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A Bioequivalence Study of Two Azithromycin Tablet Formulations in Indonesian Healthy Subjects

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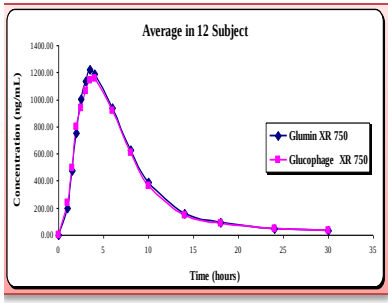
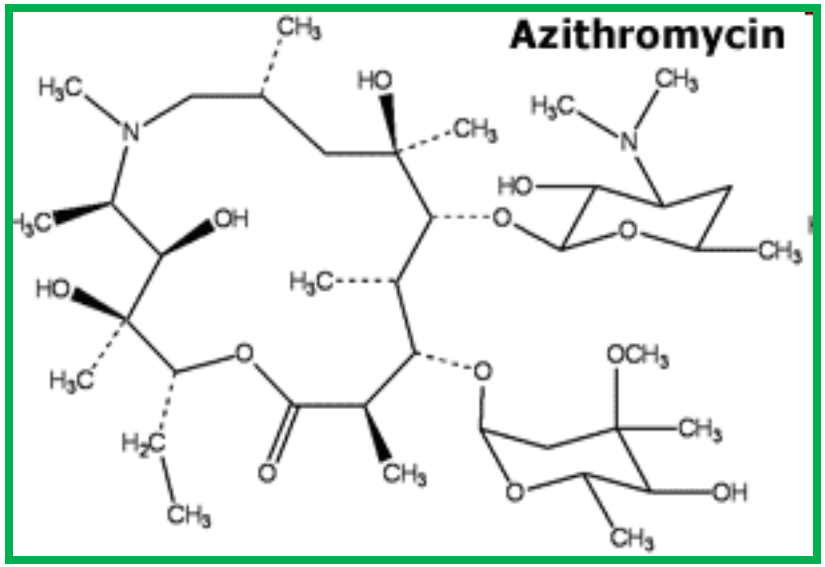
[2]Clinisindo Laboratories, Indonesia.

"European Pharma Congress, Valencia, Spain 2015"



Introduction

- **Azithromycin ⟹ is an azalide, a subclass of macrolide antibiotics related to erythromycin.**
- **Mainly to treat a variety of bacterial infections caused by respiratory pathogens ⟹ Aerobic gram-positive and gram-negative microorganisms.**



Its molecular formula is $C_{38}H_{72}N_2O_{12}$, MW is 749.00

Pharmacokinetic

- Following oral administration, azithromycin is rapidly absorbed (tmax = 2 – 3 hrs) and distributed throughout the body.
- Rapid movement of azithromycin from blood into tissue ⟶ significantly higher azithromycin concentrations in tissue than in plasma (up to 50 times the maximum observed concentration in plasma)
- Plasma concentrations of azithromycin decline in a polyphasic pattern ⟶ average terminal half-life of 68 hours.
- A single 500 mg dose, the t1/2 of azithromycin is 40–50 hours.
- The absolute bioavailability is approximately 37%.

Bioequivalence Study??

- Due to regulatory (NADFC) ⟹ It is compulsory for the generic/copy products of antibacteria to conduct the bioequivalence study
- The purpose of bioequivalence study ⟹ to demonstrate equivalence in biopharmaceutics quality between generic/copy medicinal product and a reference medicinal product ⟹ to allow bridging of preclinical tests and of clinical trials associated with the reference medicinal product

Azithromycin Tablet Formula

Active ingredient:

Azithromycin dihidrate

Auxiliary substances:

Microcrystalline cellulose

Crosscarmellose sodium

Maize starch

Talcum

Magnesium stearat

Colloidal silica anhydrous

Sodium lauryl sulfate

Polyethyleneglycol 6000

Hydroxypropyl methyl cellulose

Titanium dioxide

Talcum

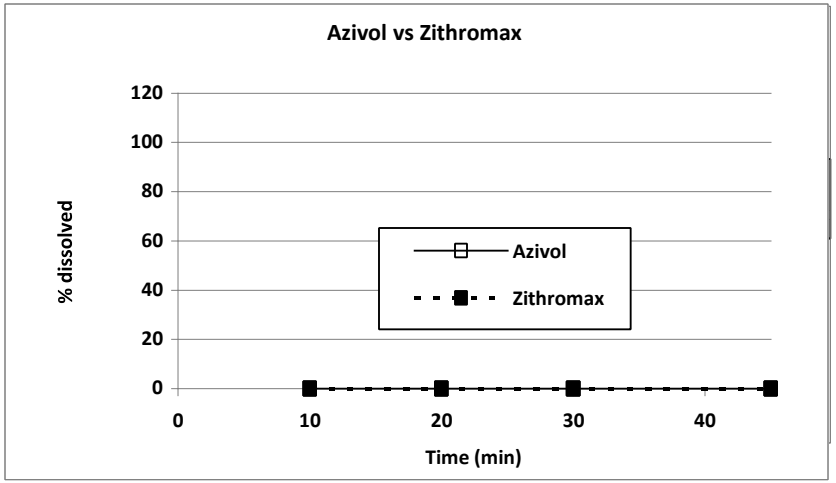
The Objective of Research

To compare the bioavailability of two azithromycin tablet formulations 500 mg Azivol® tablets (PT. Novell Pharmaceutical Laboratories, Indonesia) as test formulation and 500 mg Zithromax® tablets (Pfizer Australia Pty Ltd) as reference formulation.

DISSOLUTION PROFILE

Product	: Azivol vs Zithromax
Active ingredient	: Azithromycin
Potency	: 500.00 mg
Sample volume	: 20.00 mL
Medium volume	: 900.00 HCl pH 1.2
Sampling factor	: 0.02222
Condition	: f_1 = 0-15 f_2 = 50-100

Time (min)	% dissolved (actually)		Diff	Diff square
	Azivol	Zithromax		
10	0.00	0.00	0.00	0.00
20	0.00	0.00	0.00	0.00
30	0.00	0.00	0.00	0.00
45	0.00	0.00	0.00	0.00
		Sum	0.00	0.00
		Average	0.00	
		SD	0.00	



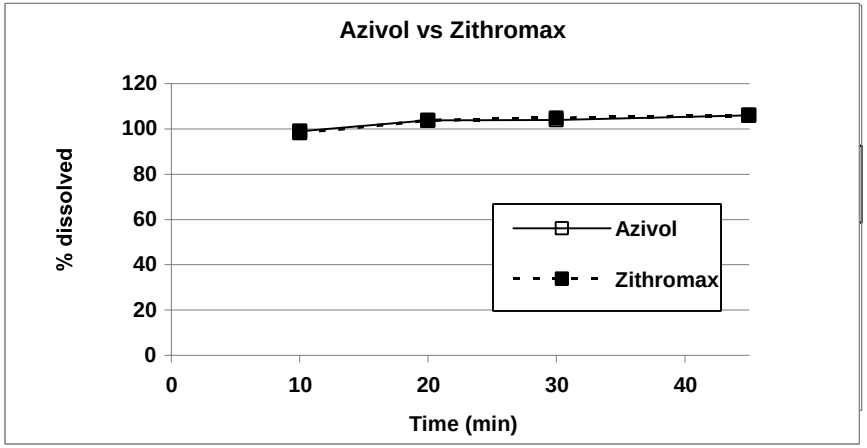
Conclusion:

Azithromycin was not soluble in medium of HCl pH 1.2

DISSOLUTION PROFILE

Product	: Azivol vs Zithromax
Active ingredient	: Azithromycin
Potency	: 500.00 mg
Sample volume	: 20.00 mL
Medium volume	: 900.00mL Acetate buffer pH 4.5
Sampling factor	: 0.02222
Condition	: f_1 = 0-15 f_2 = 50-100

Time (min)	% dissolved (actually)		Diff	Diff square
	Azivol	Zithromax		
10	98.86	98.16	0.69	0.48
20	103.72	103.76	0.05	0.00
30	103.81	104.84	1.04	1.07
45	105.98	105.82	0.16	0.03
		Sum	1.94	1.58
		Average	0.39	
		SD	0.46	



(*) Similarity Factor = $F_2 = 50 \times \text{Log}\,(100 : \sqrt{(1+\sum_{t=1-n}(R_t-T_t)^2/n)}$

F_2 = **96.38**

(*) Differences Factor = $F_1 = \{(\sum_{t=1-n}[R_t-T_t]) / \sum_{t=1-n}[R_t]\} \times 100$

F_1 = **0.47**

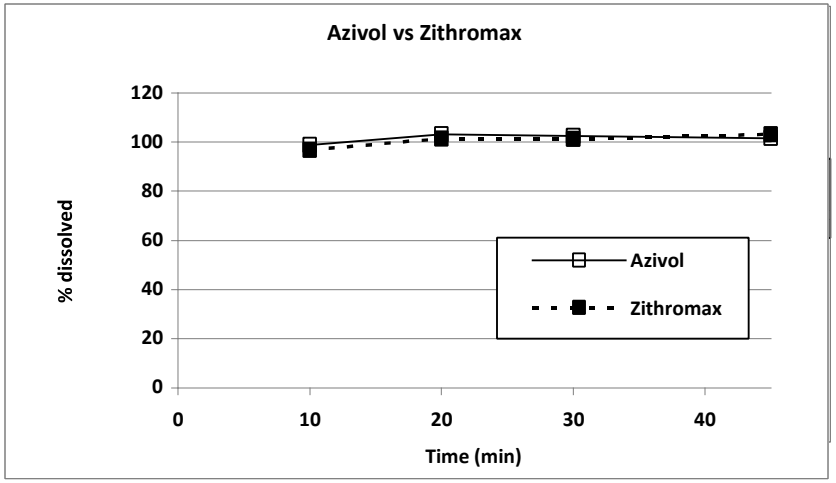
Conclusion:

Dissolution profile of Azivol and Zithromax have the similarity or have no significant difference

DISSOLUTION PROFILE

Product	**: Azivol vs Zithromax**
Active ingredient	: Azithromycin
Potency	: 500.00 mg
Sample volume	: 20.00 mL
Medium volume	: 900.00Phosphate buffer 0.1 M pH 6.0
Sampling factor	: 0.02222
Condition	: f_1 = 0-15 f_2 = 50-100

Time (min)	% dissolved (actually)		Diff	Diff square
	Azivol	Zithromax		
10	98.72	96.93	1.79	3.19
20	103.28	101.39 101.28	1.89	3.56
30	102.51	103.28	1.23	1.50
45	101.58		1.70	2.89
		Sum	6.60	11.15
		Average	1.32	
		SD	0.29	



(*) Similarity Factor = $F_2 = 50 \times \text{Log}\,(100 : \sqrt{}(1+\sum_{t=1-n}(R_t-T_t)^2/n)$

F_2 = **85.54**

(*) Differences Factor = $F_1 = \{(\sum_{t=1-n} [R_t-T_t]) / \sum_{t=1-n} [R_t]\} \times 100$

F_1 = **1.64**

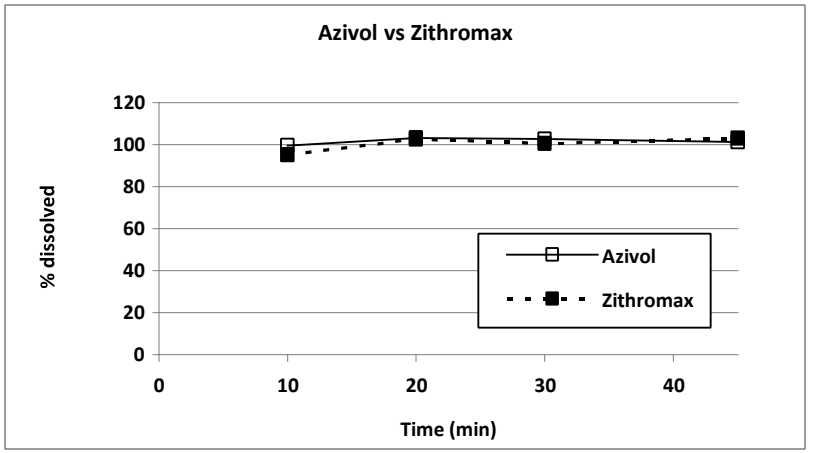
Conclusion:

Dissolution profile of Azivol and Zithromax have the similarity or have no significant difference

DISSOLUTION PROFILE

Product	**: Azivol vs Zithromax**
Active ingredient	: Azithromycin
Potency	: 500.00 mg
Sample volume	: 20.00 mL
Medium volume	: 900.00Phosphate buffer pH 6.8
Sampling factor	: 0.02222
Condition	: f1 = 0-15 f2 = 50-100

Time (min)	% dissolved (actually)		Diff	Diff square
	Azivol	Zithromax		
10	99.57	95.16	4.41	19.45
20	103.23	102.75	0.48	0.23
30	102.67	100.54	2.13	4.55
45	101.37	102.99	1.62	2.62
		Sum	8.65	26.86
		Average	2.16	
		SD	1.65	



(*) Similarity Factor = $F_2 = 50 \times \text{Log}\,(100 : \sqrt{}(1+\sum_{t=1-n}(R_t-T_t)^2/n)$

F_2 = **77.82**

(*) Differences Factor = $F_1 = \{(\sum_{t=1-n}[R_t-T_t]) / \sum_{t=1-n}[R_t]\} \times 100$

F_1 = **2.15**

Conclusion:

Dissolution profile of Azivol and Zithromax have the similarity or have no significant difference

Study Protocol → passed → **Subject screening** → **24 healthy subjects** (Signed informed consent)

single-dose, open-label, randomized, two-way crossover fasting

Committee of The Medical Research Ethics of the Faculty of Medicine, University of Indonesia and was approved by the NADFC .

Blood pressure, heart rate, body temperature and adverse events were monitored during blood sampling.

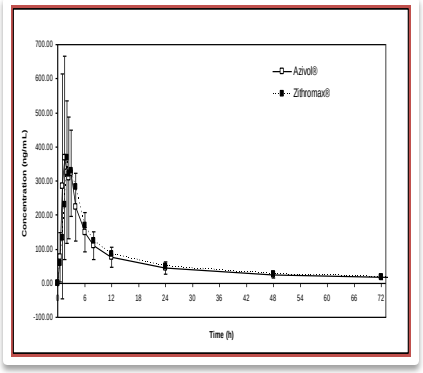
Blood sampling pre dose, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8,12, 24, 48, 72, 96 and 120 hours (two weeks wash out period)

5 mL of Blood

blood separation

Plasma was freezed at -20°C until analysis

Validated method
Linear 2-500ng/mL



Subjects

Demographic data for azithromycin bioequivalence study in 24 subjects

	Mean (± SD)	
Age (years)	**28.6 (4.9)**	**19-39**
Weight (kg)	**57.7 (9.3)**	**41-73**
Height (m)	**162.4 (7.6)**	**142.5-174**
Body mass index (kg m-2)	**21.8 (2.7)**	**18-25**

Subjects Screening

Passing Physical examination,
ECG and clinical laboratory tests (hemoglobin, hematocrite, WBC, platelets, WBC differential, blood urea nitrogen, sGPT, sGOT, alkaline phosphatase, total bilirubin, total protein, fasting glucose, albumin, creatinine, urine analysis), pregnancy test , negative results of HBsAg, anti HBC and anti HIV

Excluded :
Have a history of hepatic, renal and cardiovascular system, taken alcohol or other medications for a long period of time, had hypersensitivity to azithromycin, had received any investigation drug within four weeks, & loss more than 450 ml of blood within 3 months prior screening.

24 Subjects were selected

Before Sampling

- All subjects avoided using other drugs for at least two weeks prior to the study and until after its completion.
- They are also refrained from ingesting alcohol, caffeine, chocolate, tea or coke containing beverages at least 48 hours before each dosing and until the collection of the last blood sample.
- Subjects were confined at clinical unit of Clinisindo Laboratories one night before study to assure the fasting condition (10 hours before drug administration).
- On the study day, subjects were given one tablet of either product with 240 ml of water.
- No food was allowed until 4 hours after dose administration. Water intake was allowed 2 hours after the dose. Standard meals were served at 4 and 11 hours after drug administration. Snack was served at 8 hours after drug administration.

Safety Evaluation

- Analysis of safety-related data was considered using the more common adverse events which occurred after initiation of study treatment

Cause relation to study drug	Events	Total
Related	Abdominal discomfort	15
	Dizziness	7
	Somnolence	2
	Nausea	1
Probable	Weakness	2
	Myalgia	1
Total		28

MS condition

- Scan type : MRM
- Polarity : Positive
- Scan mode : N/A
- Ion source : Turbo Ion Spray (TIS)
- Mass Parameter :
- *Compound Dependent Parameters*

Parameter	Azithromycin	Propranolol
Detection Mass	375.0>83.1	260.2>116.2
Declustering Potential (DP) (V)	41	70
Entrance Potential (EP) (V)	6.5	12
Collision Cell Entrance Potential (CEP)	16	14
Collision Energy (CE)	33	23
Collision Cell Exit Potential (CXP)	4	4
Dwell Time (msec)	150	150

Chromatographic Condition

- **Column** : Synergy 4µ POLAR-RP-80A, 50x2.00 mm, 4µm (Phenomenex®, USA)
- **Guard column** : AQ C18, 4 x 2.0 mm (Phenomenex®, USA)
- **Mobile Phase** : Gradient,

  0.1% formic acid in acetonitrile

  0.1% formic acid in water

Time	B (%)
0.01	80
1.30	5
2.00	5
2.10	80
2.50	Controller (stop)

- **Flow rate** : 0.7 mL/min
- **Injection vol** : 5µL
- **Run time** : 2.50 minutes
- **Retention time** : 0.95 min for azithromycin and 1.10 min for propranolol

Sample Preparation

250µL of human plasma sample was added with 20µL of Propranolol (10µg/mL)

-Mixing
-250µL of acetonitrile was added
-Vortex mixed for 30'' and centrifuged at 3000rpm for 10 minutes

Supernatant was transferred into vial

centrifuged at 3000 rpm for 5 minutes

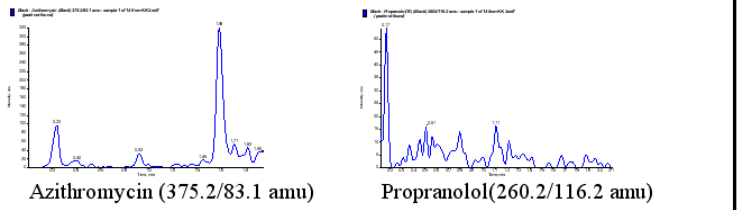
5µL supernatant was injected into LC-MS/MS system

Pharmacokinetic & Statistical Analysis

- The bioequivalence parameters, AUC0-t, AUC0-∞, and Cmax.
- Cmax and tmax were obtained directly by inspection of the individual drug plasma concentration time data, and were used as measures of rate of absorption. The area under the plasma concentration time curve up to the last time (t) showing a measurable concentration (Ct) of the analyte (AUC0-t) was calculated using the trapezoidal rule.
- For the parameters of AUC0-t, AUC0-∞ and Cmax a multiplicative model was assumed, and analysis of variance (ANOVA) was applied using the respective ln-transformed data.
- All statistical analyses were performed using EquivTest version 2.0 software (Statistical Solution, Cork, Ireland).

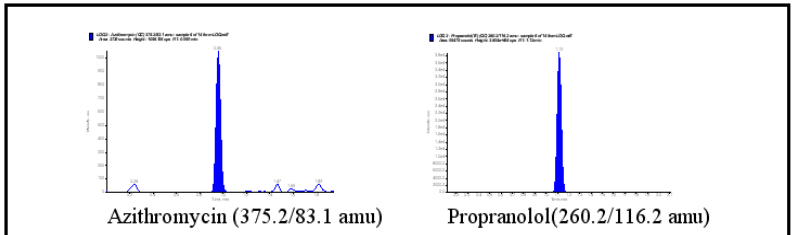
RESULT AND DISCUSSION

Chromatogram



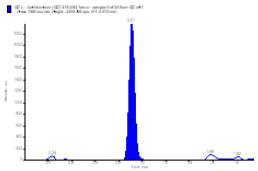
Sample Name:	Blank (375.2/83.1 amu)
Analyte Area:	0.00
Analyte RT:	0.00
IS Area:	0.00
IS RT:	0.00
Analyte/IS Ratio:	-7.00
Cal Conc (ng/mL)	N/A (ng/mL)

Figure 1. Chromatogram of blank plasma

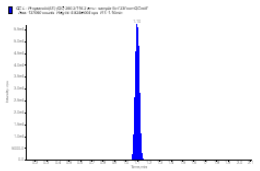


Sample Name:	LOQ 2 (375.2/83.1 amu)
Analyte Area:	2729.00
Analyte RT:	0.959
IS Area:	95475.00
IS RT:	1.12
Analyte/IS Ratio:	0.03
Cal Conc (ng/mL)	2.3197 (ng/mL)

Figure 2. Chromatogram of LOQ of Azithromycin



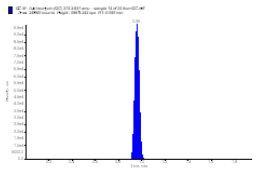
Azithromycin (375.2/83.1 amu)



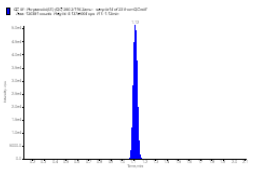
Propranolol(260.2/116.2 amu)

Sample Name:	QC L (375.2/83.1 amu)
Analyte Area:	7580.00
Analyte RT:	0.912
IS Area:	137060.00
IS RT:	1.10
Analyte/IS Ratio:	0.06
Cal Conc (ng/mL)	5.339 (ng/mL)

Figure 3. Chromatogram of QC Low of Azithromycin



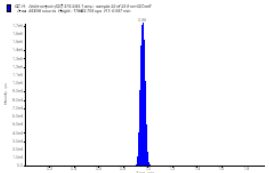
Azithromycin (375.2/83.1 amu)



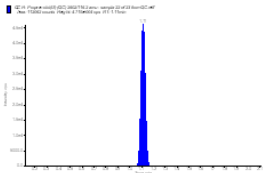
Propranolol(260.2/116.2 amu)

Sample Name:	QC M (375.2/83.1 amu)
Analyte Area:	249069.00
Analyte RT:	0.959
IS Area:	120381.00
IS RT:	1.12
Analyte/IS Ratio:	2.07
Cal Conc (ng/mL)	217.4299 (ng/mL)

Figure 4. Chromatogram of QC Medium of Azithromycin



Azithromycin (375.2/83.1 amu)



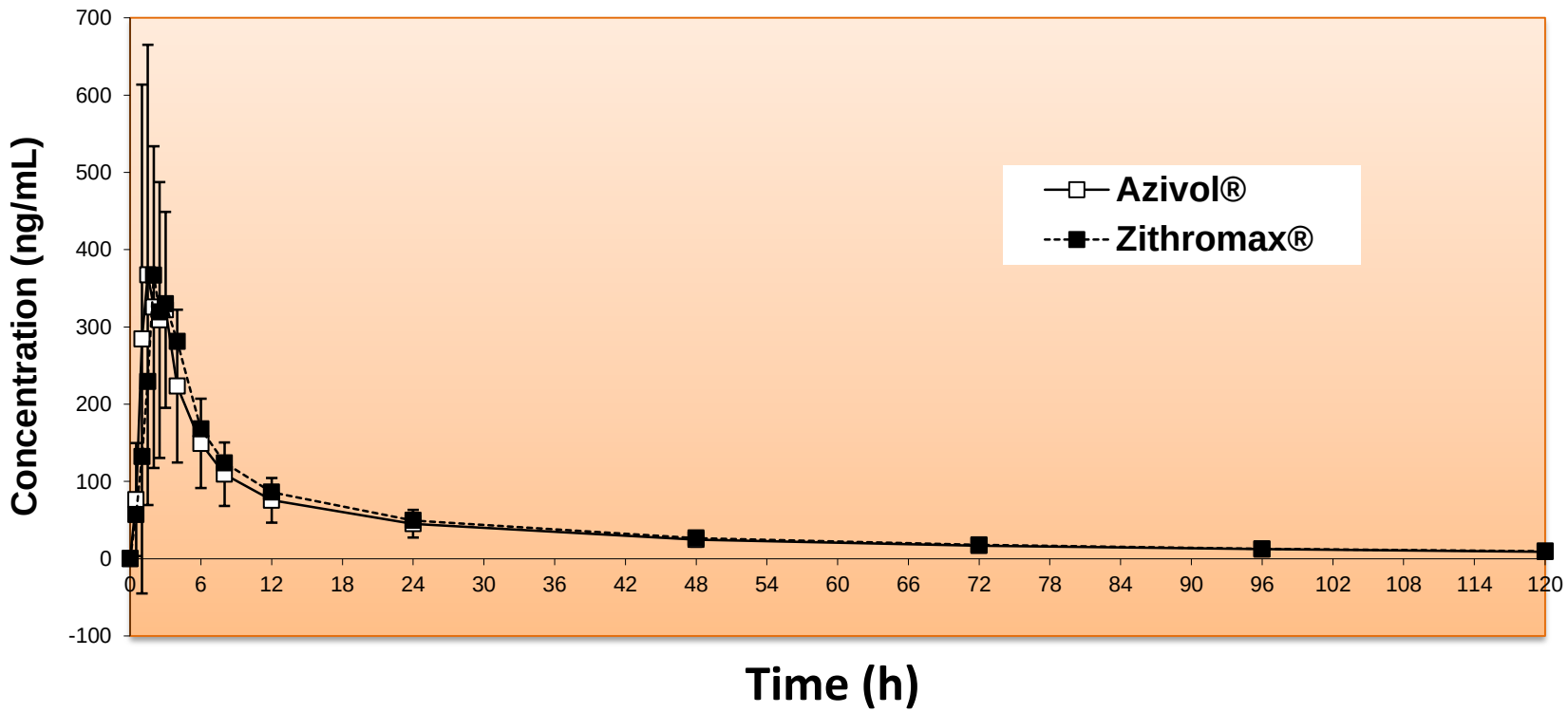
Propranolol(260.2/116.2 amu)

Sample Name:	QC H (375.2/83.1 amu)
Analyte Area:	453096.00
Analyte RT:	0.957
IS Area:	112062.00
IS RT:	1.11
Analyte/IS Ratio:	4.04
Cal Conc (ng/mL)	425.3689 (ng/mL)

Figure 5. Chromatogram of QC High of Azithromycin

Pharmacokinetic evaluation

The mean azithromycin concentration versus time profiles for both formulations



Pharmacokinetic evaluation

Table 1. Mean pharmacokinetic characteristic for Azithromycin after administration for the two formulations

Parameter	Test Formulation	Reference Formulation
Geometric mean C max (ng/mL) Range	534.67 193.87 – 1423.04	567.82 298.80 – 1585.02
Geometric mean AUC_{0-t} ngxh/ml) Range	4443.48 2178.76 – 8148.69	4695.61 2452.39 – 8203.54
Geometric mean $AUC_{0-\infty}$ (ngxh/ml) Range	5075.61 2615.24 – 9154.81	5323.31 2722.78 – 9340.76
Geometric mean $t_{1/2}$ (h) range	49.99 36.29 – 65.73	47.40 36.63 – 63.81
Median t_{max} (h) range	1.75 1.00 – 6.00	2.25 1.00 – 4.00

Pharmacokinetic evaluation

Table 2. Parametric 90% confidence interval for the mean pharmacokinetic characteristic of Azithromycin formulations

Parameter	T/R Point Estimate	Confidence Limits Range
Geometric mean C max (ng/mL)	94.16	80.31 – 110.41
Geometric mean AUC_{0-t} ngxh/ml)	94.63	86.27 – 103.81
Geometric mean $AUC_{0-\infty}$ (ngxh/ml)	95.35	87.15 – 104.31
Geometric mean $t_{1/2}$ (h)	105.46	99.59 – 111.67

Discussion

- 90% confidence intervals (CI) of AUC_t, $AUC_{0-\infty}$, C_{max}, and $t_{½}$ log-transformed individual ratios of azithromycin were included into the range of bioequivalence, i.e. 80-125%. The individual t_{max} was not statistically different between the two formulations.
- The mean ratio of $AUC_{0-t}/AUC_{0-\infty}$ for all individuals and for both products was around 12%, indicate an adequate sampling time since the extrapolated portion of the total AUC is less than 20%.
- $t_{½}$ in the study (50.50 ± 7.33 h for test product and 47.89 ± 7.23 h for reference product) were consistent with the results reported in the literatures (~ 40-50 h).
- The intra-subject variability of azithromycin in the AUC0-t, AUC0-∞ , Cmax, and t½ estimates from the coefficient of variables were 18.65%, 18.11%, 32.09%, and 11.53%, respectively.

Conclusion

- The two azythromycin tablets (test and reference drugs) were bioequivalent in terms of the rate and extent of absorption.
- Thus the two formulations are therapeutically equivalent and therefore can be used interchangeably

University of Indonesia

Thank You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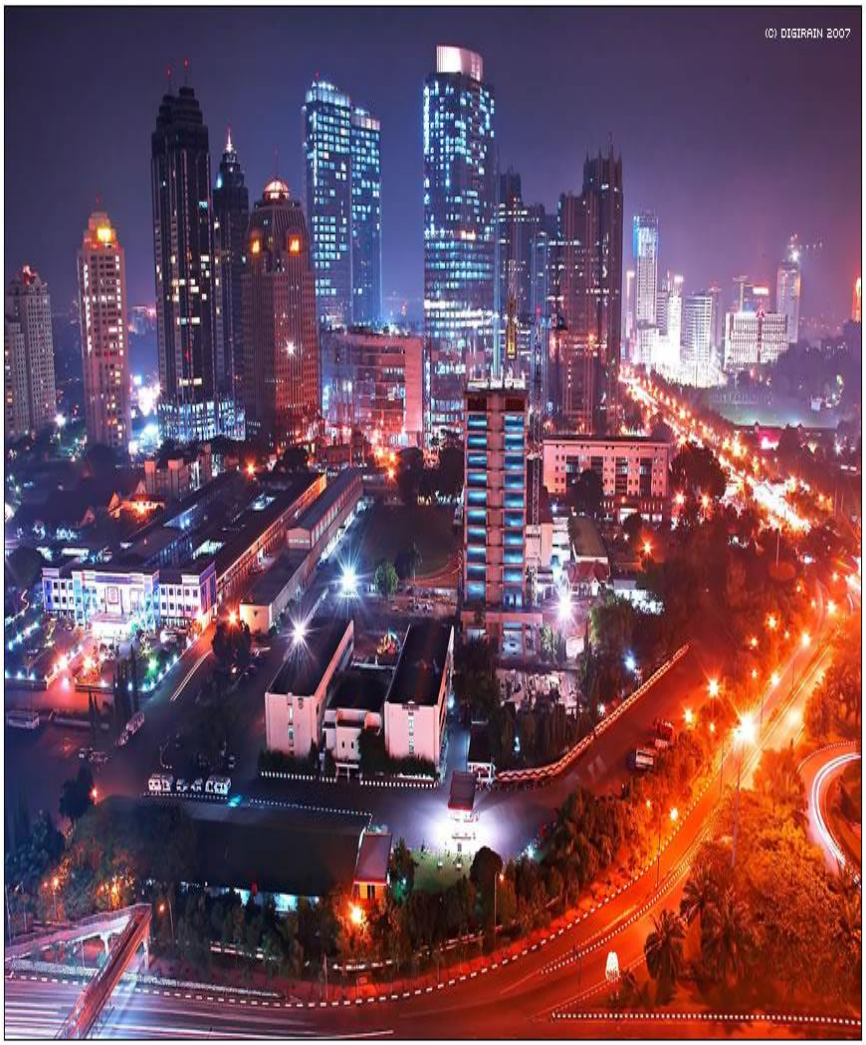
165°
South Pacific Ocean
Australia
Perth
Sydney
30°
30°
Wellington
135°
Antarctica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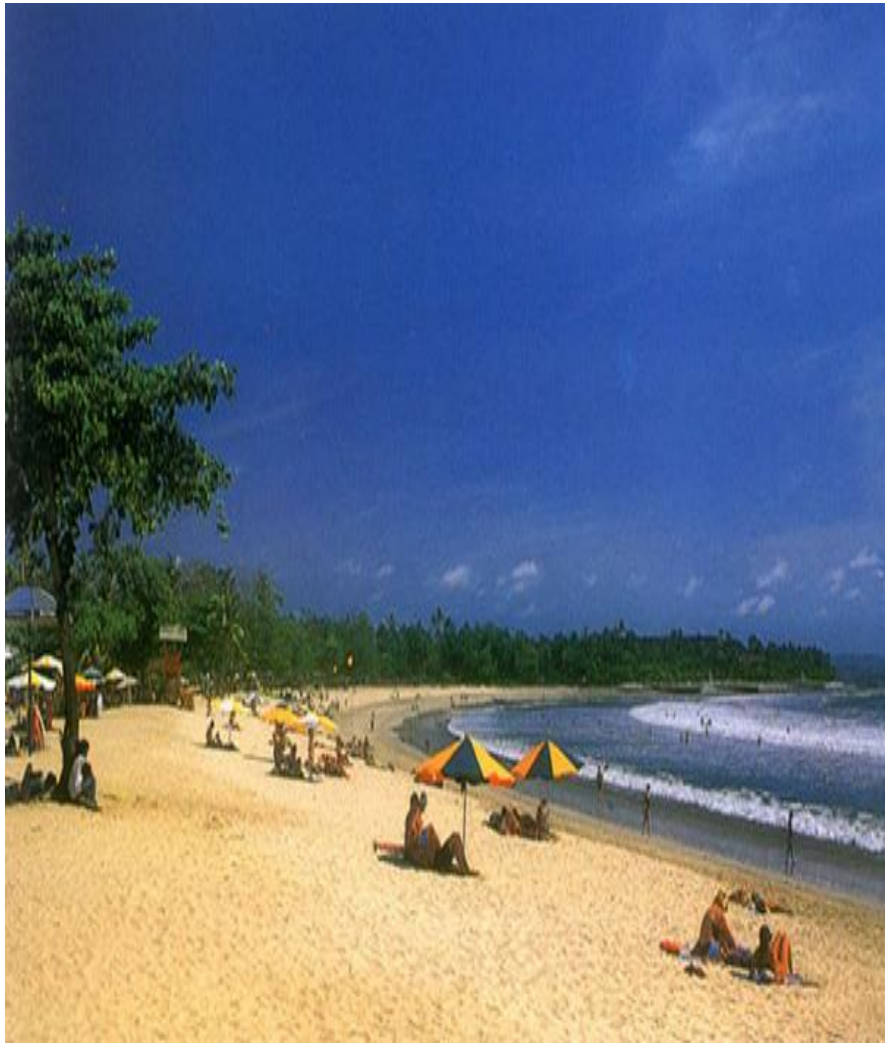


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We welcome you all to our future conferences of OMICS International

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