A pharmacokinetic model for evaluating the impact of hepatic and intestinal first-pass loss of saquinavir in the rat

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Relevance of intestinal first-pass loss of saquinavir

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List of nonstandard abbreviations of the paper:

SQV: Saquinavir.
NONMEM: Nonlinear mixed effects model.
RTV: Ritonavir.
HAART: highly active antiretroviral therapy.
IV: Intravenous administration.
IP: Intraperitoneal administration.
PO: Oral administration.
HPLC: High performance liquid chromatography.
CV: Coefficient of variation.
SD: Standard deviation.
MOFV: Minimum value of objective function.
Cl: Clearance.
Vc: Volume of central compartment.
Abstract

The aim of this study was to quantify the intestinal and hepatic first-pass loss of Saquinavir and to assess the effect of coadministration of Ritonavir on this first-pass loss. Single doses of 12, 24 and 48 mg Saquinavir and a dose of 24 mg Saquinavir/6 mg 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 (NONMEM) was developed to analyze plasma concentration-time profiles following administration via each of the three abovementioned routes. This model confirmed that a 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 an intestinal rather than a 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 criticised in recent years due to their high pill burden and side effects, especially in long-term patients though they continue to be an essential component of highly active antiretroviral therapy (HAART), SQV exhibits a 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 (MRP) (Hsu et al., 1998; Plosker and Scott, 2003; Mouly et al., 2004; Park and Sinko, 2005). The absolute oral bioavailability of SQV has been determined at 4% (Williams and Sinko, 1999; Washington et al., 2000). Ninety-seven % binds to plasma proteins, primarily to the alpha 1 acid glycoprotein (Holladay et al., 2001). The inhibitor undergoes an important first-pass metabolism that is both intestinal and hepatic, and in which CYP 3A4 is the principal isoenzyme involved. The role of CYP3A5 in the metabolism of SQV is also under investigation (Mouly et al., 2004). Other isoenzymes known to be involved to a lesser extent are CYP2A6, CYP2C9 & CYP4A1 (Williams and Sinko, 1999). SQV has also been described as a weak inhibitor of CYP3A4 and a substrate and inhibitor of the p-glycoprotein (Eagling et al., 1997).

Until now there has been lack of consensus regarding the roles of the liver and intestine in SQV first-pass metabolism. It has been postulated that, in humans, a first-pass gut metabolism was the principal cause of the low bioavailability of SQV (Devine et al., 1997; Lown et al., 1997; Kupferschmidt et al., 1998). However Tam-Zamam N et al have demonstrated that the gut-wall does not play an important role in the low bioavailability of SQV in dogs (Tam-Zaman et al., 2004). Therefore, the primary aim of this study was to assess the nonlinear processes of SQV pharmacokinetics in rats, as 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 (IV), intraperitoneal (IP) or oral (PO) routes. Gut absorption and hepatic and gut first-pass metabolism of SQV were evaluated in order to throw light on the mechanism responsible for low SQV bioavailability. The secondary aim was to study the interaction of SQV and RTV when co-administered by IV and PO routes. For this
purpose, a semi-physiological 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 Faculty of Pharmacy Ethics Commission (Valencia, Spain). Male Wistar rats weighing between 260 and 300 g were subjected to jugular vein cannulation using a previously described technique (Lledo-Garcia et al., 2007).

Drug administration and blood sample collection

SQV was obtained from F.Hoffmann-La Roche Ltd.® (Welwyn Garden City, Hertfordshire, UK), and RTV was obtained from Abbot Laboratories® (Illinois, USA). A total of 94 animals and 915 plasma samples were employed 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 I.

SQV administration

Rats were randomly allocated into eight groups according to the administration route to be employed (IV, PO and IP).

IV 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 UI/mL) in order to drag any remaining traces of the drug. The exteriorized cannula was then replaced with a new one so as to avoid possible contaminations when sampling.

IV bolus: doses of 24 and 12 mg were administered via the jugular cannula. After administration, the above mentioned procedure was repeated.

IP 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 IV infusion (30 min infusion) or orally.

After IV administration samples were collected at 8-10h, which was considered to represent the terminal phase, as previous studies have estimated half-life (t1/2) of SQV to be 1.6 h in rats (Shibata et al., 2003). However, after IP and PO 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. As longer periods of sampling times were necessary for accurately determining the terminal phase of the curves after IP and oral administration, each group was divided into two subgroups from which samples were drawn for the first 12 hours after administration from one, and during the second 12 hour 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 24h 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 5min and the plasma was transferred to an unused polypropylene tube and stored at −30ºC until it was assayed for SQV content.

**Analytical procedures**

SQV plasma levels were measured by reverse phase high performance liquid chromatography (HPLC) with UV detection (235 nm), which provided an adequate separation and quantification of the drug.

SQV content was extracted from the plasma with organic solvents, as previously described for RTV (Lledo-Garcia et al., 2007). The mobile phase consisted of a combination of acetonitrile and an aqueous solution of phosphate buffer (99% bidistilled water and 1% phosphate buffer 1/15 M) (pH 6.9), 57:43 (vol/vol). A flow rate of 1mL/min was employed.

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>0.999). Accuracy and precision were evaluated by calculating the relative error and coefficient of variation, which were always less than 14.46% and 8.4%, respectively. The limit of quantification was 0.051 ug/mL. The results obtained were considered to be completely reliable (Karnes and March, 1993).

**Pharmacokinetic calculations and statistical analysis**
A stepwise population pharmacokinetic approach was followed by employing a nonlinear mixed effects model and the first order estimation method (FO), implemented with NONMEM, version VI (Beal and Sheiner, 1989) in conjunction with a G77 FORTRAN compiler and Wings for NONMEM (http://wfn.sourceforge.net). Different subroutines were used; namely ADVAN 3, 11 and 9. The experimental data used to build the model was total plasma concentration of SQV.

The model was developed sequentially, so that whenever modifications had to be made to the base structure of the model, a backwards procedure was performed. Data were also incorporated in a sequential fashion. The analysis consisted of the following steps:

- **Step 1:** SQV IV data from groups 1,2,3,9.
- **Step 2:** SQV IV data from groups 1,2,3 and 9 & IP data from groups 7 and 8.
- **Step 3:** SQV IV & IP data from groups 1,2,3,9,7 and 8 & oral data from groups 4,5 and 6.
- **Step 4:** SQV IV & IP data from groups 1,2,3,9,7 and 8 & oral data from groups 4,5,6 and 10.

As a first approximation to the data, a more empirical analysis was performed at each step using the linear pharmacokinetic model, which allowed different parameter values to be estimated for each of the groups assayed. Based on the information obtained, a more mechanistic analysis was subsequently 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 IP absorption were fixed at those obtained in step 2, as the addition of oral data did not affect them.

The differences between individual parameters were regarded as random and were modelled in terms of eta (\(\eta\)) variables. \(\eta\) variables were assumed to be normally distributed with a mean of zero and to have an estimated variance of \(\omega^2\). The need to include interindividual variability (IIV) terms was evaluated for all parameters. The differences between the observed plasma concentrations and the predicted plasma concentrations were regarded as random and were modelled in terms of epsilon (\(\epsilon\)) variables. Each \(\epsilon\) variable was assumed to have a mean of zero and an estimated variance \(\sigma^2\). A slope-intercept error model was employed where the residual variability (\(\sigma\)) of the exponential term was interpreted as a coefficient of variation (CV) and the added component as a standard deviation (SD).
In the selection of the best model, the minimum value of objective function (MOFV) provided by NONMEM was adopted. For hierarchical models, the difference between objective function values is distributed as $\chi^2$, which allows the best model to be selected. A $p$ level of 0.005 was chosen as the criterion for accepting a more complex model over a reduced one. The difference between the objective function values of hierarchical models varying by one or two parameters was 7.879 and 10.596, respectively. The precision of the estimation of the parameter values, quantified as the relative standard error (%), was also evaluated, and a graphical goodness of fit analysis was performed using SPlus for Windows, version 7.0 (Insightful).
Results

The SQV total plasma concentration-time profiles obtained after IV, IP and PO administration are shown in Figure 1.

The experimental data used in the development of the model were those of total plasma concentration. Table II shows the key models of a series of tested models at different stages of the data analysis. The parameters considered by the model - random estimated variables (IIV) and minimum objective function values (MOFV) - are also shown.

A Michaelis-Menten elimination process was carried out to throw light on the distribution, metabolism and excretion processes (disposition phase, step 1) in which the empirical model had revealed nonlinear phenomena, but failed to provide any relevant information (Models 1 & 2-Table II). Taking into account that 97% of SQV binds to plasma proteins (Holladay et al., 2001), a dynamic and saturable plasma protein binding could explain the variability in clearance ($\text{Cl}_{G9}<\text{Cl}_{G1}<\text{Cl}_{G3}<\text{Cl}_{G2}$) highlighted by the empirical model, which showed that clearance (Cl) and volume of central compartment (Vc) differed in each group (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. Similarly, with the 48 mg dose, there could be saturation of both the plasma protein binding and elimination processes.

After incorporating the IP data (step 2), and taking into account the low solubility of the drug, a precipitation of the drug in the peritoneal cavity was considered. Experimental observation confirmed this (data not shown), which suggests that dissolution limited the absorption of the drug (Model 6-Table II). In this model, the amount of precipitation depends on the dose administered ($Q_{\text{pp}(Gi)}$), as the amount of drug dissolved is the same as that administered ($Q_{\text{dis}(Gi)}$). Subsequently, the precipitated fraction ($FR_{(Gi)}$) and dissolution rate constant ($k_D$) are estimated for each group.

Finally, after incorporating the oral data (step 3), a more semi-physiological approach was considered to define the pharmacokinetic profile of SQV (Models 8 to 11, Table II). Physiological expressions were incorporated into the model; $Q_h$ represents hepatic flow, $Q_{ah}$ represents arterio-hepatic flow and $Q_e$ represents mesenteric flow. Based on data in the literature, $Q_{ah}$ and $Q_e$ were considered to constitute 13% and 87% of the total hepatic flow respectively, and the hepatic flow for a 300g 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 (Q_h, V_h, V_e) (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), while the transit compartment rate constant (K_T) 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 (K_{m_{\text{se}}}) of 0.02 mg/L and an intrinsic blood clearance of intestinal secretion (C_{l_{\text{se}}}) of 2.83·10^{-6} L/h. In addition, a metabolic process was detected in the liver and intestine, with a Michaelis-Menten constant (K_m) of 72.0 mg/L in both. Due to RTV being a potent inhibitor of intestinal and hepatic metabolism and intestinal secretion processes, a factor was introduced in order to quantify the modification of K_m when RTV was coadministered. A value of 5.16 was estimated for group 9 (FK_m(G9)) and a value of 10.2 was estimated for group 10 (FK_m(G10)). Precipitation of the drug in the IP cavity and dissolution constants k_D for the 24 and 12 mg were also estimated. The dissolution constants k_D varies from one dose to another indicating that different sizes of the solid particle precipitated from each of the doses administered.

Finally, plasma protein binding also was takes into consideration, which occurs through a dynamic equilibrium characterized by the maximum amount of drug that binds to proteins (Q_{MA}). Table III reports the estimated values for the pharmacokinetic parameters of the final model (Model 9, covariance step aborted). Individual SQV plasma concentration values predicted with the selected model (Model 9) are shown in Figure 1. Individual and population predicted versus observed SQV plasma concentrations are plotted in Figure 3. Weighted residuals (WRES) versus time, and absolute individual weighted residuals |IWRES| versus individual predictions are also shown.

Finally, Figure 4 shows the predicted fractions of the drug dose that escaped the effects of the intestinal and hepatic first-pass loss (first stage), which depended on the hepatocyte and enterocyte concentrations of the drug.
Discussion

Evidence in the literature indicates that SQV is a substrate of CYP3A4, gp-P and CYP3A5 (Frohlich et al., 2004; Munoz et al., 2005) and that the low and variable bioavailability of SQV is not only a result of hepatic metabolism. Moreover, contradictory data exists with respect to the effect of intestinal first-pass loss on the oral bioavailability of SQV. Such discrepancies may be explained, at least in part, by the extent to which this process varies depending on the animal model employed.

It is well documented that CYP 3A4 is the major enzyme responsible for about 90% of SQV metabolism. Due to the relative abundance of CYP 3A4 enzyme in the human gut, a 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, 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 around 4% due to the intestinal metabolism and secretion processes in these animals (Sinko et al., 2004). In contrast, CYP 3A4 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 modellistic approach. The influence of RTV on the metabolism SQV was also assessed, since the combination SQV/RTV has been shown to be clinically effective because of the way in which the former augments exposure to the latter (Hoffman et al., 2007).

In order to imitate clinical practice, we selected a 24 mg dose of SQV, as, when scaled in rats (FDA, 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) (F.D.A., 2005).

The non-compartmental analysis showed nonlinearities in the absorption and elimination process (data not shown). However, results of area under the curve (AUC), clearance (Cl), and half-life (t1/2) were contradictory and was not possible a consistent interpretation. Moreover, neither was possible to distinguish the roles of the liver and intestine in the elimination process. In this scenario, the individual analysis of data was
aborted and a population analysis by means of the non-linear mixed effects modeling was performed, since has proved that it is a very useful tool to model different kinetic processes (Ruiz-Carretero et al., 2004; Munoz et al., 2005; Campos Moreno et al., 2007). The advantage of the non linear 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 while 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 IP administration and protein binding kinetics. The intrinsic blood clearance of intestinal secretion (Cl_{int}) indicate a low affinity for the intestinal efflux carrier and the fact that the metabolic process detected in the liver and intestine is governed by a enzymatic system characterized by the same value of the Michaelis-Menten constant (Km) indicate 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 Km when RTV was coadministered indicate 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 (PO) than for group 9 (IV) suggests that inhibition of the first-pass intestinal metabolism was greater than that of the first-pass hepatic metabolism. In line with this, the blood intrinsic clearance in hepatocytes (Cl_{hi}) and enterocytes (Cl_{ie}) again indicated that the main metabolic process of SQV occurs in the intestine in rats.

By employing the semiphysiologic model it is possible to calculate the extraction rate according to the corresponding concentration in the enterocyte or hepatocyte compartments at any time-point (Figure 4). In this sense, the fraction of dose escaping from the intestine (F_{i}) 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- 86%, which are far from that of 56% reported by Sinko et al. (Sinko et al., 2004).
When RTV was coadministered, values of 85-93% and 96-98% 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. (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-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 the opposite trend to that observed in the present study. Oral bioavailability was 4.1%, with a fraction of absorption of 8.3. Conversely, Shen et al. reported that the considerable presystemic losses of SQV could be due to an intestinal first-pass metabolism (Shen et al., 1997), 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 maximum amount of drug that binds to proteins (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 $Cl_{ISE}$ lacked physiological significance, and as a consequence, the model was reevaluated without plasma protein binding and intestinal secretion processes (model 10, Table II). This new model provided a value for intrinsic blood clearance in hepatocytes ($Cl_i$), which represented 0.27% of the enterocyte clearance ($Cl_e$). This 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 as a potent competitive inhibitor of this process, as the increase in $Km$ was higher when the two protease inhibitors were co-administered orally (model 11; $FRKmG10=38$ and $FRKmG9 =5$, by oral and intravenous route, respectively). However, the statistical criteria (MVOF) for model 11 (Table II) show that eliminating plasma protein binding, intestinal secretion and hepatocyte metabolism processes from consideration was not justified.
Pharmacokinetic model developed in this study could be only 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 parameters values could be only used in the animal species in which has 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 appears to be the main cause of its poor oral bioavailability. Nevertheless, we believe that the application of the non-linear mixed effect technique allows a robust estimation of the population pharmacokinetic parameters of SQV and their variability, and in spite of the complexity is a useful method for evaluating the impact of hepatic and intestinal first-pass loss on oral bioavailability in rats.
Authorship contribution

Rocío Lledó-García carried out the animal experiments and was responsible for the collection of data and the presentation and interpretation of the results. Amparo Nácher contributed to the data collection, and the preparation of the manuscript. Vicente G. Casabó participated in the design of the study, contributed to the interpretation of the results and critically reviewed the paper. Matilde Merino-Sanjuán participated in the design of the study, contributed to the presentation and interpretation of the results, and coordination and critically reviewed the paper. All authors contributed to the writing of the manuscript.
References


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Legends of figures

Figure 1.

Graphical representation of experimental concentrations (Cpexp) (mg/L) vs time (h) and the individual predicted concentrations (Cppred) (mg/L) according to model 9, after applying it to IV, IP and PO SQV data (groups 1, 2, 3, 4, 5, 6, 7, 8, 9 & 10).

Figure 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 & 9 are central, peripheral, plasma protein-binding, first intestinal transit lumen, IP drug-dissolved, IP drug-precipitated, hepatocyte, second intestinal transit lumen and enterocyte compartments, respectively. Ai and Ci are the amounts (mg) of SQV and concentrations (mg/L) in each compartment (i), respectively. CMA (mg/L) 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 (mg/L). K_B (l·mg/h) and K_U (h^(-1)) are the rate constants of binding and unbinding to proteins. Cp is the total SQV plasma concentration (mg/L). Q, Q_h, Q_{ah} & Q_e are inter-compartmental, hepatic, arterio-hepatic and mesenteric flows (L/h), respectively. E_h, E_{se}, E_{me} and E_E 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). F_h and F_E are the fractions of drug escaping from the liver and intestine, respectively. K_{AIP}, K_a1 and K_a2 are the absorption rate constants after IP and oral administration (h^(-1)). K_T is the intestinal transit rate constant (h^(-1)). F_R is the fraction of dose which precipitated after IP administration. K_D is the dissolution rate constant after IP administration (h^(-1)). K_{12} and K_{21} the inter-compartmental rate constants (h^(-1)). Vc and Vp the volume of distribution in central and peripheral compartments (l). K_m, K_me and K_mse are the Michaelis-Menten constants (mg/L) in the metabolic process in the liver and in the metabolic and secretor processes in the intestine, respectively. V_m, V_me and V_mse are the maximal rate for an enzyme (mg/h) in the metabolic process in the liver and in the metabolic and secretor processes in the intestine, respectively.
Figure 3.
Goodness of fit plots (Model 9). The upper panels show observed concentrations versus predicted concentrations for the typical individual on the left and versus predictions for each individual on the right, together with the identity line. The lower panels show the weighted residuals for the typical individual (WRES) versus time and the absolute individual weighted residuals |IWRES| versus predictions for each individual.

Figure 4
Graphical representation of the relationship between the fractions of dose escaping from the intestine ($F_E$) and liver ($F_h$) and the concentrations detected in those compartments. Population pharmacokinetic parameters and the range of concentrations of SQV in hepatocyte ($C_7$) and enterocyte ($C_9$) compartments are those predicted by model 9. The interaction was considered when calculating $F_h$ and $F_E$ for groups 9 and 10, so that fractions were calculated taking into account that clearance was affected by the empirical factor, which multiplied $K_m$. This is a constant empirical factor and depends on the RTV concentration in these two compartments.
Table I. Experimental design.

<table>
<thead>
<tr>
<th>Group</th>
<th>Route and administration method</th>
<th>Drug and Dose (mg) administered</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Intravenous (infusion) (0.5 h)</td>
<td>SQV 48</td>
</tr>
<tr>
<td>2</td>
<td>Intravenous (bolus)</td>
<td>SQV 24</td>
</tr>
<tr>
<td>3</td>
<td>Intravenous (bolus)</td>
<td>SQV 12</td>
</tr>
<tr>
<td>4</td>
<td>Oral</td>
<td>SQV 48</td>
</tr>
<tr>
<td>5</td>
<td>Oral</td>
<td>SQV 24</td>
</tr>
<tr>
<td>6</td>
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<td>7</td>
<td>Intraperitoneal</td>
<td>SQV 24</td>
</tr>
<tr>
<td>8</td>
<td>Intraperitoneal</td>
<td>SQV 12</td>
</tr>
<tr>
<td>9</td>
<td>Intravenous (infusion) (0.5 h)</td>
<td>SQV 24 &amp; RTV 6</td>
</tr>
<tr>
<td>10</td>
<td>Oral</td>
<td>SQV 24 &amp; RTV 6</td>
</tr>
</tbody>
</table>

[a] Infusion time.
### Table II

The table shows some of models tested and the selected models in each step of the analysis (in bold). Parameters considered in the model, number (N) of parameters estimated in the step, random variables estimated (IIV) and minimum objective function value obtained (MOFV) are shown.

<table>
<thead>
<tr>
<th>Model</th>
<th>Parameters &amp; comments</th>
<th>N parameters</th>
<th>N IIV$^{[a]}$</th>
<th>MOFV</th>
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<td></td>
<td>Two-compartments; MM elimination + Km different G9</td>
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<td>3</td>
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<td>8</td>
<td>4</td>
<td>514.9</td>
</tr>
<tr>
<td></td>
<td>Two-compartments; plasma protein dynamic binding + MM elimination (Km different G9)</td>
<td>9</td>
<td>6</td>
<td>483.5</td>
</tr>
<tr>
<td>STEP II: IP data added</td>
<td>K$_{aIP}$ and F different for each IP group (F for G8 fix to 1; (rest as in Model 4)</td>
<td>12</td>
<td>6</td>
<td>357.9</td>
</tr>
<tr>
<td></td>
<td>Dissolution as limitative factor for absorption (rest as in Model 4)</td>
<td>14</td>
<td>Restrict.</td>
<td>334.8</td>
</tr>
<tr>
<td></td>
<td>Same absorption (k$_a$ and F) parameter values for all PO groups (rest as in Model 6)</td>
<td>2</td>
<td>Restrict.</td>
<td>192.5</td>
</tr>
<tr>
<td></td>
<td>Considers a portal-space compartment with a MM elimination process &amp; two intestinal transit compartments+ efflux process to one of lumen compartments+ two different k$_a$s from each intestinal transit compartment; (rest as in Model 6)</td>
<td>7</td>
<td>Restrict.</td>
<td>163.2</td>
</tr>
<tr>
<td></td>
<td>Considers hepatocyte and entorocyte compartments with MM elimination processes &amp; two intestinal transit compartments+ efflux process to one of lumen compartments+ two different Ka from each intestinal transit compartment; (rest as in Model 6)</td>
<td>13</td>
<td>Restrict. (estimated in this step:8)</td>
<td>195.3</td>
</tr>
<tr>
<td></td>
<td>Takes also into account the interaction between SQV and RTV at the enzymatic level.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Considers hepatocyte and entorocyte compartments with MM elimination processes &amp; one intestinal transit compartments+ one Ka from intestinal compartment; dissolution as limitative factor for IP absorption; (rest as model 2)</td>
<td>8</td>
<td>Restrict. (estimated in this step:6)</td>
<td>342.8</td>
</tr>
<tr>
<td></td>
<td>Takes also into account the interaction between SQV and RTV at the enzymatic level.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hepatocyte compartment is not considered. Rest as Model 10</td>
<td>6</td>
<td>Restrict. (estimated in this step:5)</td>
<td>342.8</td>
</tr>
</tbody>
</table>

$^{[a]}$ Restrict. Restriction of the random variables, were fixed to the values obtained in prior steps.

F: bioavailability; G: group; IP: intraperitoneal administration; Km: constant of Michaelis-Menten kinetics; k$_a$: intestinal absorption rate constant; k$_{aIP}$: intraperitoneal absorption rate constant; MM: Michaelis-Menten kinetic; MOFV: Minimal objective function value; PO: oral administration; RTV: ritonavir; SQV: Saquinavir.
Table III. Population PK model parameters (fixed and random effects) for models 9 and 11.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Model 9</th>
<th>IIV %</th>
<th>Model 11</th>
<th>IIV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clι (L/h)</td>
<td>Intrinsic blood clearance in hepatocytes</td>
<td>0.31</td>
<td>91</td>
<td>NE</td>
<td></td>
</tr>
<tr>
<td>Km=Kmι (mg/L)</td>
<td>Michaelis–Menten constant of metabolic process in liver and enterocyte</td>
<td>72.0</td>
<td>NE</td>
<td>148</td>
<td>NE</td>
</tr>
<tr>
<td>k12 (h-1)</td>
<td>Inter compartmental rate constant</td>
<td>2.80[a]</td>
<td>NE</td>
<td>2.31[a]</td>
<td>NE</td>
</tr>
<tr>
<td>k21 (h-1)</td>
<td>Inter compartmental rate constant</td>
<td>1.14[a]</td>
<td>37[a]</td>
<td>0.63[a]</td>
<td>71[a]</td>
</tr>
<tr>
<td>Vc (L)</td>
<td>Volume of distribution in central compartment</td>
<td>0.24[b]</td>
<td>73[b]</td>
<td>0.38[a]</td>
<td>60[a]</td>
</tr>
<tr>
<td>k8 (L/mg.h)</td>
<td>Protein binding rate constant</td>
<td>1.12·10^-6</td>
<td>40[a]</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ku (h-1)</td>
<td>Protein unbinding rate constant</td>
<td>0.20</td>
<td>78[a]</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>QMA (mg)</td>
<td>Maximum binding capacity of the proteins for SQV</td>
<td>21,700</td>
<td>51[a]</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ka IP (h-1)</td>
<td>Absorption rate constant for intraperitoneal administration</td>
<td>1[a]</td>
<td>NE</td>
<td>1[a]</td>
<td>NE</td>
</tr>
<tr>
<td>Bio IP</td>
<td>Fraction of amount absorbed by intraperitoneal route</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FR(G8)</td>
<td>Fraction of dose which precipitate after intraperitoneal administration in group 8</td>
<td>0.97[a]</td>
<td>NE</td>
<td>0.87[a]</td>
<td>NE</td>
</tr>
<tr>
<td>FR(G7)=(1+FR(G8))/2</td>
<td>Fraction of dose which precipitate after intraperitoneal administration in group 7</td>
<td>0.98[a]</td>
<td>NE</td>
<td>0.93[a]</td>
<td>NE</td>
</tr>
<tr>
<td>kD(G7) (h-1)</td>
<td>Dissolution rate constant for group 7</td>
<td>0.04[a]</td>
<td>NE</td>
<td>0.04[a]</td>
<td>NE</td>
</tr>
<tr>
<td>kD(G8) (h-1)</td>
<td>Dissolution rate constant for group 8</td>
<td>0.48[a]</td>
<td>NE</td>
<td>4.46[a]</td>
<td>NE</td>
</tr>
<tr>
<td>Ka1 (h-1)</td>
<td>Absorption rate constant for oral administration from intestinal compartment</td>
<td>0.24</td>
<td>56</td>
<td>0.24</td>
<td>1.5</td>
</tr>
<tr>
<td>kT (h-1)</td>
<td>Intestinal transit rate constant</td>
<td>0.58</td>
<td>61</td>
<td>0.12</td>
<td>126</td>
</tr>
<tr>
<td>Qh (L/h)</td>
<td>Hepatic flow</td>
<td>1.15[b]</td>
<td>6.5[b]</td>
<td>1.15[b]</td>
<td>6.5[b]</td>
</tr>
<tr>
<td>Vh (L)</td>
<td>Volume of distribution in hepatocyte compartment</td>
<td>0.01[b]</td>
<td>NE</td>
<td>0.01[b]</td>
<td>NE</td>
</tr>
<tr>
<td>Ve (L)</td>
<td>Volume of distribution in enterocyte compartment</td>
<td>0.006[b]</td>
<td>NE</td>
<td>0.006[b]</td>
<td>NE</td>
</tr>
<tr>
<td>Clιe (L/h)</td>
<td>Intrinsic blood clearance due to metabolism in enterocytes</td>
<td>4.35</td>
<td>92</td>
<td>2.35</td>
<td>113</td>
</tr>
<tr>
<td>Clιie (L/h)</td>
<td>Intrinsic blood clearance of intestinal secretion</td>
<td>2.83·10^-6</td>
<td>6.9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Kmιe (mg/L)</td>
<td>Michaelis Menten constant of enterocyte secretion process</td>
<td>0.02</td>
<td>NE</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Ka2 (h-1)</td>
<td>Absorption rate constant after oral administration from intestinal compartment</td>
<td>0.17</td>
<td>59</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>FKm (G9)</td>
<td>Factor that modified the Michaelis Menten constant for group 9</td>
<td>5.16</td>
<td>5.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FKm (G10)</td>
<td>Factor that modified the Michaelis Menten constant for group 10</td>
<td>10.2</td>
<td>38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>σ1 (CV%)</td>
<td>Residual variability. Exponential component</td>
<td>12.2</td>
<td>24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>σ2 (mg/L)</td>
<td>Residual variability. Additive error</td>
<td>0.29</td>
<td>0.23</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

IIV: inter individual variability; NE: Not estimated; [a] Parameters fixed to the estimated values obtained in previous steps. [b] Parameters fixed to physiological values from literature.
Figure 1
\[
\begin{align*}
\frac{dA_1}{dt} &= -K_D \cdot Cu \cdot (CMA - Cb) \cdot Vc + K_a \cdot Cb \cdot Vc + K_{21} \cdot A_2 - K_{12} \cdot A_1 \\
&\quad + Q_h \cdot C_7 \cdot F_h - Q_h \cdot Cu \\
\frac{dA_2}{dt} &= K_{12} \cdot A_1 - K_{21} \cdot A_2 \\
\frac{dA_3}{dt} &= K_B \cdot (CMA - Cb) \cdot Cu \cdot Vc - K_a \cdot Cb \cdot Vc \\
&\quad \text{being} \quad C_p = Cu + Cb \\
\frac{dA_4}{dt} &= -K_{a1} \cdot A_4 - K_T \cdot A_4 \\
\frac{dA_5}{dt} &= -K_{AIP} \cdot A_5 + K_D \cdot A_6 \\
\frac{dA_6}{dt} &= -K_D \cdot A_6 \\
\frac{dA_7}{dt} &= Q_{ah} \cdot Cu + Q_e \cdot C_9 \cdot F_E - Q_h \cdot C_7 \cdot F_h - Q_h \cdot C_7 \cdot E_h + K_{AIP} \cdot A_5 \\
\frac{dA_8}{dt} &= -K_{a2} \cdot A_8 + K_T \cdot A_4 + Q_e \cdot C_9 \cdot E_{se} \\
\frac{dA_9}{dt} &= -Q_e \cdot C_9 \cdot F_E - Q_e \cdot C_9 \cdot E_E + Q_e \cdot Cu + K_{a1} \cdot A_4 + K_{a2} \cdot A_8 
\end{align*}
\]