

## BIOEQUIVALENCE STUDY OF 500 MG CEFUROXIME AXETIL FILM-COATED TABLETS IN HEALTHY VOLUNTEERS

MICHAŁ KAZA<sup>1\*</sup>, ANDRZEJ LEŚ<sup>1,2</sup>, KRYSZYNA SERAFIN-BYCZAK<sup>1</sup>, HANNA KSYCIŃSKA<sup>1</sup>, PIOTR J. RUDZKI<sup>1</sup>, PIOTR GUTKOWSKI<sup>3</sup>, TOMASZ DREWNIAK<sup>3</sup>, ANNA GUTKOWSKA<sup>3</sup>, ANDRZEJ TARASIUK<sup>4</sup>, EWA PIĄTKOWSKA-CHABUDA<sup>4</sup>, MAŁGORZATA SKOWROŃSKA-SMOLAK<sup>4</sup> and EWA WILKOWSKA<sup>4</sup>

<sup>1</sup> Pharmaceutical Research Institute, Pharmacology Department, Rydygiera 8, 01-793 Warszawa, Poland

<sup>2</sup> Warsaw University, Faculty of Chemistry, Pasteura 1, 02-093 Warszawa, Poland

<sup>3</sup> TRIAL – Clinical Research, Czecha 5, 04-555 Warszawa, Poland

<sup>4</sup> Tarchomińskie Zakłady Farmaceutyczne Polfa S.A., A. Fleminga 2, 03-176 Warszawa, Poland

**Abstract:** The aim of the study was to investigate the bioavailability of a generic product of 500 mg cefuroxime axetil film-coated tablets (test) as compared to that of a branded product (reference) at the same strength to determine bioequivalence and to apply for regulatory approval. The secondary objective of the study was to evaluate tolerability of both products. A double blinded, randomized, crossover, two-period, single-dose, comparative study was conducted in Caucasian healthy volunteers in fasting conditions. A single oral dose administration of the test or reference product was followed by 7-day wash-out period. The cefuroxime concentration was determined using a validated HPLC-UV method. The results of the single-dose study in healthy volunteers indicated that the film-coated tablets of Tarsime 500 mg manufactured by Tarchomińskie Zakłady Farmaceutyczne Polfa S.A. (test product) are bioequivalent to those of Zinnat<sup>®</sup> manufactured by GlaxoSmithKline Export Ltd. (reference product). Both products were well tolerated.

**Keywords:** cefuroxime axetil, bioequivalence, relative bioavailability, second-generation cephalosporin, tolerability

Cefuroxime is a second-generation cephalosporin used against different kinds of bacterial infections. Cefuroxime axetil is its 1-acetyloxyethyl ester, which has improved oral bioavailability. After oral administration, cefuroxime axetil is absorbed from the gastrointestinal tract and rapidly hydrolyzed by nonspecific esterases in the intestinal mucosa and blood to cefuroxime. Therefore, cefuroxime axetil cannot be measured in human plasma. Cefuroxime is subsequently distributed throughout the extracellular fluids. Following oral administration of cefuroxime axetil tablets, maximum cefuroxime concentration in plasma occurs after 1–4 h. The elimination half-life is 1–2 h (1, 2).

The reference product in the present study was an already approved and commercially available Zinnat<sup>®</sup> film-coated tablets (manufactured by GlaxoSmithKline Export Ltd.). For the registration purposes, the efficacy and safety of this product has been proven already in clinical trials. This

drug has therefore served as a reference and a basis for comparison to a cefuroxime test product (Tarsime 500 mg film-coated tablets manufactured by Tarchomińskie Zakłady Farmaceutyczne Polfa S.A.).

The aim of the study was to investigate the bioavailability of a generic product of 500 mg cefuroxime film-coated tablets (test) as compared to that of a branded product (reference) at the same strength to determine bioequivalence and to apply for regulatory approval. The secondary objective of the study was to compare tolerability of both products.

### EXPERIMENTAL

#### Subjects and Methods

##### Study products

The test product was Tarsime 500 mg film-coated tablets (500 mg of cefuroxime axetil, batch

\* Corresponding author: e-mail: m.kaza@ifarm.eu, phone +48 22 456 38 57, fax +48 22 456 38 38

no: 011109, expiration date: November 2011) manufactured by Tarchomińskie Zakłady Farmaceutyczne Polfa S.A. The reference product was Zinnat® 500 mg film-coated tablets (500 mg of cefuroxime axetil, batch no: C429205, expiration date: July 2012) manufactured by GlaxoSmithKline Export Ltd.

### ***In vitro* dissolution**

Comparison of dissolution profiles of the test and reference products is required for the generic product application and may be relevant to the prediction of the *in vivo* study results. An *in vitro* dissolution profile comparison was performed using analytical methods according to the current USP requirements. Samples of test and reference tablets were evaluated ( $n = 12$ ) using 900 mL of phosphate buffers of pH 1.2; 4.5 and 6.8. Temperature was maintained at  $37 \pm 0.5^\circ\text{C}$ . Samples of aliquot were collected after 15, 30 and 45 min. Concentration of cefuroxime axetil was determined spectrophotometrically at 278 nm.

### **Subjects**

The sample size for the study was determined assuming a significance level  $\alpha = 0.05$ , power of the study  $1 - \beta = 0.80$  and expected point estimate between 0.90 and 1.10. The intrasubject variability of the primary pharmacokinetic parameters after a single-dose administration of cefuroxime axetil tablets was estimated as  $CV_{\text{intra}} = 16\%$  (2). Based on these assumptions, it was calculated that the group of 24 subjects should complete the study.

All 24 male and female Caucasian healthy subjects enrolled into the study were examined to verify their healthy status. These examinations included medical history, vital sign measurements, 12-lead electrocardiogram (ECG), blood sample analysis (basic profile, complete blood cell count, viral serology) and urinalysis (sediment, drugs). Subjects with relevant clinical, analytical, or ECG abnormalities were excluded from the trial. Additional exclusion criteria were as follows: smoking, history of alcohol or drug abuse, consumption of any medication within one month prior to study commencement, participation in a clinical trial in the 3 months before enrollment; history of clinically important illness or major surgery in the 6 months before enrollment; inability to relate to and/or cooperate with the investigators; medication allergy; illness or disorders that could affect the absorption, distribution, metabolism, and/or excretion of drugs (e.g., malabsorption, edemas, renal and/or hepatic failure); history of positive serology for hepatitis B or C (not due to immu-

nization); or HIV and blood or blood-derivative transfusion in the 6 months before enrollment.

### **Study design**

The study was designed according to the respective European Medicines Agency (EMA) guidelines on bioequivalence investigation (3, 4). It was conducted between April 2010 and July 2010 by TRIAL Clinical Research Clinic in Warszawa in compliance with the International Conference of Harmonization (ICH) guideline for Good Clinical Practice (GCP) and the Declaration of Helsinki and its amendments (5, 6). Ethical approval was received from the Ethics Committee of Regional Medical Chamber in Warszawa. The clinical trial registration number of the study was EudraCT 2009-017755-97. All eligible subjects provided written informed consent to participate and were free to withdraw from the study at any time without any obligation. Subjects were compensated for the study participation.

The study was a single-dose, randomized-sequence, double-blind, two-period cross-over design with 7 day washout period. A single 500 mg of either product (Tarsime 500 mg or Zinnat® 500 mg) was administered with 200 mL of water to swallow after an overnight fast. The order of administration was randomized prior to the start of the study. Food intake was strictly controlled and all subjects received the same food to minimize the effects of food on the study outcomes. The standardized breakfast and lunch were served at 4 and 8 h after drug administration, respectively. All meals were planned by a nutritionist, and standardized meals consisted of the same food in both phases of the study. The consumption of alcohol, grapefruit juice, and beverages was not permitted for 72 h prior to the study, or after drug administration, until final blood samples were collected. During the study period, the subjects were under medical surveillance by two registered physicians.

### **Blood sample collection**

A 18 GA catheter (Venflon, Becton Dickinson, Sweden) was inserted into suitable forearm vein and 7.5 mL of blood was withdrawn at different time intervals. Venous blood samples were obtained prior to dosing (baseline) and 0.25, 0.50, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0, 16.0 and 24.0 h after dosing in each period. The samples were collected in pre-labeled lithium heparine plasma tubes (Monovette® Sarstedt). Within 30 min after sampling, the blood samples were centrifuged at room temperature at approximately 2500 rpm for 10

min. The separated plasma was stored below  $-20^{\circ}\text{C}$  until analysis.

#### Determination of cefuroxime plasma concentrations

Due to rapid deesterification of cefuroxime axetil to cefuroxime in the intestinal mucosa, plasma cefuroxime concentrations were measured to assess bioequivalence of studied products. The cefuroxime plasma concentrations were determined at the GLP-certified laboratory of the Pharmacology Department, Pharmaceutical Research Institute by means of high performance liquid chromatography (HPLC) coupled with UV detection. The validated bioanalytical method was previously described [7]. A protein precipitation was applied as the sample preparation technique and cefalexin monohydrate was used as the internal standard (IS). Cefuroxime sodium salt (reference standard) was obtained from Sigma-Aldrich (St. Louis, MO, USA) and cefalexin monohydrate was supplied by Tarchomińskie Zakłady Farmaceutyczne Polfa S.A. The liquid chromatography Shimadzu system consisted of a controller SCL-10AVP, a pump LC-10ADVP, an autosampler SIL-10A, a column oven and an UV detector SPD 10-AVP. The data were processed using Shimadzu Class-VP version 6.0 software. The chromatographic separation was performed at  $40^{\circ}\text{C}$  on a Supelcosil LC-18-DB ( $250 \times 4.6$  mm,  $5 \mu\text{m}$ ) column (Supelco, Bellefonte, PA, USA). The mobile phase consisted of 8.5% of acetonitrile in 0.07 M potassium dihydrogen phosphate solution ( $\text{pH} = 3.0$ ) at a flow rate 1.7 mL/min. The retention times of cefuroxime and IS were around 13.9 min and 9.6 min, respectively. The run time was 17.0 min. The UV wavelength  $\lambda = 275$  nm was selected. The plasma sample (500 mL) was mixed with 50 mL of IS (200  $\mu\text{g}/\text{mL}$ ), 100 mL of 5% acetic acid and 1000 mL of cooled acetonitrile. After centrifugation, the organic layer was separated and evaporated to dryness. The dry residue was reconstituted in 250  $\mu\text{L}$  of acetonitrile: water solution (1:9, v/v), centrifuged and 50  $\mu\text{L}$  aliquot was injected onto the HPLC system.

#### Pharmacokinetic and statistical analyses

The pharmacokinetic parameters for cefuroxime were determined from the plasma concentration *vs.* time curve with the aid of the WinNonlin software (version 5.0.1., Pharsight Corp.). The parameters selected as primary endpoints of the study were the area under the plasma concentration *vs.* time curve ( $\text{AUC}_{0-t}$ ), the area under the plasma concentration *vs.* time curve extrapolated to the infinity ( $\text{AUC}_{0-\infty}$ ) and the maximum plasma concentration of

the drug ( $C_{\text{max}}$ ). The time to reach maximum plasma concentration of the drug ( $t_{\text{max}}$ ), the elimination half-life ( $t_{1/2}$ ) and the mean residence time (MRT) were selected as secondary parameters.

$C_{\text{max}}$  and  $t_{\text{max}}$  were obtained directly from the experimental data. The elimination rate constant ( $k_{\text{el}}$ ) was estimated from the data of 3 or 4 points of each plasma concentration *vs.* time curve by least square regression analysis. The  $t_{1/2}$  was calculated as  $\ln 2/k_{\text{el}}$ . The  $\text{AUC}_{0-t}$  was calculated by the trapezoidal rule up to the last measurable plasma concentration ( $C_{\text{last}}$ ). The  $\text{AUC}_{0-\infty}$  was automatically obtained within the program by summing up the  $\text{AUC}_{0-t}$  and the extrapolated area ( $\text{AUC}_{\text{rest}}$ ). The latter parameter was calculated as  $C_{\text{last}}/k_{\text{el}}$ . The MRT was calculated as  $\text{AUMC}/\text{AUC}_{0-\infty}$ , where AUMC was the area under the time course of the statistical first moment curve.

The statistical calculations were performed using the SAS/STAT Software (version 9.1.3. for Windows, SAS Institute). The tests for normality of ln-transformed pharmacokinetic parameters were performed with the use of the Shapiro-Wilk, Kolmogorov-Smirnov, Cramer-von Mises and Anderson-Darling tests. The analysis of variance (ANOVA) was performed on the ln-transformed data of  $\text{AUC}_{0-t}$ ,  $\text{AUC}_{0-\infty}$ ,  $C_{\text{max}}$ ,  $t_{1/2}$  and MRT applying General Linear Models (GLM) procedure and the fixed-effects model with sequence, subject within sequence, product and period effects. The statistical significance of effects was determined on basis of the calculated p-values with value larger than 0.05 meaning no statistical significance.

Based on the ANOVA results, 90% CI for the  $\mu_T/\mu_R$  (ratio of geometric means for the test and the reference product) of the analyzed pharmacokinetic parameters was constructed. Bioequivalence is assumed when 90% CI of the point estimate (test over reference products) for  $\text{AUC}_{0-t}$ ,  $\text{AUC}_{0-\infty}$  and for  $C_{\text{max}}$  falls within the 80.00–125.00% range and when the Schuirmann's TOST test (two one-sided *t*-test) is complied ( $p < 0.05$ ) (3, 4, 8, 9). The statistical analysis for  $t_{\text{max}}$  was performed on the untransformed data using the non-parametric Wilcoxon test.

#### Tolerability analysis

In order to prevent the occurrence of an adverse events during the study, the following measures have been taken:

- the drug administration was limited to a single oral dose of 500 mg/study period;
- only healthy adult volunteers with no history of hypersensitivity reactions to cefuroxime or other related molecules were enrolled;

- the investigator has checked each volunteer's well being prior to his/her discharge from the clinic.

Tolerability was determined by monitoring vital signs (blood pressure, heart rate, body temperature) at baseline and at the end of each period. Laboratory results (hematology, urinalysis, blood biochemistry) collected before and after the study of all the subjects were also considered. The participants were interviewed by the physician as well as nonspecific questioning. All the subjects were advised to report any adverse event or undesirable sign or symptom at any time during the study period.

## RESULTS

### In vitro dissolution

Cefuroxime axetil is slightly soluble in water. At least 55% of the drug was dissolved within 10 min, 65% within 20 min and 85% within 30 min. The dissolution profiles of the test product matched those of the reference product under various pH conditions. Similarity factors calculated for the dissolution profiles in all buffers indicated similarity between dissolution profiles of the test and reference products. The results are presented in Figure 1.

Table 1. Demographic data of the population included in the study (n = 24).

Variable		Data
Gender	male [n]	17
	female [n]	7
Caucasian volunteers [n]		24
Age [years]*		25 <18 – 37>
Height [cm]*		178 <154 – 192>
Weight [kg]*		77 <62 – 90>
BMI [kg/m <sup>2</sup> ]*		23 <18 – 25>

\* Mean <range>

Table 2. Plasma pharmacokinetic parameters of cefuroxime after single 500 mg cefuroxime axetil dose administration of the test and the reference products.

Test product (n = 24)					
Pharmacokinetic parameter	Arithmetic mean	Median	Geometric mean	SD	CV [%]
AUC <sub>0-t</sub> [ng·h/mL]	15.6	16.0	14.8	4.9	31.6
AUC <sub>0-8</sub> [ng·h/mL]	16.3	17.0	15.5	5.0	30.7
C <sub>max</sub> [ng/mL]	5.24	4.92	5.01	1.64	31.3
t <sub>max</sub> [h]	1.68	1.50	1.57	0.60	35.8
t <sub>1/2</sub> [h]	1.29	1.23	1.27	0.23	17.5
MRT [h]	2.78	2.75	2.75	0.39	14.0
Reference product (n = 24)					
Pharmacokinetic parameter	Arithmetic mean	Median	Geometric mean	SD	CV [%]
AUC <sub>0-t</sub> [ng·h/mL]	15.1	14.3	14.5	4.7	30.9
AUC <sub>0-8</sub> [ng·h/mL]	15.8	15.1	15.1	4.7	30.1
C <sub>max</sub> [ng/mL]	4.86	4.53	4.71	1.33	27.3
t <sub>max</sub> [h]	1.69	1.50	1.50	0.91	53.7
t <sub>1/2</sub> [h]	1.28	1.24	1.26	0.27	21.4
MRT (h)	2.89	2.73	2.84	0.58	19.9

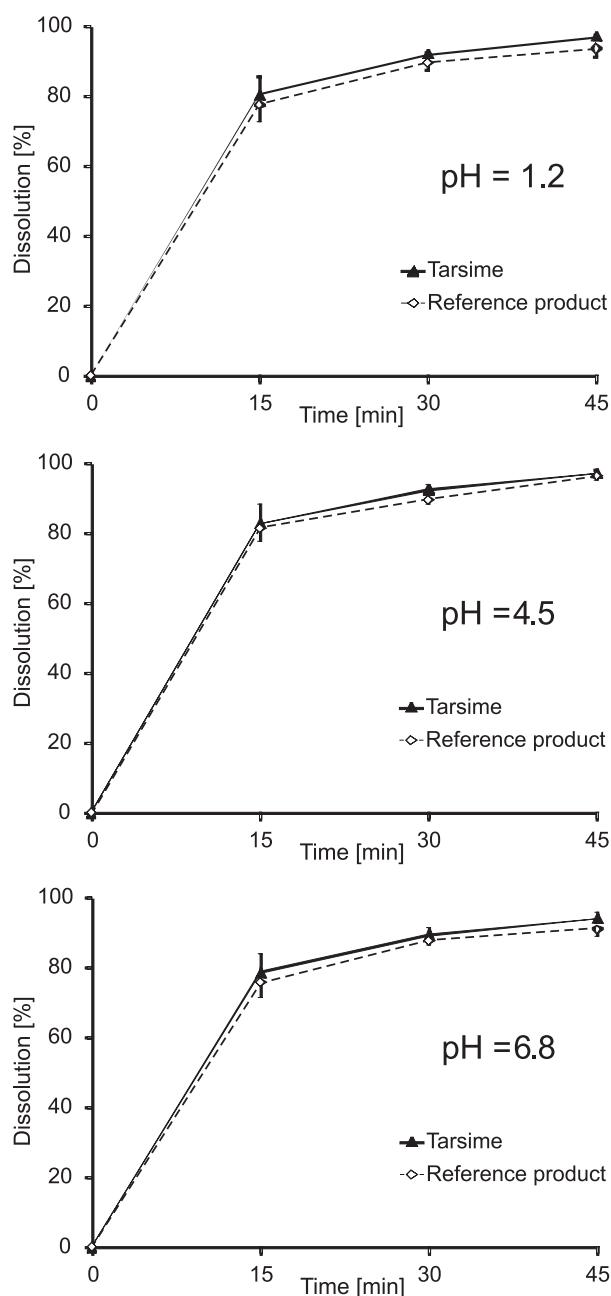


Figure 1. Dissolution profiles of cefuroxime axetil in phosphate buffers of different pH

### Study population

The study was conducted in 24 Caucasian non-smoking healthy male and female subjects. The results of the physical examination for all subjects during the pre-study visit and post-study visit were found to be normal. No subject abandoned the study for any reason. The summary of the demographic data of the population is presented in Table 1.

### Method validation

The method validation covered all required tests, including evaluation of the carry-over effect, selectivity, extraction recovery, limit of quantification, linearity, accuracy and precision, stability, dilution integrity and system suitability tests. The validation parameters were defined according to the EMA and the FDA (10, 11). All parameters met pre-defined acceptance criteria (7).

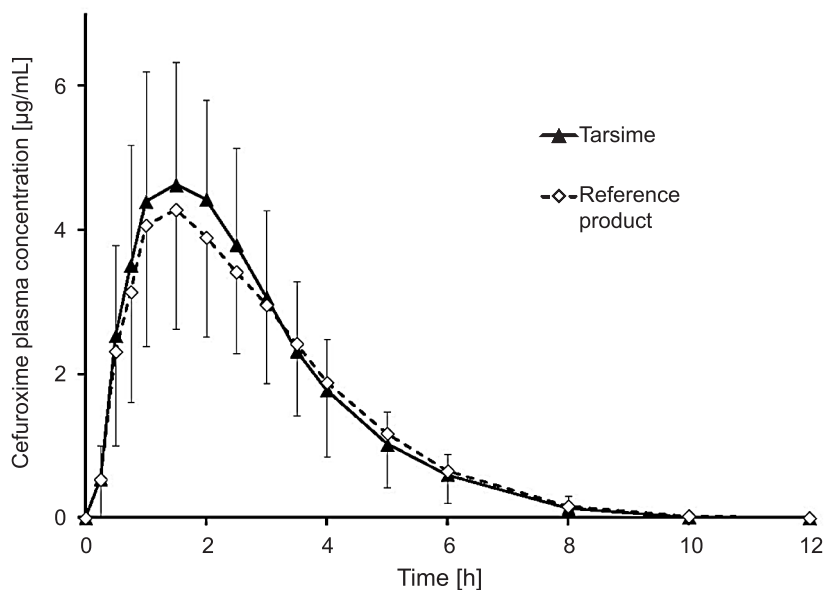


Figure 2. Mean (SD) cefuroxime plasma concentration vs. time profile following a single 500 mg oral dose of Tarsime film-coated tablets (test product) and Zinnat® film-coated tablets (reference product) truncated at 12 hours

Table 3. The 90% confidence intervals based on the Schuirmann's TOST test and using mean square error (MSE) estimated from ANOVA analysis of pharmacokinetic parameters.

Pharmacokinetic parameter (n = 24)	Point estimate [%]	90% Confidence interval [%]	Estimated intrasubject CV [%]
AUC <sub>0-t</sub>	102.58	94.52–111.33	16.6
AUC <sub>0-8</sub>	102.56	94.76–110.99	16.0
C <sub>max</sub>	106.38	98.25–115.19	16.1

### Pharmacokinetics and bioequivalence analysis

The mean plasma concentrations vs. time profiles after a single oral administration of both products with cefuroxime axetil are shown in Figure 2. The descriptive statistics of primary and secondary pharmacokinetic parameters is shown in Table 2.

It appeared that one cannot reject the hypothesis on the ln-normal distribution of the AUC<sub>0-t</sub>, AUC<sub>0-8</sub>, C<sub>max</sub>, t<sub>r</sub>, and MRT for both test and reference products at the significance level  $\alpha = 0.05$ . The t<sub>max</sub> distribution was significantly different from the normal distribution; therefore, in the subsequent analysis non-parametric tests were used for its evaluation. All primary pharmacokinetic parameters, i.e., AUC<sub>0-t</sub>, AUC<sub>0-8</sub> and C<sub>max</sub>, met the bioequivalence regulatory criteria (Table 3). The intrasubject variability of the primary pharmacokinetic parameters were higher than parameters estimated by Pistos et al. (2) with the exception of the C<sub>max</sub> which was similar to the published data. The descriptive statistics for MRT

and t<sub>1/2</sub> were similar for the test and reference products (Table 2).

The descriptive statistics of t<sub>max</sub> were similar for the test and reference products (Table 2). However, lower variability in t<sub>max</sub> was observed for the test product (CV = 35.8%) than for the reference product (CV = 53.7%). The histogram of t<sub>max</sub> for both products is presented in Figure 3. The distribution of the t<sub>max</sub> non-transformed values were analyzed with the model already employed for C<sub>max</sub>, and AUC<sub>0-8</sub>, according to the procedure suggested by Hauschke et al. (12). The differences of the t<sub>max</sub> values of the test and reference products were tested with the non-parametric Wilcoxon test. It appeared that the point estimate of this difference of 0.25 h belongs to the acceptance interval of [–0.30 h; 0.30 h]. The 90% confidence interval of [–0.25 h; 0.50 h] exceeds the upper limit of the acceptance interval by 0.20 h. Based on the statistical grounds only, one can conclude that the t<sub>max</sub> values of the test and ref-

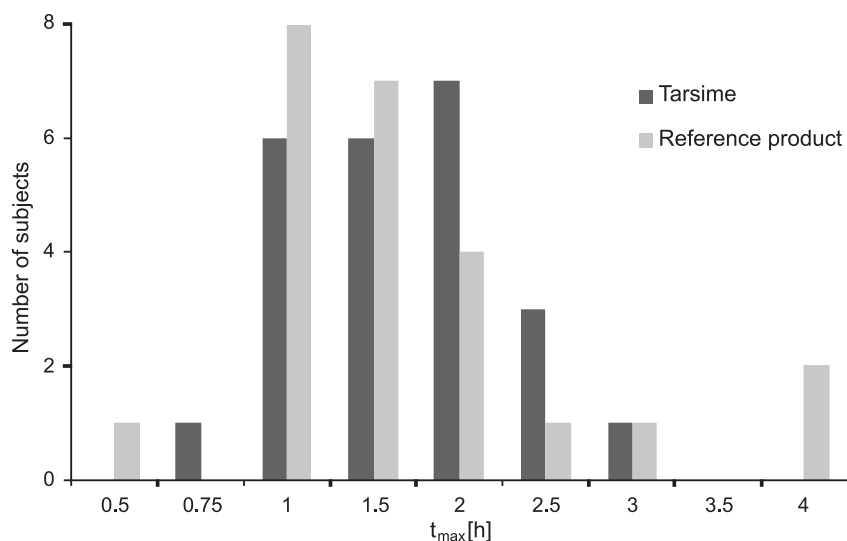


Figure 3. Histogram of  $t_{\max}$  ( $n = 24$ ) following a single 500 mg oral dose of Tarsime film-coated tablets (test product) and Zinnat® film-coated tablets (reference product)

reference products are significantly different. However, from the clinical point of view such an interval excess of 0.20 h is likely negligible. In comparison to the 1.50 h of the reference median  $t_{\max}$  as well as variability in the reference  $t_{\max}$  values, observed excess should be considered as not relevant.

### Tolerability

Both products were well tolerated. All the 24 subjects completed the study without any significant adverse events (in two cases headache was observed). No clinically significant abnormalities on physical examination including vital signs measurement, ECG recordings and laboratory results were observed.

### DISCUSSION

Cefuroxime axetil tablets have been available on the market since 1987. An extensive search in biomedical literature databases retrieved several papers on the cefuroxime axetil bioequivalence studies (1, 2, 13–17). In the most cases, bioequivalence studies were carried out as two-period crossover studies following a single dose administration in over 20 healthy volunteers, similar to the study presented in this paper. Moreover, cefuroxime plasma pharmacokinetic parameters observed in the study described above were similar to the literature data.

The aim of the study was to evaluate the bioequivalence between the test (Tarsime manufactured

by Tarchomińskie Zakłady Farmaceutyczne Polfa S.A.) and the reference (Zinnat® manufactured by GlaxoSmithKline Export Ltd.) products. The clinical part of the study was designed in compliance with the respective EMA guidances: valid in 2009 as well as revised and coming into effect in 2010 (3, 4). Based on the cefuroxime elimination half-life of 1–2 h (1, 2), a wash-out period of 7 days, i.e., over 7 cefuroxime elimination half-lives, was selected for the study to allow complete elimination of the drug before subsequent dosing and avoid carry-over effects. The cefuroxime concentrations above the lower limit of quantification (0.200 µg/mL) were not observed in any of the subject pre-dose samples, which confirmed the proper selection of the wash-out period.

For each of the primary pharmacokinetic parameters *a posteriori* calculated power of the study was higher than the assumed power of 0.80, which confirmed the proper selection of the number of volunteers participating in the study.

Generally, the proposed sampling schedule enabled proper assessment of  $t_{\max}$ , which ranged 0.5–4.0 h, and  $C_{\max}$  for both investigated products. There were no profiles, i.e., specific periods for specific volunteers, where  $C_{\max}$  was the first sampling point after the drug administration. For all profiles the  $AUC_{0-t}$  was at least 80% of the  $AUC_{0-\infty}$ , which confirmed the proper duration of sampling.

All the above arguments indicate that the study was designed and conducted according to the respective EMA guidances and enabled proper eval-

uation of the bioequivalence between the test and the reference product (3, 4).

## CONCLUSION

The results of this single-dose study in healthy white volunteers indicated that Tarsime 500 mg film-coated tablets manufactured by Tarchomińskie Zakłady Farmaceutyczne Polfa S.A. (test product) are bioequivalent to Zinnat® 500 mg film-coated tablets manufactured by GlaxoSmithKline Export Ltd. (reference product). Both products were well tolerated.

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