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Unveiling the Potential of C_{max} f_2 Factor Applied to Pilot Bioavailability/Bioequivalence Studies—A Case Study with Pazopanib Drug Products

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Abstract: Background: When companies are uncertain about the potential of a new formulation to be bioequivalent to a Reference product, it is common practice to carry out downsized pilot studies as a gatekeeping in vivo strategy to decide whether to move forward or not with a full-size pivotal study. However, due to the small study size, these studies are inarguably more sensitive to variability. **Objectives:** To address and mitigate the uncertainty of the conclusions of pilot studies concerning the maximum observed concentration (C_{max}), the f_2 factor was proposed as an alternative approach to the average bioequivalence statistical methodology. **Methods:** In this work, the alternative methodology is applied to pharmacokinetic data from pilot bioequivalence trials performed with pazopanib 200 mg and 400 mg. **Results:** Despite the small sample size, and very high intra-subject variability, the f_2 factor demonstrated the potential for predicting bioequivalence. The positive results were confirmed in the full sized pivotal studies. **Conclusions:** In conclusion, this alternate methodology shows promise in reducing uncertainty associated with pilot studies and aiding in decisions to go forward with pivotal bioequivalence studies.

Keywords: bioequivalence; generic medicinal products; pilot studies; f_2 factor; pharmacokinetics; highly variable drugs; pazopanib

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1. Introduction

Pilot bioavailability/bioequivalence (BA/BE) studies are downsized trials that can be conducted prior to pivotal trials. In these trials, 12 to 30 subjects are usually enrolled, although, in principle, a sample size is not formally calculated. Analysis and interpretation of results from pilot studies usually rely on the application of the average bioequivalence approach. However, due to the small study size, these studies are inarguably more sensitive to variability [1–3].

In order to overcome and reduce the uncertainty of the conclusions of pilot studies and on the potential of test formulations, Henriques et al. (2023) [1,2] proposed the f_2 factor as an alternative approach to the average bioequivalence methodology to assess the potential bioequivalence for the maximum observed concentration (C_{max}). C_{max} metric usually shows a higher variability in comparison to exposure metrics (area under the

plasma concentration-time curve [AUC]), and therefore a demonstration of bioequivalence is commonly more difficult for C_{max}. For the evaluation of pilot BA/BE studies, the authors suggest the application of average bioequivalence analysis, and additionally, the application of the f_2 factor method in the case where the 90% confidence interval (CI) for geometric least square means ratio (GMR) is outside the [80.00–125.00]% regulatory acceptance bioequivalence interval. Using this alternative approach, and depending on f_2 values and variability scenarios (20–60% IOV), the certainty levels to proceed with pivotal studies are highlighted. This is expected to assist companies in the decision-making process for proceeding with pivotal studies [1,2].

The proposed assessment was, however, solely based on simulated concentration-time profiles, and it is limited to drugs following a one compartment model, with median time of C_{max} (t_{max}) ranging from 0.75 to 8 h, a short elimination half-life of (approximately 4.6 h), and a mean volume of distribution of approximately 60 L [1,2].

Hence, in order to validate the f_2 factor method in a more diverse and realistic setting, the methodology was applied to real world pharmacokinetic data from BA/BE studies performed with pazopanib formulations.

Pazopanib is an ATP-competitive, second-generation inhibitor of tyrosine kinase activity associated with human vascular endothelial growth factor receptor (VEGFR)-1, VEGFR-2, VEGFR-3, platelet-derived growth factor receptor (PDGFR)-α and -β, fibroblast growth factor receptor (FGFR)-1 and -3, cytokine receptor (Kit), interleukin-2 receptor-inducible T-cell kinase (Itk), lymphocyte-specific protein tyrosine kinase (Lck), and transmembrane glycoprotein receptor tyrosine kinase (c-Fms). The drug is indicated for the treatment of patients with advanced renal cell carcinoma and for the treatment of patients with advanced soft tissue sarcoma who have received prior chemotherapy [4,5].

Pazopanib is a hydrochloride salt, being slightly soluble at pH 1 and insoluble above pH 4 in aqueous media. From in vitro caco-2 studies, pazopanib showed to be a highly permeable compound. Therefore, pazopanib is considered to be a class 2 compound (low solubility, high permeability) in the Biopharmaceutical Classification System (BCS).

Low pH dependent solubility is hypothesized to support an incomplete absorption of pazopanib, as well as a low and variable bioavailability (median was determined as 21% [range 14–39%]) [6].

From the literature, pazopanib is characterized by a low volume of distribution (approximately 25 L) [7,8]. Pharmacokinetic data from cancer patients were fitted to both monocompartment [7] or a two-compartment [8] population pharmacokinetic models, which highlights a non-extensive distribution into tissues. Following oral administration, pazopanib C_{max} is attained at approximately 2–8 h [6,9–11], which is within the f_2 factor validation range. Moreover, pazopanib also shows high inter- and intra-subject variability for C_{max} and AUC [12,13], as tested in simulations for the validation of f_2 factor [1,2].

However, pazopanib has a substantially longer elimination half-life, averaging approximately 30 h [6,9–11], deviating from the short half-life of approximately 4.6 h assumed in the simulated data [1,2]. Nevertheless, pazopanib pharmacokinetics was considered a suitable candidate to test the robustness of the f_2 method in real-world pilot bioequivalence settings, as the main objective of this work. Results derived from f_2 methodology were intended to assist the decision-making process in proceeding to pivotal studies.

2. Materials and Methods

Analyzes were performed on pharmacokinetic data obtained from two pilot and two pivotal BA/BE trials:

- Pazopanib 200 mg pilot study (BLCL-PAZ-PIL01, EudraCT No. 2020-00586-16):
  This study was a single-center, single-dose, open label, laboratory blinded, randomized, two-treatment, two-sequence, two-period ($2 \times 2 \times 2$) crossover pilot study in healthy male and nonpregnant female volunteers, with pazopanib 200 mg under fasting conditions. Doses of investigational product were separated by a washout of 14 days.

A total of 24 healthy subjects received at least one dose of investigational product and 23 subjects completed this study and were included in the statistical analysis.

- Pazopanib 200 mg pivotal study (BLCL-PAZ-EU-02, EudraCT No. 2021-002053-29):
  This study was a single-center, single-dose, open label, laboratory blinded, randomized, two-treatment, two-sequence, two-period ($2 \times 2 \times 2$) crossover pivotal study in healthy male and nonpregnant female volunteers, with pazopanib 200 mg under fasting conditions. Doses of investigational product were separated by a washout of 21 days.
  In total, 116 healthy subjects received at least one dose of investigational product. From these, 106 subjects completed this study and were included in the statistical analysis.
- Pazopanib 400 mg pilot study (BLCL-PAZ-PIL02, EudraCT No. 2020-00514-17):
  This study was a single-center, single-dose, open label, laboratory blinded, randomized, two-treatment, two-sequence, two-period ($2 \times 2 \times 2$) crossover pilot study in healthy male and nonpregnant female volunteers, with pazopanib 400 mg under fasting conditions. Doses of investigational product were separated by a washout of 21 days.
  A total of 24 healthy subjects received at least one dose of investigational product and 23 subjects completed this study and were included in the statistical analysis.
- Pazopanib 400 mg pivotal study (BLCL-PAZ-EU-03, EudraCT No. 2021-003534-36):
  This study was a single-center, single-dose, open label, laboratory blinded, randomized, two-treatment, two-sequence, two-period ($2 \times 2 \times 2$) crossover pivotal study in healthy male and nonpregnant female volunteers, with pazopanib 400 mg under fasting conditions. Doses of investigational product were separated by a washout of 21 days.
  In total, 122 healthy subjects received at least one dose of investigational product. From these, 98 subjects completed this study and were included in the statistical analysis.

The current pivotal BA/BE trials were performed to support the marketing authorization application (MAH) of a generic pazopanib formulation (Test product), using Votrient® as the Reference product. According to the European Medicines Agency (EMA)'s Guideline on the Investigation of Bioequivalence [14], for drugs with a less than proportional increase in area under the concentration-time curve (AUC) with an increasing dose over the therapeutic dose range, which is the case for pazopanib [15], bioequivalence should be established both at the highest strength and at the lowest strength. Hence, two single dose pivotal BA/BE studies under fasting condition were performed, with 200 mg and 400 mg to support MAH within EMA ambiance [15].

Before conducting the pivotal studies, the company performed two pilot studies under fasting conditions to obtain exploratory information on the relative bioavailability of the two developed formulations of pazopanib (200 mg and a 400 mg) in comparison to the Reference products, and to assist on the decision to go forward with pivotal studies.

Both pilot studies showed a Test-to-Reference GMR within the [80.00–125.00]% comparable bioavailability acceptance limits for C_{max}; however, the corresponding 90% CI were outside the acceptance limits, hence, failing to show bioequivalence. The a posteriori pivotal studies, performed with an increased number of subjects, showed bioequivalence between the Test and Reference formulations.

The pharmacokinetic analysis was performed in the parent drug, as specified in the EMA's pazopanib product-specific bioequivalence guidance [15]. Pazopanib plasma concentrations were measured using a validated liquid chromatography with tandem mass spectrometry (LC-MS/MS) analytical method in compliance with Good Laboratory Practices (GLP). The analytical method used a calibration range of 50 to 30,000 ng/mL, for the 200 mg dose, and a range of 100 to 60,000 ng/mL, for the 400 mg dose.

For each study, a total of 20 venous blood samples (volume of 6 mL each) per study period were scheduled for the quantification of pazopanib in plasma, at the following

timepoints: pre-dose (t = 0 h); 0.50, 1.00, 1.50, 2.00, 2.50, 2.75, 3.00, 3.25, 3.50, 4.00, 4.50, 5.00, 6.00, 8.00, 10.00, 12.00, 24.00, 48.00, and 72.00 h post-dose.

The four studies were conducted at BlueClinical Phase I, Hospital da Prelada, Porto, Portugal.

Pharmacokinetic and average bioequivalence analyzes were performed with Phoenix® WinNonlin® 8.2 (Certara USA Inc., Princeton, NJ, USA). Additional analyzes and graphics were performed with R version 4.3.2 (R Foundation for Scientific Computing, Vienna, Austria, 2013).

As defined by the EMA's Guideline on the Investigation of Bioequivalence [14], only subjects who provided evaluable pharmacokinetic data for both test and reference products were used for statistical analysis. A summary of the main demographic characteristics of the subjects included in the statistical analysis, for each study, is presented in Table 1.

Table 1. Summary of demographic characteristics of subject included in the statistical analysis for each study.

Demography	Pazopanib 200 mg		Pazopanib 400 mg	
	Pilot Study (n = 23)	**Pivotal Study (n = 106)**	**Pilot Study (n = 23)**	**Pivotal Study (n = 98)**
Sex				
Male	6 (26.1%)	48 (45.3%)	14 (60.9%)	43 (43.9%)
Female	17 (73.9%)	58 (54.7%)	9 (39.1%)	55 (56.1%)
Race				
Black or African American	1 (4.3%)	7 (6.6%)	0 (0.0%)	6 (6.1%)
Multiple	5 (21.7%)	12 (11.3%)	2 (8.7%)	19 (19.4%)
White	17 (73.9%)	87 (82.1%)	21 (91.3%)	73 (74.5%)
Age (years)	30 (22–42)	29 (18–48)	29 (19–50)	31 (18–51)
Height (cm)	168 (144–193)	168 (152–190)	172 (157–190)	168 (150–191)
Weight (kg)	69.0 (47.6–106.2)	68.3 (47.7–94.2)	69.1 (52.0–97.8)	69.8 (45.2–94.0)
BMI (kg/m^2)	24.3 (18.8–29.6)	24.2 (18.6–29.9)	23.2 (18.9–28.9)	24.6 (15.5–30.0)

n—Number of subjects with information for both the Test and Reference formulations; BMI—Body mass index. Categorical data are summarized as n, and the percentage is within the parenthesis. Continuous data are summarized as mean with the range within the parenthesis.

2.1. Average Bioequivalence Analysis

Pharmacokinetic metrics C_{max}, time of occurrence of C_{max} (t_{max}), AUC from time of dosing (t = 0 h) truncated at 72 h ($AUC_{0–72}$), apparent terminal elimination rate constant (λ_z), and apparent terminal elimination half-life ($t_{1/2}$) were estimated from individual pharmacokinetic profiles for each formulation, by using a non-compartmental analysis (NCA) approach with a *ln*-linear terminal phase assumption.

For each study, the assessment of the similarity on the rate of drug absorption, following the administration of Test and Reference products, was performed using an analysis of variance (ANOVA) applied to the *ln*-transformed C_{max} and estimating the Test-to-Reference least square-means (LSM) ratio (GMR) and the corresponding 90% confidence interval (CI). The ANOVA model included Sequence, Subject nested within Sequence, and Period and Formulation as fixed effects, assessed at a 5% significance level ($\alpha = 0.05$) [14]. The intra-subject coefficient of variation (ISCV) was estimated from the mean square error (s^2) of the ANOVA model, as:

$$\text{ISCV} = 100 \cdot \sqrt{e^{s^2} - 1} \quad (1)$$

As per EMA's bioequivalence guideline, bioequivalent between the Test and Reference products is demonstrated if the GMR and corresponding 90% CI fell within the [80.00–125.00]% acceptance interval [14].

2.2. Alternative f_2 Factor Approach

As an alternative to the average bioequivalence approach, the f_2 factor was used to assess the similarity on the rate of drug absorption as proposed by Henriques et al. (2023) [1,2], using the R package 'f2PilotBE' [16].

The f_2 factor was calculated by normalizing the mean Test and Reference concentration-time profiles to the C_{max} of the mean Reference profile, until C_{max} of the mean Reference profile was observed (Reference t_{max}) [1,2]:

$$C_t^N = 100 \cdot \frac{\overline{C}_t}{C_{max\,R}} \quad \text{where } 0 \leq t \leq t_{max\,R}, \tag{2}$$

$$C_{max} f_2 = 50 \cdot \log\left(100 \cdot \left[1 + \frac{1}{n}\sum_{t=1}^{t=n}\left(R_t^N - T_t^N\right)^2\right]^{-0.5}\right) \tag{3}$$

A cut-off of 35 was considered to indicate a similarity between the Test and Reference products C_{max} (assessing the rate of absorption) [1,2]. Considering that pazopanib is a highly variable drug, the following conclusions can be taken based on the magnitude of the estimated f_2 factor [2]:

- If f_2 factor $\geq$ 35 and ISCV > 40%, the confidence in proceeding to a pivotal study is >60%.
- If f_2 factor $\geq$ 41 and ISCV $\geq$ 50%, the confidence in proceeding to a pivotal study is >80%.
- If f_2 factor $\geq$ 50, the confidence in proceeding to a pivotal study is high (>90%) regardless of ISCV.

3. Results

3.1. Pazopanib 200 mg

Following the administration of Test and Reference products in the pilot study, the mean pharmacokinetic profiles are illustrated in Figure 1, and the estimated pharmacokinetic metrics are presented in Table 2. The distribution of the estimated C_{max} after the 200 mg administration of the Test and Reference formulation of pazopanib is visually depicted in Figure 2.

Table 2. Summary statistics of pharmacokinetic metrics following the administration of pazopanib 200 mg, in pilot (n = 23) and pivotal (n = 106) studies.

	Pazopanib 200 mg			
	Pilot Study		**Pivotal Study**	
Metric (Unit)	**Test (n = 23)**	**Reference (n = 23)**	**Test (n = 106)**	**Reference (n = 106)**
C_{max} (ng/mL)	12,153.87 (45.6%)	12,377.02 (59.9%)	12,342.44 (46.5%)	11,713.78 (41.2%)
t_{max} (h)	3.25 (1.50–8.00)	4.00 (1.50–6.00)	3.25 (1.00–10.00)	3.13 (1.00–10.00)
$AUC_{0–72}$ (ng·h/mL)	354,866.48 (44.6%)	354,966.79 (51.2%)	359,463.36 (44.9%)	350,094.25 (43.0%)
λ_z (1/h)	0.017 (30.3%)	0.018 (28.6%)	0.017 (21.3%)	0.017 (21.6%)
$t_{1/2}$ (h)	44.91 (28.4%)	42.69 (30.5%)	42.84 (22.5%)	42.17 (23.7%)

C_{max}—Maximum observed concentration; t_{max}—Time to maximum observed concentration; $AUC_{0–72}$—Area under the concentration-time curve (AUC) truncated at 72 h (AUC from time of dosing up to 72 h); λ_z—apparent terminal elimination rate constant; $t_{1/2}$—apparent terminal elimination half-life. Values are the arithmetic mean with the coefficient of variation (CV%) within the parenthesis. For t_{max}, values are the median with the range between the parentheses.

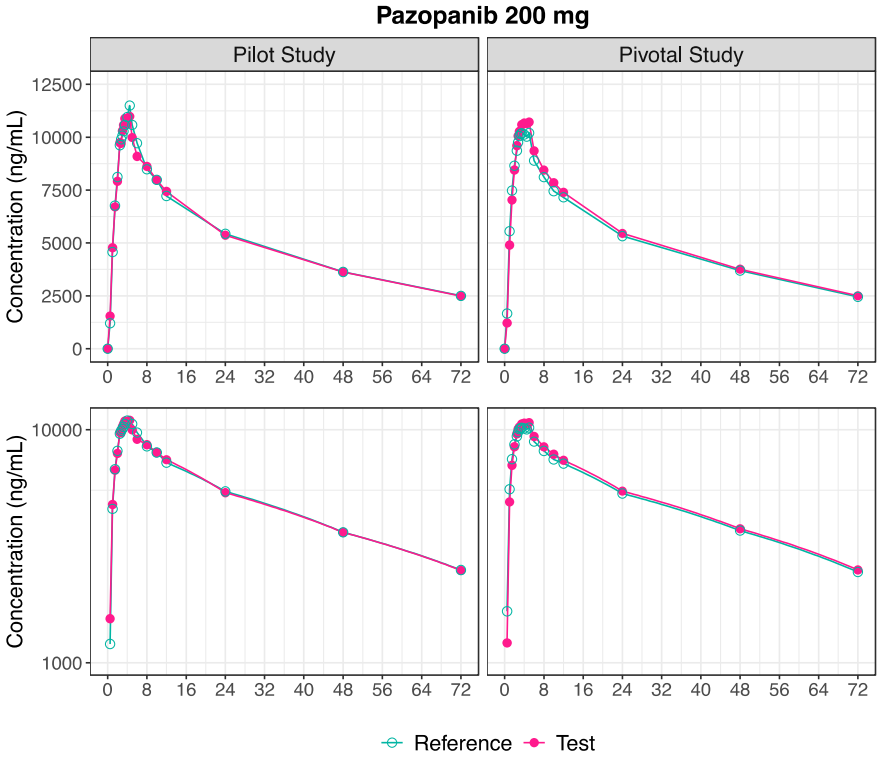


Figure 1. Plasma concentration-time profiles following the administration of pazopanib 200 mg Test and Reference formulation, in pilot (n = 23, **Left**) and pivotal (n = 106, **Right**) studies, in linear (**Above**) and semi-logarithmic (**Below**) scales.

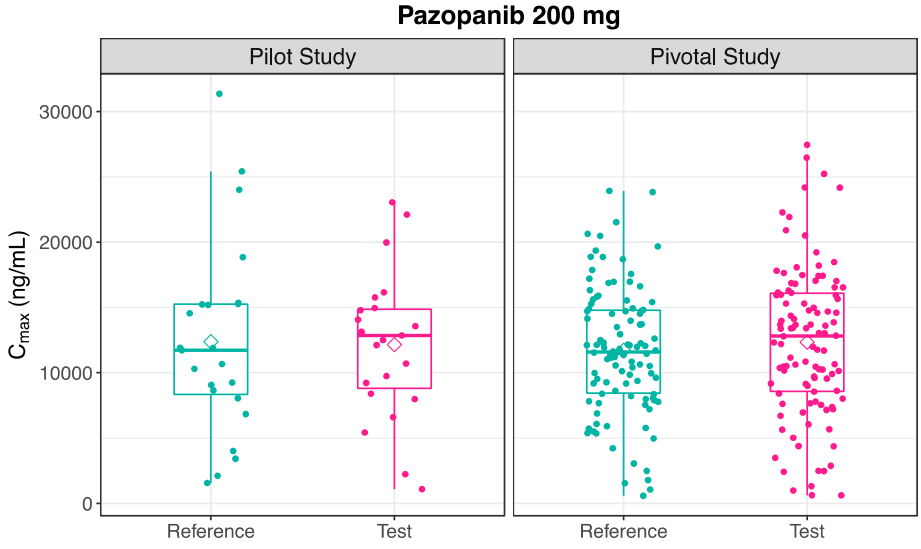


Figure 2. Box plots of distribution of C_{max} following the administration of pazopanib 200 mg Test and Reference formulation, in pilot (n = 23, **Left**) and pivotal (n = 106, **Right**) studies. Individual values are represented as points, while median and mean values are represented as a line and a diamond shape, respectively.

The point estimate calculated from the average bioequivalence for C_{max} was close to 100%, and within the [80.00–125.00]% comparable bioavailability acceptance limits. Likewise, the lower limit of the GMR 90% CI fell within the [80.00–125.00]% comparable bioavailability acceptance limits. However, the upper limit of the GMR 90% CI was above the [80.00–125.00]% comparable bioavailability acceptance limits (Figure 3 and Table 3). Moreover, the estimated ISCV was high (50.1%) (Table 3).

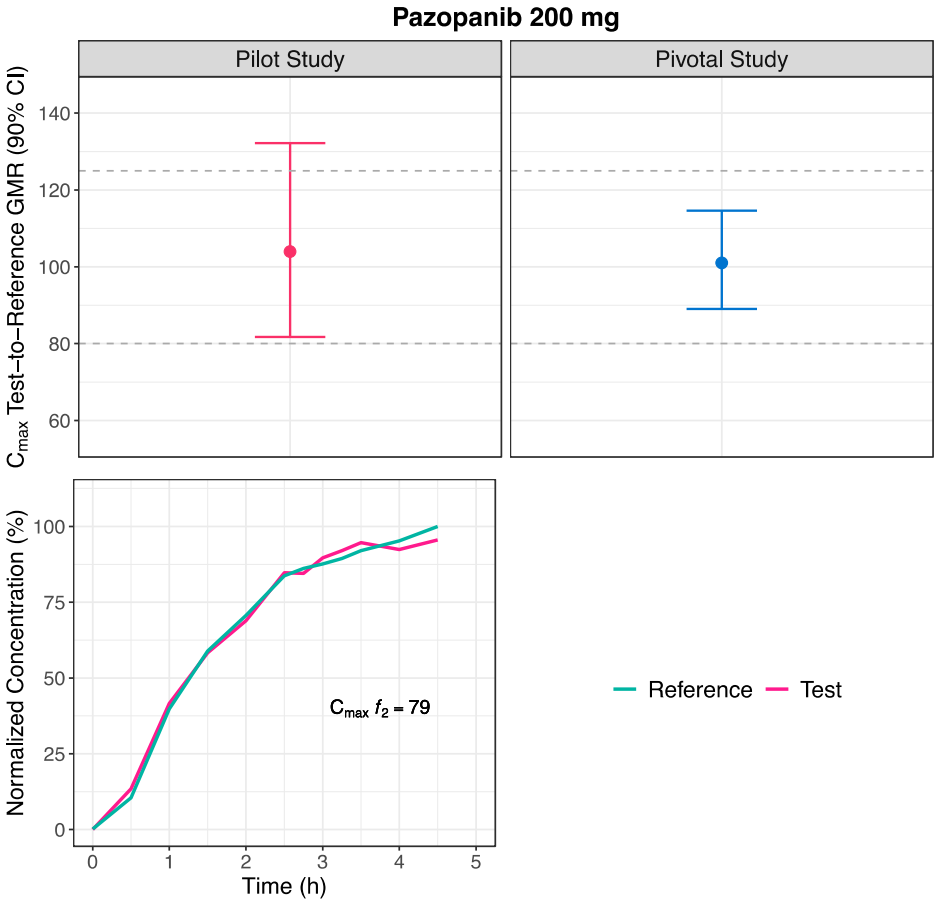


Figure 3. Test-to-Reference geometric least square means ratio (GMR) and corresponding 90% confidence interval (CI) estimated for C_{max} (**Above**), and normalized Test and Reference mean concentration-time curves, to the Reference C_{max}, until the Reference t_{max}, for the estimation of the similarity f_2 factor (**Below**), following the administration of pazopanib 200 mg, in pilot (n = 23, **Left**) and pivotal (n = 106, **Right**) studies.

Table 3. Average Bioequivalence and f_2 Factor Analysis Results for C_{max} Following the Administration of Pazopanib 200 mg.

	Pazopanib 200 mg	
	Pilot Study	**Pivotal Study**
n	23	106
ISCV (%)	50.1	59.8
Geometric LSM (ng/mL)		
Test	10,398.64	10,427.44
Reference	10,001.77	10,322.74
GMR [90% CI] (%)	103.97 [81.76–132.21]	101.01 [89.04–114.60]
f_2 Factor	79.00	NC

n—Number of subjects with information for both the Test and Reference formulations; ISCV—Intra-subject coefficient of variation (calculated from the average bioequivalence); LSM—Least square mean (calculated from the average bioequivalence); GMR—Test-to-Reference geometric coefficient of variation (calculated from the average bioequivalence); CI—Confidence interval of the GMR (calculated from the average bioequivalence); NC—Not calculated.

Using the alternative f_2 factor approach, the mean Test and Reference concentration-time profiles (Figure 1) were normalized to the C_{max} of the Reference mean profile. The normalization was performed until 4.5 h for the pilot study (Figure 3). The calculated f_2 factor from the normalized profiles was 79.00 (Figure 3 and Table 3).

Following the administration of the Test and Reference products in the pivotal study, the mean pharmacokinetic profiles are illustrated in Figure 1 and the estimated pharmacokinetic metrics are presented in Table 2. The pharmacokinetic outcomes for the 200 mg pazopanib were consistent across both pilot and pivotal studies. Figure 2 shows a similar distribution of C_{max} values following the administration of pazopanib 200 mg Test and Reference formulations between the pilot and pivotal studies.

For the pivotal study, the estimated GMR for C_{max} was close to 100% and the corresponding 90% CI fell within the [80.00–125.00]% comparable bioavailability acceptance limits (Figure 3 and Table 3). Likewise, the estimated ISCV was high (59.8%) (Table 3).

3.2. Pazopanib 400 mg

Following the oral administration of 400 mg pazopanib in the pilot study, the mean plasma concentration-time profile for the Test product was slightly higher than the Reference mean profile (Figure 4). The estimated pharmacokinetic metrics are presented in Table 4, and the distribution of the estimated C_{max} after the 400 mg administration of the Test and Reference formulation of pazopanib is visually depicted in Figure 5.

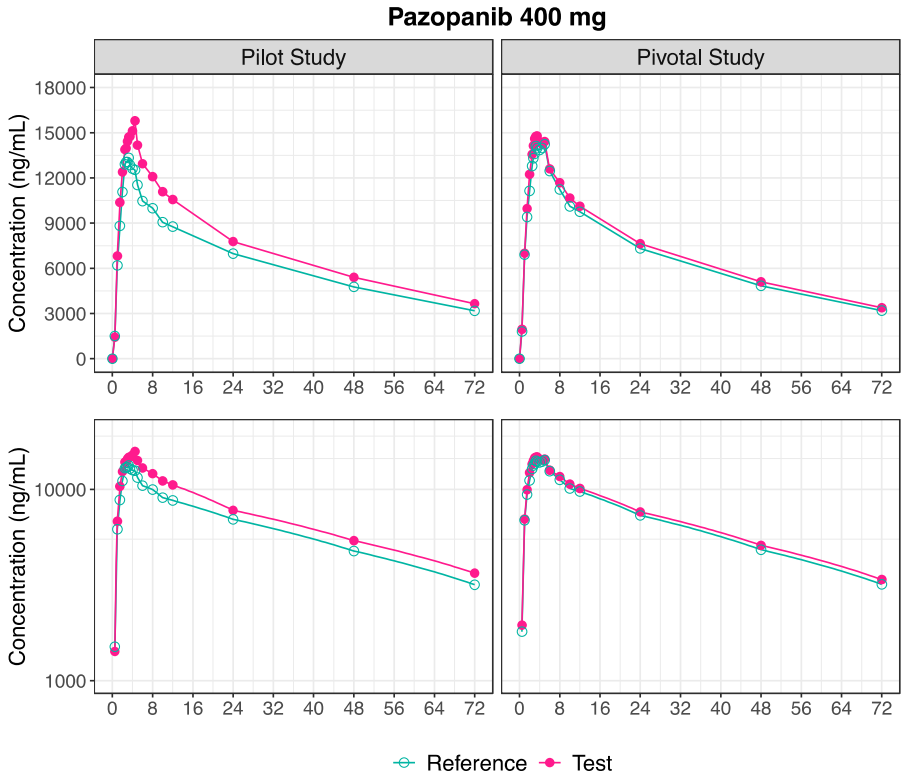


Figure 4. Plasma concentration-time profiles following the administration of pazopanib 400 mg Test and Reference formulation, in pilot (n = 23, **Left**) and pivotal (n = 98, **Right**) studies, in linear (**Above**) and semi-logarithmic (**Below**) scales.

In the pilot study, the point estimate calculated from the average bioequivalence for C_{max} was above 100%, but within the [80.00–125.00]% comparable bioavailability acceptance limits. Likewise, the lower limit of the GMR 90% CI fell within the [80.00–125.00]% comparable bioavailability acceptance limits. Nevertheless, the upper limit of the GMR 90% CI was above the [80.00–125.00]% comparable bioavailability acceptance limits (Figure 6 and Table 5). Moreover, the estimated ISCV was high (41.7%) (Table 5).

Table 4. Summary Statistics of Pharmacokinetic Metrics Following the Administration of Pazopanib 400 mg, in Pilot (n = 23) and Pivotal (n = 98) Studies.

	Pazopanib 400 mg			
	Pilot Study		**Pivotal Study**	
Metric (Unit)	**Test (n = 23)**	**Reference (n = 23)**	**Test (n = 98)**	**Reference (n = 98)**
C_{max} (ng/mL)	17,413.79 (51.1%)	14,894.93 (51.7%)	17,031.50 (57.4%)	16,552.74 (42.8%)
t_{max} (h)	3.00 (1.50–4.50)	2.75 (1.00–4.50)	3.50 (1.00–10.00)	3.25 (1.00–6.00)
AUC_{0-72} (ng·h/mL)	512,126.67 (57.3%)	444,128.55 (58.0%)	492,949.12 (59.0%)	469,368.48 (46.5%)
λ_z (1/h)	0.018 (21.3%)	0.018 (23.9%)	0.018 (23.0%)	0.018 (25.6%)
$t_{1/2}$ (h)	41.56 (21.1%)	41.02 (24.7%)	41.49 (35.0%)	40.86 (25.4%)

C_{max}–Maximum observed concentration; t_{max}—Time to maximum observed concentration; AUC_{0-72}—Area under the concentration-time curve (AUC) truncated at 72 h (AUC from time of dosing up to 72 h); λ_z—apparent terminal elimination rate constant; $t_{1/2}$—apparent terminal elimination half-life. Values are the arithmetic mean with the coefficient of variation (CV%) within the parenthesis. For t_{max}, values are the median with the range between the parentheses.

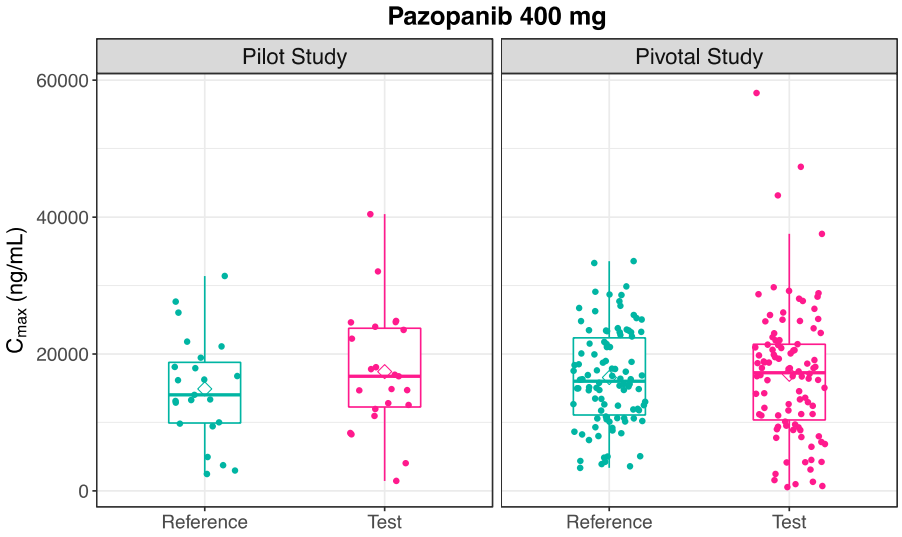


Figure 5. Box Plots of Distribution of C_{max} Following the Administration of Pazopanib 400 mg Test and Reference Formulation, in Pilot (n = 23, **Left**) and Pivotal (n = 98, **Right**) Studies. Individual Values are Represented as Points, While Median and Mean Values are Represented as a Line and a Diamond Shape, respectively.

Table 5. Average Bioequivalence and f_2 Factor Analysis Results for C_{max} Following the Administration of Pazopanib 400 mg.

	Pazopanib 400 mg	
	Pilot Study	**Pivotal Study**
n	23	98
ISCV (%)	41.7	53.2
Geometric LSM (ng/mL)		
Test	14,667.79	13,429.92
Reference	12,509.20	14,739.56
GMR [90% CI] (%)	117.26 [95.69–143.68]	91.11 [80.91–102.61]
f_2 Factor	53.51	NC

n—Number of subjects with information for both the Test and Reference formulations; ISCV—Intra-subject coefficient of variation (calculated from the average bioequivalence); LSM—Least square mean (calculated from the average bioequivalence); GMR—Test-to-Reference geometric coefficient of variation (calculated from the average bioequivalence); CI—Confidence interval of the GMR (calculated from the average bioequivalence); NC—Not calculated.

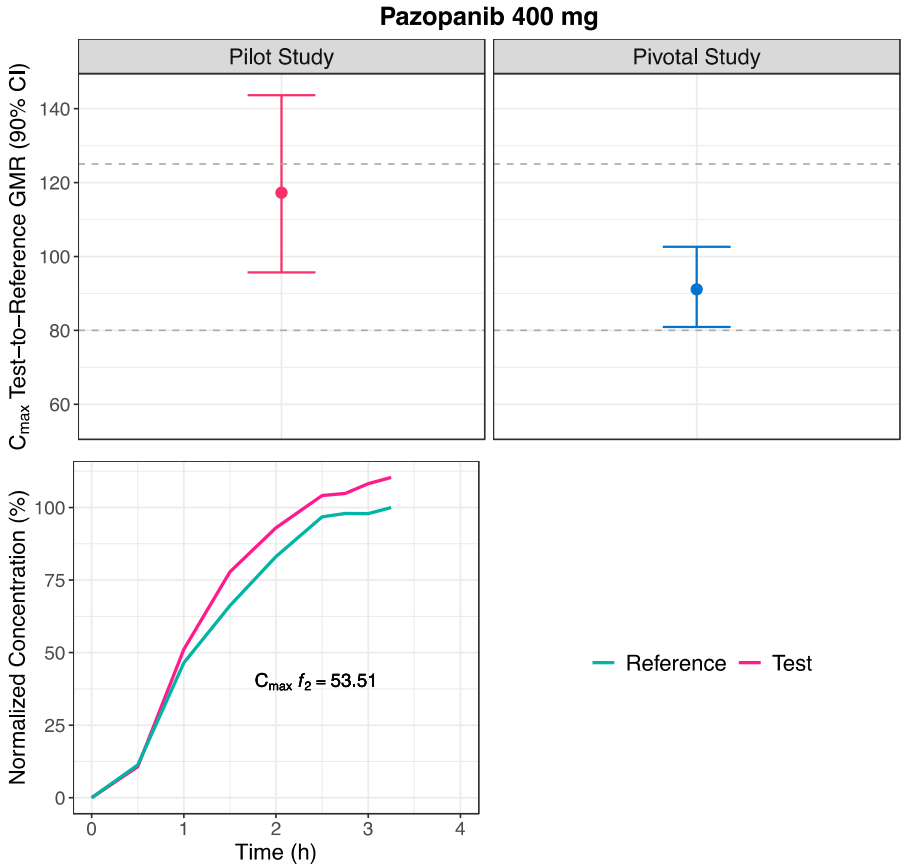


Figure 6. Test-to-Reference geometric least square means ratio (GMR) and corresponding 90% confidence interval (CI) estimated for C_{max} (**Above**), and normalized Test and Reference mean concentration-time curves, to the Reference C_{max}, until the Reference t_{max}, for the estimation of the similarity f_2 factor (**Below**), following the administration of pazopanib 400 mg, in the pilot (n = 23, **Left**) and pivotal (n = 98, **Right**) studies.

Using the alternative f_2 factor approach, the mean Test and Reference concentration-time profiles (Figure 4) underwent normalization to the C_{max} of the Reference mean profile. The normalization was carried out until 3.25 h (Figure 6). The calculated f_2 factor from the normalized profiles was 53.51 (Figure 6 and Table 5).

However, the pharmacokinetic outcomes for the 400 mg pazopanib were slightly different between the pilot and pivotal studies. With the increment of subjects in the pivotal study (n = 98), the mean plasma concentration-time profiles following the administration of the Test and Reference products were almost superimposable. These profiles are visually depicted in Figure 4. The estimated pharmacokinetic metrics are presented in Table 4, and Figure 5 shows a similar distribution of C_{max} values following the administration of the Test and Reference formulations of 400 mg pazopanib.

The increment in the number of subjects to 98 allowed the GMR and the corresponding 90% CI to fall within the [80.00–125.00]% comparable bioavailability acceptance limits. Nevertheless, in opposition to the pilot study, in the pivotal study, the GMR decreased to below 100% (Figure 6 and Table 5). Likewise, the estimated ISCV was high (53.2%) (Table 5).

4. Discussion

Pazopanib is recognized as a highly variable drug [12,13], which was also observed in the current pivotal bioequivalence studies, with an ISCV of approximately 50% for both C_{max} and AUC bioequivalence metrics. Such high variability is probably due to the variable absorption as a consequence of a variable and low pH dependent solubility of the compound on the form of hydrochloride salt [6].

4.1. Pazopanib 200 mg

In the pilot study performed with 200 mg pazopanib formulations, bioequivalence between the Test and Reference products was not demonstrated for the 23 subjects included in the statistical analysis. However, the point estimate was centered, indicating proximity to 100%. Nevertheless, it is important to note that, based on prior simulations, a centered GMR alone suggests only a ~60% probability of the Test product being truly bioequivalent, particularly in scenarios with high ISCV [1,2].

Upon applying the alternative approach, the estimated f_2 factor was 79.00, which, in accordance with conclusions drawn from previous simulations, i.e., for an f_2 factor of this dimension (>50), indicates a 90% probability that the Test product is truly bioequivalent to the Reference product in terms of C_{max}. Therefore, the sponsor could be recommended to proceed with a full-size pivotal study [2].

Considering the results from the pilot study, sponsor conducted a pivotal study with 116 subjects. A sample of 96 subjects would allow an a priori statistical power of at least 80% to meet the [80.00–125.00]% bioequivalence range, assuming an ISCV of 50% for AUC metric, a true GMR of 105%, and a 5% significance level (α = 0.05). To compensate for possible dropouts and variation in ISCV, a sample size of 116 subjects was defined. From the 116 enrolled subjects, 106 completed this study and had evaluable pharmacokinetic data for bioequivalence analysis. The increment of the number of subjects to 106 allowed the GMR and the corresponding 90% CI to fall completely within the [80.00–125.00]% acceptance interval; hence, showing bioequivalence.

The values derived for C_{max}, t_{max}, and $t_{1/2}$ for both pilot and pivotal studies are in agreement with results from a bioequivalence study performed in healthy Chinese subjects [13]. However, the AUC parameter is not comparable due to different last sampling times.

4.2. Pazopanib 400 mg

Similarly to the 200 mg pazopanib, in the pilot study performed with 400 mg pazopanib formulations, bioequivalence between the Test and Reference products was not demonstrated for the 23 subjects included in the statistical analysis. Hence, the point estimate was above 100% and not centered, but within the [80.00–125.00]% comparable bioavailability acceptance limits, along with its lower limit of the 90% CI.

Upon applying the alternative approach, the estimated f_2 factor was 53.51. According with conclusions from previous simulations, an f_2 factor of this dimension (>50) indicates a 90% probability that the Test product is truly bioequivalent to the Reference product in terms of C_{max}. Therefore, the sponsor could be recommended to proceed with a full-size pivotal study [2].

The sponsor decided to conduct a pivotal study with 122 subjects. A sample of 106 subjects would allow an a priori statistical power of at least 80% to meet the [80.00–125.00]% bioequivalence range, assuming an ISCV of 35%, a true GMR falling within [90.00–111.11]%, and a 5% significance level (α = 0.05). To compensate for possible dropouts and variation in ISCV, a sample size of 122 subjects was defined. From the 122 enrolled subjects, 98 completed this study and had evaluable pharmacokinetic data for bioequivalence analysis. The increase in the number of subjects to 98 allowed the GMR and the corresponding 90% CI to fall completely within the [80.00–125.00]% acceptance interval; hence, showing bioequivalence.

To date, no bioequivalence studies have been published with 400 mg strength. Nevertheless, the elimination half-life observed is similar to values found for the 200 mg strength in the performed pivotal study and the literature [13].

Moreover, when comparing results derived from the 400 mg and 200 strengths, a less than dose proportional behavior in C_{max} and $AUC_{0\text{-}72}$ was observed, as described [6,9], primarily driven by limited solubility and saturation of absorption processes at higher doses.

5. Conclusions

The f_2 factor has shown to be capable of predicting bioequivalence between two pazopanib formulations for both 200 mg and 400 mg strengths, using data from pilot studies, despite the limited sample size of only 23 subjects that have been involved, considered to be low facing the high intra-subject variability known for the drug.

The methodology is intended to be applied to data from other bioequivalence trials covering BCS class 2 and class 4 drugs. Nevertheless, the f_2 factor has been exhibiting the potential to reduce the uncertainty associated with pilot studies and to be helpful in terms of making the decision to go forward with pivotal bioequivalence studies.

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Institutional Review Board Statement: For each study, the clinical study protocol (CSP) and written subject information with informed consent form (ICF) were submitted to the Portuguese Ethics Committee for Clinical Research (CEIC—Comissão de Ética para a Investigação Clínica, Avenida do Brasil, Lisboa, Portugal) for evaluation and approval, prior to the start of this study. The studies were submitted to the review and approval of the National Authority for Medicines and Health Products (INFARMED—Autoridade Nacional do Medicamento e Produtos de Saúde, I.P.) according to the rules in force. Final versions of the CSP and ICF were approved on: Pazopanib 200 mg pilot study (BLCL-PAZ-PIL01, EudraCT No. 2020-00586-16): 23 March 2020; Pazopanib 200 mg pivotal study (BLCL-PAZ-EU-02, EudraCT No. 2021-002053-29): 21 May 2021; Pazopanib 400 mg pilot study (BLCL-PAZ-PIL02, EudraCT No. 2020-00514-17): 15 January 2021; Pazopanib 400 mg pivotal study (BLCL-PAZ-EU-03, EudraCT No. 2021-003534-36): 24 September 2021. These studies were implemented and conducted according to the CSP, International Council for Harmonisation (ICH) Good Clinical Practices (GCP), the Declaration of Helsinki, EMA's guidelines, and the applicable European and Portuguese laws and regulations. Clinical conduction was performed on the following dates: Pazopanib 200 mg pilot study (BLCL-PAZ-PIL01, EudraCT No. 2020-00586-16): 25 November 2020 (first screening)—4 January 2021 (last completion); Pazopanib 200 mg pivotal study (BLCL-PAZ-EU-02, EudraCT No. 2021-002053-29): 21 June 2021 (first screening)—20 September 2021 (last completion); Pazopanib 400 mg pilot study (BLCL-PAZ-PIL02, EudraCT No. 2020-00514-17): 18 February 2021 (first screening)—2 April 2021 (last completion); Pazopanib 400 mg pivotal study (BLCL-PAZ-EU-03, EudraCT No. 2021-003534-36): 22 December 2021 (first screening)—23 March 2022 (last completion). All analyzes were authorized by Bluepharma-Indústria Farmacêutica, S.A., and rigorous anonymization measures were applied to safeguard participant confidentiality.

Informed Consent Statement: Written informed consent was obtained from each prospective subject prior to any study procedure, after the physician in charge had the conviction that each subject was aware of the implications of participating in this study and the subject confirmed their willingness to participate. Subjects were assured that they might abandon this study at any time without any prejudice.

Data Availability Statement: The data that support the findings of this study are available on request to the corresponding authors. The data are not publicly available due to privacy or ethical restrictions.

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Conflicts of Interest: S.C.H. is conducting her PhD research project at BlueClinical Ltd. and at iMed.ULisboa, Faculty of Pharmacy, Universidade de Lisboa. A.L. is the Principal Investigator of the studies. S.C.H., A.L., M.F., F.L.P. and L.A. are employees of BlueClinical Ltd. S.S. is an employee of Bluepharma-Indústria Farmacêutica, S.A. N.E.S. declares no conflict of interest.

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