v. 2, n. 2, 2022

International Journal of Health Science

RELATIVE BIOAVAILABILITY OF TWO FORMULATIONS CONTAINING 40MG OF PANTOPRAZOLE UNDER FASTING AND POSTPRANDIAL CONDITIONS

Alessandra Ferreira dos Santos
https://orcid.org/0000-0002-5620-8535

Quevellin Alves dos Santos Francisco
https://orcid.org/0000-0002-6368-8231

Carlos Eduardo Melo Correa
https://orcid.org/0000-0001-7954-6871

Edvaldo Capobiango Coelho
https://orcid.org/0000-0002-3218-7952

Abstract: The aim of this study was to evaluate the bioequivalence between two formulations of delayed-release tablets containing 40 mg of pantoprazole. The products were administered to healthy volunteers as a single dose under fasting (2x2 inbred; n=48) and postprandial (2x4 fully replicated inbred; n=80) conditions with *washout* of 7 days. Plasma pantoprazole concentrations were determined by ultra-performance liquid chromatography coupled with tandem mass spectrometry. After logarithmic transformation of the experimentally found plasma concentrations, the pharmacokinetic parameters used in the bioequivalence calculation were obtained: Cmax e ASC0-t. The 90% confidence interval for the ratios of the geometric means between the test drug and the reference drug, for the parameters Cmax e ASC0-t, are comprised in the IC 90% between 80%-125%. It is concluded that the formulations are bioequivalent, in terms of speed and extent of absorption of the active in the body, in both conditions studied, therefore interchangeable..
Keywords: Bioequivalence, postprandial and fasting, delayed release, CLUE-EM/EM

INTRODUCTION

Pantoprazole is a benzimidazole sulfoxide derivative that inhibits the proton pump of parietal cells in the gastric mucosa, leading to reduced stomach acidity and increased gastrin. The development of a proton pump inhibitor drug should provide chemical stability at neutral pH to increase its selectivity, activation in strongly acidic media and certain stability in weakly acidic conditions to avoid activation in other cellular compartments. Opantoprazole is highly ionized at low pH and has the property of accumulating in the lumen of parietal cells stimulated by being a weak base. In this acidic microenvironment, this drug is rapidly converted into a reactive intermediate that forms covalent bonds with luminal surface cysteine residues, causing an irreversible inhibition of enzyme function. The proton pump represents the final step in the secretory process, and therefore inhibition of this enzyme suppresses gastric acid secretion regardless of the primary stimulus. (CHEER, et al., 2003).

It is indicated for the treatment of erosive esophagitis associated with gastroesophageal reflux disease, peptic ulcers, and Zollinger-Ellison syndrome. Pantoprazole is also indicated in the treatment of ulcers resistant to histamine H2 receptor antagonists, in gastroduodenal lesions induced by non-steroidal anti-inflammatory drugs and in therapy for the eradication of Helicobacterpylori, in combination with antimicrobial agents. (CHALLA, et al., 2010; CHEER, et al., 2003).

Pantoprazole is rapidly and completely absorbed after dissolution of the enteric-coated tablet, and maximum plasma concentration (Cmáx) is reached approximately 2.8 h after administration of 40 mg, with an average value of 2,5µg/mL (CHALLA, et al., 2010). Its oral bioavailability is 77%, it is 98% bound to plasma proteins, and it has a low volume of distribution (mean value of 0.16 L/kg at steady state), which suggests limited tissue distribution. It is almost exclusively metabolized in the liver, via oxidation, by cytochrome CYP2C19, followed by sulfate conjugation. Renal excretion represents the main route of elimination and the remainder is excreted with the feces. The plasma half-life is approximately 2 hours, although short, once the inhibition of acid secretion is established, the effect is long-lasting, persisting even after the drug has been cleared from the bloodstream. (CHEER, et al., 2003). Concomitant food intake had no influence on the area under the curve. (ASC) and the Cmax, only about the time to reach the maximum concentration (Tmax) which

is increased under these conditions, that is, delayed (MENDES, et al., 2008).

The generic drug policy was one of the guidelines of the Ministry of Health, and intended to provide consumers with interchangeable products at different prices; equally safe and effective. In order for a drug to be registered as a generic or to obtain its registration as a similar drug, it is necessary to carry out a series of regulated tests, including bioequivalence studies, which can only be carried out by research centers duly authorized by the ANVISA. The pharmacokinetic assay conducted with the aim of determining the behavior of different formulations in vivo is defined as relative bioavailability. Relative bioavailability is defined as the quotient of the amount and rate of active ingredient that reaches the systemic circulation from the extravascular administration of a preparation and the amount and rate of active ingredient that reaches the systemic circulation from the extravascular administration of a drug product. reference containing the same active ingredient. However, the term bioequivalence is used exclusively for the designation of relative bioavailability studies for the registration of generic drugs. The concepts of quantity and speed of absorption presented in the above definition are related, respectively, to the pharmacokinetic parameters ASC e Cmax.

The purpose of this bioequivalence study was to compare the pharmacokinetic profiles of two delayed-release 40 mg pantoprazole tablet formulations under fasting and postprandial conditions in healthy male and female volunteers. Pantoprazole plasma levels were measured by ultra performance liquid chromatography coupled to mass spectrometry in *tandem* (CLUE-EM/EM).

CASUISTRY AND METHODS

CLINICAL PROTOCOL

Research projects were approved by the Research Ethics Committee (CEP) from the Universidade Vale do Sapucaí and the studies conducted in accordance with the Declaration of Helsinki and its revisions, the Good Clinical Practices, Resolutions of the CNS nº 466/12 and nº 251/97, in addition to the regulatory contribution established by the ANVISA for conducting bioequivalence studies. All steps were carried out at the Instituto Cláudia Marques of Research and Development. After explaining the nature and purpose of the research, all research participants signed an informed consent form. The volunteers included in the study were considered healthy after medical consultation and laboratory tests, described below: complete blood count, creatinine, total cholesterol, triglycerides, uric acid, total bilirubin and fractions, total protein and fractions, fasting glucose, alkaline phosphatase, TGO, TGP, urine I, serology for hepatitis B, hepatitis C and HIV. In the case of female volunteers, a serological test for pregnancy was performed (β-HCG). All volunteers underwent a previous electrocardiogram. Men and women over 18 years of age and body mass index between 18 and 30 participated in the study kg/m^2; Volunteers with chronic diseases, who used medication, pregnant women and smokers were excluded, and all those considered eligible to participate in the study met the inclusion and exclusion criteria. This is an applied, explanatory, quantitative and experimental research that presents two distinct designs.

The design of the study under fasting conditions was an open-label, 2x2, randomized crossover, with two treatments, two sequences and a sample of 48 volunteers; The study in postprandial (fed) conditions was an open-label, fully replicated 2x4 crossover, two-sequence and four-period study and a sample of 80 volunteers.

CLINICAL STAGE

The clinical stage of the studies was carried out in the ICMP&D, the volunteers were hospitalized the night before the start of the study. For the study under fasting conditions, a fasting period of at least 8 hours was determined, the proposed medications were administered to the research participants, who remained for another 4 hours in fasting, when they then received a first standard meal - lunch, followed by a afternoon and dinner. In the study under postprandial conditions, the same fasting of at least 8 hours was followed, however, 30 minutes before the administration of the proposed drugs, the research participants had a standardized hypercaloric and hyperlipidic breakfast. The research participants received, in each of the hospitalization periods, 01 tablet of each of the test or reference formulations containing 40 mg of pantoprazolem gastro-resistant tablet, orally in a single dose, administered with a glass of drinking water (200 ml) following the previously established randomization. Blood samples were collected before the administration of pantoprazole preparations, through a heparinized catheter introduced into a superficial vein of the volunteer's forearm, followed by other collections. (4,5 mL) at the following intervals from administration: 0,5; 1,0; 1,33; 1,66; 2,0; 2,25; 2,5; 2,75; 3,0; 3,33; 3,66; 4,0; 4,5; 5,0; 5,5; 6,0; 8,0; 10,0; 12,0 hours for fasting study. The postprandial study followed another collection schedule: baseline, 1,0; 2,0; 2,5; 3,0; 3,5; 4,0; 4,5; 5,0; 5,5; 6,0; 7,0; 8,0; 9,0; 10,0; 11,0; 12,0; 13,0; 14,0; 15,0; 16,0; 18,0; 20,0; 22,0; 24,0; 26,0; 28,0; 30,0; 32,0 e 36,0 hours. The heparin tubes containing the blood samples were centrifuged to obtain plasma at 3.500 rpm for 10 min to 4°C and stored at -20°C. Immediately after blood collection, samples were centrifuged at 3.500 rpm for 10 minutes to 4°C. Then the plasma was separated and transferred to cryogenic tubes previously identified and later stored in a freezer -20°C, on the unit itself. The amount of plasma obtained from centrifugation is divided into two cryogenic tubes, one of which is stored in the ICMP&D and maintained as a backup. Participants remained in the clinical unit for 24 hours after medication administration in the fasting study and 48 hours in the postprandial study and had access to medical care and specialist care during all study periods.

SECURITY ASSESSMENT

For safety monitoring purposes, volunteers were supervised throughout the study in order to detect adverse events. Volunteers were asked about the occurrence of adverse events at the time of admission, before administration of investigational products and during the conduct of the study in each period. Additionally, all were instructed to immediately report any occurrence of adverse events to the clinical team. During hospitalizations, blood pressure, pulse and body temperature were monitored and recorded before (-1) and 2; 4 and 12 hours after drug administration. At the end of the study, the results of laboratory tests (except serology) and ECG, were repeated and evaluated during the medical consultation in a comparative way to those obtained in the volunteer selection phase.

ANALYTICAL STEP

Plasma concentrations of pantoprazole were measured by a validated bioanalytical method, through ultra-performance liquid chromatography coupled to mass spectrometry in *tandem* (CLUE-EM/EM) using omeprazole as an internal standard. The analytical step developed a method for the quantification of unchanged drug in plasma and followed the validation according to Resolution RDC n° 27/12; The quantification of the samples

International Journal of Health Science ISSN 2764-0159 DOI 10.22533/at.ed.159222217018

was controlled through calibration curves covering the desired concentration range and through quality controls.

SAMPLE QUANTIFICATION

Quantification was performed blindly, with regard to randomization, thus avoiding bias in the bioequivalence analysis. For the determination of pantoprazole in plasma samples, a calibration curve was performed, whose interval between the minimum concentration and the maximum concentration was prepared according to the characteristics of the drug and the dose administered in the study, in order to encompass all concentrations at to be obtained in the quantifications. To guarantee and monitor the precision and sensitivity of the quantification method during the tests, samples of known concentration, called quality control samples, were injected. Four quality controls were used (CQ), and each one of them was injected during the quantification of the samples in the order of the lowest concentration to the highest concentration, interspersed every 10 analyzed samples. The concentrations of CQ used in the study were: 30 ng/mL (3 times the value of LQ), 3000 ng/mL (intermediate value between low CQ and high CQ) and 4500 ng/mL (75 % of the highest concentration of the calibration curve) and CQD (dilution control) 2750 ng/mL. Calibration curve standards and quality controls were prepared in the same biological matrix (plasma). The limit of quantification (LIQ) used in the study was 10 ng/mL.

The calibration curve consisted of a blank (matrix processed without the internal standard), sample zero (matrix processed with the internal standard) and the standard control samples. The control standard samples were prepared by adding to human plasmas the working standard solutions with the analyte to be quantified at the following concentrations: 10 / 20 / 100 / 500 / 1000 / 2000 / 5500 / 6000 ng / mL.

If a sample has an estimated concentration lower than the LIQ, its value should not be extrapolated, but considered to be zero, even if the drug has been detected, that is, it has an area 3 times greater than that detected in a white plasma. . Estimation of concentrations above the upper detection limit by extrapolation is not recommended. Instead, samples should be diluted and reanalyzed after performing the dilution integrity test (CQD).

The analytical run consisted of the calibration curve, quality controls and quantification of the analyte in the collected samples. The maximum coefficient of variation allowed for the QCs was 15%, and there could be 2 rejections per analytical run, as long as they were not of the same concentration. For LIQ, the maximum coefficient of variation allowed was 20%. For the calibration curve, at least 5 of the 7 concentrations should have a coefficient of variation of at most 15% (provided it was not the lowest and/or highest concentration). For the lowest concentration of the calibration curve, the coefficient of variation could not exceed 20%. The coefficient of linearity of the curve was at least 0,99.

SAMPLE EXTRACTION

The extraction process was applied to volunteer samples, calibration standard curve samples and quality control samples. In eppendorf tubes of 2 mL properly numbered according to the samples to be quantified, were added 100 µL of plasma samples and 25µL of omeprazole solution (5,0 µg/mL) prepared in methanol (degree HPLC). The tubes were shaken for one minute. Then were added 500µL of hexane and 500µL of ethyl acetate, and the tubes were shaken for three minutes. After this period, the samples were centrifuged for 15 minutes, at 4ºC and 14000 rpm. an aliquot of 300µL of the organic

phase was transferred to another tube and evaporated with compressed air. The sample was then resuspended with 1000µL of the mobile phase and stirred for one minute. The resulting solution was transferred to *inserts*de disposable and injected glass, in a volume of 2,0 µl, in the chromatographic system.

DETERMINATION OF PANTOPRAZOLE IN PLASMA

Chromatographic analyzes were performed using a chromatographic column Waters Acquity UPLC BEH C8 1,7 µm – (2,1 x 50,0) mm and a pre-column VanGuard UPLC BEH C8 1,7 µm – (2,1 x 5,0) mm, packaged at a temperature of 40°C ± 5°C. Used a liquid chromatograph Acquity UPLC (Ultra Performance Liquid Chromatography – Waters) coupled to a mass spectrometer, Waters TQ Detector with ionization by *positive electrospray* (ES+) and triple quadrupole analyzer. The mobile phase consisted of acetonitrile (grau HPLC) : ammonium acetate solution 10mM + 0,025% of formic acid (95:5, v/v) in separate channels) with programmed flow of 0,35 mL/min and the running time was 1.00 minutes for the detection of the analyte (pantoprazole) and the PI (omeprazole) in EM/EM mode the transitions were monitored (m/z) in 384,11> 200,05 and 346,15>198,17, respectively. The acquisition of data in the system CLUE-EM/EM was carried out through the program MicromassMassLynxTM 4.1 (Waters Corporation) and the bioanalytical method was determined by modifying the one described by Challa et al., 2010.

PHARMACOKINETIC AND STATISTICAL ANALYSIS

All pharmacokinetic and statistical analyzes were processed using the software Phoenix WinNonlin version 5.3 (Pharsigh Corporation, NC, USA), using the Bioequivalence module.

The following pharmacokinetic parameters were determined after quantification of plasma concentrations performed in the analytical stage of the study and application of the non-compartmental kinetic model:

Cmáx = Maximum concentration detected in plasma after each treatment (Test and Reference);

Tmáx = Time corresponding to maximum plasma concentration;

ASC0-t = Area under the concentration versus time curve from zero to the last experimentally determined concentration;

ASC0-inf = Area under the concentration versus time curve from time zero to infinity;

T1/2 = Drug elimination half-life;

Ke = terminal phase elimination constant.

The pharmacokinetic parameters used in the bioequivalence analysis were Cmáx and ASC. Cmax and Tmax were obtained directly from the plasma concentration versus time curve. The ASC0-t was calculated using the trapezoid method and the ASC0-inf was calculated by the equation: ASC0-t + Cn/Ke (where Cn is the last quantifiable concentration of the drug).

Multivariate analysis was performed to evaluate the effects of product, sequence and period using analysis of variance ANOVA for the parameters Cmáx, ASC 0-t and ASC 0-inf on a logarithmic scale. The traditional criterion of mean bioequivalence was used, that is, a confidence interval was constructed (IC) of 90% for the difference of the means of the transformed data of the test and reference drugs for the parameters Cmáx and ASC0-t. The antilogarithm of the IC obtained constituted the 90% IC for the ratio of the geometric means of the parameters: (Cmax test / Cmax reference and ASC0-t test / ASC0-t reference). Formulations were considered statistically bioequivalent if these intervals were between 80% and 125%.

RESULTS

PARTICIPANTS AND TOLERABILITY

48 research participants were selected for the fasting study and 80 participants for the postprandial study, thus the studies were completed with 47 and 75 volunteers, respectively; which were part of the statistical analyses. The most common adverse event reported by 20% of research participants in both studies was headache. One fasted study participant reported epigastric pain and three postprandial study participants reported nausea. In general, the formulations were well tolerated at the administered dose, no serious adverse events were reported or observed during the study. No clinical and laboratory parameters showed clinically relevant changes after physical examinations, vital signs, electrocardiogram and laboratory tests during the post-study consultation..

PHARMACOKINETIC PARAMETERS AND BIOEQUIVALENCE

The mean curves of pantoprazole plasma concentration profiles versus time are shown in Figure 1. The pharmacokinetic profiles were different between the administration conditions (fasting and postprandial) but similar for the test and reference formulations within the same study. Table 1 shows the pharmacokinetic parameters: Cmáx, ASC 0-t, ASC 0-inf, Tmáx and T1/2 obtained after administration of 40 mg of pantoprazole under fasting and postprandial conditions and the 90% confidence intervals for the pharmacokinetic parameters relevant to the determination of bioequivalence between the formulations, obtained through the application of the non-compartmental model, for the parameters ASC0-t, ASC0-inf and Cmax.

DISCUSSIONS

The generic drug policy in Brazil has among its strategies the promotion of the purchase of cheaper drugs and the contribution to access to quality, safe and effective drugs. And these can only be recorded after performing bioequivalence studies.

The bioanalytical method developed and validated was suitable for the quantification of the drugpantoprazole in human plasma through the technique of coupled liquid chromatography in mass spectrometry in *tandem*. The validation of the method followed the criteria established in resolution of the ANVISA, being the same sensitive, linear, accurate, precise and robust. No interference from endogenous plasma components or other sources was found. The bioanalytical method used was highly specific due to the inherent selectivity of mass spectrometry and has significant advantages over other techniques previously described to quantify pantoprazole in biological matrices. The method described was simple, robust, precise, accurate, sensitive and fast; each sample required 1 (one) minute of analysis time; thus proving its feasibility for routine pharmacokinetic analysis.

Food had no relevant effect on pantoprazole absorption and Cmax values observed after oral administration to volunteers were similar to those reported in the literature. On the other hand, the Tmax values were very different between the two administration conditions, according to previous findings. (DE CAMPOS, et al., 2007; MENDES, et al., 2008). In this study, the Cmax and ASC values of pantoprazole under postprandial conditions were slightly reduced when compared to the study under fasting conditions (Table 1). The intra-individual variability was between 27-32% in postprandial conditions and 20% in fasting conditions. The collection schedule was considered adequate to obtain ASC and Cmax, and long enough to fully characterize

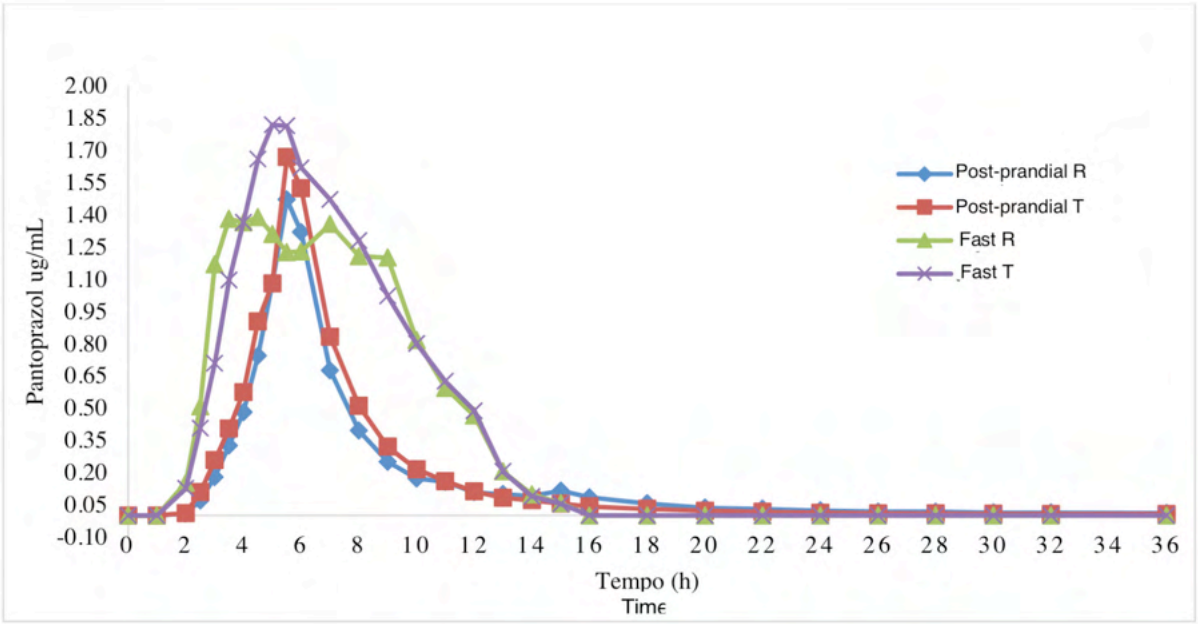

Figure 1.

Pharmacokinetic Parameters	Fast		Post-prandial		Fast	Post-prandial
	Reference	Test	Reference	Test	IC 90%	
Cmax (µg/mL)	3,31 ± 1,29	3,27 ± 1,07	2,76 ± 0,08	2,83 ± 0,08	94,0-110,5	94,6-107,4
ASC0-t (µg.h/mL)	6,30 ± 3,83	6,50 ± 3,76	6,00 ± 0,42	5,96 ± 0,42	99,1-111,6	94,6-104,1
ASC0-inf (µg.h/mL)	6,58 ± 4,33	6,78 ± 4,30	6,12 ± 0,46	6,14 ± 0,50	99,2-111,5	94,9-104,3
Tmax (h)	2,74 ± 1,11	2,71 ± 1,01	5,16 ± 0,17	5,39 ± 0,19		
$T_{1/2}$ (h)	1,37 ± 0,74	1,39 ± 0,79	1,63 ± 0,17	1,46 ± 0,10		

Table1. Geometric means of pharmacokinetic parameters and their respective 90% IC.

the elimination phase of pantoprazole. T1/2 is in agreement with the values reported in the literature and proves the adequate choice of the period of *washout*de 7 days, avoiding effects of *carryover.*

Two pharmaceutical forms are considered bioequivalent when there are no significant differences in terms of their bioavailability, which refers to the speed and extent of absorption of the active ingredient. The confidence interval was constructed (IC) of 90% for the ratio of the geometric means of the parameters Cmáx, ASC0-t e ASC0-inf. Both studies demonstrated that the values obtained for the IC of 90% for the parameters Cmáx and ASC0-t are between 80% and 125%, therefore the two pantoprazole formulations are bioequivalent according to the technical legislation of the ANVISA (Brasil, 2003; Brasil, 2006).

CONCLUSIONS

The bioanalytical method described is simple, fast, sensitive, selective, reproducible. Each sample requires approximately 1.0 minutes of analysis for the entire run. The sensitivity of the analysis is sufficient to accurately track the pharmacokinetics of pantoprazole in plasma. This analytical procedure can be easily applied to the routine analysis of plasma samples for pantoprazole bioavailability, bioequivalence and pharmacokinetic studies. The test and reference formulations were considered statistically bioequivalent in healthy Brazilian volunteers of both sexes under fasting and postprandial conditions according to the criteria of the ANVISA. The drug pantoprazole was evaluated in both formulations and in both administration conditions; according to the results obtained they are therapeutic equivalents and, therefore, interchangeable. The availability of generic drugs allows patients to have broad access to treatment and lower cost of therapy. The 90% confidence intervals were within the limits of 80-125%, concluding that the formulations are bioequivalent and, therefore, interchangeable..

THANKS

This work is scientifically supported by the Instituto Claudia Marques of research (ICMP&D).

REFERENCES

Brasil. Ministério da Saúde. Conselho Nacional de Saúde. Resolução CNS nº 251, de 07 de agostode 1997.

Brasil. Ministério da Saúde. Agência Nacional de Vigilância Sanitária. Resolução (RE) nº 898, de29 de maio de 2003.

Brasil. Ministério da Saúde. Agência Nacional de Vigilância Sanitária. Resolução (RE) nº 1170, de19 de abril de 2006.

Brasil. Ministério da Saúde. Agência Nacional de Vigilância Sanitária. Resolução de diretoriaColegiada (RDC) n° 27, de 17 de maio de 2012a.

Brasil. Ministério da Saúde. Conselho Nacional de Saúde. Resolução CNS nº 466, de 12 dedezembro de 2012b.

CHALLA BR, BODDU SH, AWEN BZ, et al. Development and validation of a sensitive bioanalytical method for the quantitative estimation of pantoprazole in human plasma samples by LC-MS/MS: application to bioequivalence study. **J Chromatogr B Analyt Technol Biomed Life Sci**. v.878, n.19, p.1499-1505, 2010. doi:10.1016/j.jchromb.2010.03.049

CHEER, S. M.; PRAKASH, A.; FAULDS, D.; LAMB, H. M. Pantoprazole. An update of its pharmacologicalpropertiesandtherapeutic use in the management of acidrelateddisorders. Drugs, Auckland, v. 63, n. 1, p. 101-132, 2003.

DE CAMPOS DR, VIEIRA NR, BERNASCONI G, et al. Bioequivalence of two enteric coated formulations of pantoprazole in healthy volunteers under fasting and fed conditions. **Arzneimittelforschung**. 2007;57(6):309-314. doi:10.1055/s-0031-1296624

InternationalConference on Harmonisation (ICH). *Guideline for goodclinicalpractice E6(R1)*,1996. 53p. Disponível em:<https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E6/E6_R1_Guideline.pdf >. Acesso em: dez.2021

MENDES FD, PATNI AK, REYER S, et al. Comparative bioavailability study with two pantoprazole delayed-released tablet formulations administered with and without food in healthy subjects. **Arzneimittelforschung**. 2008;58(3):141-148. doi:10.1055/s-0031-1296484