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BIOPHARMACY

BIOEQUIVALENCE OF RIVAROXABAN HARD CAPSULES VS. FILM-COATED TABLETS IN HEALTHY CAUCASIAN VOLUNTEERS

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Abstract: Rivaroxaban is an oral anticoagulant that is a selective, direct factor Xa inhibitor. It is used to prevent thrombotic events of atherosclerotic etiology and to prevent stroke and peripheral embolism in adult patients with nonvalvular atrial fibrillation. The aim of the studies was to assess the bioequivalence of two orally administered products: test (Zarixa hard capsules) vs. reference (Xarelto® film-coated tablets). Two crossover, two-period, randomized, open-label, laboratory-blinded studies were conducted in healthy Caucasian male and female volunteers. A single oral dose (Study 1: 10 mg fasting, Study 2: 20 mg fed) of the test or reference product was followed by a minimum seven-day washout. Blood was collected up to 48 h after administration. Plasma concentrations of rivaroxaban were measured using a validated LC-MS/MS method. The bioequivalence criteria for 90% confidence intervals (CI) of the log-transformed geometric mean ratios (test/reference) for the two primary pharmacokinetic parameters ($AUC_{(0-t)}$ and C_{max}) were set at 80.00-125.00%. Vital signs, laboratory parameters, and adverse events were monitored. A total of 34 out of 36 volunteers completed Study 1, and the geometric mean ratios were 97.96% (90% CI 93.69-102.42%) for $AUC_{(0-t)}$, and 89.35% (90% CI 84.28-94.72%) for C_{max}. All 36 volunteers completed Study 2, and the geometric mean ratios were 103.57% (90% CI 98.75-108.63%) for $AUC_{(0-t)}$, and 95.17% (90% CI 87.35-103.70%) for C_{max}. All 90% CIs for the primary pharmacokinetic parameter ratios met the acceptance criteria. There were no serious adverse events. Results of both studies indicate that the test product (Zarixa) is bioequivalent to the reference product (Xarelto®). Both products were well tolerated.

Keywords: rivaroxaban; pharmacokinetics; bioequivalence; anticoagulant; Xa inhibitor

Rivaroxaban is an orally administered direct factor Xa inhibitor with well-established pharmacokinetics and pharmacodynamics in different patient populations [1]. It represents direct oral anticoagulants developed in recent years. This group overcomes the limitations of vitamin K antagonists thanks to predictable pharmacokinetics and pharmacodynamics, as well as low potential for drug-drug interactions. Rivaroxaban is indicated: (A) to prevent stroke and peripheral embolism in adults with nonvalvular atrial fibrillation; (B) to prevent thrombotic events of atherosclerotic etiology; (C) to treat deep vein thrombosis (DVT) and pulmonary embolism (PE), and to prevent recurrent DVT and PE in adults; and (D) as prophylaxis of venous thromboembolism in adults following elective hip or knee replacement surgery [2]. The evidence of rivaroxaban treatment in Poland includes successful secondary stroke prevention in patients with non-valvular atrial fibrillation [3] and reduction of the duration of hospital stay of patients with acute pulmonary embolism [4].

Rivaroxaban is rapidly absorbed and reaches maximum concentrations within 2-4 h [1, 2]. Its oral bioavailability in fasting conditions is 80-100% for 2.5 mg and 10 mg doses but decreases to 66% for 20 mg doses. Food does not influence rivaroxaban's rate and extent of absorption for 2.5 mg and 10 mg doses, but increases its mean AUC by 39% when compared to 20 mg tablet intake under fasting conditions. Thus, rivaroxaban 15 mg and 20 mg should be taken with a meal [2]. In human plasma, the parent drug is the most abundant compound and no major or active metabolites are present. The plasma

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terminal half-lives of rivaroxaban vary from 5-9 h in the young [5] to 11-13 h in the elderly [6].

We aimed to evaluate the bioequivalence of rivaroxaban hard capsules (Zarixa, test) vs. film-coated tablets (Xarelto®, reference) following a single oral administration in healthy subjects according to the European Medicines Agency (EMA) guidelines [7,8]. Due to the diverse posology of different doses of the reference product regarding food intake [2], two separate clinical studies were needed [8]: in fasting conditions for the 10 mg dose (Study 1) and in fed conditions for the 20 mg dose (Study 2).

EXPERIMENTAL

Study Products

The rivaroxaban products were administered orally in both studies. The reference product was Xarelto® [2] film-coated tablets (Bayer AG – marketing authorization holder): in Study 1 at a dose of 10 mg (lot no. ITA5CUK), in Study 2 at a dose of 20 mg (lot no. BT14BF2). The test product was Zarixa hard capsules (manufactured by Celon Pharma S.A., Kazuń Nowy, Poland): in Study 1 at a dose of 10 mg (lot no. 372001422), in Study 2 at a dose of 20 mg (lot no. 374001422).

In Vitro Dissolution Testing

Dissolution profiles for the test and reference products were determined according to the European Pharmacopeia Monograph for rivaroxaban tablets (3021), as a standard quality control to confirm the fulfilling of product quality before the planned clinical study. Briefly, dissolution was tested in sink conditions using a type II dissolution apparatus at a rotation speed of 75 rpm and sinkers for capsules. The dissolution medium (900 mL) was a mixture of acetate buffer solution at pH = 4.5 with 0.2% sodium dodecyl sulfate (SDS) for 10 mg and 0.4% SDS for 20 mg doses. The medium was sampled at 5, 10, 15, 20, 30, 45, and 60 min. After sampling, no fresh portion of the medium was added. The chromatographic analysis was performed with an Agilent Technologies 1200 series HPLC system provided with a UV-vis detector. Chromatographic column C18 (60 x 4.0 mm, 3 μm) was purchased from MZ Analysentechnik.

Study Participants

The sample size was calculated assuming:

– a significance level of $\alpha = 0.05$ and power of test $1 - \beta = 0.80$,
– geometric mean true relative ratio of primary pharmacokinetic parameters (test/reference) varying between 90% and 110%,
– intra-subject variability (expressed as coefficient of variation, CV) of the primary pharmacokinetic parameters would not exceed 25% [9-11].

Based on the above assumptions, 32 subjects should have completed each study. However, taking into account possible drop-out (assumed not to exceed 10%), there were 36 subjects included in each study.

Healthy women and men aged 18-55 years, whose body mass index (BMI) was 18.5-29.9 kg/m^2, were invited for the screening. Each of them underwent a medical examination and additional tests: electrocardiography (ECG), urine testing (general and toxicological, pregnancy test in women), blood testing (hematology, blood chemistry), and serology (human immunodeficiency virus HIV, hepatitis B and C).

The key exclusion criteria were:

– evidence or suspected pathology assessed on the conducted tests;
– suspected hypersensitivity to rivaroxaban or other ingredients of the medicinal product;
– any known significant current or past acute or chronic disease;
– congenital or acquired bleeding disorders;
– gastrointestinal disease without active ulceration that could potentially lead to bleeding;
– a medical history of vascular retinopathy, bronchiectasis, pulmonary bleeding, hypotension, hypertension;
– current disease of the alimentary tract, liver, or kidneys;
– any medications in the four weeks preceding the first product administration and during the study;
– participation in another study in the last 90 days, smoking cigarettes, abusing alcohol, a special diet (e.g. vegetarians, to avoid difficulties with diet standardization) or a specific lifestyle (professional sports).

Before admission to the clinical center, the volunteers were tested for the presence of prohibited substances in the urine and alcohol in the exhaled air.

Study Design

The studies were designed according to the EMA guidelines [7, 8] as a randomized, laboratory-blinded, two-period cross-over trial. A single administration of the test and reference products was conducted in fasting conditions (Study 1, EudraCT 2022-001878-63) or fed conditions (Study 2, EudraCT 2022-001879-13). A minimum seven-day washout period was planned between the administration of both

products. The study documents were approved by the Committee on Bioethics at the Regional Medical Chamber (Warsaw, Poland) and the Office for the Registration of Medicinal Products, Medical Devices, and Biocidal Products (Warsaw, Poland).

The clinical part was conducted at the BioResearch Group Ltd. (Kajetany, Poland) in 2022. The ethical principles of the Declaration of Helsinki and its amendments [12], current Good Clinical Practice (GCP) guidelines [13] as well as Polish Pharmaceutical Law were followed. The volunteers were informed before the screening about the procedures, restrictions, possible risks, and insurance. Each volunteer signed an informed consent form before any study procedures.

Study Products Administration

The volunteers arrived at the clinical site the day before the administration of the study products (Day 0). The next day (Day 1) in the morning, after at least 10 h of fasting (Study 1), each volunteer received a single rivaroxaban dose of 10 mg (1 test capsule or 1 reference tablet, according to the randomization table) with 240 mL water.

In Study 2, a high-fat (ca. 50% of total caloric content of the meal) and high-calorie (888 kcal) breakfast was served 30 minutes before product administration. This breakfast derived approximately 150 kcal from protein, 250 kcal from carbohydrate, and 500 kcal from fat, respectively [7]. Then, each volunteer received a single rivaroxaban dose of 20 mg (1 test capsule or 1 reference tablet, according to the randomization table) with 240 mL water.

In both studies, a doctor checked whether the products were swallowed. One hour after the administration of a product, the subjects were allowed to drink water. Participants stayed at the clinical site for at least 12 h before and 24 h after the administration, in both periods. During the stay, participants ate standardized meals. There were two ambulatory visits, 36 h (Day 2) and 48 h post-administration (Day 3), in each period.

Blood Sampling

To determine rivaroxaban concentrations in Study 1, in each period 17 blood samples were collected from each volunteer: pre-dose, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 8, 12, 16, 24, 36, and 48 h after product administration. In Study 2, 18 blood samples were collected with additional sampling at 6 h after product administration. Less than 205 mL of blood was collected from each volunteer. Samples were collected in vacuum tubes with a Venflon, an intravenous cannula, placed in the forearm venous vessel. After centrifugation for 10 min at 4°C at 1500 x g, the separated plasma was transferred to polypropylene tubes (primary and backup) and frozen at ≤-65 °C.

Bioanalysis

Rivaroxaban plasma concentrations were determined by high-performance liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) in the GLP-certified Bioanalytical Laboratory of Celon Pharma S.A. (Kazuń Nowy, Poland). Samples were prepared for analysis by protein precipitation with acetonitrile. QTrap 6500+ mass spectrometer (Sciex) and 1290 Infinity II liquid chromatograph (Agilent Technologies) were operated by Analyst 1.7.2 software (Sciex). The chromatographic separation was performed on a Synergi Fusion-RP column (80A, 50 x 2 mm, 4 µm, Phenomenex). Isocratic elution was applied with a mixture of 0.02% aqueous acetic acid with acetonitrile (65 : 35, v/v) at 0.6 mL/min. Positive electrospray ionization (ESI) was used to monitor rivaroxaban at 436 > 231 m/z and the isotope-labeled internal standard (rivaroxaban-d4) at 440 > 235 m/z. The run time was 3 min. The lower limit of quantification (LLOQ) was set at 2 ng/mL. The calibration range was fitted to the administered dose: 2-400 ng/mL for 10 mg and 2-600 ng/mL for 20 mg.

Pharmacokinetics and Statistics

The following rivaroxaban plasma pharmacokinetic parameters were selected:

– primary:
  - $AUC_{(0-t)}$ – the area under the concentration-time curve from time zero to the last measurable concentration, calculated using the linear trapezoidal method
  - C_{max} – maximum concentration
– secondary:
  - $AUC_{(0-\infty)}$ – area under the concentration-time curve extrapolated to infinity: $AUC_{(0-\infty)} = AUC_{(0-t)} + C_t/\lambda_z$, where C_t is the last measurable concentration
  - t_{max} – time to reach maximum concentration
  - λ_z – terminal elimination rate constant, calculated using the linear least squares regression using at least the last three non-zero concentrations selected manually
  - $t_{1/2}$ – the apparent plasma elimination half-life: $\ln 2 / \lambda_z$
  - t_{lag} – time of observation prior to the first observation with a measurable (non-zero) concentration.

The logarithmically transformed C_{max}, $AUC_{(0-t)}$, and $AUC_{(0-\infty)}$ were subjected to analysis of variance (ANOVA), which included Sequence, Period, and Formulation as fixed effects and Subject (Sequence) as a random effect. An F-test was performed to determine the statistical significance of the effects involved in the model at $\alpha = 0.05$. If the 90% confidence intervals (CI) for the ratio of geometric means (test/reference)—calculated based on the results of the ANOVA model—for both C_{max} and $AUC_{(0-t)}$ were included entirely within 80.00-125.00%, the test product was assumed as bioequivalent to the reference product [7, 8, 14]. The difference in t_{max} and t_{lag} between the test and reference product was assessed using a non-parametric Wilcoxon signed-rank test. Noncompartmental pharmacokinetic analysis and statistical calculations were performed using validated scripts in R 4.1.3 software (R Core Team 2021, R Foundation for Statistical Computing, Vienna, Austria). Additionally, t_{lag} and $t_{1/2}$ were calculated using Phoenix WinNonlin software (version 8.4, Certara L.P., Princeton, NJ, US).

Safety Analysis

Subjects were monitored for clinically relevant physical changes, changes in vital signs and deviations from normal clinical laboratory test results, tolerability, and safety. They were closely observed to assure maximum safety and to collect adverse events. During the entire study, subjects could contact the Principal Investigator or other medical staff. The intensity of adverse effect was classified according to the Common Terminology Criteria for Adverse Events (CTCAE) v. 5.0.

RESULTS

Sixty-five (65) and 69 healthy volunteers signed the informed consent form and participated in the screening visit during Study 1 and Study 2, respectively (Figure 1). Finally, 36 healthy volunteers were enrolled in each study (demographic data in Table 1). The clinical part was completed by 34 (Study 1) and 36 (Study 2) volunteers (Figure 1). In Study 1, two volunteers withdrew from participation before period 2 due to personal reasons.

Table 1. Demographic data

Variable	Study 1	Study 2
White	36	36
Males / Females	19 / 17	21 / 15
Age [years][a]	38 ± 11	37 ±10
Height [cm] [a]	173 ± 11	172 ± 9
Weight [kg] [a]	74 ± 13	72 ± 14
BMI [kg/m^2][a]	24.6 ± 3.0	23.7 ± 3.2

[a] arithmetic mean (SD), BMI, body mass index

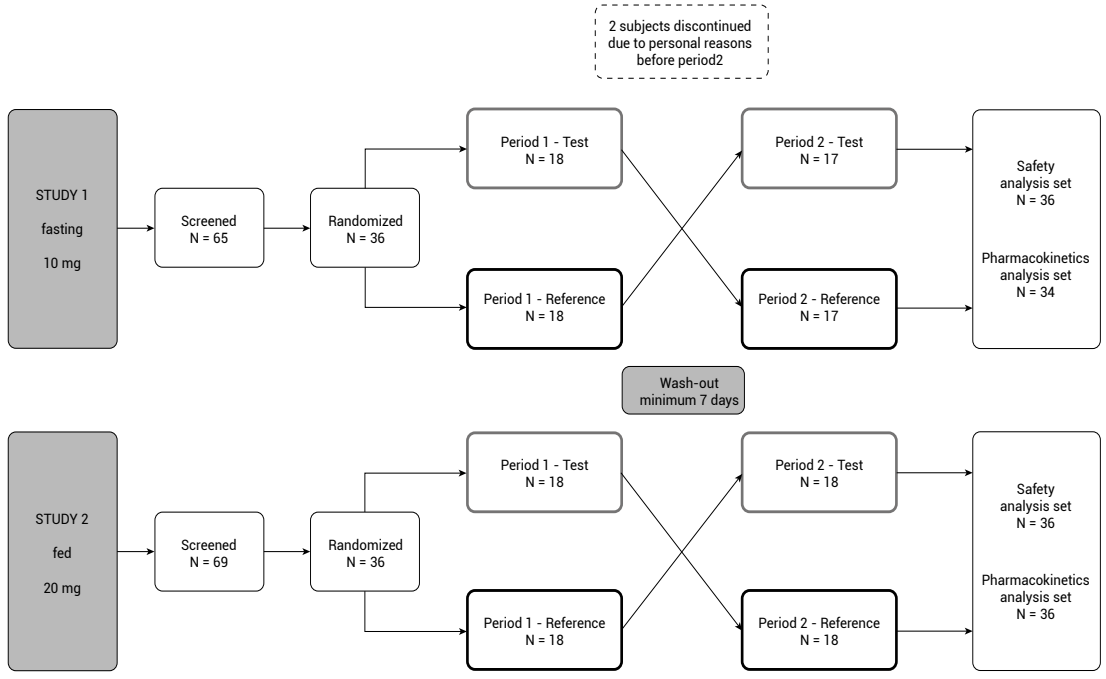

Figure 1. Bioequivalence studies flowchart

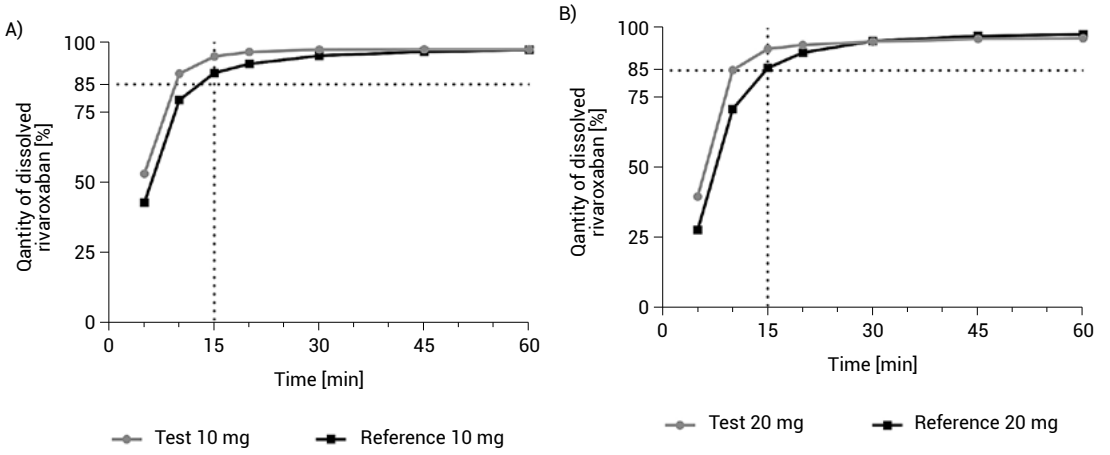

Figure 2. In vitro dissolution profiles at pH = 4.5 with the addition of sodium dodecyl sulfate (SDS) for products administered in Study 1–0.2% SDS (A) and in Study 2–0.4% SDS (B). Test – hard capsules (Zarixa); Reference – film-coated tablets (Xarelto®)

In Vitro Dissolution Testing

For both doses of the test and reference products, more than 85% of the active pharmaceutical ingredient was dissolved within 15 minutes (Figure 2). Thus, complete dissolution was expected before gastric emptying. Both products met the criteria for immediate-release formulations [7].

Validation of the Bioanalytical Method

The method was validated according to the respective EMA [15] and Food and Drug Administration (FDA) guidelines [16]. The results met the acceptance criteria for intra- and between run accuracy (80-120% for LLOQ, 85-115% for QCs) and precision (≤20% for LLOQ, ≤15% for QCs). The stability of rivaroxaban was confirmed in solutions and human plasma. The incurred sample reanalysis (ISR) results at 99% and 97% confirmed the reliability of the method (Table 2).

Evaluation of Bioequivalence

Some differences in the mean rivaroxaban concentration-time profiles may be observed between the test and reference products (Figure 3). It seems that in fasting conditions equilibrium between absorption and elimination was reached at the lower concentration level for the test than for the reference product (Figure 3A). In fed conditions, the C_{max} region for the test product was sharper (as expected for capsule) although absorption was somewhat delayed (Figure 3B), contrary to *in vitro* dissolution test results. This phenomenon is further described in the discussion section.

Table 2. Bioanalytical method validation

Parameter	Study 1, fasting 10 mg	Study 2, fed 20 mg
Calibration range	2-400 ng/mL	2-600 ng/mL
Quality control (QC) concentrations	6, 30, 160, 240 ng/mL	6, 30, 240, 480 ng/mL
Within-run accuracy	103.1-111.6%	
Within-run precision	1.99-6.56%	
Between-run accuracy	105.5-108.4%	
Between-run precision	3.01-5.37%	
Long-term stability at ≤ -65°C	62 days	
Short-term stability at 15-30°C	48 hours	
Number of ISR samples	110	116
%ISR	99%	97%

ISR, incurred sample reanalysis,%ISR, percentage of individual ISR within ±20% acceptance criteria

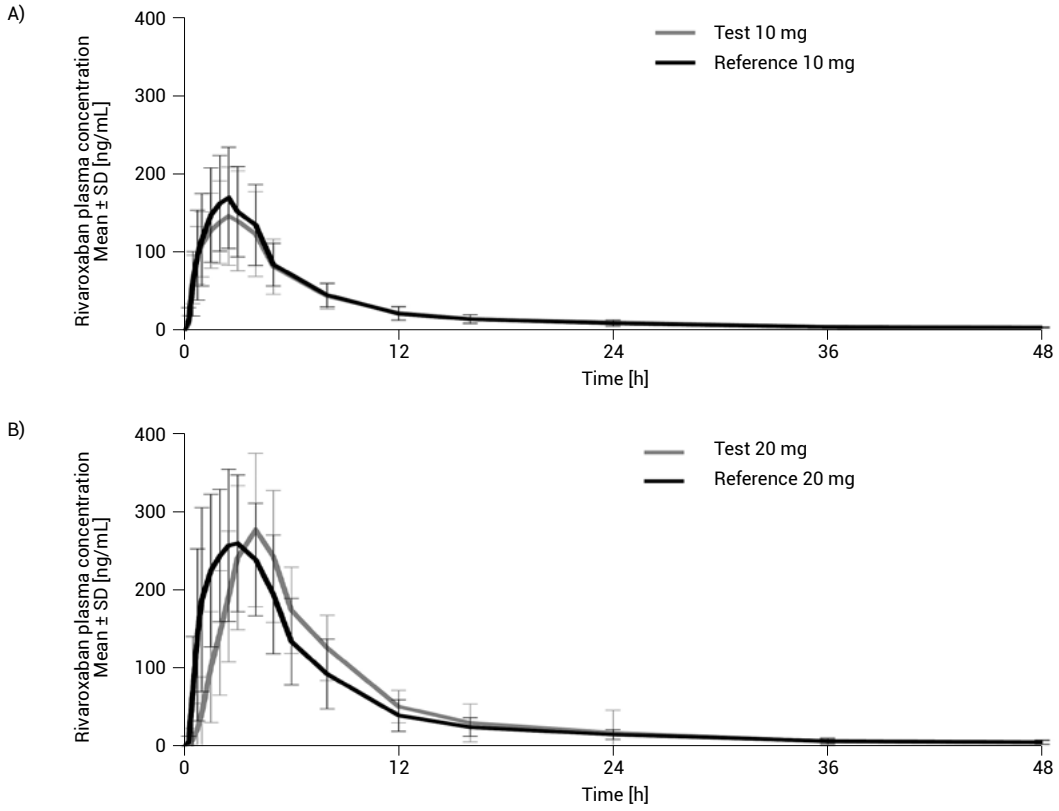

Figure 3. Arithmetic mean (SD) rivaroxaban plasma concentration–time profiles after a single administration of the test (hard capsules, Zarixa) and the reference (film-coated tablets, Xarelto®) products: (A) Study 1, n = 34; (B) Study 2, n = 36.

Primary pharmacokinetic parameters C_{max} and $AUC_{(0-t)}$ were similar (Table 3, Figure 4, Figure 5) and met the bioequivalence criteria. The power of the study exceeded 0.90 for C_{max} and $AUC_{(0-t)}$ (Table 4).

The Wilcoxon signed-rank test revealed no statistically significant differences in t_{max} in fasting conditions, but indicated a significantly longer t_{max} for the test product in fed conditions (Study 2,

Table 3. Pharmacokinetic parameters of rivaroxaban after a single administration of the test (Zarixa, hard capsules) and the reference (Xarelto®, film-coated tablets) products.

Study	**Study 1, fasting 10 mg (n = 34)**			**Study 2, fed 20 mg (n = 36)**		
Parameter	Test mean ± SD	Reference mean ± SD	Geometric mean T/R (90% CI)	Test mean ± SD	Reference mean ± SD	Geometric mean T/R (90% CI)
$AUC_{(0-t)}$ [ng/mL×h]	1141 ± 339	1173 ± 374	97.96% (93.69-102.42%)	2180 ± 582	2112 ± 588	103.57% (98.75-108.63%)
$AUC_{(0-\infty)}$ [ng/mL×h]	1194 ± 337	1225 ± 380	98.30% (94.06-102.73%)	2194 ± 515 [b]	2194 ± 611	102.61% (97.88-107.58%) [b]
C_{max} [ng/mL]	164 ± 65	182 ± 65	89.35% (84.28-94.72%)	301 ± 80	315 ± 83	95.17% (87.35-103.70%)
t_{max} [a] [h]	2.50 [0.50-4.00]	2.25 [0.75-4.00]	-	4.00 [3.00-23.62]	2.00 [0.75-5.00]	-
t_{lag} [a] [h]	0.00 [0.00-0.25]	0.00 [0.00-0.50]	-	0.50 [0.00-1.00]	0.25 [0.00-0.75]	-
$t_{1/2}$ [h]	10.5 ± 3.7	10.0 ± 3.0	-	10.2 ± 5.6[b]	11.2 ± 5.2	-

[a] Median and [min–max] for t_{max} and t_{lag}; [b] n = 35; CI, confidence interval; T, test; R, reference; pharmacokinetic parameters – see text.

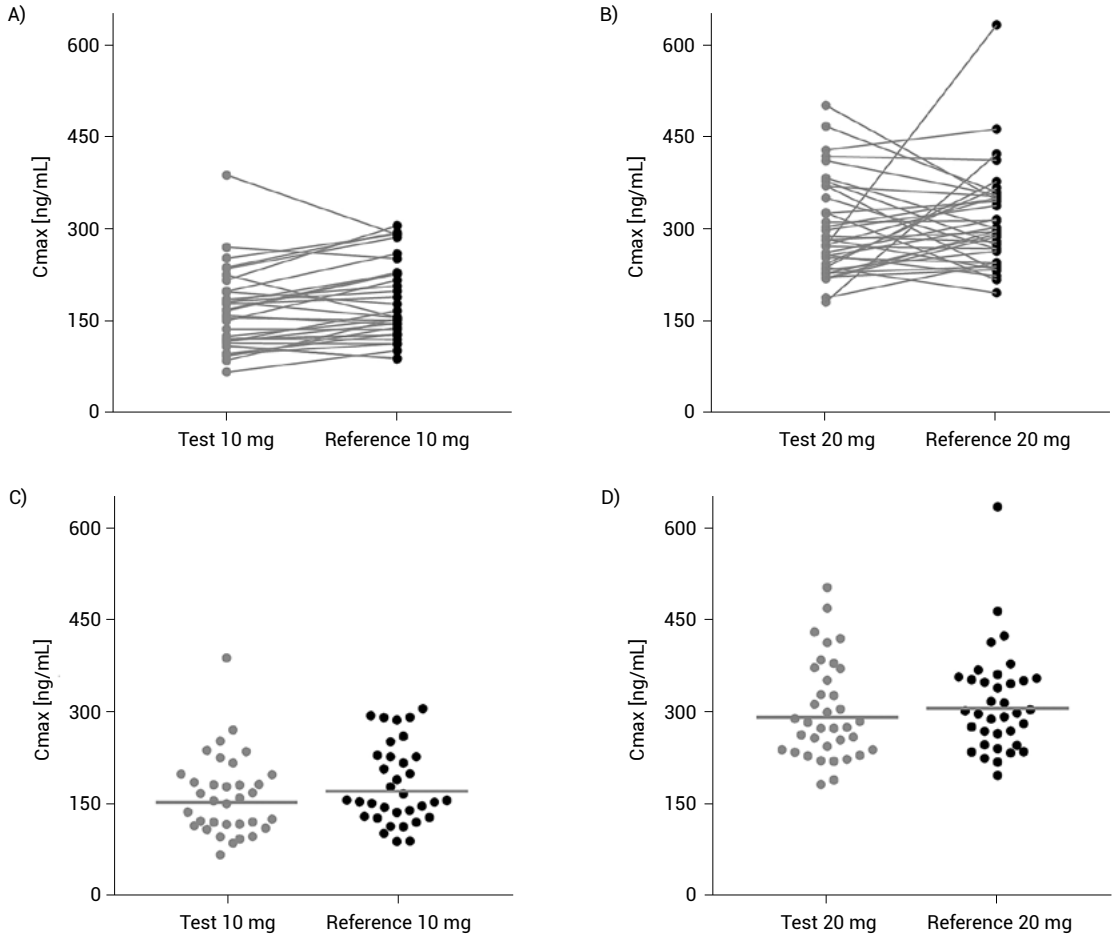

Figure 4. Visual presentation of C_{max} – paired dot plots for Study 1, n = 34 (A) and Study 2, n = 36 (B), scatter plots with a geometric mean (grey line) for Study 1 (C) and Study 2 (D). Test – hard capsules (Zarixa); Reference – film-coated tablets (Xarelto®)

$p<0.001$). Similarly, the statistically significant differences in t_{lag} were observed only in fed conditions (Study 2, $p<0.001$), indicating longer t_{lag} for the test product.

Safety Results

In both studies, all 36 participants who received at least one dose of the test or reference product were included in the safety assessment (Table 5). There were no serious adverse events recorded. During Study 1, there were 14 adverse events not related to the study products, reported in 10 subjects (11 for the reference and three for the test product). The intensity of 10 adverse events was classified as mild and four as moderate. The most frequently reported adverse event was a headache (n = 11).

During Study 2, there were six adverse events not related to the study products and two possibly related, reported in eight subjects (three for the reference and five for the test product). The intensity of three adverse events was classified as mild and five as moderate. Again, the most frequently reported adverse event was a headache (n = 6).

DISCUSSION

EMA bioequivalence guideline considers various immediate-release oral pharmaceutical forms as one pharmaceutical form [7]. However, different oral pharmaceutical forms have their technological and biopharmaceutical characteristics and limitations. The influence of food intake on the behavior of tablets and capsules may vary [17, 18]. Thus, developing a bioequivalent product in different oral forms (e.g. capsule vs. tablet) is a demanding challenge. Generally, the release of active ingredients from tablets starts immediately and is faster in the initial stages, but then it is slowed by the dissolution of the tablet itself. Release from a hard capsule does not start until the gelatine shell is dissolved or

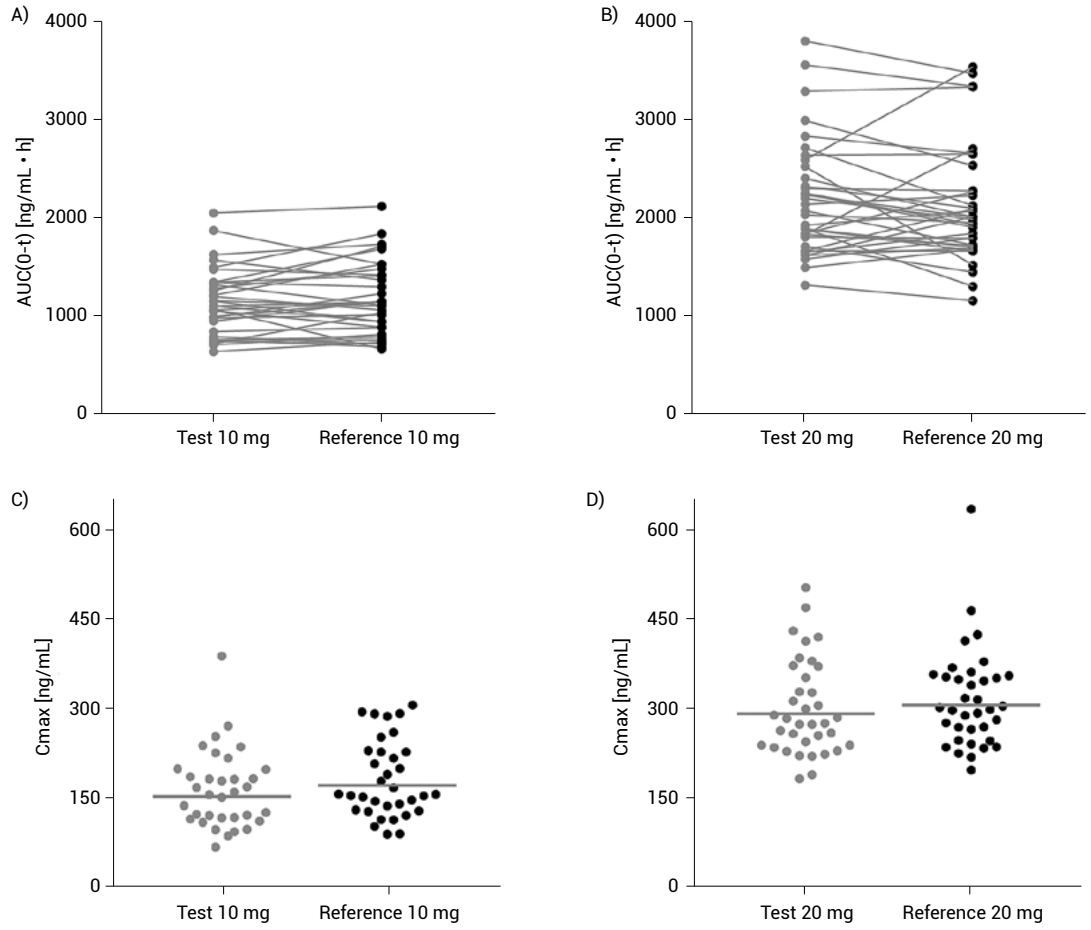

Figure 5. Visual presentation of $AUC_{(0-t)}$ – paired dot plots for Study 1, n = 34 (A) and Study 2, n = 36 (B), scatter plots with a geometric mean (grey line) for Study 1 (C) and Study 2 (D). Test – hard capsules (Zarixa); Reference – film-coated tablets (Xarelto®)

disintegrated, but once it happens the release is rapid and complete.

The key finding of both studies is the bioequivalence of hard capsules (test) and film-coated tablets (reference) based on 90% CI for geometric mean ratios (test/reference, Table 3) completely lying within the acceptance criteria [7, 8]. Changes in pharmaceutical formulation influenced the shape of mean pharmacokinetic profiles (Figure 3), but still resulted in similar primary pharmacokinetic parameters of the test and reference products (Figures 4-5). Among the ANOVA results, the most important was a significant effect of the formulation on C_{max} in Study 1. Clinical relevance of this finding is not expected as the 90% CI was within the acceptance criteria (Table 3) and in spite of the observed inter-subject variability for C_{max} (Figure 4C, 4D). Statistically significant differences in t_{max} and t_{lag} between the products were observed in fed conditions. For the test product in Study 2, the median t_{max} was delayed for 2 h (Table 3). Thirty-four subjects had a t_{max} of up to 5 h, while the remaining two subjects had a t_{max} of 8 h and 23.6 h. For the test product in Study 2, the median t_{lag} was 0.25 h longer then for

Table 4. Within subject variation (WSV) and *a posteriori* power for primary pharmacokinetic parameters of rivaroxaban after a single administration of the test and the reference products.

Study	**Study 1 fasting 10 mg (n = 34)**		**Study 2 fed 20 mg (n = 36)**	
Parameter	WSV	Power	WSV	Power
$AUC_{(0-t)}$	10.9%	>0.999	12.0%	>0.999
C_{max}	14.3%	0.932	21.8%	0.956

Table 5. Adverse events observed after a single administration of the test and the reference products.

No. of adverse events (AEs)	Study 1, fasting 10 mg		Study 2, fed 20 mg	
	Test n = 35	Reference n = 35	Test n = 36	Reference n = 36
Adverse events - total	3	11	5	3
Headache	2	9	4	2
Increased alanine transaminase (ALT) level	0	1	0	0
Diarrhea	1	0	0	0
Menstrual pain	0	1	0	0
Increased d-dimer level	0	0	0	1
Increased urine leukocyte level	0	0	1	0

Note: in Study 1, two subjects were discontinued due to personal reasons before Period 2 (each was administered a different product in Period 1). Thus, there were 36 subjects included in the adverse event analysis, but only 35 subjects for each product.

reference product (Table 3). Clinical relevance of the above observations is not expected as rapid release is not claimed to be of importance for the onset of rivaroxaban action.

Proper study design enabled reliable evaluation of bioequivalence. The wash-out periods of 12 days (Study 1) and seven days (Study 2) were appropriate as rivaroxaban concentrations in all the pre-dose samples in period 2 were below the lower limit of quantification (2 ng/mL). The blood sampling schedule allowed for the proper characterization of pharmacokinetics: (A) there were no C_{max} observed in the first sample after product administration, (B) each individual $AUC_{(0-t)}$ was greater than 80% of $AUC_{(0-\infty)}$. Appropriate sample size resulted in the *a posteriori* power above 0.90 for each primary pharmacokinetic parameter (Table 4).

The pharmacokinetic parameters observed in both studies are in line with previous reports [9–11, 17]. Compared with another bioequivalence study between rivaroxaban capsules and film-coated tablets, we obtained a slightly higher extent of exposure for the reference product (arithmetic mean of $AUC_{(0-t)}$ for 10 mg was 1013 [18] vs. 1173 ng/mL×h in our study; for 20 mg 1856 [18] vs. 2112 ng/mL×h;). The shape of the mean pharmacokinetic profiles was similar to our study, indicating that different types of immediate release pharmaceutical forms may influence changes of rivaroxaban concentrations over time. Geometric mean $AUC_{(0-t)}$ after administration of the 20 mg dose for capsules developed by Sanovel ˙Ilaç Sanayi ve Ticaret A.S. was 116.5% of the value observed for the reference tablets [18]. In comparison, in the case of capsules developed by Celon Pharma, the respective geometric mean T/R for $AUC_{(0-t)}$ was 103.6%. It should also be noted that the population studied by Sözer et al. [18] consisted of male subjects only, which does not meet EMA recommendations [7, 8].

We observed faster rivaroxaban release *in vitro* from the test product (85% release achieved in 10 min) than from the reference product (85% release in 15 min, Figure 2). On the other hand, pharmacokinetic profiles indicate a bit faster *in vivo* absorption of rivaroxaban from the reference product than from the test product (Figure 3). This phenomenon may be explained by the difference in dosage forms. *In vitro* dissolution was conducted in a high volume of medium (900 mL), where wetting of the capsule shell and dissolving of the uncompressed capsule filling is faster than release from compressed coated tablets. The medium is used for quality control (to detect any potential failure of product), thus it does not need to simulate the physiological situation. Liberation of the active pharmaceutical ingredient *in vivo* takes place at a different pH and solvent volume, in the presence of digestion enzymes. In this environment, the capsule shell may behave unlike the tablet due to differences in the composition of both products. As more than 85% of the rivaroxaban was released within 15 minutes (Figure 2), the dissolution profiles were considered similar as defined by the EMA bioequivalence guidelines [7].

The capsule development was also driven by unmet patients' needs. The reference product contains lactose, thus it is contraindicated in patients with total lactase deficiency, hereditary problems of galactose intolerance, or glucose-galactose malabsorption [2]. The test product does not contain lactose, which extends the possibility to administer rivaroxaban to patients with lactose intolerance. Also, in the case of patients with difficulties in swallowing,

the tablet needs to be crushed before administration, e.g. to prepare a suspension in water or mix it with apple puree [2]. On the other hand, the capsule content may be used directly as a capsule shell is easy to open.

Numerous published PK/PD models for rivaroxaban suggest a strong association between pharmacokinetics and anticoagulant efficacy. Some examples include Zdovc et al. describing the linear association of both prothrombin time and partial thromboplastin time to the logarithm of the rivaroxaban plasma concentration [19], and Liu et al. describing a comparable exposure–response relationship between Asians and Caucasians [20]. Modeling approaches were extended to connect pharmaceutical formulation performance with a probability of lack-of-response, e.g., Romański et al. on the relationship between biorelevant fed-state dissolution tests and lack of response to rivaroxaban in nonvalvular atrial fibrillation patients [21]. However in the context of bioequivalence, the most relevant model may be the one by González-Sales et al. [22]. The study aimed to assess the safety and efficacy of rivaroxaban therapy in patients undergoing hip or knee replacement. The authors, all affiliated with the FDA, concluded that a generic product passing the current bioequivalence criteria is expected to have similar safety and efficacy as the reference product.

Study Limitations

The studies were conducted in healthy subjects, and one may consider the application of their results to patients as a major limitation. It should be emphasized that bioequivalence of orally administered medicines is a well-established procedure based on pharmacokinetic endpoints. A key concept of designing bioequivalence studies is to decrease all sources of pharmacokinetic variability except formulation. Disease may influence pharmacokinetics and in turn mask differences between formulations. Thus, it is preferred to conduct bioequivalence studies in healthy subjects [7]. In the case of rivaroxaban, PK/PD models further justify this approach [22].

At the time of the conducted study, the harmonized ICH M10 guidelines on bioanalytical method validation [23] had not yet been implemented. However, validation in line with the EMA [15] and FDA [16] guidelines as well as the method's in-study performance meets the criteria set by the ICH M10 guidelines.

At the time of writing this manuscript, the global harmonization of bioequivalence rules was ongoing [24]. However, we predict that significant changes in standard two-way crossover design studies of orally administered instant-release products are rather unlikely.

CONCLUSIONS

The challenging task to develop a bioequivalent medicine in different oral forms (e.g. capsule vs. tablet) was successfully completed. The results of both studies, conducted in healthy subjects after a single dose administration, confirmed that hard capsules (test product, Zarixa 10 mg and 20 mg manufactured by Celon Pharma S.A.) are bioequivalent to film-coated tablets (reference product, Xarelto® 10 mg and 20 mg). Both products were well tolerated. The results indicate that hard capsules can be interchangeable in clinical practice with film-coated tablets.

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Authors' Contributions

Conceptualization, A.G.-P., K.J.-D., G.H. and P.J.R.; Methodology, A.G.-P., M.K., K.J.-D, G.H., and K.S.; Software, A.S.-Ś. and D.R.; Validation, M.K., O.C.-B. and K.S.; Formal Analysis, M.K., K.J.-D., and A.S.-Ś.; Investigation, M.K., K.J.-D., O.C.-B., G.H., and K.S.; Data Curation, A.G.-P., M.K., K.J.-D., A.S.-Ś., G.H., D.R., and P.J.R.; Writing – Original Draft Preparation, A.G.-P., O.C.-B., A.S.-Ś., G.H., K.S., and P.J.R.; Writing – Review & Editing, M.K.; K.J.-D., and D.R., Visualization, P.J.R. and G.H.; Supervision, A.G.-P., P.J.R.; Project Administration,

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