# Relative bioavailability/bioequivalence between two oral formulations of rivaroxaban (20 mg) in healthy brazilian subjects, under fasting and fed conditions

# Biodisponibilidade relativa/bioequivalência entre duas formulações orais de rivaroxabana (20 mg) em participantes brasileiros saudáveis, em condições de jejum e alimentação

DOI:10.34119/bjhrv5n4-208

Recebimento dos originais: 14/04/2022
Aceitação para publicação: 30/06/2022

**Juliana Loschi**
Pós-Graduação em Pesquisa Clínica pela Harvard T.H. Chan School of Public Health
Institution: Brainfarma Indústria Química e Farmacêutica S/A
Address: Rua Bonnard, 980, Alphaville Empresarial, Barueri - SP
E-mail: bqv@brainfarma.ind.br

**Luciana Montes Rezende**
Doutorado em Ciências, Área de Concentração Genética Médica pela Universidade Estadual de Campinas (UNICAMP)
Institution: Centro Avançado de Estudos e Pesquisas Ltda (CAEP)
Address: R. José Geraldo Cerebino Christófaro, 245, Fazenda Santa Cândida, Campinas - SP
E-mail: luciana.rezende@synovahealth.com

**Rodolfo Rodrigo Pereira Santos**
Mestrado em Saúde Baseada em Evidências pela Universidade Federal de São Paulo (UNIFESP)
Institution: Centro Avançado de Estudos e Pesquisas Ltda (CAEP)
Address: R. José Geraldo Cerebino Christófaro, 245, Fazenda Santa Cândida, Campinas - SP
E-mail: rodrigostat@gmail.com

**Silvana Aparecida Calafatti Carandina**
Doutorado em Ciências com ênfase em Química Analítica pela Universidade Federal de São Carlos (UFSCAR)
Institution: Centro Avançado de Estudos e Pesquisas Ltda (CAEP)
Address: R. José Geraldo Cerebino Christófaro, 245, Fazenda Santa Cândida, Campinas - SP
E-mail: silvana.carandina@synvia.com

**José Jorge Gabbai**
Pós-Graduação em Gestão Industrial Farmacêutica pela Universidade Estácio de Sá
Institution: Centro Avançado de Estudos e Pesquisas Ltda (CAEP)
Address: R. José Geraldo Cerebino Christófaro, 245, Fazenda Santa Cândida, Campinas - SP
E-mail: jorge.gabbai@synvia.com

**Giulia Minuti**
Mestranda em Farmacologia pela Universidade Estadual de Campinas (UNICAMP)
Institution: Brainfarma Indústria Química e Farmacêutica S/A
Address: Rua Bonnard, 980, Alphaville Empresarial, Barueri - SP
E-mail: bqv@brainfarma.ind.br

**Abrão Abuhab**
Doutor em Medicina Faculdade de Medicina da Universidade de São Paulo (FMUSP – InCor)
Institution: Brainfarma Indústria Química e Farmacêutica S/A
Address: Rua Bonnard, 980, Alphaville Empresarial, Barueri - SP
E-mail: abrao.abuhab@hypera.com.br

**José Pedrazzoli Júnior**
Livre Docência em Farmacologia Clínica pela Universidade Estadual de Campinas (UNICAMP)
Institution: Centro Avançado de Estudos e Pesquisas Ltda (CAEP)
Address: R. José Geraldo Cerebino Christófaro, 245, Fazenda Santa Cândida, Campinas - SP
E-mail: unifag@uol.com.br

**ABSTRACT**
The study aimed to evaluate the bioequivalence of two rivaroxaban coated tablets in healthy Brazilian subjects after single dose under fasting and fed conditions. Two studies were conducted using the open, randomized, 2-sequence, 4-period crossover design separated by a 7-day washout. Serial blood samples were collected to measure plasma concentration of rivaroxaban and to perform pharmacokinetic analysis. Bioequivalence was confirmed if 90% confidence intervals (90% CI) for geometric mean ratio between two formulations fell within 80-125% equivalence limits for area under the concentration-time curve from time 0 to last determined concentration ($AUC_{0-t}$) and maximum concentration ($C_{max}$). Moreover, the upper limit of the 90% CI for intra-subject variability ratio ($\sigma WT/\sigma WR$) between both formulations should be ≤ 2.5. Points estimates and 90% CI for $AUC_{0-t}$ (102,4; 97,3-107,9), $C_{max}$ (97,5; 91,6-103,7), $\sigma WT/\sigma WR$ for $AUC_{0-t}$ (0,70; 0,51-0,95) and $\sigma WT/\sigma WR$ for $C_{max}$ (0,94; 0,69-1,28) demonstrated that both formulations were bioequivalent in the fasting study. The similar results were obtained for $AUC_{0-t}$ (99,5; 96,1-103,1), $C_{max}$ (98,9; 95,1-102,9), $\sigma WT/\sigma WR$ for $AUC_{0-t}$ (0,94; 0,70-1,25) and $\sigma WT/\sigma WR$ for $C_{max}$ (0,97; 0,73-1,30) in the fed study. Thus, the formulations have equivalent systemic bioavailability under both fasting and fed conditions, and a similar clinical performance can be expected.

**Keywords:** factor xa inhibitors, antithrombotics, rivaroxaban, pharmacokinetics, bioequivalence.

**RESUMO**
O estudo teve como objetivo avaliar a bioequivalência entre dois comprimidos revestidos de rivaroxabana em participantes brasileiros saudáveis após administração em dose única em condições de jejum e alimentação. Dois estudos foram conduzidos com um desenho aberto, randomizado, cruzado com duas sequências, quatro períodos e *washout* de sete dias entre os períodos. Foram realizadas coletas seriadas de sangue para quantificar a concentração plasmática de rivaroxabana e realizar a análise farmacocinética. A bioequivalência é confirmada se os intervalos de confiança de 90% (IC de 90%) para a razão entre as médias geométricas das duas formulações estiverem dentro dos limites de bioequivalência de 80-125%

para a área sob a curva do tempo 0 à última concentração mensurável (AUC0-t) e concentração máxima (Cmax). Além disso, o limite superior do IC de 90% para a razão da variabilidade intra-sujeito ($\sigma WT/\sigma WR$) entre ambas as formulações deve ser ≤ 2,5. Os pontos estimados e o IC de 90% para AUC0-t (102,4; 97,3-107,9), Cmax (97,5; 91,6-103,7), $\sigma WT/\sigma WR$ para AUC0-t (0,70; 0,51-0,95) e $\sigma WT/\sigma WR$ para Cmax (0,94; 0,69-1,28) demonstraram que ambas as formulações foram bioequivalentes no estudo jejum. Resultados semelhantes foram obtidos para AUC0-t (99,5; 96,1-103,1), Cmax (98,9; 95,1-102,9), $\sigma WT/\sigma WR$ para AUC0-t (0,94; 0,70-1,25) e $\sigma WT/\sigma WR$ para Cmax (0,97; 0,73-1,30) no estudo alimentado. Assim, as formulações têm biodisponibilidade sistêmica equivalente sob condições de jejum e alimentação, e um desempenho clínico semelhante pode ser esperado.

**Palavras-chave:** inibidores do fator xa, antitrombóticos, rivaroxabana, farmacocinética, bioequivalência.

## 1 INTRODUCTION

Rivaroxaban was the first medicine of a new class of small molecules developed as factor Xa inhibitors. Exhibiting a selectivity for factor Xa 10,000 times higher than other serine proteases, rivaroxaban binds directly to the factor Xa active site, blocking the interaction with its substrate. The small size and non-basic characteristic allow the inhibition of the free factor Xa, the clot-associated factor Xa and the prothrombinase complex (Ageno *et al*., 2012; Steinberg, Becker, 2014).

The factor Xa plays a central role in coagulation, and it is activated by the intrinsic and extrinsic pathways, directly converting prothrombin to thrombin through the prothrombinase complex, leading to fibrin clot formation and platelet activation by thrombin. Data from preclinical and clinical studies confirm that factor Xa and thrombin are suitable targets for effective anticoagulation (Steinberg, Becker, 2014; Becker, 2021).

Direct oral anticoagulants, such as rivaroxaban, were developed to overcome the limitations of vitamin K antagonists, which are available for clinical use for some decades. Although effective, vitamin K antagonists have a slow onset of action, numerous food-drug, and drug-drug interactions, and an unpredictable pharmacodynamic response. Therefore, frequent monitoring of coagulation parameters and dose adjustment are required. On the other hand, direct oral anticoagulants demonstrate predictable pharmacokinetic and pharmacodynamic, and low potential for drug-drug interactions. Also, it can be administered in fixed doses and the frequent monitoring of coagulation parameters is not necessary (Ageno *et al*., 2012; Mueck *et al*., 2014).

The pharmacokinetic profile of rivaroxaban in healthy subjects exhibits rapid absorption within 2 to 4 hours after oral administration, bioavailability of 80 to 100% for the 10 mg dose,

distribution volume of approximately 50 L at steady state, and half-life time of 7 to 11 hours. Approximately two-thirds of the rivaroxaban dose undergoes metabolic degradation in the liver by CYP3A4/5 and CYP2J2 enzymes and by CYP-independent mechanisms (Kreutz, 2012; Kvasnicka *et al*., 2017). Inactive metabolites are excreted by the kidneys and by hepatobiliary route. The residual unchanged dose is eliminated in the urine, mainly by active renal secretion or glomerular filtration (Weinz *et al*., 2009; Kreutz, 2012).

In Brazil, rivaroxaban is currently indicated for adult use in the treatment of deep vein thrombosis (DVT) and pulmonary embolism. It is also indicated for the prevention of stroke, DVT, pulmonary embolism, and systemic embolism in adults with non-valvular atrial fibrillation. The use in children and adolescents is approved for the treatment and prevention of venous thromboembolism (Xarelto®, package insert). The 15 and 20 mg tablets should be taken with food, while the 10 mg tablets can be taken with or without food. Under feed condition, the bioavailability of the 20 mg dose is about 39% higher compared to administration under fasting condition (Kubitza *et al*., 2006; Zhang *et al*., 2017).

Therefore, two studies were conducted - one of them in fasting condition and the other after standardized food - to compare the bioavailability of two drugs containing rivaroxaban, Xarelto® 20 mg from Bayer AG (reference drug) and rivaroxaban 20 mg from Brainfarma Indústria Química e Farmacêutica S/A (test drug). Thus, the formulations were administered in a single dose to healthy subjects in both studies, followed by the collection of serial blood samples for rivaroxaban quantification, to prove bioequivalence between the two formulations.

## 2 MATERIAL AND METHODS

### 2.1 STUDY POPULATION

The Relative Bioavailability/Bioequivalence of rivaroxaban was assessed in two clinical trials conducted at Centro Avançado de Estudos e Pesquisas Ltda. - CAEP (Campinas-SP, Brazil), one of them in fasting condition and the other one in fed condition. The studies were previously approved by the Ethics Committee (EC) - Investiga Instituto de Pesquisas and conducted in accordance with the ICH Guidelines for Good Clinical Practice GCP/ICH, Americas Document (2005) and applicable regulatory requirements. All the study procedures were explained to the subjects, and all signed the Informed Consent Form (ICF) before included in the studies.

Brazilian subjects of both genders, aged between 18 and 50 years old, with body mass index (BMI) between 18.5 and 29.9 kg/m$^2$ and able to understand the nature, objectives, and risks related to the studies were selected for both clinical trials. In addition, the subjects had to

be in good health or without significant disease, as confirmed by clinical history, evaluation of vital signs, anthropometric data, physical examination, electrocardiogram with 12 variations, and laboratory tests (hemogram, urea, creatinine, creatinine clearance, total bilirubin and fractions, total proteins and fractions, glycemia, alkaline phosphatase, alanine aminotransferase, arginine aminotransferase, total cholesterol, triglycerides, uric acid, prothrombin time, activated partial thromboplastin time, urine routine, RT-PCR for SARS-CoV-2, and serology for HIV and hepatitis B and C). Female participants had beta-HCG testing at screening and before hospitalization in each period.

Subjects with positive response to any of the following criteria were excluded: history of allergy to rivaroxaban or the formula components; history of liver, gastrointestinal, kidney, respiratory, hematologic, cardiologic, neurologic, neoplastic, or psychiatric disease, considered clinically significant at the physician's discretion; history of heart, kidney, gastrointestinal, liver, or pancreatic surgery; history of drug or alcohol abuse; and women who were pregnant, breastfeeding, or planning to become pregnant during the study.

## 2.2 STUDY DESIGN

Both fasting and fed studies was open-label, randomized, crossover design with 2-sequences (TRTR and RTRT) and 4-periods. The periods were separated by a 7-day washout, and in each period the subjects received a single oral dose of rivaroxaban 20 mg (test or reference) with 200 mL of water at room temperature. Fluid intake was not allowed from two hours before to two hours after drug administration.

For all periods of both studies, subjects were admitted to the research center 24 hours prior to rivaroxaban administration for pre-hospitalization laboratory testing, including RT-PCR for SARS-CoV-2, and clinical examination. The test or reference drug was administered at 07:00 am the day after admission. For the fasting study, subjects fasted for at least eight hours overnight before rivaroxaban administration and for at least four hours after the administration. Subjects remained at the research center for approximately 12 hours after rivaroxaban administration, completing 36 hours of hospitalization in each period. In the fed study, subjects ate a standardized hypercaloric breakfast (~ 800 kcal), served 30 minutes before rivaroxaban administration. The duration of hospitalization at the research center was approximately 24 hours after rivaroxaban administration, completing 48 hours of hospitalization in each period.

A total of 21 blood samples of 4.9 mL each, in tubes containing EDTA (ethylenediaminetetraacetic acid) anticoagulant, were collected from each subject per period. Blood collections during the hospitalization of fasting study were performed following the

predefined schedule, at the times: -01:00 (baseline) and after 00:20 / 00:40 / 01:00 / 01:20 / 01:40 / 02:00 / 02:20 / 02:40 / 03:00 / 03:20 / 03:40 / 04:00 / 04:30 / 06:00 / 08:00 / 10:00 and 12:00 hours after rivaroxaban administration. In addition, the subjects returned to the research center for collection at 24:00, 36:00 and 48:00 hours. In the fed study, samples were collected at -01:00 (baseline) and after 00:30 / 01:00 / 01:30 / 02:00 / 02:30 / 03:00 / 03:20 / 03:40 / 04:00 / 04:20 / 04:40 / 05:00 /06:00 / 08:00 / 10:00 / 12:00 / 16:00 / 24:00 of rivaroxaban administration. Besides, the subjects returned to the research center for the collections at times 36:00 and 48:00.

## 2.3 RIVAROXABAN QUANTIFICATION

Blood samples with EDTA anticoagulant were centrifuged at 3500 rpm for 10 minutes at approximate temperature of 4°C, and the resultant plasma was separated, transferred to 2 mL cryogenic tubes, and stored in ultrafreezer until analysis.

Initially, the plasma samples were submitted to protein precipitation with 100% acetonitrile. A total 700 µL of acetonitrile was added to 200 µL of plasma, followed by agitation for 1 min and centrifugation at 15,000 rpm for 3 min at 4°C. After this extraction, the samples were analyzed using reverse-phase ultra-performance liquid chromatography with mass spectrometry detection (UPLC - MS/MS).

The chromatographic conditions included an Acquity UPLC BEH C18 2.1 x 50 mm, 1.7 µm chromatographic column (Waters, USA), a mobile phase with solvent A (1 mM ammonium formate + 0.1% formic acid) and solvent B (100% acetonitrile), a column temperature of 28 ºC, an autoinjector temperature of 15 ºC, and a flow rate of 0.42 mL/min. A volume of 3 µL of sample was fully injected into the UPLC MS/MS system and the run time was 1.7 minutes.

Detection of rivaroxaban at fasting study was performed in the ACQ-TQD model triple quadrupole mass spectrometer (Waters, USA). The detection of rivaroxaban at fed study was executed in the Xevo-TQS model triple quadrupole mass spectrometer (Waters, USA). The ionization mode set for both mass spectrometers was the electrospray positive (ESI+). The detection parameter employed was the *m/z* ratio between the precursor and product ions. The quantification parameter was the area under the chromatogram peak identified at the retention time, operating in Multiple Reaction Monitoring (MRM) mode. The MRM transitions were *m/z* 436.20→145.0 for rivaroxaban and *m/z* 440.20→145.0 for the internal standard (IP) rivaroxaban-d4. The analyte concentration in the subject samples were calculated by interpolation on the calibration curve. The linearity range used was 1 to 500 ng/mL. The

software MassLynx - Version 4.2 (Waters, USA) was used for the quantification of rivaroxaban using the internal standard addition method.

The bioanalytical method used was validated for selectivity, concomitant selectivity, matrix effect, residual effect, calibration curve, accuracy, precision, reinjection validation and stability, according to the current regulatory requirements. After validation, the method was used for the subject's samples quantification. For each analytical run, calibration standards, high, medium, and low control samples, and samples from one or more subjects were included, and all samples from the same subject were analyzed in a single run, except in cases of reanalysis.

## 2.4 SAFETY AND TOLERABILITY

Clinical examination with evaluation of the eyes, mouth, ear, nose, throat, neck (including thyroid), head, heart, abdomen (including liver and spleen), skin, lymph nodes, nervous/psychiatric system, respiratory, appearance, extremities, chest, spine, lungs, skeleton, and muscle were performed before and after the study. All laboratory exams, except for hepatitis and HIV serology and creatinine clearance, were repeated within 15 days after the last study period. Vital signs, blood pressure, heart rate and body temperature were measured before rivaroxaban administration and during hospitalization after rivaroxaban administration at 6 and 11 hours in the fasting study and 6, 11 and 23 hours in the fed study.

Any unexpected medical occurrence in a subject after signing the ICF, such as abnormal findings on exams or vital signs, unfavorable and unintended symptoms, and diseases temporally associated or not with rivaroxaban were recorded as adverse events. For each adverse event occurrence, the intensity (mild, moderate, severe) and causality relationship were recorded, according to WHO-defined categories (definite, probable, possible, unlikely, conditional/unclassified, not accessible/not classifiable).

## 2.5 STATISTICAL ANALYSIS

The pharmacokinetic parameters of maximum plasma concentration ($C_{max}$), area under the curve *vs* time from time zero to the last determined concentration ($AUC_{0-t}$), area under the curve *vs* time from time zero to infinity ($AUC_{0-\infty}$), time corresponding to maximum plasma concentration ($T_{max}$), terminal phase elimination constant ($K_{el}$), drug elimination half-life ($T_{½}$), volume of distribution (Vd) and clearance (CL) were determined using Software R© (The R Foundation, Austria), as well as Relative Bioavailability/Bioequivalence analysis.

For the evaluation of Relative Bioavailability/Bioequivalence between the two formulations of rivaroxaban (test and reference) in both studies (fasting and fed), the values of $C_{max}$ and $AUC_{0-t}$ were transformed into natural logarithm and evaluated by means of the ratio between the geometric means of the test and reference drug. The 90% confidence intervals (CI) for the ratios between the geometric means of $C_{max}$ and $AUC_{0-t}$ were calculated in both studies and the formulations would be considered bioequivalent if the 90% CI was contained in the interval 0.80 to 1.25 (RE No. 1170/2006, Brazil).

Analysis of variance (ANOVA) was performed for the evaluation of sequence, treatment group and period effects for the parameters of $C_{max}$, $AUC_{0-t}$ and $AUC_{0-\infty}$. The significance levels set for the fixed effects in the ANOVA were 0.05 for period and treatment group effects and 0.10 for sequence effects.

The intra-subject variability for the log-transformed parameters $AUC_{0-t}$ and $C_{max}$ of test and reference drugs, obtained for the two formulations in both studies, was performed as a complementary analysis according to the Food and Drug Administration - FDA Guidance for Bioequivalence of Rivaroxaban. The FDA guidance states that the upper limit of the 90% CI for variability ratio between the test and reference drugs intra-subject ($\sigma WT / \sigma WR$) should be $\leq 2.5$.

## 3 RESULTS AND DISCUSSION

### 3.1 STUDY POPULATION

The demographic features of the subjects in both studies (fasting and fed) are shown in **Table I**.

Table I. Demographics of the subjects in the fasting and fed studies.

| Demographic features | Fasting (N = 48) | Fed (N = 44) |
|---|---|---|
| Sex | | |
| Male | 24 | 22 |
| Female | 24 | 22 |
| Age (years) | | |
| Mean ± SD | 30,5 ± 7,7 | 30,9 ± 7,5 |
| Interval | 18 – 45 | 18 – 49 |
| Weight (kg) | | |
| Mean ± SD | 67,1 ± 10,7 | 68,3 ± 11,2 |
| Interval | 48,0 – 92,0 | 47,2 – 95,0 |
| Height (m) | | |
| Mean ± SD | 1,69 ± 0,10 | 1,69 ± 0,10 |
| Interval | 1,49 – 1,87 | 1,52 – 1,91 |
| BMI ($kg/m^2$) | | |
| Mean ± SD | 23,4 ± 2,1 | 23,7 ± 2,4 |
| Interval | 18,7 – 26,9 | 19,2 – 28,2 |

BMI, body mass index; N, number of subjects; SD, standard deviation.

Among the selected 48 subject for the fasting study, 32 of them (12 women and 20 men) completed the four periods of hospitalization as well as the respective outpatient collections and were included in the analyses. Six subjects were removed from the study due to positive RT-PCR test result for SARS-CoV-2; one subject due to the presence of flu-like symptoms (dry cough, runny nose, and sore throat); one subject due to vomiting after medication intake; and one subject due to the occurrence of an adverse event (generalized pruritus) after the second hospitalization period. Also, seven subjects dropped out of the study due to personal reasons or absence in one of the periods/collections after hospitalization.

In the feed study, 36 subjects out of 44 selected completed the study. Two subjects were removed from the study due to a positive RT-PCR test result for SARS-CoV-2; one subject due to antibiotic use during the study; one subject due to being breastfeeding; and one subject due to a positive illicit drug test result. Three subjects dropped out of the study due to personal reasons or absence at one of the hospitalization periods.

## 3.2 PHARMACOKINETIC PARAMETERS AND BIOEQUIVALENCE

The mean values for the pharmacokinetic parameters $C_{max}$, $AUC_{0-t}$, $AUC_{0-\infty}$, $T_{max}$, $T_{½}$, $K_{el}$, Vd, and CL for the test and reference drugs were calculated for the fasting and fed studies and are presented in **Table II**. The mean curves of plasma concentrations of rivaroxaban (test and reference) *vs*. time for each of the studies are shown in **Figure 1** and **Figure 2**. The geometric mean ratios of $C_{max}$ and $AUC_{0-t}$, in logarithmic scale, were 97.5% and 102.4%, respectively, for the fasting study and 98.9% and 99.5%, respectively, for the fed study. All CIs of 90% were within the recommended range for bioequivalence, as presented in **Table III**.

Table II. Pharmacokinetic parameters for rivaroxaban test drug and reference drug from fasting study and fed study.

| Pharmacokinetic parameters | Fasting Mean ± SD | | Fed Mean ± SD | |
|---|---|---|---|---|
| | Test (N = 32) | Reference (N = 32) | Test (N = 36) | Reference (N = 36) |
| $C_{max}$ (ng/mℓ) | 141,3 ± 49,8 | 144,4 ± 50,5 | 276,1 ± 58,9 | 280,2 ± 69,4 |
| $AUC_{0-t}$ (h.ng/mℓ) | 1250,2 ± 373,9 | 1225,5 ± 380,8 | 1647,3 ± 414,9 | 1653,1 ± 442,5 |
| $AUC_{0-\infty}$ (h.ng/mℓ) | 1435,1 ± 512,7 | 1374,4 ± 471,4 | 1698,4 ± 425,6 | 1715,0 ± 456,1 |
| $T_{max}$ (h) | 2,0 ± 1,1 | 2,1 ± 0,89 | 2,8 ± 1,2 | 2,6 ± 1,2 |
| $T_{½}$ (h) | 14,8 ± 7,5 | 13,8 ± 6,9 | 11,8 ± 6,0 | 12,2 ± 5,5 |
| $K_{el}$ (ℓ/h) | 0,06 ± 0,03 | 0,06 ± 0,03 | 0,07 ± 0,04 | 0,07 ± 0,04 |
| Vd (ℓ) | 308,3 ± 132,1 | 306,1 ± 140,0 | 211,0 ± 110,7 | 219,6 ± 116,5 |
| CL (ℓ/h) | 15,6 ± 5,4 | 16,4 ± 6,5 | 12,5 ± 3,3 | 12,5 ± 3,4 |

$AUC_{0-\infty}$, area under the curve *vs* time from time zero to infinity; $AUC_{0-t}$, area under the curve *vs* time from time zero to the last determined concentration; $C_{max}$, maximum plasma concentration; CL, clearance; CV, coefficient of variation; $K_{el}$, terminal phase elimination constant; N, number of subjects; SD, standard deviation; $T_{½}$, drug elimination half-life; $T_{max}$, time corresponding to maximum plasma concentration; Vd, volume of distribution.

Figure 1. Concentration-time curves for rivaroxaban under fasting condition. The mean curves of the plasma concentrations of the reference drug (circles and red line) and the test drug (triangles and blue line) for the 32 participants under fasting condition were plotted.

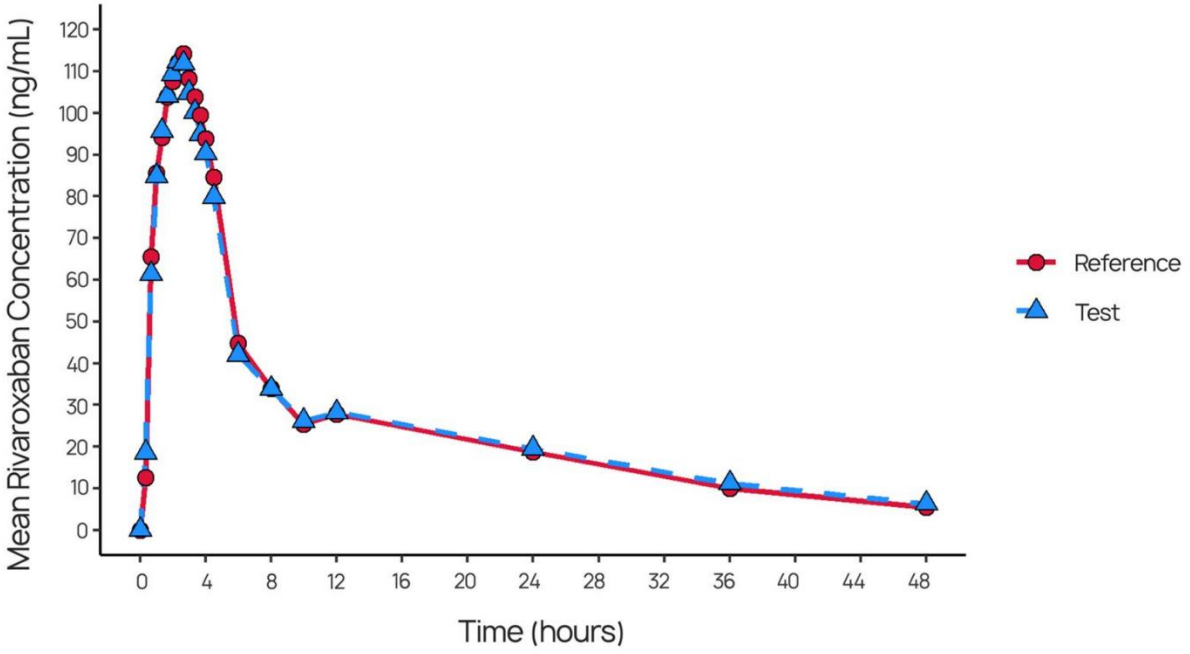

Figure 2. Concentration-time curves for rivaroxaban under fed condition. The mean curves of the plasma concentrations of the reference drug (circles and red line) and the test drug (triangles and blue line) for the 36 participants under fed condition were plotted.

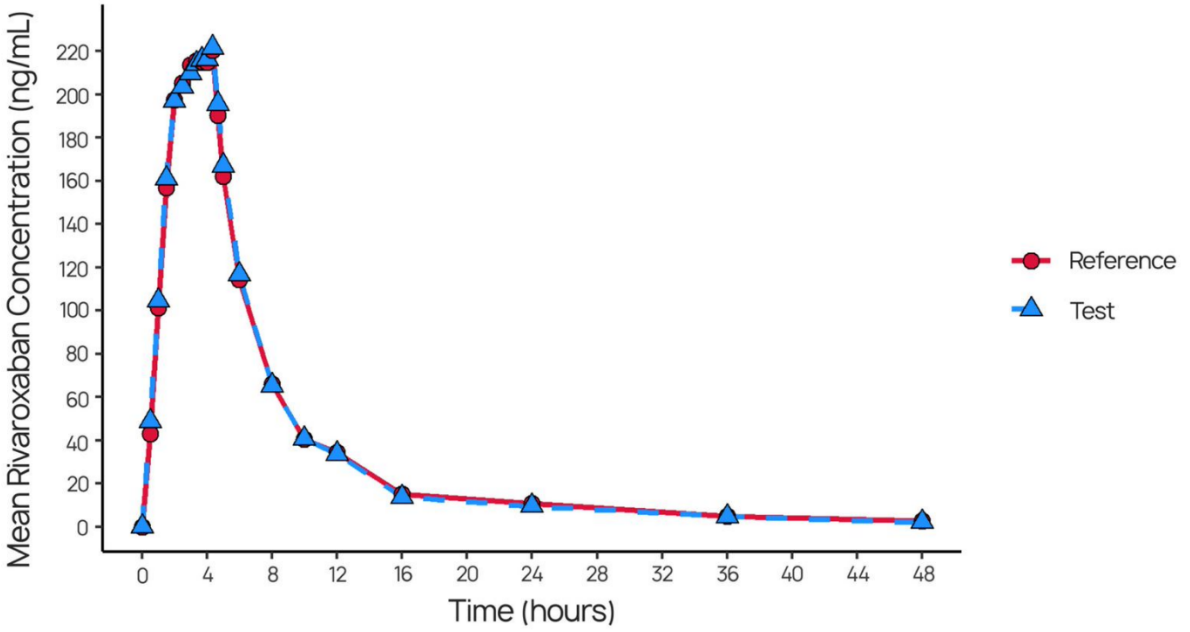


Table III. Evaluation of bioequivalence between rivaroxaban test drug and reference drug, under fasting and fed conditions, from pharmacokinetic parameters.

| Study | Pharmacokinetic parameter | N | Ratio (%) | 90% CI | CV (%) | Power (%) |
|---|---|---|---|---|---|---|
| Fasting | $C_{max}$ | 32 | 97,5 | 91,6 – 103,7 | 21,4 | 99 |
| | $AUC_{0-t}$ | 32 | 102,4 | 97,3 – 107,9 | 17,6 | 100 |
| | $AUC_{0-\infty}$ | 32 | 104,4 | 98,3 – 110,9 | 20,6 | 100 |
| Fed | $C_{max}$ | 36 | 98,9 | 95,1 – 102,9 | 14,2 | 100 |
| | $AUC_{0-t}$ | 36 | 99,5 | 96,1 – 103,1 | 12,5 | 100 |
| | $AUC_{0-\infty}$ | 36 | 98,9 | 95,6 – 102,3 | 12,2 | 100 |

$AUC_{0-\infty}$, area under the curve *vs* time from time zero to infinity; $AUC_{0-t}$, area under the curve *vs* time from time zero to the last determined concentration; $C_{max}$, maximum plasma concentration; CI, confidence interval; CV, coefficient of variation; N, number of subjects.

For the comparison of the intra-subject variability of the test and reference drugs, the $\sigma WT/\sigma WR$ ratio was evaluated from a 90% CI, as presented in **Table IV**. For the fasting study, the upper limit of the 90% CI for $C_{max}$ and $AUC_{0-t}$ were 0.95 and 1.28, respectively. For the fed study, the values were 1.25 and 1.30, respectively. Thus, $\sigma WT$ and $\sigma WR$ were considered comparable since the FDA Guidance for Bioequivalence of Rivaroxaban recommend the upper limit of the 90% CI ≤ 2.5.

Table IV. Sensitivity analysis for rivaroxaban test drug and reference drug, under fasting and fed condition.

| Study | PK Parameter | Reference | | Test | | Ratio $\sigma WT/\sigma WR$ | Lower 90% CI limit | Upper 90% CI limit |
|---|---|---|---|---|---|---|---|---|
| | | $S^2_{WR}$ | $CV_{WR}$ (%) | $S^2_{WT}$ | $CV_{WT}$ (%) | | | |
| Fasting | $C_{max}$ | 0,059 | 24,6 | 0,029 | 17,1 | 0,70 | 0,51 | 0,95 |
| | $AUC_{0-t}$ | 0,026 | 16,2 | 0,023 | 15,3 | 0,94 | 0,69 | 1,28 |
| Fed | $C_{max}$ | 0,016 | 12,6 | 0,014 | 11,9 | 0,94 | 0,70 | 1,25 |
| | $AUC_{0-t}$ | 0,015 | 12,2 | 0,014 | 11,9 | 0,97 | 0,73 | 1,30 |

$AUC_{0-t}$, area under the curve vs time from time zero to the last determined concentration; $C_{max}$, maximum plasma concentration; CI, confidence interval; $CV_{WR}$, intra-subject coefficient of variation of the reference; $CV_{WT}$, intra-subject coefficient of variation of the test; $S^2_{WR}$, intra-subject standard deviation for the reference; $S^2_{WT}$, intra-subject standard deviation for the test; $\sigma WR$, intra-subject variability of the reference; $\sigma WT$, intra-subject variability of the test.

The calculated pharmacokinetic parameters for rivaroxaban in both studies were similar to the data available in the literature (Kubitza *et al*., 2005; Mueck *et al*., 2014). As expected, the $C_{max}$, $AUC_{0-t}$ and $AUC_{0-\infty}$ values were higher when rivaroxaban was administered after a standard feeding compared to fasting administration.

Different studies have been demonstrating the effect of feeding on the pharmacokinetics of rivaroxaban (Kubitza *et al*., 2006; Zhang *et al*., 2017; Ding *et al*., 2019). At doses up to 10 mg, rivaroxaban exhibits linear pharmacokinetics, and its absorption remains unchanged, regardless of the presence of food (Kubitza *et al*., 2005; Kubitza *et al*., 2006; Zhang *et al*., 2017). However, the drug absorption is compromised for higher doses if administration occurs during fasting, with loss of pharmacokinetic linearity (Kubitza *et al*., 2005; Kubitza *et al*., 2008; Kushwah *et al*., 2021).

Kou and colleagues (2021) investigate the involvement of the xenobiotic transporters P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) in the bioavailability of rivaroxaban through a systematic review that included 9 pharmacokinetic drug-drug interaction studies, one food-drug interaction study, and one bioavailability study in relation to liver dysfunction. The results indicated that inhibition of P-gp or BCRP appears to have no significant effects on the absorption process of rivaroxaban.

In fact, Kushwah and colleagues (2021) reported an increase of ~2-fold in the solubility (in vitro) of a formulation containing rivaroxaban (20 mg) when using a dissolution medium similar to the fed condition with higher lipid concentration. The in vitro release of the formulation after 30 minutes, simulating gastric fluids in fasting and fed conditions, were 11% and 27.7%, respectively. The time to release 80% of rivaroxaban from the formulation was 360 minutes for the fasting condition and 210 minutes for the fed condition.

Demographic factors such as sex, age, ethnicity, and body weight do not appear to influence pharmacokinetics beyond the interindividual variability already observed, and there is no need for dose adjustments based on these factors. On the other hand, dose adjustments may be necessary in people with renal or hepatic impairment, given the pathways of metabolism and elimination of the drug (Kreutz, 2014; Mueck *et al*., 2014).

### 3.3 SAFETY AND TOLERABILITY

In the fasting study, 72 adverse events (AEs) were recorded in 28 subjects, with 59.7% of them occurring in the third period of the study and 81.9% being of mild intensity. Among the 72 AEs, 25 of them (34.7%) occurred after administration of the test and 47 (65.3%) after the administration of the reference drug. Fourteen (19.4%) were considered possibly related to the study drug, while 23 (31.9%) were considered probably related.

For the fed study, 32 AEs were recorded in 15 subjects, with 40.6% of them occurring in the second period of the study and 90.6% being of mild intensity. Among the 32 AEs, 18 of them (56.3%) occurred after administration of the test drug and 14 (43.7%) after the administration of reference drug. Eight (25%) were considered possibly related to the study drug, while 10 (31.2%) were considered probably related.

Headache was the most common AE in both studies for both the test and reference drug, accounting for 28% and 43% of the total AEs reported by subjects in the fasting study and fed study, respectively. One woman in the fasting study experienced generalized pruritus, hyperemia, and papular lesion in the right shoulder region after the second study period. The event was classified as mild intensity, with probable causality, and non-serious. The subject received treatment as prescribed by the study physician and showed improvement of symptoms in approximately 5 days. The other AEs had no significant impact on the quality of life for the subjects.

Overall, rivaroxaban has been demonstrating safety and tolerability when administered to healthy subjects (Kubitza *et al*., 2005; Kubitza *et al*., 2008; Zhao *et al*., 2009; Ding *et al*., 2019). In the PK/PD study published by Kubitza and colleagues (2005), which included 103 healthy men receiving single doses of 1.25 mg to 80 mg of rivaroxaban, the incidence of AEs was similar between subjects receiving placebo and those receiving rivaroxaban. Clinically relevant signs or symptoms of bleeding were not observed. In another PK/PD study with 96 healthy subjects of both sexes and aged over 60 years old, the incidence rates of adverse events were 25% for the 30 mg dose, 42% for the 40 mg dose, and 58% for the 50 mg dose and placebo,

with all doses administered after feeding. Similar to our study, headache was the most frequent AE with rivaroxaban, occurring in 22% of the subjects (Kubitza *et al*., 2008).

In summary, the results demonstrated bioequivalence between rivaroxaban 20 mg from Brainfarma Indústria Química e Farmacêutica S/A and the reference drug Xarelto® in healthy subjects. In addition to obtaining the pharmacokinetic profile of rivaroxaban (20 mg) in the Brazilian population, which can be used as a reference for further studies, this study contributes to the national drug policy, aiming to offer quality, safe and effective medication for the population, with lower market value.

## ACKNOWLEDGMENTS

To Fernando Bastos Canton Pacheco MD for conducting the clinical step of the study. To Marcela de Fátima Ferreira for her contribution in the subject's follow-up during hospitalization. To Fernanda dos Santos Barbosa for her contribution in the rivaroxaban quantification analyses. To Lucas Mattos Souza for his contribution to statistical analyses. To Alessandra Couto Boava for her contribution in the study planning and ethical procedures. To Adriana Thiemi Yamaguchi for her support in the document review.

## REFERENCES

Agência Nacional de Vigilância Sanitária (Brazil). Resolução- RE nº 1170, de 19 de abril de 2006. Guia para provas de biodisponibilidade relativa / bioequivalência de medicamentos. Diário Oficial da União 24 abr 2006; nº 77.

Ageno W, Gallus AS, Wittkowsky A, Crowther M, Hylek EM, Palareti G. Oral anticoagulant therapy. Chest. 2012;141(2):e44S-e88S.

Becker RC. Factor Xa inhibitors: critical considerations for clinical development and testing. J Thromb Thrombolysis. 2021;52(2):397–402.

Ding S, Wang L, Xie L, Zhou S, Chen J, Zhao Y, et al. Bioequivalence study of 2 formulations of rivaroxaban, a narrow-therapeutic-index drug, in healthy chinese subjects under fasting and fed conditions. Clin Pharmacol Drug Dev. 2019;9(3):346–52.

Food and Drug Administration (FDA). Draft Guidance on Rivaroxaban. [cited 2022 fev 21]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/psg/ Rivaroxaban_oral%

20tablet_22406_RC09-15.pdf.

Kou W, Sodhi JK, Wu X, Benet LZ. Investigating intestinal transporter involvement in rivaroxaban disposition through examination of changes in absorption. Pharm Res. 2021;38(5):795–801.

Kreutz R. Pharmacokinetics and pharmacodynamics of rivaroxaban – an oral, direct factor Xa inhibitor. Curr Clin Pharmacol. 2014;9(1):75–83.

Kubitza D, Becka M, Voith B, Zuehlsdorf M, Wensing G. Safety, pharmacodynamics, and pharmacokinetics of single doses of BAY 59-7939, an oral, direct factor Xa inhibitor. Clin Pharmacol Ther. 2005;78(4):412-21.

Kubitza D, Becka M, Zuehlsdorf M, Mueck W. Effect of food, an antacid, and the H2 antagonist ranitidine on the absorption of BAY 59-7939 (rivaroxaban), an oral, direct factor Xa inhibitor, in healthy subjects. J Clin Pharmacol. 2006;46(5):549-58.

Kubitza D, Becka M, Roth A, Mueck W. Dose-escalation study of the pharmacokinetics and pharmacodynamics of rivaroxaban in healthy elderly subjects. Curr Med Res Opin. 2008;24(10):2757-65.

Kubitza D, Berkowitz SD, Misselwitz F. Evidence-based development and rationale for once-daily rivaroxaban dosing regimens across multiple indications. Clin Appl Thromb Hemost. 2016;22(5):412–22.

Kushwah V, Arora S, Tamás Katona M, Modhave D, Fröhlich E, Paudel A. On absorption modeling and food effect prediction of rivaroxaban, a BCS II drug orally administered as an immediate-release tablet. Pharmaceutics. 2021;13(2):283.

Kvasnicka T, Malikova I, Zenahlikova Z, Kettnerova K, Brzezkova R, Zima T, et al. Rivaroxaban - metabolism, pharmacologic properties and drug interactions. Curr Drug Metabolism. 2017, 18:636-42.

Mueck W, Stampfuss J, Kubitza D, Becka M. Clinical pharmacokinetic and pharmacodynamic profile of rivaroxaban. Clin Pharmacokinet. 2014;53(1):1–16.

Steinberg BA, Becker RC. Structure–function relationships of factor Xa inhibitors: implications for the practicing clinician. J Thromb Thrombolysis. 2014;37(2):234–41.

Weinz C, Schwarz T, Kubitza D, Mueck W, Lang D. Metabolism and excretion of rivaroxaban, an oral, direct factor Xa inhibitor, in rats, dogs, and humans. Drug Metab Dispos. 2009;37(5):1056–64.

Xarelto®. [leaflet]. Leverkusen, Alemanha: Bayer AG; 2022. [cited 2022 fev 21]. Available from: https://consultas.anvisa.gov.br/#/bulario/q/?numeroRegistro=170560048.

Zhang L, Peters G, Haskell L, Patel P, Nandy P, Moore KT. A cross-study analysis evaluating the effects of food on the pharmacokinetics of rivaroxaban in clinical studies. J Clin Pharmacol. 2017;57(12):1607-1615.

Zhao X, Sun P, Zhou Y, Liu Y, Zhang H, Mueck W, et al. Safety, pharmacokinetics and pharmacodynamics of single/multiple doses of the oral, direct Factor Xa inhibitor rivaroxaban in healthy chinese subjects. Br J Clin Pharmacol. 2009;68(1):77-88.