A Pharmacokinetic Model for Evaluating the Impact of Hepatic and Intestinal First-Pass Loss of Saquinavir in the Rat

R. Lledó-García, A. Nácher, V. G. Casabó, and M. Merino-Sanjúan


Received May 18, 2010; accepted October 25, 2010

ABSTRACT:
The aim of this study was to quantify the intestinal and hepatic first-pass loss of saquinavir and to assess the effects of coadministration of ritonavir on this first-pass loss. Single doses of 12, 24, and 48 mg of saquinavir and a dose of 24 mg of saquinavir/6 mg of ritonavir were orally, intravenously, or intraperitoneally administered to 94 rats. Ten groups of animals were studied. A semiphysiological pharmacokinetic model incorporating a population pharmacokinetic analysis [nonlinear mixed-effects model (NONMEM)] was developed to analyze plasma concentration-time profiles after administration via each of the three above-mentioned routes. This model confirmed that saturable metabolism in hepatocytes and enterocytes and dose-dependent precipitation in the peritoneal cavity after intraperitoneal administration characterize the pharmacokinetics of SQV. It also demonstrated that low oral bioavailability of saquinavir is due mainly to intestinal rather than to hepatic first-pass metabolism. In addition, it was shown that ritonavir diminished saquinavir clearance through competitive inhibition. The present report presents a new pharmacokinetic model applied in rats to evaluate the impact of hepatic and intestinal first-pass loss on oral bioavailability.

Introduction

Saquinavir (SQV) and ritonavir (RTV) are two protease inhibitors widely used in the management of AIDS and are characterized by variable oral bioavailability, which results in an inconsistent therapeutic response (Guiard-Schmid et al., 2003; Plosker and Scott, 2003). Both drugs have been criticized in recent years because of their high pill burden and side effects, especially in patients receiving long-term therapy, although they continue to be an essential component of highly active antiretroviral therapy, SQV exhibits low oral bioavailability that increases when it is coadministered with RTV, as both are substrates of the metabolic enzymes cytochrome P450 isoform 3A4 (CYP3A4), glycoprotein P, and multidrug-resistant protein (Hsu et al., 2004) have demonstrated that the gut wall does not play an important role in the low bioavailability of SQV (Devine et al., 1997; Lown et al., 1997; Kupferschmidt et al., 1998). However, Tam-Zaman et al. (2004) have demonstrated that the gut wall does not play an important role in the low bioavailability of SQV in dogs. Therefore, the primary aim of this study was to assess the nonlinear processes of SQV pharmacokinetics in rats, because this animal model is commonly used in pharmacokinetic studies. We assessed the nonlinear processes of SQV pharmacokinetics by administering different doses of SQV via intravenous, intraperitoneal, or oral routes. Gut absorption and hepatic and gut first-pass metabolism of SQV were evaluated to throw light on the mechanism responsible for low SQV bioavailability. The secondary aim was to study the interaction of SQV and RTV when coadministered by intravenous and oral routes. For this purpose, a semiphysiological pharmacokinetic model was developed for the administration of SQV alone or in combination with RTV.

Materials and Methods

Animals and Surgery Preparation. All the assays described in the present study adhere to the Principles of Animal Care and were approved by the Institutional ethics committee of the University of Valencia (Spain) according to RD 1201/2005. Male Wistar rats weighing between 260 and 300 g were placed in metabolism cages for 5 days before being randomly divided into 10 groups of 10 rats each. The rats were housed in metabolic cages under a 12-h light/12-h dark cycle with free access to food and water. The temperature and humidity of the animal room were maintained at 22 ± 2°C and 55 ± 5%, respectively.

All the assays described in the present study adhere to the Principles of Animal Care and were approved by the Institutional ethics committee of the University of Valencia (Spain) according to RD 1201/2005. Male Wistar rats weighing between 260 and 300 g were placed in metabolism cages for 5 days before being randomly divided into 10 groups of 10 rats each. The rats were housed in metabolic cages under a 12-h light/12-h dark cycle with free access to food and water. The temperature and humidity of the animal room were maintained at 22 ± 2°C and 55 ± 5%, respectively.

The rats were fasted for 18 h before the experiment and given water ad libitum. The rats were then anesthetized with sodium pentobarbital (50 mg/kg, ip) and placed in supine position, and a 25-gauge needle was introduced into the left external jugular vein. A 10-ml syringe was used to inject the test compound. The drug dose was injected over a period of 1-2 min. The rats were then returned to their cages and allowed to recover for 48 h. The rats were maintained on a 12-h light/12-h dark cycle with free access to food and water. The temperature and humidity of the animal room were maintained at 22 ± 2°C and 55 ± 5%, respectively.

Abbreviations: SQV, saquinavir; RTV, ritonavir; NONMEM, nonlinear mixed-effects model; ILV, interindividual variability; MOFV, minimum value of objective function; QMA, maximum amount of drug that binds to proteins; $F_e$, the fraction of dose escaping from the intestine; $F_i$, the fraction escaping from the liver; Clint, clearance of intestinal secretion.
300 g were subjected to jugular vein cannulation using a previously described technique (Lledo-García et al., 2007).

**Drug Administration and Blood Sample Collection.** SQV was obtained from F. Hoffman-La Roche Ltd. (Welwyn Garden City, Hertfordshire, UK), and RTV was obtained from Abbott Laboratories (Chicago, IL). A total of 94 animals and 915 plasma samples were used in the study. Solutions were prepared by dissolving the corresponding amount of drug in 1 ml of a mixture of cosolvents containing saline solution, propylene glycol, and ethanol in proportions of 25:25:50. The study design is shown in Table 1.

**SQV Administration.** Rats were randomly allocated into eight groups according to the administration route to be used (intravenous, oral, and intraperitoneal).

**Intravenous infusion.** The 48-mg dose was administered via the jugular cannula with the aid of a volumetric pump IVAC (30-min infusion). The cannula was immediately injected with 0.4 ml of heparinized saline solution (20 IU/ml) to draw out any remaining traces of the drug. The exteriorized cannula was then replaced with a new one to avoid possible contaminations when sampling.

**Intravenous bolus.** Doses of 24 and 12 mg were administered via the jugular cannula. After administration, the above-mentioned procedure was repeated.

**Intraperitoneal administration.** Doses of 24 and 12 mg were injected 2 cm above the genital area while animals were held in a supine position.

**Oral administration.** Doses of 48, 24, and 12 mg were administered by gastric intubation.

**SQV and RTV Administration.** Rats were randomly allocated into two groups that received a 24- and 6-mg dose of SQV and RTV, respectively, by either intravenous infusion (30-min infusion) or orally.

After intravenous administration, samples were collected at 8 to 10 h, which was considered to represent the terminal phase, because previous studies have estimated the half-life ($t_{1/2}$) of SQV to be 1.6 h in rats (Shibata et al., 2003). However, after intraperitoneal and oral administration, profiles showed that the initial protocol sampling times (up to 8–10 h) had not adequately captured the terminal phase and were therefore modified. Because longer sampling periods were necessary for accurately determining the terminal phase of the curves after intraperitoneal and oral administration, each group was divided into two subgroups from which samples were drawn for the first 12 h after administration from one and during the second 12-h period from the other.

Blood samples (0.2 ml) were drawn from the jugular vein cannula with heparinized syringes at a scheduled time point. After each sample was drawn, the blood volume was replaced with the same volume of a saline solution. The number of samples processed within a 24-h period to obtain the curve of the plasma level of SQV was never higher than 11. The animals’ weight and hematocrit were recorded; losses in hematocrit during the assay period were lower than 19%, and thus pharmacokinetic parameters were not considered to be affected. After collection, each blood sample was centrifuged at 5000 rpm for 5 min, and the plasma was transferred to an unused polypropylene tube and stored at $-30^\circ$C until it was assayed for SQV content.

**Analytical Procedures.** SQV plasma levels were measured by reverse-phase high-performance liquid chromatography with UV detection (235 nm), which provided adequate separation and quantification of the drug. SQV content was extracted from the plasma with organic solvents, as described previously for RTV (Lledo-García et al., 2007). The mobile phase consisted of a combination of acetonitrile and an aqueous solution of phosphate buffer (99% bidistilled water and 1% 1/15 M phosphate buffer, pH 6.9, 57:43, v/v). A flow rate of 1 ml/min was used.

Calibration curves covering the whole range of SQV concentrations in the plasma samples were prepared and assayed in triplicate on each day of analysis. The peak area of SQV was measured in each sample and correlated with SQV concentration. Excellent linear plots of the peak areas and SQV concentrations were obtained ($r > 0.99$).

![Graphical representation of experimental concentrations (Cexp) (milligrams per liter) versus time (hours) and the individual predicted concentrations (Cpred) (milligrams per liter) according to model 9, after applying it to intravenous, intraperitoneal, and oral SQV data (groups 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10).](image-url)
consisted of the following steps: a more empirical analysis was performed. Data were also incorporated in a sequential fashion. The analysis to be made to the base structure of the model, a backward procedure was carried out. The processes involved in the pharmacokinetic profiles were described in physiological terms; namely, expressions of flow, clearances, and volumes. Physiological parameters were fixed according to data in the literature. In the last stages of the model’s development (steps 3 and 4), the values for disposition and intraperitoneal absorption were fixed at those obtained in step 2; because the addition of oral data did not affect them.

### Results

The SQV total plasma concentration-time profiles obtained after intravenous, intraperitoneal, and oral administration are shown in Fig. 1. The experimental data used in the development of the model

### Table 2

Some models tested and the models selected in each step of the analysis

<table>
<thead>
<tr>
<th>Step</th>
<th>Parameters and Comments</th>
<th>No. Parameters</th>
<th>No. IIV</th>
<th>MOFV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step 1: intravenous data</td>
<td>Two compartments; MM elimination</td>
<td>5</td>
<td>3</td>
<td>604.9</td>
</tr>
<tr>
<td>1</td>
<td>Two compartments; MM elimination + (K_{a}) different G9</td>
<td>6</td>
<td>3</td>
<td>543.5</td>
</tr>
<tr>
<td>2</td>
<td>Three compartments; MM elimination + (K_{a}) different G9</td>
<td>8</td>
<td>4</td>
<td>514.9</td>
</tr>
<tr>
<td>3</td>
<td>Two compartments; plasma protein dynamic binding + MM elimination ((K_{a}) different G9)</td>
<td>9</td>
<td>6</td>
<td>483.5</td>
</tr>
<tr>
<td>Step 2: intraperitoneal data added</td>
<td>(K_{a}) and (F) different for each intraperitoneal group ((F) for G8 fixed to 1 (rest as in model 4)</td>
<td>12</td>
<td>6</td>
<td>357.9</td>
</tr>
<tr>
<td>4</td>
<td>Dissolution as limiting factor for absorption (rest as in model 4)</td>
<td>14</td>
<td>Restriction</td>
<td>334.8</td>
</tr>
<tr>
<td>Step 3: oral data added</td>
<td>Same absorption ((k_{a}) and (F)) parameter values for all oral groups (rest as in model 6)</td>
<td>2</td>
<td>Restriction</td>
<td>192.5</td>
</tr>
<tr>
<td>7</td>
<td>Considers a portal space compartment with a MM elimination process and two intestinal transit compartments + efflux process to one of lumen compartments + two different (k_{a}) from each intestinal transit compartment (rest as in model 6)</td>
<td>7</td>
<td>Restriction</td>
<td>163.2</td>
</tr>
<tr>
<td>Step 4: oral data from G10 added</td>
<td>Considers hepatocyte and enterocyte compartments with MM elimination processes and two intestinal transit compartments + efflux process to one of lumen compartments + two different (K_{a}) from each intestinal transit compartment (rest as in model 6); also takes into account the interaction between SQV and RTV at the enzymatic level</td>
<td>13</td>
<td>Restriction (estimated in this step: 8)</td>
<td>195.3</td>
</tr>
<tr>
<td>9</td>
<td>Considers hepatocyte and enterocyte compartments with MM elimination processes and one intestinal transit compartment + one (K_{a}) from intestinal compartment; dissolution as limiting factor for intraperitoneal absorption (rest as model 2); takes also into account the interaction between SQV and RTV at the enzymatic level</td>
<td>8</td>
<td>Restriction (estimated in this step: 6)</td>
<td>342.8</td>
</tr>
<tr>
<td>10</td>
<td>Hepatocyte compartment is not considered (rest as in model 10)</td>
<td>6</td>
<td>Restriction (estimated in this step: 5)</td>
<td>342.8</td>
</tr>
</tbody>
</table>

**MM.** Michaelis-Menten kinetics; G; group; \(K_{a}\); intraperitoneal absorption rate constant; \(k_{a}\); intestinal absorption rate constant; \(F\); bioavailability.

**a** Restriction indicates restriction of the random variables (were fixed to the values obtained in prior steps).
were total plasma concentrations. Table 2 shows the key models of a series of models tested at different stages of the data analysis. The parameters considered by the model, random estimated variables (IV) and MOFV, are also shown.

A Michaelis-Menten elimination process was performed to elucidate the distribution, metabolism, and excretion processes (disposition phase, step 1) for which the empirical model had revealed nonlinear phenomena but failed to provide any relevant information (models 1 and 2, Table 2). Taking into account the fact that 97% of SQV binds to plasma proteins (Holladay et al., 2001), dynamic and saturable plasma protein binding could explain the variability in clearance (Cl) and volume of the central compartment (Vc) differed in each group (G) (Campos Moreno et al., 2007). Thus, with the 24-mg dose, saturation of binding to plasma proteins would deliver more drug, which would in turn be eliminated, so that clearance would be higher than that observed with the 12-mg dose. Likewise, with the 48-mg dose, there could be saturation of both the plasma protein binding and elimination processes.

After incorporation of the intraperitoneal data (step 2) and taking into account the low solubility of the drug, precipitation of the drug in the peritoneal cavity was considered. Experimental observation confirmed this theory (data not shown), which suggests that dissolution limited the absorption of the drug (model 6, Table 2). In this model, the amount of precipitation depends on the dose administered (Qpp(G1)), because the amount of drug dissolved is the same as that administered (Qdis(G1)). Subsequently, the precipitated fraction (FR(G1)) and dissolution rate constant (kd) are estimated for each group.

Then, after incorporation of the oral data (step 3), a more semi-physiological approach was considered to define the pharmacokinetic profile of SQV (models 8–11, Table 2). Physiological expressions were incorporated into the model; Qh represents hepatic flow, Qah represents arteriohepatic flow, and Qp represents mesenteric flow. On the basis of data in the literature, Qh and Qp were considered to constitute 13 and 87% of the total hepatic flow, respectively, and the hepatic flow for a 300-g rat was considered to be 1.15 l/h (Birnbaum et al., 1994). Physiological parameters related to flow and volumes in liver and intestine were fixed to values in the literature (Qh, Vh, and Vp) (Birnbaum et al., 1994). Figure 2 illustrates the schematic structure of the model that best defined the pharmacokinetic profile of SQV and the corresponding differential equations. It is important to emphasize that unbound and bound SQV plasma concentrations were described in different compartments (compartments 1 and 3, respectively). The absorption rate constant was different in each intestinal compartment (0.24 and 0.17 h⁻¹ in the first and second compartments, respectively), whereas the transit compartment rate constant (KT) was 0.58 h⁻¹, indicating that the drug remained in each intestinal transit compartment for 1.71 h. There was a weak process of secretion from the enterocyte to the second intestinal transit compartment, characterized by a Michaelis-Menten secretion constant (Km) of 0.02 mg/l and an intrinsic blood clearance of intestinal secretion (Clint) of 2.83 × 10⁻⁶ l/h. In addition, a metabolic process was detected in the liver and intestine, with a Michaelis-Menten constant (Km) of 72.0 mg/l in both. Because RTV was a potent inhibitor of intestinal and hepatic metabolism and intestinal secretion processes, a factor was introduced to quantify the modification of Km when RTV was coadministered. A value of 5.16 was estimated for group 9 (Fm(G9)) and a value of 10.2 was estimated for group 10 (Fm(G10)). Precipitation of the drug in the intraperitoneal cavity and dissolution constants kdp for the 24 and 12 mg were also estimated. The dissolution constant kdp

---

**Fig. 2.** Structure of the model developed: description of the pharmacokinetic profile of SQV. In the differential equations, compartments 1, 2, 3, 4, 5, 6, 7, 8, and 9 are central, peripheral, plasma protein-binding, first intestinal transit lumen, intraperitoneal drug-dissolved, intraperitoneal drug-precipitated, hepatocyte, second intestinal transit lumen, and enterocyte compartments, respectively. A and C are the amounts (milligrams) of SQV and concentrations (milligrams per liter) in each compartment (i), respectively. CMA (milligrams per liter) is the maximum binding capacity of the protein for SQV, expressed as the amount of SQV divided by central volume. Cu is the SQV unbound plasma concentration and Cb is the SQV bound plasma concentration (milligrams per liter). Km (liters per milligram per hour) and Kd (hours⁻¹) are the rate constants of binding and unbinding to proteins. Cp is the total SQV plasma concentration (milligrams per liter). Q, Qh, Qpw, and Qp are intercompartmental, hepatic, arteriohepatic, and mesenteric flows (liters per hour), respectively. Eint, Eout, Eext, and Ehp are the hepatic extraction ratio, the extraction ratio due to an intestinal secretion process, an intestinal metabolic process, and the global extraction ratio in intestine (due to metabolism and secretion processes). Fp and Fh are the fractions of drug escaping from the liver and intestine, respectively. Kaug, Kahl, and Kauh are the absorption rate constants after intraperitoneal and oral administration (hours⁻¹). KT is the intestinal transit rate constant (hours⁻¹). FR is the fraction of dose that precipitated after intraperitoneal administration. Kdp is the dissolution rate constant after intraperitoneal administration (hours⁻¹). Kaug and Kahl are the intercompartmental rate constants (hours⁻¹). Vh and Vp are the volumes of distribution in central and peripheral compartments (liters). Km, Km, and Km are the Michaelis-Menten constants (milligrams per liter) in the metabolic processes in the liver and in the metabolic and secretor processes in the intestine, respectively. Vh, Vp, and Vpw are the maximal rate for an enzyme (milligrams per hour) in the metabolic process in the liver and in the metabolic and secretor processes in the intestine, respectively.
It is well documented that CYP3A4 is the major enzyme responsible for approximately 90% of SQV metabolism. Because of the relative abundance of CYP3A4 enzyme in the human gut, first-pass gut metabolism was thought to be a significant factor in the low bioavailability of SQV in humans (Fitzsimmons and Collins, 1997; Steimer et al., 1998). In line with this thought, it has been observed that SQV and grapefruit juice coadministration produces an increase in the absolute bioavailability of SQV without affecting its clearance (Lown et al., 1997; Kupferschmidt et al., 1998), which suggests a decrease in the intestinal metabolism. A similar tendency is observed in rabbits, in which the oral bioavailability of SQV is approximately 4% because of the intestinal metabolism and secretion processes in these animals (Sinko et al., 2004). In contrast, CYP3A4 in the dog gut wall plays no significant role in clearance of SQV, and its bioavailability is greater than in any other species tested to date (Tam-Zaman et al., 2004).

In the present study, the impact of liver and intestine function on the pharmacokinetics of SQV in the rat, an animal model of the projection of human intestinal permeability (Amidon et al., 1995), was evaluated using a modelistic approach. The influence of RTV on the metabolism SQV was also assessed, because the combination of SQV and RTV has been shown to be clinically effective owing to the way in which the former augments exposure to the latter (Hoffman et al., 2007).

To imitate clinical practice, we selected a 24-mg dose of SQV, because, when scaled in rats (U.S. Food and Drug Administration,
2002), it corresponds with the most commonly used therapeutic dose in humans; namely, 1000 mg administered in combination with 100 mg of RTV (b.i.d.) (U.S. Food and Drug Administration, 2005, http://www.fda.gov/cder/).

The noncompartmental analysis showed nonlinearities in the absorption and elimination process (data not shown). However, results for area under the curve, clearance (Cl), and half-life ($t_{1/2}$) were contradictory, and a consistent interpretation was not possible. Moreover, it also was not possible to distinguish the roles of the liver and intestine in the elimination process. In this scenario, individual analyses of data were aborted, and a population analysis by means of nonlinear mixed-effects modeling was performed, because this has
proved to be a very useful tool to model different kinetic processes (Ruiz-Carretero et al., 2004; Muñoz et al., 2005; Campos Moreno et al., 2007). The advantage of the nonlinear mixed-effects models is that the data may be "sparse" with a little as one observation per subject, "rich" with many observations per subject, or a combination of both with some subjects having many samples collected in a dose interval, whereas others have only a few (Bonate, 2006).

The SQV pharmacokinetic model developed and supported by our experimental data considers two absorption sites in the gastrointestinal tract, saturable metabolism in hepatocytes and enterocytes, saturable intestinal efflux, dose-dependent precipitation in the peritoneal cavity after intraperitoneal administration, and protein binding kinetics.

The $C_{\text{lin}}$ indicates low affinity for the intestinal efflux carrier, and the fact that the metabolic process detected in the liver and intestine is governed by an enzymatic system characterized by the same $K_{m}$ value indicates that the drug has the same affinity for the metabolic enzyme regardless of where the process occurs.

The factor used to quantify the modification of $K_{m}$ when RTV was coadministered indicates that there was a loss of affinity for the enzyme when RTV was coadministered due to competitiveness between the two drugs for the enzyme. The fact that a higher factor was obtained for group 10 (oral) than for group 9 (intravenous) suggests that inhibition of the first-pass intestinal metabolism was greater than that of the first-pass hepatic metabolism. In line with this finding, the blood intrinsic clearance in hepatocytes ($C_{\text{li}}$) and enterocytes ($C_{\text{le}}$) again indicated that the main metabolic process of SQV occurs in the intestine in rats.

By using the semiphysiological model, it is possible to calculate the extraction rate according to the corresponding concentration in the enterocyte or hepatocyte compartments at any time point (Fig. 4). In this sense, the fraction of dose escaping from the intestine ($F_{p}$) was shown to undergo variations of 15 to 60% as the concentration increased and the enzymatic system became saturated. On the other hand, the fraction escaping from the liver ($F_{h}$) did not undergo considerable oscillations, maintaining values of 80 to 86%, which are far from that of 56% reported by Sinko et al. (2004).

When RTV was coadministered, values of 85 to 93% and 96 to 98% were observed for $F_{h}$ and $F_{e}$, respectively. These results indicate that there was an important inhibition of the SQV metabolism that was most evident at the intestinal level.

Shibata et al. (2002) used in vitro and in vivo data (rats) to develop a physiological model to describe the interaction between protease inhibitors, and among the combinations they assayed was that of SQV and RTV. These authors considered the enterocyte and hepatocyte compartments, each with its own metabolic process, but did not take into account the intestinal secretion process. They concluded that the main metabolic process took place in the liver ($F_{h}$ and $F_{e}$, 51.1 and 97.4%, respectively), which is opposite to the trend observed in the present study. Oral bioavailability was 4.1%, with a fraction of absorption of 8.3. In contrast, Shen et al. (1997) reported that the considerable presystemic losses of SQV could be due to intestinal first-pass metabolism, a hypothesis that the present results support.

The SQV pharmacokinetic model developed in this study also takes into consideration plasma protein binding, which occurs through a dynamic equilibrium that does not reach saturation. The QMA suggests that this binding produces a delay in the distribution process, so that it performs the role of a peripheral compartment.

The values obtained for the parameters QMA and $C_{\text{lin}}$ lacked physiological significance, and, as a consequence, the model was reevaluated without plasma protein binding and intestinal secretion processes (model 10, Table 2). This new model provided a value for $C_{\text{li}}$, which represented 0.27% of the $C_{\text{li}}$. This result indicates that the most important organ for elimination of SQV in rats is the gut wall and that the liver does not play a significant role. Consequently, in the final evaluation, the hepatocyte compartment was not considered (model 11). This model confirms that SQV intestinal metabolism is the process by which the oral bioavailability of SQV in rats is restricted and indicates that orally administered RTV acts a potent competitive inhibitor of this process, because the increase in $K_{m}$ was higher when the two protease inhibitors were coadministered orally (model 11: $FR_{K_{m}(G10)} = 38$ and $FR_{K_{m}(G0V)} = 5$, by the oral and intravenous routes, respectively).

However, the statistical criteria (MVOF) for model 11 (Table 2) show that eliminating plasma protein binding, intestinal secretion, and hepatocyte metabolism processes from consideration was not justified.

The pharmacokinetic model developed in this study could only be extrapolated to humans in terms of compartments and processes. It is interesting to note that bioavailability of SQV is very low in all animal species studied, and intestinal first-pass metabolism has been also identified in humans (Noble and Faulds, 1996; Fitzsimmons and Collins, 1997; Eagling et al., 2002) and rabbits (Sinko et al., 2004). On the contrary, first-pass metabolism associated with the liver seems to be the main cause in dogs (Tam-Zaman et al., 2004). However, pharmacokinetic parameter values could be only used in the animal species that have been characterized. Therefore, although the mean values estimated in this study for the maximum plasma concentration after oral administration of a single dose of SQV alone or coadministered with RTV could be considered comparable to those of human (Plosker and Scott, 2003; Dickinson et al., 2008), the impact on the interaction and saturation of protein binding in both species could be very different. Results obtained in this study indicate that saturation of this process in rats could be unlikely.

In conclusion, the pharmacokinetics of SQV in rats is complex and nonlinear, and intestinal first-pass loss seems to be the main cause of its poor oral bioavailability. Nevertheless, we believe that the application of the nonlinear mixed-effect technique allows a robust estimation of the population pharmacokinetic parameters of SQV and their variability and despite the complexity is a useful method for evaluating the impact of hepatic and intestinal first-pass loss on oral bioavailability in rats.

**References**


Address correspondence to: Dr. A. Nácher, Departamento de Farmacia y Tecnología Farmacéutica, Facultad de Farmacia, Avda, Vicente Andrés Estellés s/n, 46100 Burjassot, Spain. E-mail: amparo.nacher@uv.es