daily dosing with other antibiotics. Several off-patent azithromycin dihydrate 500 mg tablet formulations have proliferated the Philippines market. Most of these products are sold at highly cheaper retail prices than the innovator product, making their qualities, safety and efficacy oblivious to scrutiny among physicians and pharmacists. To ensure safety and efficacy of generic products, the conduct of expensive bio-equivalence studies using human subjects are preferred because actual pharmacokinetic data are compared against the innovator product. A cheaper alternative is the conduct of *in vitro* comparative multipoint dissolution studies as they correlate well with *in vivo* bio-equivalence studies [2]. This study compares the multi-point dissolution profiles of 3 off-patent azithromycin dihydrate 500 mg tablets currently available in the Philippines against the innovator product.

2. Materials and Methods

2.1. Product Procurement

Samples of azithromycin dihydrate 500 mg tables were provided either as gifts or were purchased at wholesale

price from medical representatives on the condition that the samples have a remaining shelf-life of at least 18 months at the time they were donated or purchased. These are as follows:

- Pfizer, Inc., Makati, Philippines (ZithromaxTM, designated as the innovator product with lot no. B141464102 and expiring on April 30, 2017);
- Farma Iberica Co., Pasig City, Philippines (OD MacTM, designated as test sample 1, with lot no. EMM401 and expiring on September 30, 2016);
- Stallion Lab., Gujarat, India (AnzalTM, designated as test sample 2 with lot no. PH240 and expiring on October 31, 2016); and
- Interphil Lab., Cabuyao, Laguna, Philippines (ZithramycinTM, designated as test sample 3 with lot no. 14ZAT-1 and expiring on August 2016).

2.2. Dissolution Testing

Each of the 6 vessels of the USP dissolution type 2 apparatus was filled with 900 mL of 0.1-N HCl. One tablet coming from any of the 3 test samples and the innovator product were randomly distributed into each vessel and the paddles were made to rotate at 75 rpm. From each vessel, 2 mL aliquot portion was taken at the end of 5, 10, 20, 30, 40, 60, 80 and 120 minutes and then filtered (Millipore No. 1). The filtrates were diluted to 100 ml with 0.1-N HCl and then assayed for azithromycin dihydrate content. Two mL of fresh medium was replaced after each aliquot withdrawal to maintain sink conditions. More cycles of dissolution tests were repeated similarly so that at the end of the study, 6 units of each of the 3 test sample and the innovator product have been subjected to multi-point dissolution testing.

2.3. Assay for Azithromycin Dihydrate by High-performance Liquid Chromatography (HPLC)

Azithromycin dihydrate reference standard was dissolved in 0.1-N HCl and then filtered (Millipore no. 1) to produce a 0.1 mg/mL standard solution. The standard solution and the finally diluted test sample solutions from 2.2 were injected at 20-μL quantities in a Merck Hitachi 24-1 (Tokyo, Japan) equipped with a reverse phase C-18 column (5 x 4.66 mm) that was heated at 50°C and a UV detector at 210 nm and eluted with HPLC grade 0.03-M phosphate buffer – methanol (20:80 v/v, pH = 7.5; Merck Co.) at a flow rate of 2 mL/minute [3].

2.4. Quality Control of the Materials Procured

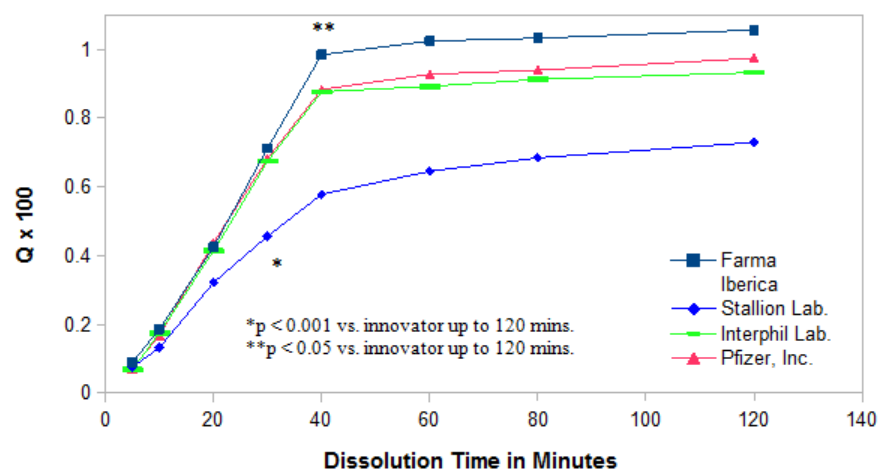
The 3 test samples and innovator products were tested for assay for azithromycin dihydrate content by the spectrophotometric method of Patil *et al* [4] (2011), friability (Roche ED-2), hardness, (Stokes-Monsanto), disintegration (USP Electrolab) in 0.1-N HCl for 2 hours at $37 \pm 2^\circ\text{C}$ and weight variation (Sartorius PG 03-S).

3. Results and Discussions

3.1. Comparative Multi-point Dissolution Profiles

The area under the dissolution-time curves for the 3 test samples were compared against the innovator product by the similarity (f_2) and difference (f_1) factors, such that $f_2 = 50 \log \left[\left\{ 1 + \frac{1}{n} \sum (R_t - T_t)^2 \right\}^{-0.5} \times 100 \right]$ and $f_1 = \left[\left\{ \frac{\sum |R_t - T_t|}{\sum R_t} \right\} \times 100 \right]$, where R_t and T_t are the percentage drug dissolved at each time point for the innovator and test products, respectively, n is the number of dissolution sampling time and t are the time points for collecting dissolution samples. Figure I compares the dissolution-time curve of the 3 test samples and the innovator product.

Figure 1: Comparative Multi-point Dissolution between the Three Test Samples and the Innovator



The dissolution-time curve of test sample 3 was comparable to the innovator product, assuming almost mirror-image super imposable dimensions. Test sample 2 failed to achieve this, with Q values being significantly lower than the innovator product and the 2 other test samples after 30 minutes. Test sample 1 gave significantly higher Q values than the innovator starting at 40 minutes. All 4 products exhibited an initial phase of high release (i.e., burst effect) with a common asymptote after 40 minutes, followed by a second phase of moderate release. To validate this finding, comparison of f_1 and f_2 values were made and are presented in Table I.

Table I: Comparative Similarity and Difference Factors Among the Three Test Products

Formulations	Similarity (f_2)	Difference (f_1)
Test Sample 1	51.16%	8.88%
Test Sample 2	51.99%	28.90%
Test Sample 3	53.00%	3.01%

* $p < 0.001$ vs. innovator up to 120 mins.

** $p < 0.05$ vs. innovator up to 120 mins.

All 3 test samples passed the Food and Drug Authority (FDA) specification for f_2 which should be at least 50% to make it equivalent to the innovator product in terms of comparative *in vivo* multi-point dissolution. However, test sample 2 exceeded the maximum f_1 value of 15% set by the FDA and that does not make it equivalent to the innovator product as far as *in vitro* bio-equivalence is concerned.

3.2. Kinetics of Dissolution

To analyze the *in vitro* dissolution release of azithromycin dihydrate, the following kinetic models were applied:

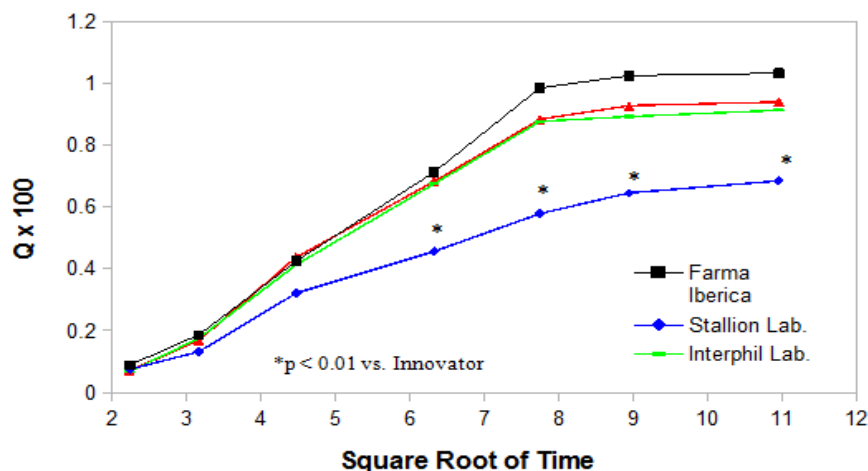
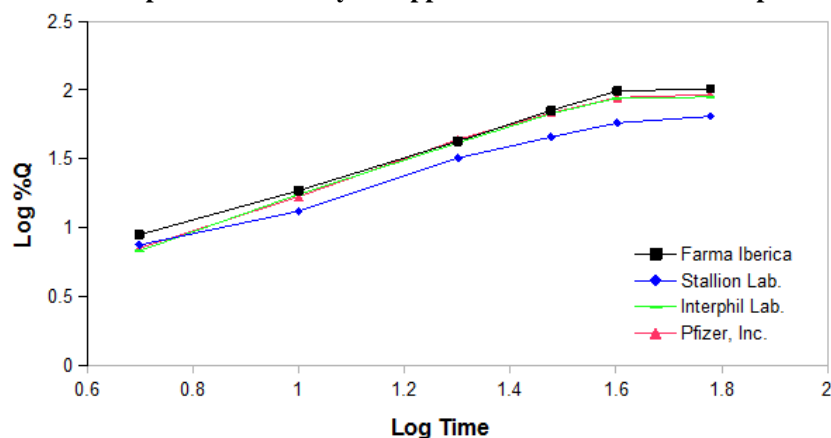
- Zero order, where cumulative concentration Q was plotted against time t;
- First order, where log Q was plotted against t;
- Higuchi model where Q was plotted against square root of time t;
- Hixson-Crowell, where $Mo^{1/3} - Mt^{1/3} = K_{HCT}$, such that Mt is the amount of drug released in time t and Mo is the initial amount of the drug in the sample (~ 500 mg), and where $Mo^{1/3} - Mt^{1/3}$ was plotted against t; and
- Korsmeyer-Peppas, where log Q, up to 60%, was plotted against log time t.

Information on the dissolution kinetics of the 3 test samples and the innovator product are provided in Table II. The plots of azithromycin dihydrate with zero order, first order and Hixson-Crowell kinetics showed low linearity, where $r < 0.9$ for zero order and $r < 0.8$ for first order and Hixson-Crowell kinetics, to signify that diffusion is independent of concentrations. The best linearity was found with Higuchi's and Korsmeyer-Peppas's kinetics ($r > 0.9$).

Table II: Comparative Dissolution Release Kinetics

Formulation	Drug Release Kinetics (r = correlation coefficient)					
	Zero Order	First Order	Higuchi	Hixson-Crowell	Korsmeyer – Peppas	
	r	r	r	r	r	n
Test Sample 1	0.82	0.64	0.91	-0.77	0.99	1.06
Test Sample 2	0.88	0.77	0.96	-0.81	0.99	0.93
Test Sample 3	0.81	0.72	0.91	-0.63	0.98	1.1
Innovator	0.82	0.72	0.92	-0.76	0.98	1.1

The Higuchi plots, shown in Figure II, assumes one-dimensional drug diffusion where matrix swelling and dissolution are negligible within a perfect sink condition [2]. It is apparent that after 30 minutes, the Higuchi plots of test sample 2 was not comparable to the innovator product and test samples 1 and 3. The Korsmeyer-Peppas model was designed to confirm whether release mechanisms of drugs from cylindrical tablets obey Fick's law of diffusion. The release exponent n, which is derived from the slope of the Korsmeyer-Peppas plots in Figure III, characterizes different release mechanisms described in Table III [2]. All 3 test samples and the innovator product gave n values higher than 0.89 which means that these products follow super case II transport diffusion due to chain entanglement as a result of swelling of hydrophobic and hydrophilic polymers which are present in the formulations to provide sustained-release properties [5].

Figure II: Comparative Higuchi Plots of the Various Samples Tested**Figure III: Comparative Korsmeyer-Peppas's Plots of the Various Samples Tested****Table III: Interpretation of Diffusional Release Mechanisms from Polymeric Films**

Release Exponent n	Drug Transport Mechanism	Rate as a Function of Time
0.5	Fickian diffusion	$t^{-0.5}$
$0.45 < n < 0.89$	Non-Fickian transport	t^{n-1}
0.89	Case II transport	Zero order release
Higher than 0.89	Super Case II transport	t^{n-1}

3.3. Quality Control of the Finish Products

Table IV compares the 3 test samples against the innovator product based on standard quality control tests for tablets. Results showed that there are no appreciable differences among the 3 test samples and the innovator product in terms of disintegration and weight variation tests. However, test sample 2 exceeded the friability limit of 1%, making it oblivious to abrasion during transport. Test sample 2 is the hardest among the samples tested at almost 7 kg, a factor that reflects the high amount of binders in the formulation. Too much abrasion and the additive effects of excessive amount of binders are factors to consider why test sample 2 was not equivalent with the innovator product, let alone test samples 1 and 3. Furthermore, it is possible that the overage of 3.2% observed with test sample 1 after assay for azithromycin dihydrate content contributed to the high Q values during dissolution as reflected in Figures 1 and 2. This overage, however, is still deemed acceptable as the upper monograph limit is set at 110% by the United States Pharmacopoeia 34.

Table IV: Quality Control Tests for the Various Samples Tested

Formulation (N = 20)	Mean \pm S.E.M. (Specification)				
	Assay	Friability	Hardness	Disintegraton	Weight Variation
Test Sample 1	103.20 \pm 0.32%	0.23 \pm 0.02%	4.7 \pm 0.21 kg	6.2 \pm 0.23 mins.	777.2 \pm 4.5 mg
Test Sample 2	98.80 \pm 0.17%	1.11 \pm 0.03%	6.8 \pm 0.11 kg	6.1 \pm 0.19 mins.	713.5 \pm 5.6 mg
Test Sample 3	99.30 \pm 0.21%	0.46 \pm 0.01%	4.2 \pm 0.02 kg	5.7 \pm 0.13 mins.	792.3 \pm 4.8 mg
Innovator	100.30 \pm 0.13%	0.25 \pm 0.03%	4.4 \pm 0.12 kg	5.9 \pm 0.14 mins.	788.3 \pm 5.8 mg

4. Discussions

Cost analysis and survey of the azithromycin dihydrate 500 mg tablets tested in this study showed the following retail prices: test sample 2 (AnzalTM) at 60 pesos per 500 mg tablet; test sample 3 (ZithramycinTM) at 40 pesos per tablet; test sample 1 (OD MACTM) at 140 pesos per tablet; and the innovator (ZithromaxTM) at 151.25 pesos per tablet. This study showed that generic branding at relatively cheaper prices than the innovator is possible by formulating preparations that are bio-equivalent through comparative *in vivo* multi-point dissolution testing. This study revealed that test samples 1 and 3 are comparable with the innovator product in terms of similarity and difference factors and exhibited similar extra-Fickian diffusion behavior as both fitted to Higuchi and Korsmeyer-Peppas release kinetics. These findings are important since azithromycin dihydrate has a relatively long shelf-life and high volume of distribution as it is primarily stored in tissues so that it is important that their tablet preparations provide sustained-release mechanisms to maintain patient compliance, particularly when these tablets are given once daily for 3 days [6].

It was found out that test sample 2 was not equivalent to the innovator in terms of multi-point comparative *in vitro* dissolution testing but these findings do not translate to poor quality in terms of bioavailability, even if it also failed friability and hardness tests. It has passed assay limits and, therefore, it should release azithromycin into the blood streams and underlying tissues at sufficient bactericidal concentrations [7].

5. Conclusions

This study showed that 2 of the 3 off-patent generic azithromycin dihydrate 500 mg tablets are equivalent to the innovator product in terms of multi-point *in vitro* dissolution profile. All 3 preparations tested exhibited extra-Fickian release mechanisms that are characteristics of drugs released from matrices that contains hydrocolloidal polymers that are intended to provide sustained-release effects.

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