Bioequivalence study of two oral tablet formulations containing saquinavir mesylate boosted with ritonavir in healthy male subjects

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Abstract

Saquinavir (SAQ) mesylate (CAS 149845-06-7) is a potent inhibitor of the HIV-1 protease indicated in combination with other antiretrovirals for the management of HIV-1 infection. The objective of this study was to compare rate and extent of absorption and to assess the bioequivalence between a new pharmaceutical equivalent tablet formulation containing 500 mg of SAQ mesylate and the innovator film coated tablet formulation. A randomized, single-center, open-label, two-treatment, two-sequence, three-period, replicated crossover bioequivalence study in 40 healthy male subjects was conducted. All subjects received 100 mg ritonavir (CAS 155213-67-5) twice daily for a run-in period of 3 days before treatment. Dosing was separated by a wash-out period of 14 days. Blood samples were collected over 72 h and plasma levels of SAQ were determined by a validated HPLC/UV assay. The 90 % confidence interval(CI) of the ratio of the geometric means for log-transformed C_{max}, AUC_{last} and AUC_{inf} values were used to assess bioequivalence using the equivalence interval of 80–125 %. Point estimate and 90 % CI of the ratios of C_{max}, AUC_{last} and AUC_{inf} values were 94.9 (80.9–111.3), 97.4 (82.4–115.4) and 97.4 (82.5–115.0), respectively. Both treatments exhibited similar tolerability and safety. It was concluded that the new pharmaceutical product was bioequivalent to the innovator.

Key words

- Antiretroviral
- Bioequivalence
- Healthy volunteers
- Saquinavir mesylate

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1. Introduction

Saquinavir (SAQ) is a potent inhibitor of the HIV-1 protease and it is a well established component of current highly active antiretroviral therapy (HAART) regimens [1–6]. SAQ is a peptide-like substrate analogue that binds to the HIV protease active site and inhibits its activity which prevents cleavage of the viral polyproteins resulting in the formation of immature noninfectious virus particles. SAQ mesylate (CAS 149845-06-7) 500 mg film coated tablet (FCT) formulation was firstly developed to support patient adherence to treatment by reducing daily pill burden and was approved by the Food and Drug Administration (FDA), USA in 2005 after demonstrating bioequivalence to SAQ 200 mg hard capsules [1].

SAQ, when given as monotherapy, has a very low systemic bioavailability due to incomplete absorption and high first pass metabolism in the gut and liver [2]. Like most other HIV protease inhibitors (PIs), SAQ is rapidly metabolized by the cytocrome P450 isoenzyme CYP3A4 and is a substrate of P-glycoprotein (P-gp) [3,4]. Absorption is enhanced by dose administration with a high-fat meal and ritonavir (RTV) (CAS 155213-67-5) is able to boost levels of SAQ *in vivo* by inhibiting CYP3A4 and P-gp, thus increasing its bioavailability by 10 fold, relative to its single administration [4–7]. The pharmacokinetics of FCT SAQ mesylate has been evaluated in healthy adult subjects and HIV-infected patients. After oral administration of 1000 mg of SAQ combined with RTV 100 mg under fed condition with a previous 2-week run-in period of RTV dosing, SAQ concentrations increase with a maximum concentration (C_{max}) of approximately 3900 ng/ml and a mean area under the curve concentration-versus time curve (AUC) of ap-

proximately 29 000 ng · h/ml with estimated intra-individual coefficients of variation of approximately 24 % and 27 %, respectively [1, 8]. Several studies have demonstrated SAQ C_{max} and AUC values in HIV-infected patients are approximately twice those observed in healthy volunteers [1, 9, 10].

The twice daily dosing schedule is supported by SAQ serum elimination half-life of 4 to 12 h [7–10]. The approved "boosted" combination regimen is 1000 mg SAQ mesylate given in combination with 100 mg RTV twice daily and has demonstrated good efficacy and tolerability in both treatment-naive and treatment-experienced HIV-infected patients [11,12].

A new pharmaceutical equivalent oral tablet formulation containing SAQ mesylate 500 mg has been developed and the objective of the present study was to evaluate and compare its rate and extent of absorption to that of the innovator product when boosted with ritonavir in healthy male subjects under fed condition.

2. Subjects and methods

2.1 Study design and methodology

This was a single-center, open-label, randomized, two-treatment, two-sequence, three-period, replicated crossover bioequivalence study carried out in 40 healthy male adults subjects under fed condition. A replicated crossover design was used to overcome the absorption variability of SAQ due to the fact of its presystemic metabolism [13]. The study design is summarized in Fig. 1. The study was conducted at the pharmacokinetic unit of F. P. Clinical Pharma (Buenos Aires, Argentina) between June and October 2010. The study was carried out in compliance with the principles enunciated in the Declaration of Helsinki (2008), ICH-GCP guidance and FDA guidance for conducting bioavailability and bioequivalence studies for oral administered drugs [14–16]. The study protocol and the Informed Consent Form (ICF) were approved by an Independent Ethic Committee (Comité Independiente de Etica Para Ensayos En Farmacología Clínica – Fundación de Estudios Farmacológicos y de Medicamentos "FEFyM", Buenos Aires, Argentina) and by the local Regulatory Agency (ANMAT) before study start-up. Written ICF was obtained from all study subjects.

All study participants received 100 mg RTV twice daily for a run-in period of 3 days before the administration of SAQ. Individuals were randomly assigned to receive the single doses of SAQ mesylate two tablets of 500 mg (as it is the usual recommended daily dose) each in one of two treatment sequences: RTR or TRT (R = Reference treatment; T = Test treatment) in compliance with the FDA guidance [13]. On the morning of days 4, 18 and 32, after an overnight fast of at least 10 h, all study participants ate a standard high-fat, high-calorie breakfast of 900–1000 calories (500–600 cal from fat, 250 cal from carbohydrates and 150 cal from proteins) before taking a single dose of 100 mg of RTV and a single dose of two tablets of 500 mg each of SAQ. Mouth checks were performed after each dosing. Subjects were not allowed either to crush or chew the study medication. A standard lunch and afternoon meal were administered after the 4th and 8th hour of dosing.

Saquinavir was administered either in two 500 mg tablets of SAQ mesylate Proteovir® as test preparation (batch No. L867) manufactured by Richmond Laboratories (Buenos Aires, Argentina), or in two 500 mg tablets of FCT SAQ mesylate of the innovator product as reference preparation (batch No. E0028B01) purchased at a local pharmacy. The treatments were administered in three different dosing periods separated by a 14-day wash-out period according to the predetermined randomized sequence of treatment.

2.2 Study population

Sample size was based on the table of Liu and Chow [17] assuming an intra-individual coefficient of variation of approximately 25 % for area under curve (AUC) as it was reported in previous studies [1,8].

A total of 40 healthy male subjects between 21 and 50 years of age were enrolled. Inclusion criteria included Body Mass Index (BMI) between 19 and 27 kg/m^2. Laboratory tests, chest x-rays and electrocardiograms had to be within normal range. Negative test for HIV, hepatitis B and C viruses were also required. Subjects were excluded if they had a history or current manifestations of gastrointestinal disease or surgery, or hepatic, renal, cardiovascular, respiratory, hematopoietic, neurological endocrine-metabolic diseases. Individuals were not allowed to use any kind of medicine within the previous two weeks and throughout the study execution, with the exception of medications used to treat adverse events.

Forty healthy male subjects were randomized to one of the two treatment sequences. One subject was excluded because of an emesis episode at 90 min after dosing, and three subjects withdrew from the study during the run-in period as a consequence of personal commitments. Demographic data and mean health parameters of all the study participants are summarized in Table 1.

2.3 Sample collection and bioanalytic procedures

Blood samples for pharmacokinetic assessments were taken on days 4, 18 and 32 over a 72 h period at the following points: 0 (predose), 1.0, 1.5, 2, 2.5, 3, 3.5, 4, 6, 12, 24 and 72 h after oral

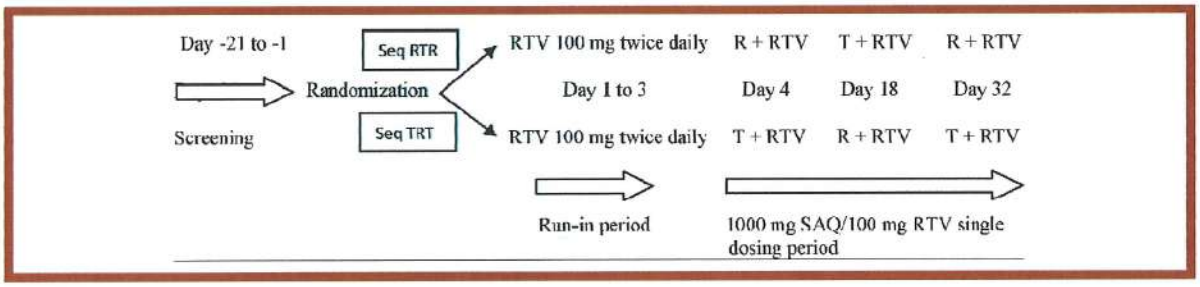

Fig. 1: Flowchart of study design.

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Table 1: Demographic data and health parameters of subjects (n = 40).

Characteristic	Results
Race (Caucasian /Non-caucasian), n (%)	39 (97.5)/1 (2.5)
Gender (male/female), n (%)	40 (100)/0 (–)
Age (yrs), mean ± SD	33.02 ± 7.92
Height (m), mean ± SD	1.74 ± 0.71
Weight (kg), mean ± SD	74.07 ± 10.05
BMI (kg/m^2), mean ± SD	24.26 ± 2.14

administration of saquinavir. A volume of 10 ml of blood was collected into vacutainers containing EDTA as an anticoagulant for each sample. Plasma was separated by centrifugation and stored at –20 °C until analysis.

Plasma concentrations of SAQ were determined by a validated specific high-performance liquid chromatography HPLC/UV method [18]. The method of analysis of SAQ in plasma was based on an alkaline extraction with a mixture of ethyl acetate 7:3 nelfinavir used as internal standard. A Knauer chromatograph consisting of a pump model K-501 with an injector loop of 50 µl and a variable UV detector model K-250 was employed. The samples were run through a column Lichrospher 5 µm, C18 (Hewlett Packard), with a guard column RP-18 (Upchurch Scientific, Inc.) with a data acquisition system: PeakSimple (SRI). The mobile phase consisted on a solution of 0.05 M monopotassium phosphate brought to pH 4.0-acetonitrile (65:35). The flow was 1.5 ml/min provided the detector to 239 mµ and a sensitivity range from 0.02 to 0.1 absorbance unit. The lower limit of quantification of SAQ was 10 ng/ml. The linearity of the method was tested between 10 and 5000 ng/ml Methodological validation was carried out in concordance with the FDA guidance for bioanalytical validation [19].

2.4 Pharmacokinetic evaluation

Pharmacokinetic parameters after oral administration of a single dose of test and reference treatment were calculated with the software WinNonlin version 5.2 (Pharsight, Mountain View, CA, USA) using a standard non-compartmental pharmacokinetic model.

The maximum plasma concentration after a dose and the time of their occurrence were defined as C_{max} and t_{max}, respectively. The slope of the log-linear regression function (λ) was the first order rate constant associated with the terminal portion of the curve estimated by linear regression of time vs. log-concentration. The terminal half-life ($t_{1/2}$) was derived from $\ln 2/\lambda$. The area under the plasma concentration-time curve from the time of dosing to the time after the last measurable concentration (AUC_{last}) was calculated using the linear trapezoidal rule. The AUC from dosing time extrapolated to infinity based on the last observed concentration was calculated as the sum of AUC_{last} and Cn/λ, where Cn is the last measurable plasma concentration and λ is the slope of the log-linear regression function.

A pharmacokinetic rule was generated to treat data coming from samples presenting values less than the lower level of quantification in bioanalytic assays. Subjects who experienced emesis at or before 2 times the median time to maximum concentration (t_{max}) for the analyte were excluded from the PK analysis set [16].

2.5 Safety assessment

Adverse events were recorded throughout the study period when reported by the study participants or observed by the investigators. The intensity of adverse events was graded on a three-point scale (mild, moderate or severe) in relationship to discomfort and disruption of daily activities. Physical examination, hematology, eritrocyte sedimentation rate, serum chemistry (liver function panel, urea, creatinin, fasting glucose), total cholesterol, triglycerides, coagulation panel and urianalysis, were performed at screening (Day –21 to –1) and at study termination for safety purposes (Day 35). A chest x-ray and a 12-lead electrocardiogram and were also carried out at screening. Vital signs (heart rate and systolic and diastolic blood pressure in supine position) were recorded during screening, immediately before drug administration, and 2, 4, 8, 12, 24 and 72 h after drug administration. An abbreviated physical examination was also performed on the morning before drug administration.

2.6 Statistical analysis

The following pharmacokinetic parameters: C_{max}, AUC_{last}, and AUC_{inf} were analyzed using natural log-transformed data. These PK variables were compared by means of ANOVA for a replicated crossover design. The model included the fixed effects of period, sequence, treatment and subjects within sequence as random effect. The average SAQ bioavailability of SAQ mesylate test formulation relative to SAQ mesylate FCT reference formulation was expressed as the ratio of respective estimated mean exposures and 90 % confidence intervals (CIs) in terms of C_{max}, AUC_{last} and AUC_{inf}.

In accordance with scientific standards and international guidelines for bioequivalence studies, bioequivalence was to be concluded if the 90 % CIs for the ratio of the geometric least-squares means (test treatment/reference treatment) was within the range of 80–125 % for the primary PK parameters. All statistical tests used a 5 % level of significance [13, 16].

3. Results

3.1 Pharmacokinetics

The pharmacokinetic analysis population comprised 36 subjects. The 72-h sampling period was sufficient to describe the concentration-time curve of SAQ after single dose administration. Fig. 2 (arithmetic scale) and Fig. 3 (semilog-transformed scale) show mean plasma concentration-time curves after single dose administration of two tablets of 500 mg of test and reference products. The curves for the two treatments were essentially similar and followed a typical profile for a conventional immediate release formulation with long half-lives. The terminal log-linear phase extended over 10–20 h for SAQ. Following the achievement of C_{max}, SAQ concentrations declined in a biphasic manner for both the test and reference products. Pharmacokinetic parameters for SAQ are summarized in Table 2. SAQ formulations showed similar mean t_{max} and half-lives values.

The analysis of variance did not show any statistically significant difference between test and reference formulations ($p < 0.05$) in relation to the fixed effect of period, sequence, treatment and subjects within sequence for

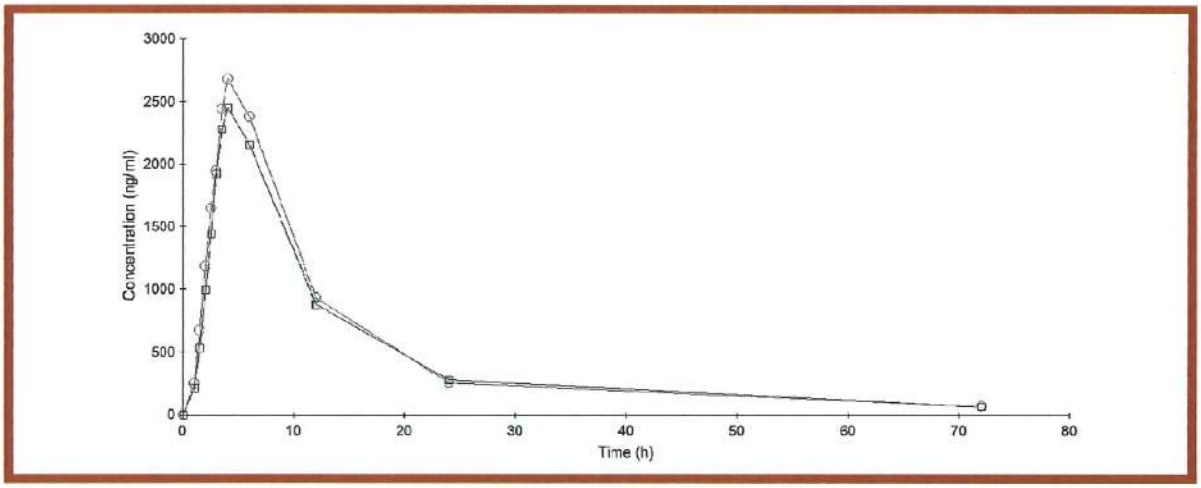

Fig. 2: Mean plasma concentration-time profile of SAQ (n = 36) following administration of SAQ mesylate (1000 mg single-dose) in combination with low-dose RTV (100 mg) as test (⊟) and reference (⊖) formulations under fed conditions.

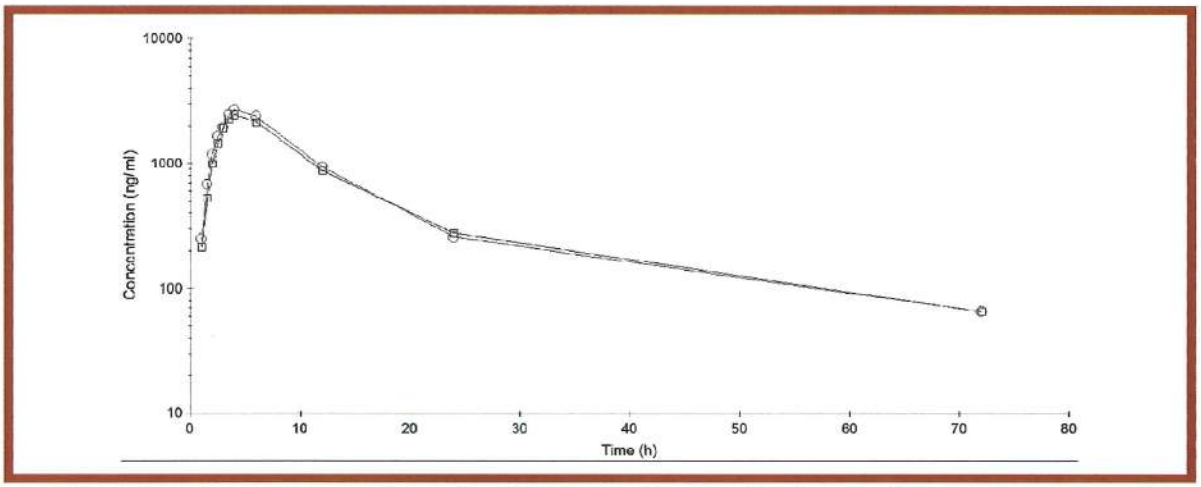

Fig. 3: Mean plasma log-concentration time profile of SAQ (n = 36) following administration of SAQ mesylate (1000 mg single-dose) in combination with low-dose RTV (100 mg) as test (⊟) and reference (⊖) formulations under fed conditions.

the pharmacokinetic parameters analyzed: ln C_{max}, AUC_{last} and AUC_{inf}.

Statistical analysis of SAQ pharmacokinetic log-transformed parameters and their geometric least squares mean ratios for the test and reference treatment are presented in Table 3. The limits of the 90 % CIs for the ratios of C_{max}, AUC_{last}, and AUC_{inf} for their log-transformed data fell well within 80–125 %. A large inter-individual variability was observed, being estimated coefficients of inter-indivudual variation for C_{max}, AUC_{last} and AUC_{inf} of 0.23, 0.24 and 0.23; respectively. Estimated coefficients of intra-individual variation for C_{max}, AUC_{last} and AUC_{inf} were 0.20, 0.17 and 0.17; respectively.

Test-Reference ratio for the geometric means (%) for all primary pharmacokinetic metrics (AUC_{last}, AUC_{inf}, C_{max}) and the corresponding two-sided 90 % CIs were contained within the predefined limits for bioequival-

Table 2: Pharmacokinetic parameters of SAQ in healthy volunteers (n = 36) after a 1000 mg single oral dose of test or reference treatment in combination with RTV.

SAQ PK parameter	Reference treatment (n = 36)	Test treatment (n = 36)
C_{max} (ng/ml), mean (SD)	3216.88 (1941.95)	2983.44 (1730.36)
t_{max} (h), mean (SD)	4.57	4.36
AUC_{last} (ng · h/ml), mean (SD)	34530.28 (19052.83)	33023.77 (18766.71)
AUC_{inf} (ng · h/ml), mean (SD)	36223.08 (19425.91)	34656.35 (786.822)
Ke (1/h)	0.05	0.05
Half-life (h)	16.66	15.07

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Table 3: Bioequivalence analysis for SAQ following single-oral dose administration of either test or reference treatment (1000 mg).

Pharmacokinetic parameter	Ref Geo LSM[1)]	Test Geo LSM[2)]	Ratio (% Ref)	CI 90 % Classical	Schuirmann's two one-sided *t* test	p	Power of the analysis
Ln (C_{max}), ng/ml	2800.51	2609.16	94.92	80.94 to 111.32	P (0 < 80 %) = 0.0390 P (0 > 125 %) = 0.0026	P < 0.05	0.75
Ln ($AUC_{0\text{-}last}$), ng · h/ml	29 980.05	28 374.53	97.46	82.39 to 115.39	P (0 < 80 %) = 0.0274 P (0 > 125 %) = 0.0085	P < 0.05	0.71
Ln ($AUC_{0\text{-}inf}$), ng · h/ml	31 651.28	29 953.91	97.42	82.51 to 115.03	P (0 < 80 %) = 0.0264 P (0 > 125 %) = 0.0079	P < 0.05	0.72

[1)] Ref Geo LSM: Reference Geometric Least Squares Mean.
[2)] Test Geo LSM: Test Geometric Least Squares Mean.

ence of 80–125 %, and the null hypothesis of the two one-sided Schuirmann's *t* test could be rejected ($p < 0.05$).

3.2 Safety

SAQ was well tolerated by all subjects. No clinically significant changes in vital signs (blood pressure, heart rate) and safety laboratory tests were observed after a 1000 mg single-oral dose administration of SAQ mesylate as test or reference products. A total of six non-serious adverse events (AEs) were reported: One case of emesis of moderate intensity which was considered related to the study drug by the investigators, and resolved without any medication. Another case of emesis of mild intensity that was considered not related to the study drug and did not require any medication. Three cases of headache of moderate intensity and not related to the study drug that resolved with the use of acetaminophen 500 mg in each case, and one case of traumatic finger pain of moderate intensity, and not related to the study drug that resolved with the use of ibuprofen 400 mg.

4. Discussion

The objective of the present study was to evaluate and compare rate and extent of absorption of a new pharmaceutical equivalent tablet formulation containing 500 mg SAQ mesylate to that from the innovator product in healthy male adult subjects when boosted with RTV under fed conditions; and secondarily to assess bioequivalence between them. Our results showed that no significance differences were found, in terms of rate and extent of absorption, between test and reference products, as indicated by C_{max} and AUC comparisons and also by the similar plasma SAQ concentration-time curves. The null hypothesis that the estimated parameters exceeded limits of acceptance for bioequivalence was rejected since the 90 % CIs of the ratios of μT/μR for the PK parameters (C_{max} and AUCs log-transformed) were found to be within the predetermined range (80 %–125 %) and the Schuirmann's two one-sided *t* test procedure (probability of exceeding limits of acceptance) found all probability values < 0.05.

To our knowledge, this is the first bioequivalence study evaluating SAQ mesylate tablets *versus* the innovator FCT as a single dosage formulation.

Parameters of bioavailability are in concordance with a previous bioequivalence study comparing the 500 mg FCT SAQ mesylate formulation with the 200 mg hard-capsule (HC) SAQ mesylate formulation in a single-dose of 1000 mg boosted with RTV 100 mg under fed conditions (high fat meal) administered to 94 healthy subjects (87 males and 7 females) in a randomized, two-sequence, four-period, replicated, crossover study [8]. In this report, the SAQ mesylate mean values for C_{max} and AUC_{Inf} were 3911 ng/ml (CV36 %) and 3322 ng/ml (CV 39 %), 29 734 ng · h/ml (CV45 %) and 27 805 ng · h/ml (CV 51 %) for the FCT and HC formulations, respectively; being the C_{max} values slightly higher than those from test and reference formulations observed in our study (2983.44 ng/ml and 3216.88 ng/ml, respectively) [8, 20]. This could be explained by the longer run-in boosting period with RTV 100 mg twice daily dosing of two weeks used in this report *versus* 3 days used in our study, and by gender-based differences in CYP3A4 expression or function resulting in differences in SAQ metabolism, since our study included only healthy men subjects [21].

Earlier data with the HC SAQ mesylate formulation demonstrated that the twice-daily SAQ/RTV (SAQ/r) regimens (1000/100 mg) taken with food (high fat meal) generate adequate concentrations, good efficacy and high tolerability both in healthy subjects and HIV-infected patients [5–7].

Published data for the new FCT formulation is limited in the HIV-infected population. However, the pharmacokinetic profile of SAQ mesylate reported in this study did not differ from a previous pharmacokinetic study carried out in HIV-infected pregnant women, using the 500 mg FCT formulation in a SAQ/r 1000/100 mg regimen under fed conditions with mean C_{max} and mean calculated AUC_{last} reported values: 3670 ng/ml and 23 650 ng · h/ml [22]. In another study carried out in HIV-infected patients with the 500 mg FCT formulation in the same regimen, an AUC_{last} of 18 760 ng · h/ml was calculated, being lower than the AUC_{last} reported in this study: 3023.77 ng · h/ml and 34 530.28 ng · h/ml for the test

and reference formulations, respectively [23]. The reduced exposure could possibly be explained by the small sample size (n = 11) and by the large inter-individual variability of SAQ [5–7]. The observed inter-individual variability of C_{max}, AUC_{last} and AUC_{inf} with both SAQ mesylate formulations in this study is known to occur with SAQ and is related to the effect of the CYP3A4 and P-gp on its absorption [2–4].

The adverse events reported in this study that were related to the drugs were confined to the gastrointestinal system and are in line with the safety profile of SAQ in general [1, 7–10]. In a previous pharmacokinetic study carried out in healthy subjects, the most common adverse events reported during the run in and treatment period were diarrhea, nausea, headache, dizziness and fatigue [8]. However, in the present study, no adverse events were observed during the run-in period and only one case of emesis with moderate intensity related to the study drug was observed during the treatment period.

Recognition of the potency of SAQ/r 1000/100 mg twice daily is reflected by its inclusion as a recommended boosted PI in published guidelines [11,12]. The use of the 500 mg FCT SAQ mesylate formulation has contributed to its widespread use since it reduces the daily pill count when compared to the HC formulation and improves the level of adherence to the treatment which is one of the most important determinants of virology response to PIs [1, 8, 11].

In conclusion, the point estimate of 90% CI for the log-transformed C_{max}, AUC_{last} and AUC_{inf} were in the range of 80–125%. No statistically significant differences were found for fixed effects when ANOVA test was applied to the ln C_{max}, ln AUC_{last} and ln AUC_{inf}. Both formulations were similar in terms of rate and extent of absorption. This study demonstrated that the new pharmaceutical equivalent 500 mg SAQ mesylate tablet formulation is also bioequivalent to the reference product. Considering that test product is pharmaceutical equivalent and bioequivalent, both products are therapeutically equivalent and interchangeable.

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Conflict of Interest

The authors state no conflict of interest in relation to the present study.

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