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The use of Healthy Volunteers to Evaluate Bioequivalence of Anti-neoplastic Drugs: Pilot Studies with Capecitabine

Gustavo Duarte Mendes^{1,2,3}, Tainah Babadopulos^{1,2}, Lu Shi Chen², Jaime O. Ilha², José Cássio de Almeida Magalhães^{1,3}, Khalid Alkharfy⁴ and Gilberto De Nucci^{4,5*}

¹Department of Pharmacology, State University of Campinas, Campinas, Brazil

²Galeno Research Unit, Campinas, Brazil

³Faculty of Odontology, University Camilo Castelo Branco (UNICASTELO), São Paulo, Brazil

⁴College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

⁵Department of Pharmacology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil

Summary

Capecitabine is a prodrug, is selectively activated by tumor cells to its cytotoxic moiety, 5-fluorouracil, by thymidine phosphorylase, which is generally expressed at high levels in tumors. Clinical and pharmacokinetics studies for capecitabine are performed in patients with cancer. The objective of the study was to evaluate the safety of a bioequivalence study (150 mg tablet) using healthy male volunteers under fasting and non-fasting conditions. The study was conducted with an open, randomized, two-period crossover design in a 2-week washout interval without food. After the study without food was completed, a new protocol was submitted to the Ethics Committee to evaluate the study with food. The volunteers were selected for the study after having their health status previously assessed by a clinical evaluation and laboratory tests (biochemical and hematological parameters, and urinalysis). A single capecitabine tablet (150mg) was given in each interment. An extra laboratory analysis was performed one week after the first administration of the drug for the safety of the subjects. Plasma capecitabine concentrations were analyzed by liquid chromatography coupled to tandem mass spectrometry (HPLC/MS/MS) with positive ion electrospray ionization using multiple reactions monitoring (MRM). The pharmacokinetic parameters were 529.38 (± 265.22) and 462.88 (± 425.85) ng.mL⁻¹ for C_{max}, 262.31 (± 75.34) and 300.49 (± 91.51) ng.hr.mL⁻¹ for AUC_{last}, and 0.66 (range 0.5 – 1.25) hr and 1.0 (range 0.33 – 1.33) hr for T_{max}, without and with food, respectively, for the reference formulation. The intra-subject CV were 42.6% and 76.3% for C_{max} and 9.64% and 20.3% for AUC_{last} without and with food, respectively. The drug was well tolerated by the volunteers, and they presented no adverse reactions. The biochemical and hematological parameters presented no clinically relevant alterations. Our results indicate that it is safe to perform capecitabine bioequivalence studies in healthy male volunteers.

Keywords: Tandem mass spectrometry; HPLC/MS/MS; Bioavailability; Pharmacokinetics; Food

Introduction

Capecitabine is an adjuvant treatment for colon cancer and for the treatment of metastatic breast cancer in particular patients whose pathology did not improve during treatment with other therapeutic agents [6-11]. Capecitabine is a prodrug is selectively activated by tumor cells to its cytotoxic moiety, 5-fluorouracil, by thymidine phosphorylase, which is generally expressed at high levels in tumors [6-11]. Clinical and pharmacokinetics studies for capecitabine are performed in both healthy volunteers and patients with cancer [1-8]. Maximum plasma concentration (C_{max}) and the area under the plasma concentration-time curve (AUC) values are decreased after the ingestion of food [4,5]. The capecitabine is administrated orally, morning and evening, for 14 consecutive days followed by 1 week of no drug (3-week cycles) [4-6]. The incidence of adverse reactions observed in breast and colorectal cancer patients after 2500 mg/m² per day (the average therapy was 127 days) was as follows: 1-10%: taste disturbance, chest pain, alopecia, gastrointestinal hemorrhage, thrombocytopenia, cough, venous thrombosis, dehydration, skin/nail discoloration, insomnia, back pain, oral discomfort, gastrointestinal motility disorder, dizziness, arthralgia/myalgia, headache; 11-20%: dyspnea, neuropathy/paresthesia, eye irritation, edema, constipation, neutropenia, pyrexia; 21-30%: lymphopenia, stomatitis, anorexia, dermatitis, vomiting; 31%-40%: abdominal pain; 41-50%: fatigue/weakness, hyperbilirubinemia, nausea; > 50%: hand-foot syndrome, diarrhea, anemia [5,6].

The aim of this study was to evaluate the safety of the use of healthy volunteers in bioequivalence studies for capecitabine. Here we report the results obtained with two capecitabine pilot studies (150 mg tablet) in healthy male volunteers. Capecitabine plasma levels were measured by high performance liquid chromatography coupled to tandem mass spectrometry (HPLC/MS/MS).

Materials and Methods

Demographic data

Group I (fasting) consisted of eight volunteers and group II (non-fasting) also eight health volunteers. The healthy male volunteers were aged between 18 and 55 years old and within the 15% of the ideal body weight. The ideal body weight was ≥ 19 and ≤ 28.75 for body mass index. It was based in Report of the Dietary Guidelines Advisory Committee on the Dietary Guidelines for Americans, 2000, U.S.

***Corresponding author:** Gilberto De Nucci, 415 Jesuino Marcondes Machado Ave., 13092-320, Campinas - SP, Brazil, Tel: +55-193251-6923; Fax: +55-19-3252-1516; E-mail: gugamendes@terra.com.br

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Department of Agriculture U.S. Department of Health and Human Services. Demographic data for group I were: age: 28.3 year (mean) \pm 10.8 (SD); 18 – 46 (range), height: 1.71 \pm 0.08; 1.58 - 1.83 cm, body weight: 68.4 \pm 6.6; 59.5 – 78.6 kg. Demographic data for group II were: age: 31.0 year (mean) \pm 11.3 (SD); 19 – 51 (range), height: 1.73 \pm 0.08; 1.61 - 1.88 cm, body weight: 73.2 \pm 9.6; 56.0 – 84.2 kg.

Laboratory tests

The volunteers did not present significant cardiac, hepatic, renal, pulmonary, neurological, gastrointestinal and hematological diseases, as assessed by the clinical examination, ECG and the following laboratory tests: blood glucose, urea, creatinine, AST, ALT, alkaline phosphatase, γ GT, total bilirubin and fractions, uric acid, total cholesterol, triglycerides, albumin and total protein, hemoglobin, hematocrit, total and differential white cell counts and urinalysis. All subjects were negative for HIV, HBV (except for serological scar) and HCV. An extra laboratory analysis was performed one week after the first dose of capecitabine. The study began with eight healthy volunteers in each group (fasting and non-fasting) and all completed the clinical study.

Clinical protocol

All subjects provided written informed consent to the Ethics Committee of the State University of Campinas, following evaluation by three oncologists, who initially approved the protocol for group I. After the submission of the final report from group I to the Ethics Committee of the State University of Campinas, the protocol for group II (non-fasting) was submitted and approved by the Ethics Committee. The studies were conducted in accordance with the provisions of the Declaration of Helsinki (1964), Tokyo (1975), Venice (1983), Hong Kong (1989), Somerset West (1996), Edinburgh (2000), Washington (2002), Tokio (2004) and Seoul (2008) revisions.

After screening and a washout period (of at least 2 weeks), the individuals who qualified were confined for 2 periods of approximately 38 hours. Each confinement was intervalled by 2 weeks. The study was conducted in an open, randomized, two-period crossover. During each period, the volunteers were hospitalized between 4:00 pm and 7:00 p.m., having already eaten a normal evening meal. Group I, after an overnight fast, received a single 150 mg dose of the appropriate capecitabine tablet formulation along with 200 mL of tap water at 7:00 a.m. No food was allowed for 2 hr following drug administration; afterwards, a standard breakfast was consumed. Group II, after overnight fasting, received at 7:00 a.m., 30 minutes after consuming a standard high-fat breakfast, a single 150 mg dose of appropriate capecitabine tablet formulation along with 200 ml of tap water. A lunch and an evening meal were provided at 5 and 10 hrs after dosing, respectively for both groups. No other food was permitted during the 'in-house' period. Liquid consumption was permitted ad libitum after lunch, but xanthine-containing drinks including tea, coffee or cola were not permitted.

Formulations

The following formulations were employed: Capecitabine test formulation 150 mg (formulation name: "Capecitabine"; lot number: 377/09; expiration date: August 2011; Eurofarma Laboratórios Ltda, Brazil) and reference formulation (Xeloda) 150 mg (lot number: U6004B04, expiration date: February-2010 (without food) and U6011B01, expiration date: March-2011).

The following formulations were employed: Capecitabine test formulation 150 mg (formulation name: Capecitabine"; lot number: 377/09; expiration date: August 2011; Eurofarma Laboratórios Ltda, Brazil) and reference formulation (Xeloda) 150 mg (lot number: U6004B04, expiration date: February-2010 (without food) and U6011B01, expiration date: March-2011 (with food)).

Drug analysis

For group I, blood samples (7 ml) from a suitable antecubital vein were collected into heparin-containing tubes before and 0:05, 0:10, 0:15, 0:30, 0:45, 1:00, 1:15, 1:30, 1:45, 2:00, 2:20, 2:40, 3:00, 3:30, 4:00, 4:30, 5:00, 5:30, 6:00, 8:00 and 10:00 hours post-dosing, after the administration of each dose of capecitabine. For group II, blood samples (7 ml) from a suitable antecubital vein were collected into heparin-containing tubes before and 0:10, 0:20, 0:30, 0:40, 0:50, 1:00, 1:10, 1:20, 1:30, 1:40, 1:50, 2:00, 2:10, 2:20, 2:30, 2:40, 2:50, 3:00, 3:15, 3:30, 3:45, 4:00, 4:20, 4:40, 5:00, 6:00 and 8:00 post-dosing hours, after the administration of each dose of capecitabine. The blood samples were centrifuged at 2500 g for 10 min at -4°C and the plasma decanted and stored at -20°C until assayed for their capecitabine contents. Capecitabine and the internal standard (IS) were extracted from plasma samples and quantified by liquid chromatography tandem mass spectrometry (HPLC/MS/MS) with positive electrospray ionization using multiple reactions monitoring (MRM).

Briefly, 0.2 mL of each plasma sample was introduced into glass tube followed by 0.05 mL of the internal standard solution (50 ng/mL d11-capecitabine). The tubes were briefly vortex-mixed and the compounds of interest were extracted with 1 mL of acetonitrile (100%). The mixture was vortex-mixed for approximately 30 s. After, 2 ml of ethyl acetate (100%) was added in the tubes. The mixture was vortex-mixed for approximately 50 s. The samples were then centrifuged at 2000 x g for 3 min. The upper phase (organic phase) was then transferred to another clean test and evaporated under N₂ at 40°C. The dry residues were reconstituted with 200 mL of acetonitrile/water (50/50; v/v) and vortex-mixed for 10s. The solutions were then transferred to the auto-injector microvials.

The high performance liquid chromatography (HPLC) was performed on C₁₈, 5 μ m (150 x 4.6 mm i.d.; Alltech Alltima HP) at a flow rate of 1.0 mL/min, however a post-column split (1:3) was introduced. Pressure of the system was approximately 70 - 75 bar. The mobile phase was acetonitrile/water (50/50; v/v). The column operated at room temperature while the temperature of the auto sampler was maintained at 8 \pm 2°C and the injection volume was 10 μ L.

Typical standard retention times were 2.27 \pm 0.3 min for capecitabine and 2.25 \pm 0.3 min for the IS, and the total run-time was 3.4 min. The mass spectrometer (API 3000) equipped with an electrospray source using a crossflow counter electrode was run in positive mode (ES+) with multiple reaction monitoring (MRM). The mass spectrometer was set as follows: 359.93>244.15 and 371.08>255.20, for capecitabine and d11-capecitabine, as the precursor ions *m/z* and the respective product ions. The source block temperature was set at 500°C and the turboionspray voltage to 5.5 kV. Nitrogen was used as collision gas. For capecitabine, the declustering potential, collision energy and collision exit potential were 56 (V), 15 (eV) and 18 (V), respectively. The corresponding values for I.S. were 41 (V), 17 (eV) and 16 (V), respectively. Data were acquired by Analyst software (1.4.1, Applied Biosystems, Foster City, CA, USA).

Linearity

Linearity was determined to assess the performance of the method. A linear regression with a weighting index of $1/x^2$ was applied to the peak area ratios of and internal standard vs. the concentrations of the plasma standards of capecitabine in duplicate to generate a calibration curve. Weight regression was employed since it enhances the accuracy in the lower part of the concentration curve.

Limit of detection and quantification

The limit of detection (LOD) and the lower limit of quantification (LOQ) were determined calculated as the concentrations with a signal-to-noise ratio of 3 and 10, respectively. Each back-calculated concentration standard should meet the following acceptable criteria: no more than 20% deviation.

Precision and accuracy

Within- and between-run precision were determined as the coefficient of variation, (CV) (%) = $100(\text{SD}/\text{M})$ and the accuracy (%) by $100(\text{M}/\text{T})$, where M is the mean, SD is the standard deviation of M, and T is the theoretical concentration.

Pharmacokinetics and statistical analysis

The first-order terminal elimination rate constant (k_e) was estimated by linear regression from the points describing the elimination phase in a log-linear plot, and the half-life ($t_{1/2}$) was derived from this rate constant ($t_{1/2} = \ln(2)/k_e$).

Bioequivalence between the three formulations was assessed by calculating individual test/reference ratios for the peak of concentration (C_{\max}), the area under the curve (AUC) of the plasma concentration until the last concentration observed (AUC_{last}), and the area under the curve between the first sample (pre-dosage) and infinity ($\text{AUC}_{0-\infty}$). The C_{\max} and the time taken to achieve this concentration (T_{\max}) were obtained directly from the curves. The areas under the capecitabine plasma concentration curve vs. time curve from time zero to the last detectable concentration (AUC_{last}) were calculated by applying the linear trapezoid rule. Extrapolation of these areas to infinity ($\text{AUC}_{0-\infty}$) was done by adding the value C_{last}/k_e to the calculated AUC_{last} (where C_{last} = the last detectable concentration). The AUC and C_{\max} data for the two formulations were analyzed by ANOVA to determine whether the 90% CI of the ratios were within the 80 - 125% interval indicative of bioequivalence as proposed by the US Food and Drug Administration.

| Parameter | Unit | Normal range | PRE-STUDY | | | | INTER-STUDY | | | | POS-STUDY | | | |
|-----------------------|---------------------|---------------|-----------|--------|--------|--------|-------------|-------|--------|--------|-----------|-------|--------|--------|
| | | | MEAN | SD | MIN | MAX | MEAN | SD | MIN | MAX | MEAN | SD | MIN | MAX |
| Hemoglobin | g% | 13.5 - 17.5 | 14.74 | 0.67 | 13.5 | 15.3 | 14.65 | 0.78 | 13 | 15.3 | 14.55 | 0.60 | 13.5 | 15.3 |
| Hematocrit | % | 39 - 50 | 43.38 | 1.97 | 39.5 | 45.2 | 42.90 | 2.06 | 39 | 45.1 | 42.71 | 1.22 | 40.6 | 44.0 |
| Red Blood Count | 106/mm ³ | 4.3 - 5.7 | 5.09 | 0.26 | 4.88 | 5.70 | 5.03 | 0.27 | 4.78 | 5.66 | 5.03 | 0.20 | 4.8 | 5.47 |
| MCV | Micra ³ | 81 - 95 | 85.26 | 4.54 | 78.2 | 89.6 | 85.34 | 3.67 | 79.7 | 89.0 | 85.03 | 3.90 | 78.5 | 88.2 |
| MHbC | pg | 26 - 34 | 28.98 | 1.63 | 26.5 | 31.0 | 29.19 | 1.84 | 26.0 | 30.8 | 29.00 | 1.73 | 26.4 | 30.5 |
| MCHC | % | 31 - 36 | 34.00 | 0.65 | 33.5 | 35.5 | 34.14 | 0.77 | 32.6 | 35.0 | 34.08 | 0.57 | 33.3 | 34.8 |
| RDW | % | 11.8 - 15.6 | 12.96 | 0.37 | 12.4 | 13.4 | 13.20 | 0.49 | 12.5 | 13.8 | 13.29 | 0.60 | 12.2 | 14.0 |
| Platelet Count | - | 150k - 400k | 216625 | 44998 | 153000 | 278000 | 215875 | 27570 | 180000 | 259000 | 224000 | 18555 | 196000 | 250000 |
| Leucocytes | 103/mm ³ | 3500 - 10500 | 6362.5 | 2081 | 3200 | 8700 | 6712.5 | 1700 | 4700 | 9600 | 6462.5 | 2794 | 4000 | 1290 |
| Band | % | ≤ 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.3 | 0.7 | 0 | 2 |
| Segmented | % | 48 - 76 | 54.4 | 12.7 | 32.9 | 67.0 | 53.9 | 9.2 | 40.6 | 66.2 | 56.6 | 11.4 | 43.0 | 81.0 |
| Eosinophils | % | 40664 | 4.3 | 2.6 | 1.0 | 8.0 | 4.5 | 3.3 | 0.9 | 10.2 | 4.3 | 3.4 | 0.0 | 10.6 |
| Basophis | % | 0 - 1 | 0.5 | 0.3 | 0.0 | 1.0 | 0.5 | 0.3 | 0.1 | 1.0] | 0.4 | 0.3 | 0.0 | 0.9] |
| Lymphocytes | % | 25 - 30 | 32.8 | 12.5 | 20.2 | 55.2 | 33.2 | 8.2 | 24.3 | 43.1 | 30.4 | 9.7 | 11.0 | 41.3 |
| Monocytes | % | 40787 | 8.1 | 1.1 | 6.2 | 9.9 | 7.9 | 1.1 | 6.2 | 9.9 | 8.0 | 1.4 | 6.0 | 9.6 |
| Fasting Blood Glucose | mg/dL | 60 - 100 | 91.75 | 7.29 | 82 | 103 | 90.38 | 5.24 | 85 | 100 | 89.88 | 6.33 | 79 | 100 |
| Urea | mg/dL | 18537 | 29.50 | 8.90 | 20 | 46 | 27.25 | 5.90 | 19 | 37 | 25.00 | 9.58 | 13 | 43 |
| Creatinine | mg/dL | 0.4 - 1.3 | 1.11 | 0.08 | 1.0 | 1.2 | 1.01 | 0.12 | 0.8 | 1.2 | 1.04 | 0.12 | 0.9 | 1.3 |
| Total proteins | g/dL | 40761 | 7.79 | 0.34 | 7.4 | 8.2 | 7.45 | 0.26 | 7.2 | 7.9 | 7.33 | 0.19 | 7.0 | 7.6 |
| Albumine | g/dL | 3.5 - 5.5 | 4.21 | 0.24 | 3.9 | 4.6 | 4.16 | 0.23 | 3.9 | 4.5 | 4.09 | 0.16 | 3.9 | 4.4 |
| Total Bilirrubins | mg/dL | 0 - 1.1 | 0.83 | 0.46 | 0.3 | 1.7 | 0.75 | 0.54 | 0.4 | 2.0 | 0.66 | 0.53 | 0.3 | 1.8 |
| Total Cholesterol | mg/dL | ≤ 239 | 145.00 | 26.57 | 111 | 192 | 144.38 | 35.98 | 102 | 215 | 143.25 | 38.01 | 96 | 226 |
| Triglycerids | mg/dL | ≤ 153 | 73.00 | 9.38) | 42 | 132 | 89.63 | 7.69 | 48 | 203 | 73.88 | 9.98 | 47 | 112 |
| SGOT | U/L | £ 43 | 23.50 | 7.45 | 15 | 32 | 23.25 | 9.04 | 12 | 34 | 20.75 | 6.67 | 13 | 34 |
| SGPT | U/L | £ 43 | 23.00 | 12.29 | 10 | 43 | 21.25 | 11.61 | 10 | 40 | 18.38 | 9.74 | 7 | 39 |
| Alkaline Phosphatase | U/L | 27 - 100 | 70.00 | 16.23 | 55 | 100 | 73.38 | 19.95 | 52 | 112 | 70.00 | 15.93 | 50 | 102 |
| γ-GT | U/L | 14855 | 29.13 | 17.22 | 11 | 54 | 28.00 | 14.28 | 13 | 48 | 28.13 | 13.85 | 13 | 46 |
| Uric Acid | mg/dL | 2.5 - 7.0 | 5.34 | 0.60 | 4.1 | 6.0 | 5.73 | 1.13 | 3.7 | 7.0 | 5.25 | 1.28 | 3.5 | 6.8 |
| Specific Gravity | - | 1.015 - 1.028 | 1.019 | 4.0 | 1.015 | 1.025 | - | - | - | - | 1.018 | 5.0 | 1.010 | 1.025 |
| pH | - | 5.5 - 6.5 | 6.00 | 0.38 | 5.5 | 6.5 | - | - | - | - | 6.00 | 0.60 | 5.0 | 7.0 |
| Leucocytes | /mL | £ 10000 | 4000 | 4309 | 1000 | 14000 | - | - | - | - | 4000 | 1773 | 3000 | 8000 |
| Eritocytes | /mL | £ 5000 | 5125 | 7472.6 | 1000 | 23000 | - | - | - | - | 2000 | 534.5 | 1000 | 3000 |

Table 1: Laboratory tests in healthy male volunteers in capecitabine (150 mg tablet) bioequivalence study in fasting conditions.

| Parameter | Unit | Normal range | PRE-STUDY | | | | POS-STUDY | | | |
|-----------------------|----------------------------------|---------------|-----------|---------|----------|----------|-----------|---------|----------|----------|
| | | | MEAN | SD | MIN | MAX | MEAN | SD | MIN | MAX |
| Hemoglobin | g% | 13.5 - 17.5 | 15.3 | 0.7 | 14.1 | 16.0 | 14.5 | 0.5 | 13.9 | 15.3 |
| Hematocrit | % | 39 - 50 | 46.0 | 1.4 | 44.5 | 48.9 | 43.1 | 1.5 | 40.8 | 44.7 |
| Red Blood Count | 10 ⁹ /mm ³ | 4.3 - 5.7 | 5.4 | 0.3 | 5.0 | 5.9 | 5.0 | 0.4 | 4.4 | 5.5 |
| MCV | Micra ³ | 81 - 95 | 86.0 | 5.1 | 76.2 | 92.9 | 86.1 | 5.0 | 77.8 | 93.3 |
| MHbC | pg | 26 - 34 | 28.7 | 2.3 | 24.9 | 31.1 | 28.9 | 1.9 | 25.7 | 31.7 |
| MCHC | % | 31 - 36 | 33.3 | 1.0 | 31.7 | 34.8 | 33.5 | 0.6 | 32.9 | 34.6 |
| RDW | % | 11.8 - 15.6 | 13.3 | 0.5 | 12.7 | 14.1 | 13.5 | 0.5 | 12.9 | 14.4 |
| Platelet Count | - | 150k - 400k | 236750.0 | 48585.0 | 207000.0 | 352000.0 | 236625.0 | 61003.4 | 114000.0 | 312000.0 |
| Leucocytes | 10 ⁹ /mm ³ | 3500 - 10500 | 6400.0 | 1406.1 | 4400.0 | 8900.0 | 7000.0 | 1479.4 | 4400.0 | 9200.0 |
| Band | % | ≤ 5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.4 | 0.0 | 1.0 |
| Segmented | % | 48 - 76 | 54.4 | 6.9 | 46.0 | 65.4 | 55.1 | 6.7 | 47.6 | 67.7 |
| Eosinophils | % | 1 - 5 | 5.4 | 5.4 | 1.7 | 18.0 | 5.1 | 5.6 | 1.3 | 17.6 |
| Basophis | % | 0 - 1 | 0.4 | 0.3 | 0.0 | 0.9 | 0.4 | 0.3 | 0.0 | 0.9 |
| Lymphocytes | % | 25 - 30 | 31.0 | 5.9 | 24.0 | 40.6 | 31.2 | 5.7 | 22.1 | 39.7 |
| Monocytes | % | 1 - 9 | 8.8 | 1.6 | 4.9 | 10.1 | 8.2 | 1.5 | 5.4 | 10.0 |
| Fasting Blood Glucose | mg/dL | 60 - 100 | 87.6 | 7.5 | 74.0 | 100.0 | 97.4 | 7.9 | 88.0 | 115.0 |
| Urea | mg/dL | 10 - 50 | 28.9 | 6.8 | 19.0 | 39.0 | 27.8 | 6.5 | 20.0 | 42.0 |
| Creatinine | mg/dL | 0.4 - 1.3 | 1.0 | 0.1 | 0.8 | 1.1 | 1.1 | 0.2 | 0.9 | 1.3 |
| Total proteins | g/dL | 6 - 8 | 7.6 | 0.3 | 7.2 | 8.0 | 7.6 | 0.4 | 7.1 | 8.3 |
| Albumine | g/dL | 3.5 - 5.5 | 4.0 | 0.1 | 3.8 | 4.2 | 4.0 | 0.2 | 3.6 | 4.1 |
| Total Bilirrubins | mg/dL | 0 - 1.1 | 0.6 | 0.2 | 0.4 | 1.1 | 0.5 | 0.1 | 0.4 | 0.7 |
| Total Cholesterol | mg/dL | ≤ 239 | 165.5 | 18.7 | 144.0 | 199.0 | 164.6 | 25.9 | 137.0 | 209.0 |
| Triglycerids | mg/dL | ≤ 150 | 103.1 | 56.0 | 45.0 | 201.0 | 113.5 | 39.1 | 73.0 | 184.0 |
| SGOT | U/L | ≤ 43 | 19.5 | 6.2 | 12.0 | 31.0 | 36.1 | 36.5 | 13.0 | 124.0 |
| SGPT | U/L | ≤ 43 | 26.0 | 15.8 | 13.0 | 57.0 | 40.6 | 30.3 | 12.0 | 87.0 |
| Alkaline Phosphatase | U/L | 27 - 100 | 62.9 | 15.9 | 40.0 | 90.0 | 71.4 | 19.5 | 45.0 | 100.0 |
| γ-GT | U/L | 9 - 40 | 27.6 | 12.2 | 15.0 | 47.0 | 41.4 | 28.9 | 15.0 | 89.0 |
| Uric Acid | mg/dL | 2.5 - 7.0 | 5.0 | 0.9 | 3.5 | 6.6 | 5.4 | 1.3 | 3.4 | 7.6 |
| Specific Gravity | - | 1.015 - 1.028 | 1.017 | 5.9 | 1005.0 | 1025.0 | 1020.0 | 6.5 | 1010.0 | 1025.0 |
| pH | - | 5.5 - 6.5 | 6.0 | 1.0 | 5.0 | 7.0 | 5.9 | 1.0 | 5.0 | 8.0 |
| Leucocytes | /mL | ≤ 10000 | 8125.0 | 14932.6 | 1000.0 | 45000.0 | 19875.0 | 30591.5 | 3000.0 | 92000.0 |
| Eritocytes | /mL | ≤ 5000 | 1500.0 | 534.5 | 1000.0 | 2000.0 | 2750.0 | 3058.9 | 1000.0 | 10000.0 |

Table 2: Laboratory tests in healthy male volunteers in capecitabine (150 mg tablet) bioequivalence study in non-fasting conditions.

| | Intra-batch Validation (n=7) | | | |
|-------------------------------|-------------------------------|---------|---------|---------|
| | 1 | 3 | 30 | 400 |
| Nominal concentration (ng/mL) | 1 | 3 | 30 | 400 |
| Mean (ng/mL) | 0.95 | 3.17 | 31.74 | 423.57 |
| SD (ng/mL) | 0.04 | 0.15 | 0.66 | 4.79 |
| Precision (%) | 4.49% | 4.77% | 2.08% | 1.13% |
| Accuracy (%) | 94.79% | 105.57% | 105.81% | 105.89% |
| | Inter-batch Validation (n=21) | | | |
| | 1 | 3 | 30 | 400 |
| Nominal concentration (ng/mL) | 1 | 3 | 30 | 400 |
| Mean (ng/mL) | 0.96 | 3.17 | 31.29 | 421.24 |
| SD (ng/mL) | 0.05 | 0.11 | 0.68 | 5.00 |
| Precision (%) | 5.03% | 3.41% | 2.16% | 1.19% |
| Accuracy (%) | 96.23% | 105.79% | 104.29% | 105.31% |

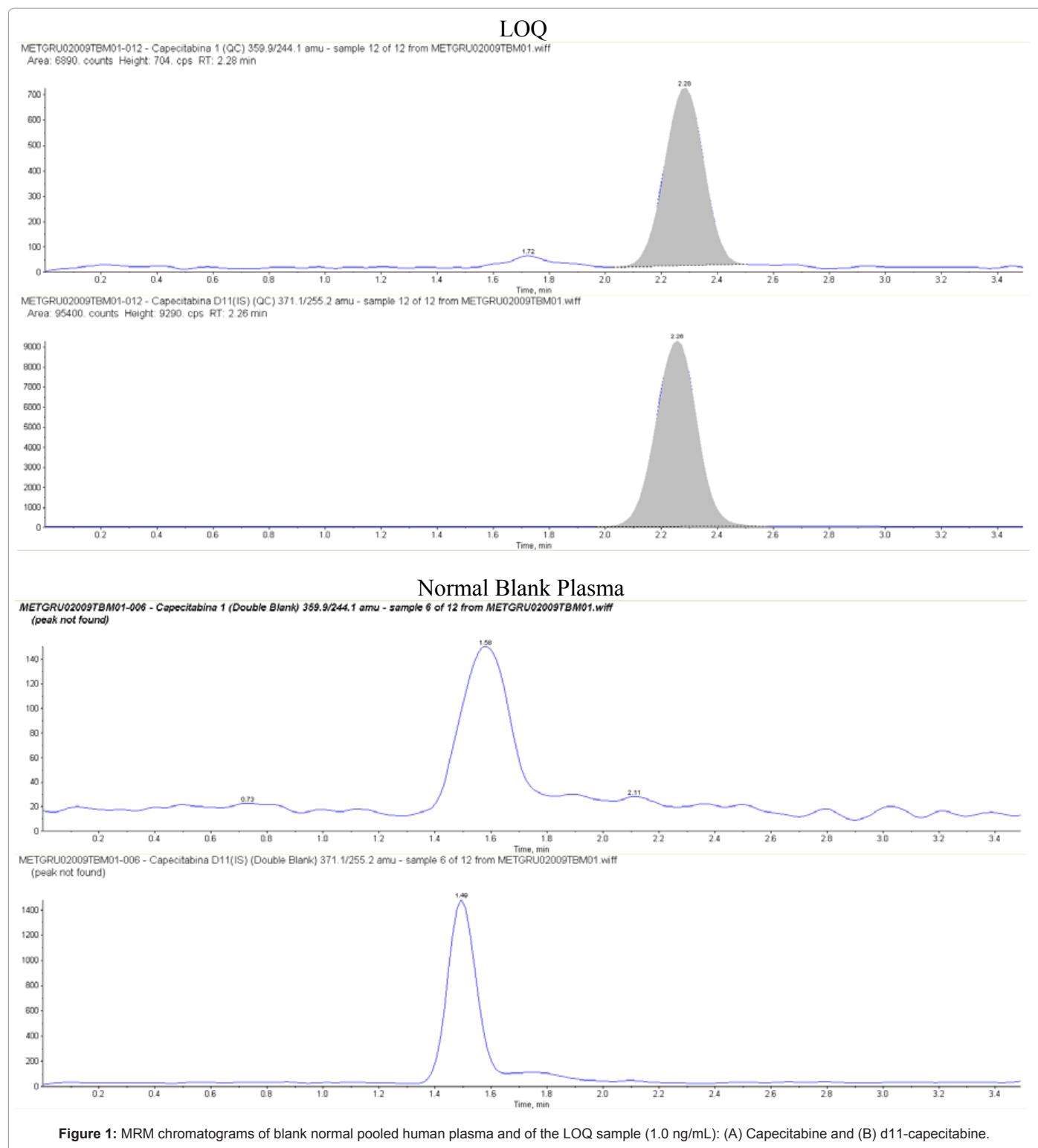
Table 3: Accuracy and precision data for ciprofibrate from the pre-study validation in human plasma.

Results

The drug was well tolerated by the volunteers, and they presented no adverse event. The biochemical and hematological parameters

presented no clinically relevant alterations ([Tables 1 and 2](#)).

The method was linear regression for capecitabine concentrations from 1 to 500 ng/mL (calibration curve $y=9.65e+003x + 1.21e+003$,



$r^2 > 0.9979$). A linear regression with a weighting index of $1/x^2$ was performed on the peak area ratios of capecitabine and the internal standard versus the capecitabine concentrations of the eight human plasma standards (in duplicate) to generate a calibration curve. The limit of quantification (LOQ), defined as the lowest concentration at which both the precision and accuracy were $<20\%$, was 1.0 ng/mL. The

within- and between-run precision and accuracy for the LOQ and QCs are summarized in Table 3.

No endogenous peak was observed in the mass chromatogram of blank plasma. Figure 1 shows a chromatogram for the standard LOQ sample in which the retention times for capecitabine and the internal standard was 2.27 ± 0.3 and 2.25 ± 0.3 min, respectively.

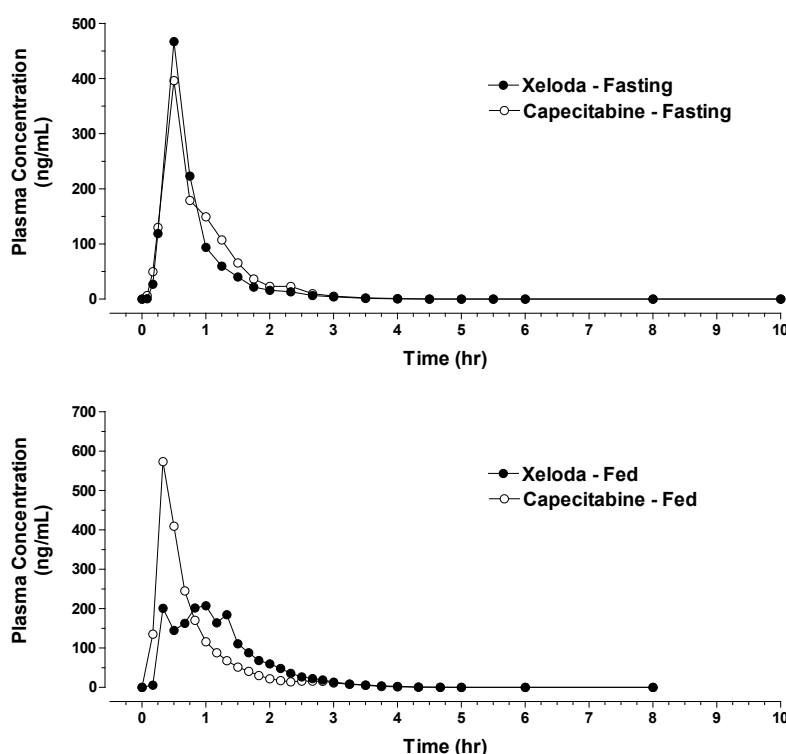


Figure 2: Mean pharmacokinetic parameters of the capecitabine tablet formulations under fasting and non-fasting conditions.

The mean capecitabine plasma concentration vs. time profiles after a single oral dose of each 150 mg Tablet formulation of capecitabine in fasting (group I) and non-fasting (group II) are shown in Figure 2. Table 4 shows the mean pharmacokinetic parameters obtained from volunteers after the administration of 150 mg capecitabine tablet groups I and II.

Table 5 shows geometric mean of the individual C_{max} and AUC_{last} (test/reference formulation), the respective 90% confidence intervals CI power, Intra-subject CV for the volunteers in group I with and group II.

Discussion

Many different methods have been used for capecitabine quantification in human plasma samples by HPLC/MS/MS (LOQ 1 - 150 ng/mL, retention time 2.4 - 11.5 min, run time of 4.5 -15 min, liquid-liquid extraction) [9-13]. Our method has good sensitivity (LOQ of 1 ng/mL), retention time of 2.27 min, run time of 3.4 min, liquid-liquid extraction and deuterated internal standard, permitting a high throughput. As demonstrated in this study, the present HPLC/MS/MS method is simple and selective for the determination of capecitabine in human plasma.

After the oral administration of capecitabine tablets (150 mg) to the fasting and non-fasting groups, the observed capecitabine peak plasma concentration (C_{max}) values for reference formulation are similar to those reported in the literature [4].

In accordance with to the Food and Drug Administration (FDA) guidelines, the capecitabine bioequivalence study should be performed

in cancer patients in non-fasting conditions [14]. The reason for the FDA guideline is due to the toxicity of capecitabine; indeed, it also states that pregnant and geriatric cancer patients (> 80 years), and cancer patients with a prior history of coronary artery disease, receiving concomitant therapy of warfarin, and dihydropyrimidine dehydrogenase deficiency, should be excluded to avoid serious adverse events [14]. Cancer patients with hepatic impairment and severe renal impairment should be excluded from the study due to possible effect on pharmacokinetics of capecitabine and its metabolites and due to the potential adverse events [14].

Our results demonstrate that a single administration of capecitabine was well tolerated by the volunteers, with no evidence of adverse reactions. This should not be surprising, for the following reasons:

i - The toxicity reported with capecitabine is paralleled to the therapeutic doses used for treatment of metastatic cancer. Indeed, in case of adverse events, it is recommended that the dose should be reduced to 50% or 25% if necessary. The usual therapeutic scheme employed consists of two grams twice daily, for two weeks, followed by one week interval. This scheme can be performed for six months or longer, depending on the therapeutic response observed. The dose administered to the healthy volunteer was a single administration of one tablet of 150 mg, which represents less than 4% of the dose one patient would receive in one day, or less than 0.3% of the dose received in one single cycle.

ii - Capecitabine acts as a prodrug. It needs to be converted to 5-fluorouracil through the action of thymidine phosphorylase and this occurs preferentially in tumours, since high activity of this enzyme is found in malignant tissue. Comparison of fluorouracil

| Reference - Fasting | | | | | | | | |
|---------------------|--------------|---|--------|--------|--------|--------|---------|--------|
| Parameter | Unit | N | Mean | DP | Min | Median | Max | CV% |
| AUC % extrap | (%) | 8 | 0.36 | 0.14 | 0.21 | 0.30 | 0.55 | 38.37 |
| AUC _{all} | ([ng*hr]/mL) | 8 | 262.66 | 75.38 | 158.57 | 274.48 | 364.17 | 28.70 |
| AUC _{inf} | ([ng*hr]/mL) | 8 | 263.21 | 75.43 | 159.11 | 275.36 | 364.60 | 28.66 |
| AUC _{last} | ([ng*hr]/mL) | 8 | 262.31 | 75.34 | 158.24 | 273.99 | 363.85 | 28.72 |
| C _{last} | (ng*/mL) | 8 | 1.47 | 0.33 | 1.21 | 1.29 | 2.08 | 22.51 |
| C _{max} | (ng*/mL) | 8 | 529.38 | 265.22 | 107.00 | 504.00 | 936.00 | 50.10 |
| K _e | (1/hr) | 8 | 1.73 | 0.34 | 1.25 | 1.66 | 2.41 | 19.85 |
| T _{1/2} | (hr) | 8 | 0.41 | 0.08 | 0.29 | 0.42 | 0.56 | 18.96 |
| T _{last} | (hr) | 8 | 3.40 | 0.60 | 2.70 | 3.25 | 4.50 | 17.79 |
| T _{max} | (hr) | 8 | 0.66 | 0.27 | 0.50 | 0.50 | 1.25 | 40.41 |
| Test – Fasting | | | | | | | | |
| Parameter | Unit | N | Mean | DP | Min | Median | Max | CV% |
| AUC % extrap | (%) | 8 | 0.30 | 0.14 | 0.17 | 0.27 | 0.62 | 46.95 |
| AUC _{all} | ([ng*hr]/mL) | 8 | 281.79 | 82.46 | 202.16 | 260.19 | 449.09 | 29.26 |
| AUC _{inf} | ([ng*hr]/mL) | 8 | 282.30 | 82.47 | 202.50 | 261.06 | 449.59 | 29.21 |
| AUC _{last} | ([ng*hr]/mL) | 8 | 281.51 | 82.44 | 201.99 | 259.87 | 448.80 | 29.28 |
| C _{last} | (ng*/mL) | 8 | 1.25 | 0.14 | 1.04 | 1.29 | 1.39 | 11.21 |
| C _{max} | (ng*/mL) | 8 | 458.63 | 134.09 | 169.00 | 462.00 | 638.00 | 29.24 |
| K _e | (1/hr) | 8 | 1.72 | 0.45 | 0.82 | 1.78 | 2.15 | 26.12 |
| T _{1/2} | (hr) | 8 | 0.44 | 0.17 | 0.32 | 0.39 | 0.85 | 39.48 |
| T _{last} | (hr) | 8 | 3.67 | 0.77 | 2.67 | 3.75 | 5.00 | 21.00 |
| T _{max} | (hr) | 8 | 0.56 | 0.18 | 0.50 | 0.50 | 1.00 | 31.43 |
| Reference - Fed | | | | | | | | |
| Parameter | Unit | N | Mean | DP | Min | Median | Max | CV% |
| AUC % extrap | (%) | 8 | 0.24 | 0.10 | 0.11 | 0.22 | 0.36 | 40.05 |
| AUC _{all} | ([ng*hr]/mL) | 8 | 300.74 | 91.40 | 183.74 | 287.15 | 433.11 | 30.39 |
| AUC _{inf} | ([ng*hr]/mL) | 8 | 301.16 | 91.51 | 183.69 | 287.56 | 433.46 | 30.38 |
| AUC _{last} | ([ng*hr]/mL) | 8 | 300.49 | 91.51 | 183.02 | 286.97 | 432.98 | 30.45 |
| C _{last} | (ng*/mL) | 8 | 1.21 | 0.14 | 1.03 | 1.15 | 1.44 | 11.53 |
| C _{max} | (ng*/mL) | 8 | 462.88 | 425.85 | 117.00 | 331.50 | 1420.00 | 92.00 |
| Ke | (1/hr) | 8 | 1.94 | 0.36 | 1.08 | 2.03 | 2.17 | 18.50 |
| T _{1/2} | (hr) | 8 | 0.38 | 0.11 | 0.32 | 0.34 | 0.64 | 29.17 |
| T _{last} | (hr) | 8 | 4.14 | 0.61 | 3.25 | 4.00 | 5.00 | 14.63 |
| T _{max} | (hr) | 8 | 1.00 | 0.37 | 0.33 | 1.09 | 1.33 | 36.74 |
| Test - Fed | | | | | | | | |
| Parameter | Unit | N | Mean | DP | Min | Median | Max | CV% |
| AUC % extrap | (%) | 8 | 0.27 | 0.20 | 0.12 | 0.22 | 0.75 | 75.57 |
| AUC _{all} | ([ng*hr]/mL) | 8 | 344.67 | 114.50 | 204.15 | 332.19 | 549.40 | 33.22 |
| AUC _{inf} | ([ng*hr]/mL) | 8 | 345.22 | 114.57 | 204.77 | 332.77 | 549.93 | 33.19 |
| AUC _{last} | ([ng*hr]/mL) | 8 | 344.42 | 114.63 | 203.23 | 332.05 | 549.25 | 33.28 |
| C _{last} | (ng*/mL) | 8 | 1.25 | 0.26 | 1.01 | 1.15 | 1.84 | 21.08 |
| C _{max} | (ng*/mL) | 8 | 704.89 | 556.95 | 94.10 | 605.00 | 1760.00 | 79.01 |
| K _e | (1/hr) | 8 | 1.69 | 0.44 | 1.18 | 1.68 | 2.47 | 25.97 |
| T _{1/2} | (hr) | 8 | 0.43 | 0.11 | 0.28 | 0.41 | 0.59 | 25.23 |
| T _{last} | (hr) | 8 | 3.67 | 0.66 | 3.00 | 3.50 | 5.00 | 18.13 |
| T _{max} | (hr) | 8 | 0.83 | 0.85 | 0.33 | 0.50 | 2.83 | 102.62 |

Table 4: Mean pharmacokinetic parameters obtained after administering of 150 mg capecitabine tablet formulation in fasting and non-fasting conditions.

| Capecitabine / Xeloda 150 mg (n = 8) | Capecitabine in fasting conditions | | | |
|---|------------------------------------|----------------|--------|------------------|
| | Parametric Analysis | | | |
| | Geom. Mean | 90% CI | Power | Intra-subject CV |
| C_{max} | 95.71 | 64.35 - 142.35 | 0.2251 | 42.61% |
| AUC_{last} | 107.99 | 98.36 - 118.57 | 0.9825 | 9.64% |
| AUC_{inf} | 107.93 | 98.34 - 118.45 | 0.9829 | 9.60% |
| Capecitabine / Xeloda 150 mg (n = 8) | Capecitabine in fed conditions | | | |
| | Parametric Analysis | | | |
| | Geom. Mean | 90% CI | Power | Intra-subject CV |
| C_{max} | 147.67 | 76.47 - 285.17 | 0.1434 | 76.27% |
| AUC_{last} | 113.92 | 93.7 - 138.51 | 0.6069 | 20.31% |
| AUC_{inf} | 113.95 | 93.83 - 138.39 | 0.6115 | 20.19% |

Table 5: Geometric mean of the individual AUC_{last} , AUC_{inf} , and C_{max} ratios (test/reference), the respective 90% confidence intervals (CI), power and CV.

concentrations in primary colorectal tumor and adjacent healthy tissues after capecitabine administration demonstrates that the average concentration of fluorouracil is 3.2 higher in the malignant tissue [5].

Capecitabine is employed for the treatment of metastatic cancer, therefore the prognosis of these patients is usually reserved. To expose these critical patients to either ineffective or toxic doses of the drug (due to unforeseen infra or supra-bioavailability of the test formulation), to experimental procedures such as internment, venous puncture and blood collection to evaluate bioequivalence should be ethically re-evaluated. Furthermore, studies performed in patients are with therapeutic doses, using several tablets for each administration (generally a combination of both 150 and 500 mg tablets), a procedure that limits the discrimination between dosage forms.

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