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Pharmacokinetic modeling of gastric emptying and small intestinal transit time in dogs using paracetamol and sulfasalazine as markers

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Abstract

In this thesis, two pharmacokinetic models are evaluated with respect to their capacity to quantify gastric emptying and small intestinal transit in dogs. The experiments are based on the double marker technique, which uses two drug substances (paracetamol and sulfasalazine) that are absorbed in the duodenum and the colon, respectively. A standard model is compared to a more sophisticated transit rate model. It is shown that both models provide reasonable predictions for the plasma levels of paracetamol, but for the sulfasalazine data, the transit rate model produce considerably better fit than the standard model. Based on this study, the transit rate model seems to be the best choice for the analysis of double marker experiments.

Keywords

Pharmacokinetic models, double marker technique, gastric emptying, intestinal transit time.

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Sammanfattning

Det är vanligt att läkemedel påverkar magtarmkanalen och ger biverkningar såsom diarré eller förstoppning. Därför är det önskvärt att finna metoder som redan på ett tidigt stadium i utvecklingen av nya läkemedel kan avgöra huruvida de påverkar mag- eller tarmfunktion.

Genomloppstiden för mage och tarm kan mätas med hjälp av t.ex. radioaktiva isotoper. Denna studie behandlar istället den så kallade “double marker”-metoden som baseras på blodprov, vilket anses vara ett mindre ingrepp. Paracetamol och sulfapyridin tas upp i olika delar av magtarmkanalen (tolvfingertarm respektive tjocktarm). Genom att använda dem som markörer och jämföra deras plasmanivåer vid olika tidpunkter kan genomloppstider bestämmas.

För att tillförlitligt beräkna när ett läkemedel först dyker upp i plasman med direkta mätningar krävs täta blodprov, vilket ofta inte är etiskt försvarbart i djurförsök. Målet med detta examensarbete är att studera hur farmakokinetiska modeller kan användas för mer robust analys av ett begränsat antal mätpunkter. Två olika modeller implementeras och utvärderas med avseende på hur väl de beskriver magsäckstömningshastigheten respektive genomloppstiden i tarmkanalen hos hundar. Den mer avancerade “transit rate”-modellen där tarmkanalen delats upp i flera delsystem visar sig ge bättre resultat än den enklare standardmodellen, framför allt vid studien av sulfapyridin.

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Uppsala universitet November 2005

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1 Introduction

The development of new drug substances is a complex, time consuming and expensive process. Reducing the time for drug development and avoiding late termination of candidate drugs are important challenges for the pharmaceutical industry.

Adverse effects due to altered gastrointestinal function have been reported to account for around 18% of all adverse drug reactions (Lewis, 1986). This includes diarrheas, constipation and other reactions directly related to gastrointestinal transit. Altered gastrointestinal transit or gastric emptying rates may also affect the total uptake of other drug substances. As a consequence, it is desirable to be able to screen for drug substances that alter the gastrointestinal transit already in an early stage of the drug development process.

There are different approaches to estimate the gastric emptying rate and gastrointestinal transit time. Scintigraphic measurement uses radioactive isotopes and image analysis (Iwanaga et al., 1998). In breath tests, an isotope (for example ^{13}C) is given orally and the level of particles in the breath marked by that isotope (for example $^{13}CO_2$) is measured (Lee et al., 2000). These have disadvantages in terms of costs or invasiveness. In the present investigation, we use the double marker technique which is based on measurements of the plasma levels for two drug substances that are absorbed in different parts of the gastrointestinal system (Mizuta et al., 1990). Because it may be difficult to accurately estimate when a drug substance first appears in the plasma based on observations (at least when their number is limited), pharmacokinetic models are used.

The pharmacokinetic models used for predicting and analyzing the pharmacokinetic profiles of drug substances administered orally are simplifications of complex processes. Specifically, although gastric emptying and transit time can affect drug absorption these processes are seldom taken into account (Yu and Amidon, 1999). In this thesis, two pharmacokinetic models are considered: the standard model and the transit rate model. The standard model consists of an absorption compartment and a central compartment where the measurements are made (the plasma). It does not explicitly account for the gastric emptying rate and the intestinal transit time. The transit rate model does. It divides the gastrointestinal tract into the stomach, the small intestine and the colon. The small intestine is, in turn, further partitioned into six segments and the flow through the segments is described by first order kinetics. Simulations are also carried out for a lag time model. This model also divides the gastrointestinal tract into the stomach, the small intestine and the colon, but is based on residence times rather than transit rates.

1.1 Pharmacokinetics

Pharmacokinetics is the study of drug kinetics and the processes that affect drug substances in the body. It includes absorption, distribution, metabolism and elimination (see figure 1). The effect of a drug is often related to the concentration of the drug at the site of action and the aim of pharmacokinetics is to describe the rate of change of concentrations.

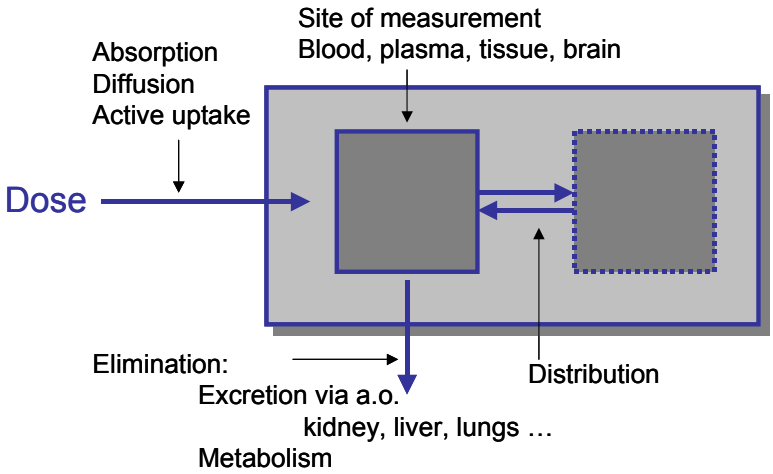


Figure 1. Pharmacokinetics is the study of drug kinetics in the body and describes the processes of absorption, distribution, metabolism and elimination. The illustration was used with permission from Sandra Visser at AstraZeneca R&D Södertälje.

Since pharmacological response is related to the concentration of a drug at the site of action, usually a receptor site, it is desirable to know the extent to which the drug substance is available at that site. However, it is generally difficult to measure drug concentrations directly at the site of action and therefore, one normally measures drug concentration in the blood instead. The bioavailability of a drug substance (F) indicates the extent to which it is absorbed and becomes available in the systemic circulation. Bioavailability ranges from 0 (0% has reached the systemic circulation) to 1 (100%). A drug given intravenously has a bioavailability of 1 whereas the bioavailability is typically lower for other routes of administration. For example, drugs given per orally may be degraded before reaching the plasma, they may be unabsorbable from the lumen due to permeability or solubility problems or they may be metabolized or eliminated by the liver before reaching the systemic circulation.

The volume of distribution is generally defined as the apparent volume into which a drug distributes in the body at equilibrium. It can be estimated by dividing the dose given intravenously by the plasma concentration immediately after injection (before any elimination takes place). A small volume of distribution means that a larger fraction of the dose is in the plasma while a large volume of distribution implies that more of the dose is distributed for example to the tissues or bound to plasma proteins.

Clearance is a parameter that describes how fast a drug is eliminated from the body. It is defined as the amount eliminated each time unit divided by the concentration in the blood. Thus, it corresponds to a volume of plasma completely cleared of the drug.

The absorption rate indicates the speed with which a drug substance is taken up by the body. It depends on, for example, the gastric emptying rate, the gastrointestinal transit time, permeability and solubility.

1.2 Double marker technique

The double marker technique is a method to determine the gastrointestinal transit time using paracetamol (acetaminophen) and sulfasalazine (salicylazosulfapyridine) as markers (Mizuta et al., 1990).

Paracetamol is a marker compound for the estimation of the gastric emptying rate (Mizuta et al., 1990, Sagara et al., 1995, Levein et al., 1999). The substance is poorly absorbed from the stomach but rapidly so from the small intestines. Therefore, the time of appearance of paracetamol in the plasma correlates with the gastric emptying time.

Sulfasalazine, on the other hand, is used as a marker compound to estimate the small intestine transit time. In the colon, sulfasalazine is metabolized by bacteria to sulfapyridine, which in turn is absorbed (Mizuta et al., 1990). Hence, the time of first appearance of sulfapyridine in the plasma correlates with the time it takes for sulfasalazine to reach the colon.

Together these substances are expected to provide information about gastric emptying and small intestinal transit time in Beagle dogs. The gastrointestinal tract of dogs resembles that of humans with a simple, one compartment stomach, similar gastric motility patterns to those found in man and with approximately exponential emptying of non-nutrient liquids in the fasted state (de Zwart et al., 1999). Figure 2 shows a schematic illustration of the gastrointestinal tract of a dog from the stomach via the different parts of the small intestine to the colon.

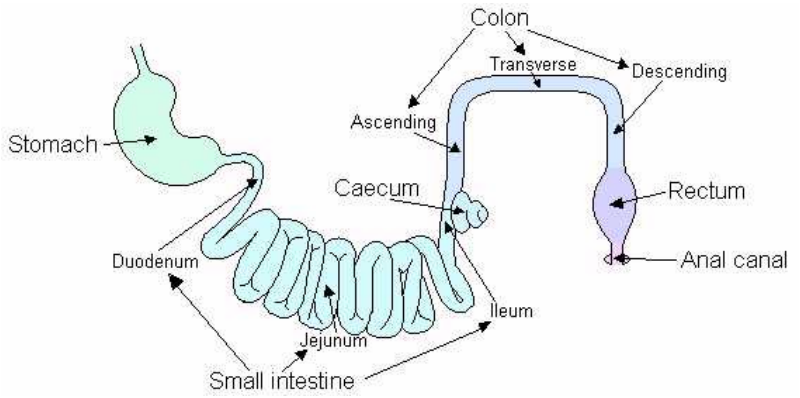


Figure 2. A schematic illustration is shown over the gastrointestinal of a dog. The illustration was used with permission from Sandra Visser at AstraZeneca R&D Södertälje.

1.3 Aim

The aim of this thesis is to evaluate two pharmacokinetic models, the standard model and a transit rate model, with respect to their capacity to quantify drug-induced changes in gastric emptying rate and intestinal transit time *in vivo*.

2 Materials and methods

2.1 *In vivo* experiments and analytical procedure

The *in vivo* experiments were conducted at the Department of General Pharmacology and the analytic procedures of the blood samples were performed at the Department of DMPK and Bioanalytical Chemistry, both at AstraZeneca R&D Södertälje, Sweden.

2.1.1 Animals

Six male Beagle dogs weighing 16.5 - 21.5 kg were used (supplier Kennel Rååhöjden, Sweden). The dogs were housed in animal rooms, and the dogs were exercised together daily in a yard. The animal rooms and the exercise yards were illuminated by daylight from skylights. As a supplement, fluorescent tubes were used between 07.00 and 17.00. The target value for the temperature was 20°C with permitted deviations in the interval from 15°C to 25°C. Once daily at around 15:00, the dogs received 200-350 grams of a dog diet for laboratory use (Specific CXD, from Leo Pharmaceuticals in Denmark). Municipal tap water for human consumption was available to the dogs at all times via an automatic watering system. The dogs were acclimatized to laboratory conditions for at least 1 month before the experiments.

2.1.2 Drug substances and dosing

Single doses of atropine (0.06 mg/kg), erythromycin (1 mg/kg), morphine (0.05 mg/kg) or placebo (saline) were administered intravenously via the cephalic vein. Each infusion was given in a volume of 1 mL/kg over a period of 15 minutes. All dogs received each treatment in randomized order at four different occasions. The order of administration is listed in table 1.

Atropine inhibits the effect of acetylcholine and reduces the motor activity of the stomach, intestine and colon. Erythromycin acts as a motilin agonist and induces strong contractions in the stomach and the duodenum. Morphine is an opioid and can affect gastrointestinal function via both central and peripheral sites of action. Opioids delay gastric emptying and prolong small and large intestinal transit. An intravenous injection of morphine may however initially stimulate intestinal motility followed by a subsequent later long-lasting suppression of motility.

Fifteen minutes after the infusion of a test substance (saline, atropine, erythromycin or morphine) a solution of paracetamol (24 mg/kg, 1 mL/kg) and sulfasalazine (20 mg/kg, 1 mL/kg) was administrated per orally directly to the stomach, by intubation using rubber tubing connected to a 30 mL syringe.

Table 1. Dosing overview. Each of the six dogs received atropine (0.06 mg/kg), erythromycin (1 mg/kg), morphine (0.05 mg/kg) and saline with at least 3 days between the doses.

Dog	Dose 1	Dose 2	Dose 3	Dose 4
Dog 1 Ior	Saline	Erythromycin	Atropine	Morphine
Dog 2 Tarzan	Morphine	Atropine	Saline	Erythromycin
Dog 3 Atlas	Erythromycin	Atropine	Saline	Morphine
Dog 4 Basset	Atropine	Morphine	Erythromycin	Saline
Dog 5 Hubert	Atropine	Morphine	Erythromycin	Saline
Dog 6 Måns	Erythromycin	Saline	Morphine	Atropine

2.2 Models

2.2.1 The standard model

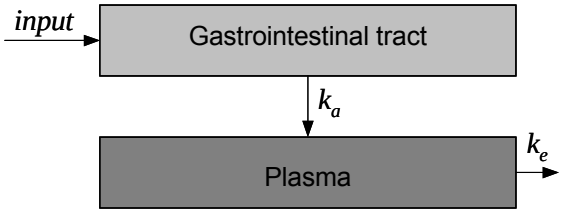


Figure 3. The standard model is composed of two compartments: the gastrointestinal tract and the plasma. k_a is the absorption rate constant and k_e is the elimination rate constant.

The standard model consists of an absorption compartment (the gastrointestinal tract) and a central compartment (the plasma) as is illustrated in figure 3. It may incorporate a lag time before the dose appears in the absorption compartment. The absorption is assumed to follow a first order process with absorption rate constant k_a and the elimination follow a first order process with elimination rate constant k_e.

The differential equation for the absorption process from the gastrointestinal compartment is:

$$\begin{cases} \dfrac{dA(t)}{dt} = -k_a \cdot A(t) \\ A(0) = input \end{cases} \tag{1}$$

$$input = \begin{cases} 0 & time < t_{lag} \\ dose & time \geq t_{lag} \end{cases} \tag{2}$$

where *A(t)* is the amount of a drug at time *t*, k_a is the absorption rate constant, *dose* denotes the dose administered per orally and t_{lag} is the time before absorption can take place. If there is no lag time (t_{lag} = 0) the dose given is available for absorption at time zero (t = 0). If a nonzero lag time is included, there is a delay and no absorption takes place before a certain time (t_{lag} time units).

The differential equation for the change in plasma drug concentration is:

$$\begin{cases} \dfrac{dC(t)}{dt} = \dfrac{k_a \cdot A(t)}{V} - k_e \cdot C(t) \\ C(0) = 0 \end{cases} \tag{3}$$

where V denotes the volume of distribution. k_e is the elimination rate constant which can be defined as

$$k_e = \frac{CL}{V} \tag{4}$$

where CL is the plasma clearance.

2.2.2 The transit rate model

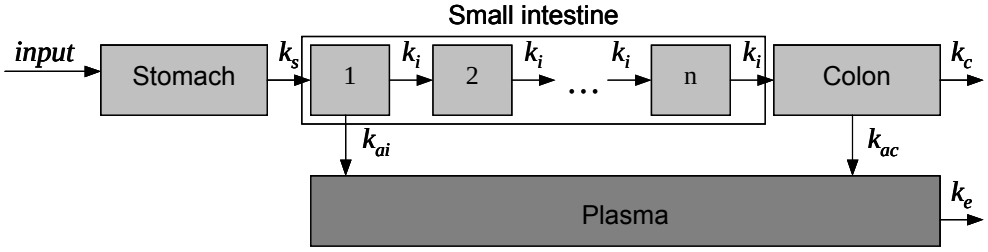


Figure 4. In the transit rate model the gastrointestinal tract is partitioned into the stomach, the small intestine and the colon. The small intestine is subsequently divided into n segments. k_s, k_i and k_c are the transit rate constants, k_{ai} and k_{ac} are the absorption rate constants and k_e is the elimination rate constant.

In the transit rate model the gastrointestinal tract is divided into three segments: the stomach, the small intestine and the colon, as shown in figure 4. The small intestine, in turn, is partitioned into *n* segments. Different choices for *n* are possible. The small intestine could be divided into three parts: the duodenum, the jejunum and the ileum. Another possibility, chosen here, is to let *n* be 6 and divide the small intestine into the duodenum, the upper jejunum, the lower jejunum, the upper ileum, the lower ileum and the caecum like Sawamoto et al (1997). Yu et al (1996) showed that the transit flow in the human small intestine could be described by first order kinetics through seven serial compartments.

The rate of gastric emptying is expressed by

$$\begin{cases} \dfrac{dA_s(t)}{dt} = -k_s \cdot A_s(t) \\ A_s(0) = dose \end{cases} \tag{5}$$

where k_s represent the gastric emptying rate constant. The amount in the stomach at time zero (t = 0) equals the dose of the orally administered drug. It is assumed that no absorption takes place in the stomach and that no delay is present.

The small intestine is divided into *n* compartments where compartment 1 represents the first intestine compartment. How the amount of a drug changes with time in the first compartment is defined as

$$\begin{cases} \dfrac{dA_{i1}(t)}{dt} = k_s \cdot A_s(t) - (k_{ai} + k_i) \cdot A_{i1}(t) \\ A_{i1}(0) = 0 \end{cases} \tag{6}$$

where k_{ai} is the absorption rate constant from the small intestine. k_i represent the transit rate constant for the small intestine, which is assumed to be the same in all the small intestine compartments. The absorption and the transit processes are assumed to follow a first order process. The transit rate through the remainder of the small intestine is described as

$$\begin{cases} \dfrac{dA_{iN}(t)}{dt} = k_i \cdot A_{iN-1}(t) - k_i \cdot A_{iN}(t) \\ A_{iN}(0) = 0 \end{cases} \qquad N = 2, 3, \ldots, n \tag{7}$$

Since paracetamol is absorbed from the first part of the small intestine (Reppas et al, 1998) it is here assumed that absorption is only present in compartment 1 in the small intestine, but this can easily be modified if desired and equations 6 and 7 can be replaced by the more general form:

$$\begin{cases} \dfrac{dA_{iN}(t)}{dt} = k_i \cdot A_{iN-1} - (k_{aiN} + k_i) \cdot A_{iN}(t) \\ A_{iN}(0) = 0 \end{cases} \qquad N = 1, 2, \ldots, n \tag{8}$$

When $N = 1$, the term $k_i \bullet A_{i0}(t)$ is replaced by $k_s \bullet A_s(t)$.

The change in drug amount in the colon is expressed by the following equation:

$$\begin{cases} \dfrac{dA_c(t)}{dt} = k_i \cdot A_{in}(t) - (k_{ac} + k_c) \cdot A_c(t) \\ A_c(0) = 0 \end{cases} \tag{9}$$

where k_{ac} is the absorption rate constant from the colon and k_c denotes the transit rate constant for the colon.

The change in plasma concentration is expressed by equation

$$\begin{cases} \dfrac{dC(t)}{dt} = \dfrac{k_{ai} \cdot A_{i1}(t) + k_{ac} \cdot A_c(t)}{V} - k_e \cdot C(t) \\ C(0) = 0 \end{cases} \tag{10}$$

where V and k_e are the volume of distribution and the elimination rate constant, respectively. For the definition of k_e, see equation 4. For a substance that is only absorbed from the colon (for example sulfapyridine) k_{ai} is zero. Similarly, a substance that is only absorbed from the small intestine (for example paracetamol) k_{ac} is zero.

2.2.3 The lag time model

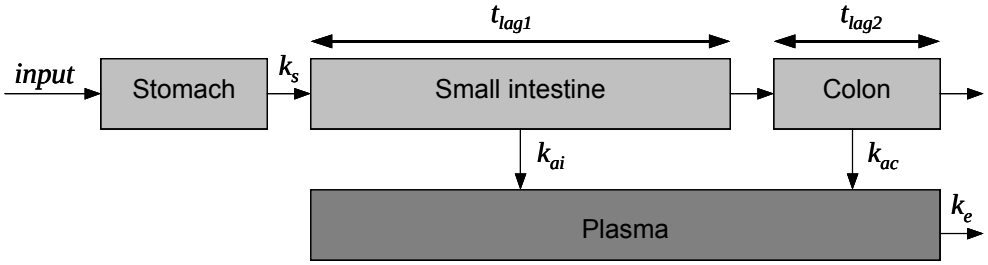


Figure 5. In the lag time model, the gastrointestinal tract is divided into the stomach, the small intestine and the colon. k_s is the gastric emptying rate constant, k_{ai} and k_{ac} are the absorption rate constants, k_e is the elimination rate constant and t_{lag1} and t_{lag2} are time constants that describe the time the drug resides in the small intestine and the colon, respectively.

In the lag time model the gastrointestinal tract is divided into the stomach, the small intestine and the colon, shown in figure 5. The gastric emptying is assumed to follow a first order process and k_s denotes the gastric emptying rate constant. The drug substance then enters the small intestine and can eventually enter the colon after t_{lag1} time units. After t_{lag2} time units in the colon the drug substance can exit from the body. Absorption takes place in the small intestine and/or the colon with first order kinetics and k_{ai} and k_{ac} represent the absorption rate constants from the small intestine and the colon, respectively.

The gastric emptying rate is defined as

$$\begin{cases} \dfrac{dA_s(t)}{dt} = -A_{out,s}(t) \\ A_s(0) = dose \end{cases} \tag{11}$$

where $A_{out,s}(t)$ is defined as

$$A_{out,s}(t) = k_s \cdot A_s(t) \tag{12}$$

k_s represents the gastric emptying rate constant and the amount in the stomach at time zero (t = 0) equals the dose of the orally administered drug. It is assumed that no absorption takes place in the stomach and that no delay is present.

Assuming that the inflow to the small intestine equals the outflow from the stomach

$$A_{in,i}(t) = A_{out,s}(t) \tag{13}$$

the change of drug amount in the small intestine can be described as

$$\begin{cases} \dfrac{dA_i(t)}{dt} = A_{in,i}(t) - k_{ai} \cdot A_i(t) - A_{in,i}(t - t_{lag1}) \cdot e^{-k_{ai} \cdot t_{lag1}} \\ A_i(0) = 0 \end{cases} \tag{14}$$

where k_{ai} is the absorption rate constant from the small intestine and t_{lag1} is a time constant. The outflow from the small intestine is the same as the inflow to the small intestine t_{lag1} time units earlier, but a factor smaller (exp(-$k_{ai} \cdot t_{lag1}$)) due to absorption.

By defining $A_{out,i}(t)$ as the outflow from the small intestine

$$A_{out,i}(t) = A_{in,i}(t - t_{lag1}) \cdot e^{-k_{ai} \cdot t_{lag1}} \tag{15}$$

the equation 14 can be rewritten as

$$\begin{cases} \dfrac{dA_i(t)}{dt} = A_{in,i}(t) - k_{ai} \cdot A_i(t) - A_{out,i}(t) \\ A_i(0) = 0 \end{cases} \tag{16}$$

Assuming that the inflow to the colon is equal to the outflow from the small intestine

$$A_{in,c}(t) = A_{out,i}(t) \tag{17}$$

the change of drug amount in the colon can be represented by the following expression

$$\begin{cases} \dfrac{dA_c(t)}{dt} = A_{in,c}(t) - k_{ac} \cdot A_c(t) - A_{in,c}(t - t_{lag2}) \cdot e^{-k_{ac} \cdot t_{lag2}} \\ A_c(0) = 0 \end{cases} \tag{18}$$

where k_{ac} is the absorption rate constant from the colon and t_{lag2} is a time constant. The outflow from the colon is the same as the inflow to the colon t_{lag2} time units earlier but a factor smaller (exp(-$k_{ac} \cdot t_{lag2}$)).

By defining $A_{out,c}(t)$ as the outflow from the colon

$$A_{out,c}(t) = A_{in,c}(t - t_{lag2}) \cdot e^{-k_{ac} \cdot t_{lag2}} \tag{19}$$

the equation 18 can be rewritten as

$$\begin{cases} \dfrac{dA_c(t)}{dt} = A_{in,c}(t) - k_{ac} \cdot A_c(t) - A_{out,c}(t) \\ A_c(t) = 0 \end{cases} \tag{20}$$

The change in plasma concentration is expressed by

$$\begin{cases} \dfrac{dC(t)}{dt} = \dfrac{k_{ai} \cdot A_i(t) + k_{ac} \cdot A_c(t)}{V} - k_e \cdot C(t) \\ C(0) = 0 \end{cases} \tag{21}$$

where V and k_e are the volume of distribution and the elimination rate constant, respectively. For definition of k_e, see equation 4. In the same way as in the transit rate model k_{ai} is zero for a substance that is only absorbed from the colon and for substance that is only absorbed from the small intestine k_{ac} is zero.

2.3 Sensitivity analysis

The standard model, the transit rate model and the lag time model have a very similar behavior with respect to the elimination rate constant (k_e). As seen in table 2 and 3 an increase in k_e results in a decrease in the maximum concentration value (C_{max}) and the time to which C_{max} is reached (t_{max}). It also reduces the total area under the curve (*AUC*).

The absorption rate constant, on the other hand, has a different influence in the standard model than in the other two models as seen in figure 6. In the standard model the absorption process can last endlessly if the absorption constant is infinitely small. This is not true for the transit rate model or the lag time model in which the absorbable substance is moving forward to the next compartment in the gastrointestinal tract and eventually leaves the body.

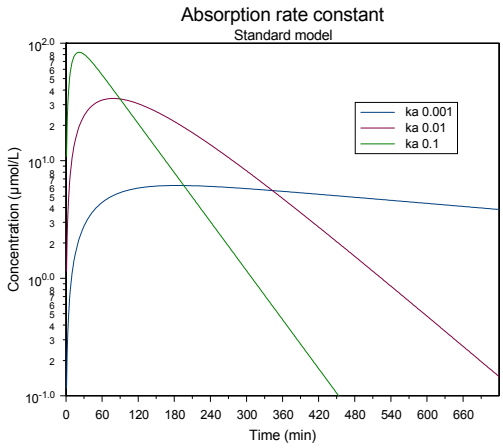
The transit rate model and the lag time model include a gastric emptying process. Both these models are influenced by the gastric emptying rate constant (k_s) in the same way: an increase in k_s leads to an increase in C_{max} and a decrease in t_{max}, but has no effect on the total *AUC* as can be seen in table 2 and 3.

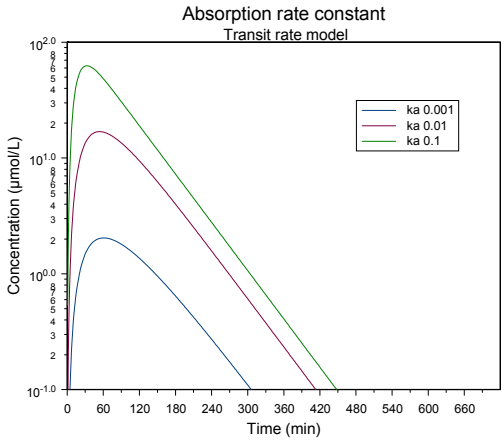
The transit rate model includes a small intestine transit rate constant (k_i) and a colon transit rate constant (k_c). k_i describes the rate with which a substance moves through the small intestine. For a substance absorbed from the upper small intestine like paracetamol, a fast transit through the small intestine leads to a smaller fraction of the dose having the time to be absorbed and hence reduces the *AUC*. Both C_{max} and t_{max} decrease with an increase in k_i (see table 2). An increase in the small intestine transit also means that a drug substance reaches the colon earlier and faster, which for sulfapyridine leads to a decrease in t_{max} and an increase in C_{max} (because the concentration curve is less spread out over time) while *AUC* remains unchanged (see table 3). k_c defines the rate with which a substance moves through the colon and a large k_c results in a small *AUC* for sulfapyridine which is absorbed from the colon. For sulfapyridine, C_{max} and t_{max} decreases with an increase in k_c while paracetamol is unaffected (table 2 and 3).

The transit rate model includes a parameter, *n*, specifying the number of compartments in the small intestine. For sulfapyridine, an increase in *n* leads to a decrease in C_{max} end an increase in t_{max} but has no effect on the *AUC*. For paracetamol, the choice of n has no effect on the pharmacokinetic profile (table 2 and 3).

The lag time model includes a parameter, t_{lag1}, that specifies the time for which a substance remains in the small intestine before it can move on to the colon. The longer paracetamol stays in the small intestine the more of the dose has the time to be absorbed and an increase in t_{lag1} results in an increase in C_{max}, t_{max} and *AUC* (table 2). A larger t_{lag1} also increases the time for any drug substance to reach the colon and thus results in a lag time before sulfapyridine will appear in the plasma but C_{max} and *AUC* are unaffected (table 3). t_{lag2} defines the time for which a substance remains in the colon. For sulfapyridine, an increase in t_{lag2} results in an increase in C_{max}, t_{max} and the total *AUC*. Paracetamol is not affected by t_{lag2} (table 2 and 3).

Additional figures are shown in appendix 1.





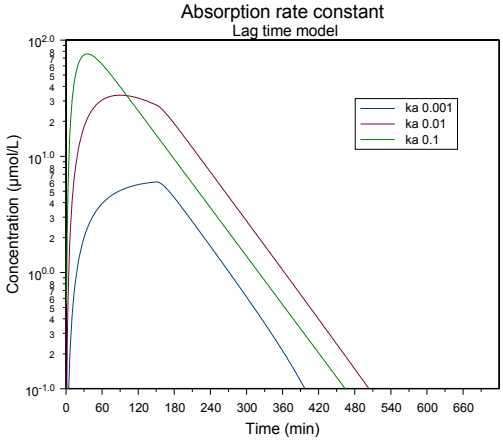


Figure 6. The impact of changing the absorption rate constant (k_a) on the pharmacokinetic profile in the standard model (upper left panel) the transit rate model (upper right panel and the lag time model (lower panel) is shown.

Table 2. Overview of the effect of the parameters used in the standard model, the transit rate model and the lag time model on C_{max}, t_{max} and AUC for paracetamol.

Paracetamol			
The standard model			
	C_{max}	t_{max}	$AUC_{0-\infty}$
$k_a \uparrow$	↑	↓	→
$k_e \uparrow$	↓	↓	↓
$F \uparrow$	↑	→	↑
The transit rate model			
	C_{max}	t_{max}	$AUC_{0-\infty}$
$k_a \uparrow$	↑	↓	↑
$k_e \uparrow$	↓	↓	↓
$F \uparrow$	↑	→	↑
$k_s \uparrow$	↑	↓	→
$k_i \uparrow$	↓	↓	↓
$k_c \uparrow$	→	→	→
$n \uparrow$	→	→	→
The lag time model			
	C_{max}	t_{max}	$AUC_{0-\infty}$
$k_a \uparrow$	↑	↓	↑
$k_e \uparrow$	↓	↓	↓
$F \uparrow$	↑	→	↑
$k_s \uparrow$	↑	↓	→
$t_{lag1} \uparrow$	↑	↑	↑
$t_{lag2} \uparrow$	→	→	→

Table 3. Overview of the effect of the parameters used in the standard model, the transit rate model and the lag time model on C_{max}, t_{max} and AUC for sulfapyridine.

Sulfapyridine			
The standard model			
	C_{max}	t_{max}	$AUC_{0-\infty}$
$k_a \uparrow$	↑	↓	→
$k_e \uparrow$	↓	↓	↓
$F \uparrow$	↑	→	↑
The transit rate model			
	C_{max}	t_{max}	$AUC_{0-\infty}$
$k_a \uparrow$	↑	↓	↑
$k_e \uparrow$	↓	↓	↓
$F \uparrow$	↑	→	↑
$k_s \uparrow$	↑	↓	→
$k_i \uparrow$	↑	↓	→
$k_c \uparrow$	↓	↓	↓
$n \uparrow$	↓	↑	→
The lag time model			
	C_{max}	t_{max}	$AUC_{0-\infty}$
$k_a \uparrow$	↑	↓	↑
$k_e \uparrow$	↓	↓	↓
$F \uparrow$	↑	→	↑
$k_s \uparrow$	↑	↓	→
$t_{lag1} \uparrow$	→	↑	→
$t_{lag2} \uparrow$	↑	↑	↑

2.4 Simulations and sampling design

Pilot data about gastrointestinal transit time for paracetamol and sulfapyridine in dogs were available. The mean plasma concentration for the 6 dogs was calculated at each time point. Simulations were made in the WinNonlin (Version 4.0, Pharsight Corporation, Mountain View CA, USA) and Berkeley Madonna (Version 8.0, Macey and Oster, University of California, Berkeley, USA) software packages using the standard model and a pharmacokinetic profile of the plasma concentration versus time, was obtained for each substance (figure 7). Based on these profiles, a sampling scheme was designed in order to ensure that measurements were taken in the absorption and elimination phase (table 4).

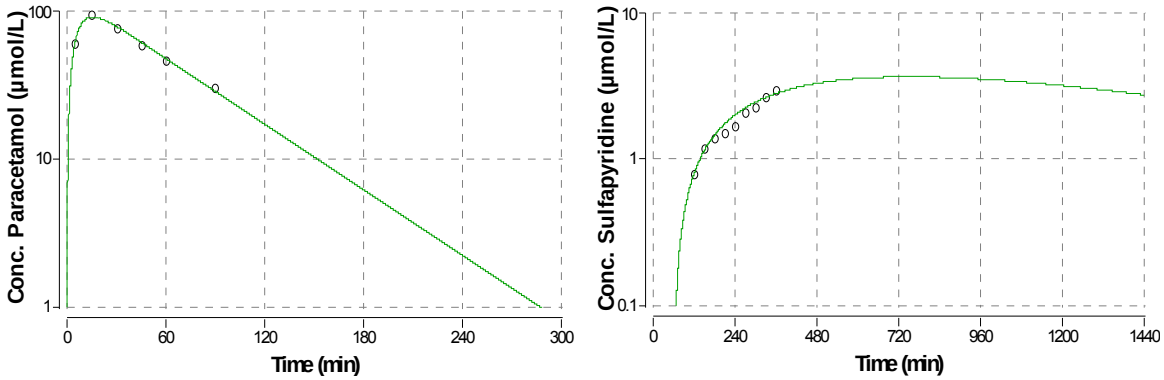


Figure 7. Predicted average serum concentration of paracetamol (left) and sulfapyridine (right) versus time plots based on pilot data. Six dogs received an oral dose of 159 µmol/kg paracetamol and 50.2 µmol/kg sulfasalazine.

Table 4. Scheme for blood sampling and data collection. Time is relative to dosing (0 min).

Paracetamol										
Time (min)	-15	10	20	30	60	90	120	200	300	400
Sulfasalazine										
Time (min)	-15	30	60	90	120	200	300	400	1440	1500

3 Results

3.1 Data

The mean observed plasma paracetamol and sulfapyridine concentrations (± SD) after different treatments are shown in figure 8 and figure 9, respectively. For paracetamol, treatment with erythromycin resulted in a higher maximum concentration compared to saline (placebo). Treatment with atropine on the other hand resulted in a lower maximum concentration appearing at a later time. The paracetamol profile after treatment with morphine resembles the profile after saline treatment. For sulfapyridine, a large variability was observed and no clear tendencies with respect to treatment can be seen. The concentrations increased up to 400 minutes and decreased between 1440 and 1500 minutes. No observations were made between 400 and 1440 minutes, due to practical reasons.

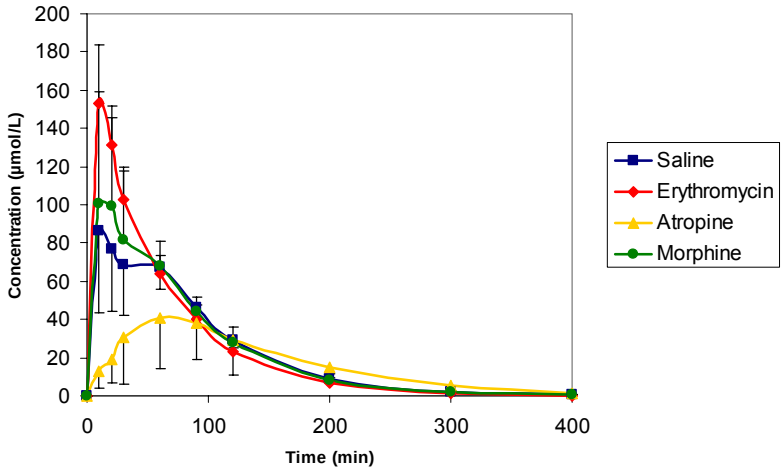


Figure 8. Observed mean paracetamol concentrations (± SD). An oral dose of 159 µmol/kg paracetamol was given to six dogs 15 minutes after a 15 minutes intravenous infusion of erythromycin (1 mg/kg), atropine (0.06 mg/kg), morphine (0.05 mg/kg) or saline.

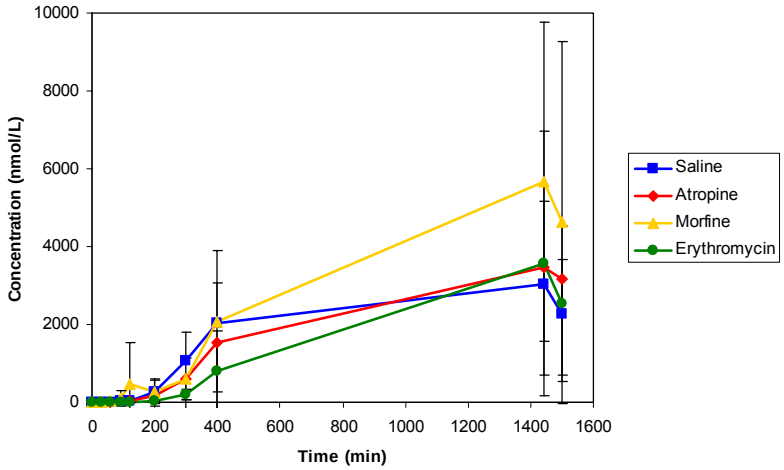


Figure 9. Observed mean sulfapyridine concentrations (± SD). An oral dose of 50.2 µmol/kg sulfapyridine was given to six dogs 15 minutes after a 15 minutes intravenous infusion of erythromycin (1 mg/kg), atropine (0.06 mg/kg), morphine (0.05 mg/kg) or saline.

3.2 Standard model

3.2.1 Paracetamol

The standard model was able to describe the pharmacokinetic profiles of paracetamol adequately. The mean observed and fitted plasma concentrations of paracetamol are presented in figure 10 and the fitted profiles of individual dogs are shown in figure 11. The fitted profiles (mean and individual) are based on equations 1-3. The elimination rate constant (k_e) and the apparent volume of distribution (V/F) were estimated for each dog by fitting the observed paracetamol concentrations after the four treatments simultaneously.

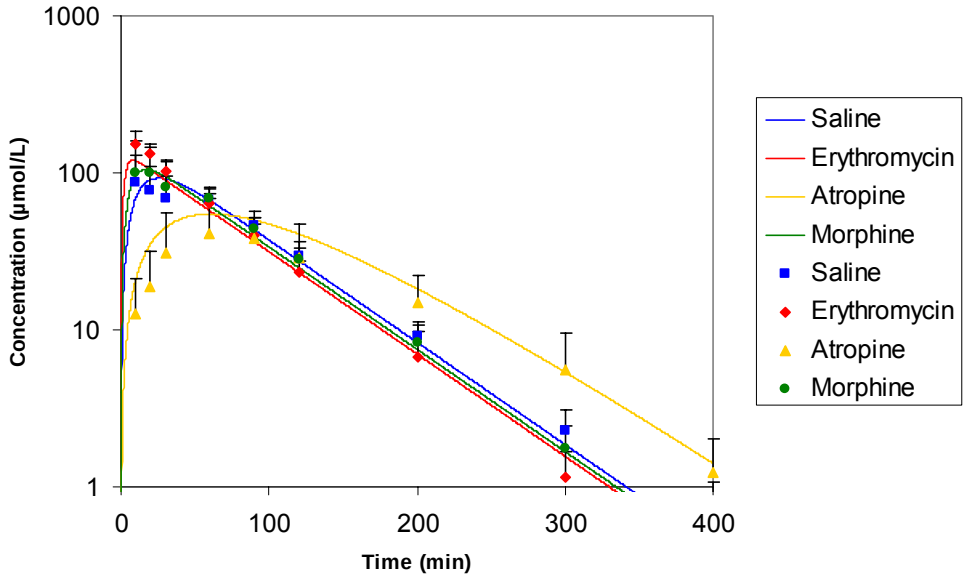


Figure 10. Mean observed (markers) and predicted (lines) paracetamol concentrations (± SD). The predicted lines are based on the standard model fitting the curves for different treatments simultaneously. An oral dose of 159 µmol/kg paracetamol was given to the six dogs after intravenous infusion of different treatments.

The absorption rate constant for paracetamol when treated with erythromycin (k_{aE}) was larger in all the six dogs than the absorption rate constant achieved with any other treatment. Similarly, treatment with atropine resulted in the lowest absorption rate constant for all dogs. In all the dogs, treatment with erythromycin resulted in the largest C_{max} and the smallest t_{max} while the opposite was true for treatment with atropine (figure 11 and table 5).

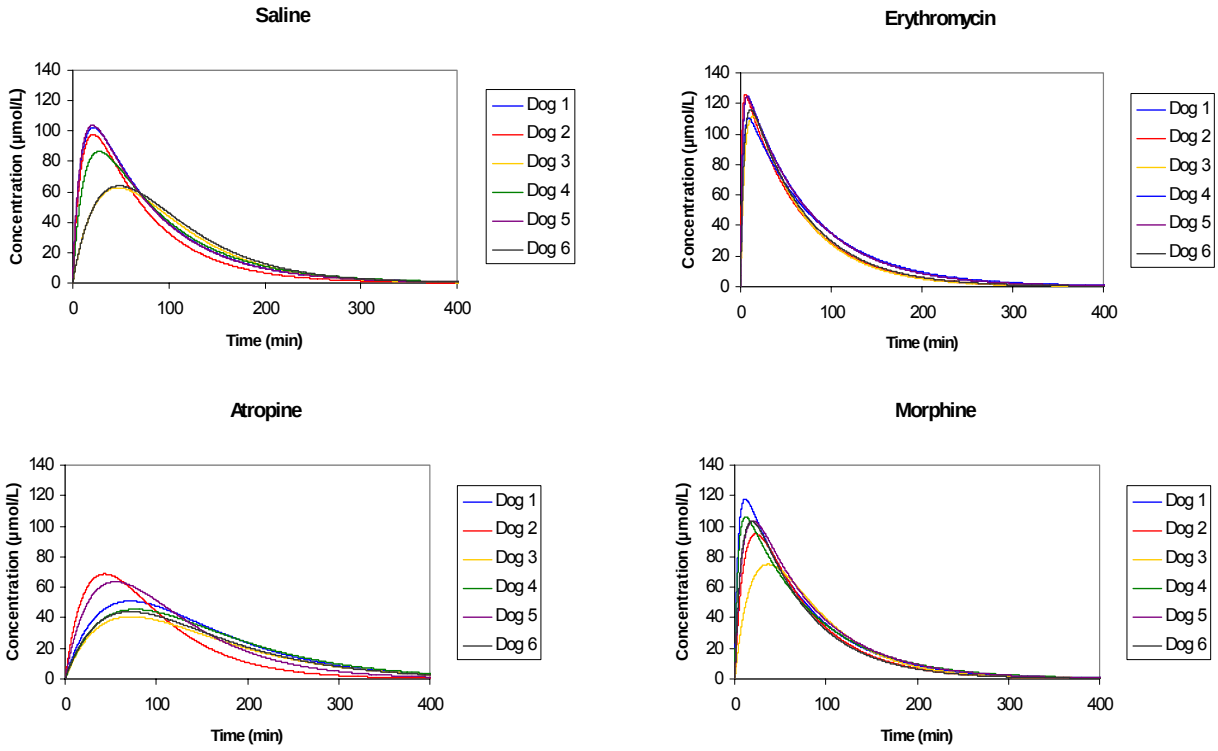


Figure 11. Predicted paracetamol concentrations in the individual dogs. The predicted concentrations are achieved using the standard model and the parameter estimates are presented in table 5. An oral dose of 159 µmol/kg paracetamol was given to the six dogs after intravenous infusion of different treatments.

3.2.2 Sulfapyridine

There were difficulties to fit the observed sulfapyridine concentrations because of limited data (in particular around the maximum concentration and in the elimination phase). In the literature, however, a more complete profile was found (Lefebvre et al., 2001) and the standard model (with lag time) was used to fit this data set. As can be seen from figure 12 the model underestimated the first concentrations and had difficulties to fit the absorption phase. The parameter estimates obtained for the absorption rate constant, the elimination rate constant, the apparent volume of distribution and the lag time were 0.007 1/min, 0.003 1/min, 5.2 L/kg and 322 minutes, respectively.

The parameters obtained above were used as initial values when fitting curves to the individual (table 5) and average sulfapyridine concentrations (figure 13). The volume of distribution was held fixed at 5.2 L/kg. The absorption and the elimination rate constant were obtained for each dog by fitting the sulfapyridine concentrations after four treatments simultaneously but the lag time was estimated for each treatment and dog. The sulfapyridine profile after treatment with erythromycin showed the largest lag time (349 minutes) and treatment with saline resulted in the smallest lag time value (249 minutes) (see table 5).

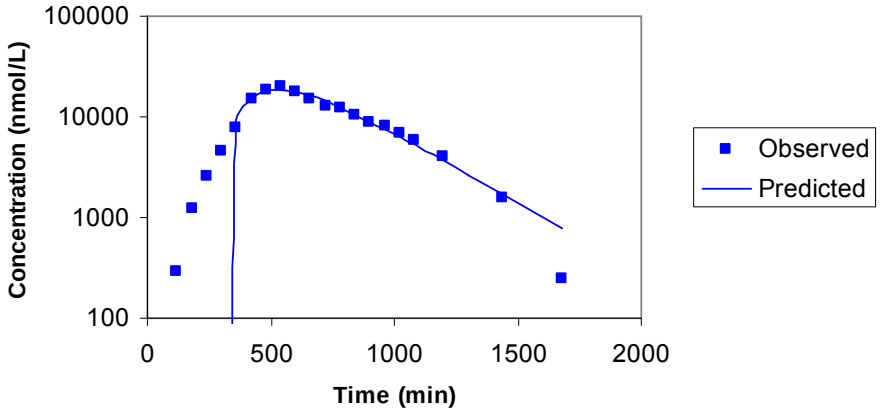


Figure 12. The observed (markers) sulfapyridine concentrations from an article written by Lefebvre et al. (2001) and the predicted (line) concentrations obtained from the standard model with lag time.

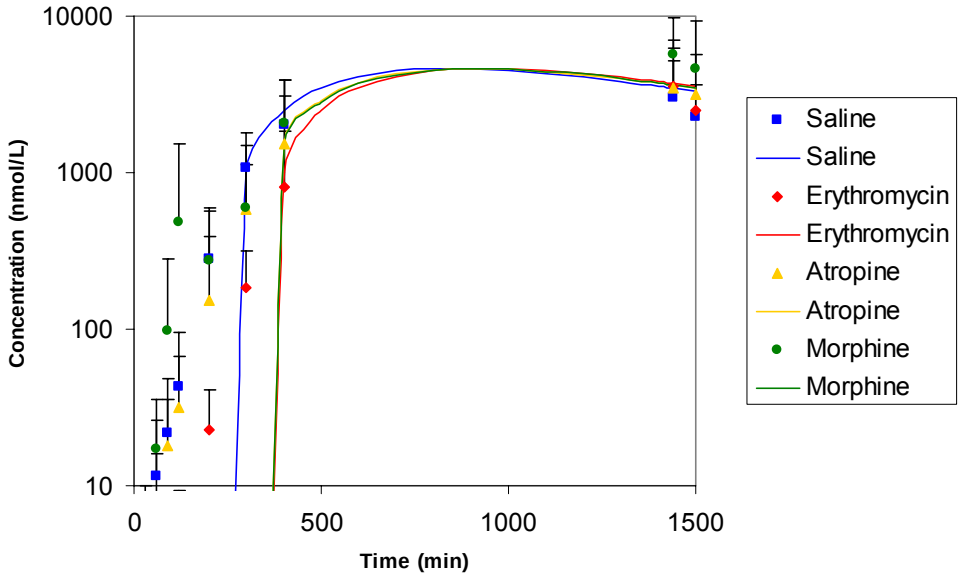


Figure 13. Predicted (lines) and observed (markers) sulfapyridine concentrations (mean ± SD). The fitted lines are obtained using the standard model with lag time. An oral dose of 50.2 µmol/kg sulfapyridine was given to the six dogs after intravenous infusion of different treatments.

Table 5. Pharmacokinetic parameters of paracetamol and sulfapyridine obtained using the standard model. S, E, A and M represents saline (placebo), erythromycin, atropine and morphine, respectively. k_a denotes the absorption rate constant and k_e is the elimination rate constant. CL/F and V/F are the apparent clearance and the apparent volume of distribution, respectively. t_{lag} represent the lag time.

Parameter	Unit	Dog 1 Ior	Dog 2 Tarzan	Dog 3 Atlas	Dog 4 Basset	Dog 5 Hubert	Dog 6 Måns	Mean	SD
Paracetamol									
k_{aS}	1/min	0.11	0.10	0.03	0.08	0.12	0.03	0.08	0.04
k_{aE}	1/min	0.50	0.72	0.21	0.39	0.52	0.27	0.43	0.19
k_{aA}	1/min	0.014	0.032	0.011	0.013	0.023	0.012	0.018	0.008
k_{aM}	1/min	0.27	0.09	0.04	0.27	0.12	0.13	0.15	0.09
k_e	1/min	0.014	0.016	0.017	0.013	0.014	0.016	0.015	0.002
CL/F	L/kg/min	0.016	0.019	0.019	0.017	0.016	0.018	0.018	0.001
V/F	L/kg	1.1	1.2	1.1	1.3	1.2	1.1	1.2	0.1
Sulfapyridine									
k_a	1/min	0.002	0.010	0.003	0.005	0.003	0.004	0.004	0.003
k_e	1/min	0.002	0.004	0.002	0.001	0.001	0.001	0.002	0.001
CL/F	L/kg/min	0.011	0.019	0.008	0.007	0.003	0.003	0.008	0.006
V/F [a)]	L/kg	5.2	5.2	5.2	5.2	5.2	5.2	5.2	-
t_{lagS}	min	261	376	251	257	277	372	299	59
t_{lagE}	min	354	394	383	351	367	387	373	18
t_{lagA}	min	500	392	500	225	359	384	393	102
t_{lagM}	min	354	297	500	349	366	278	357	78

[a)] The parameter is fixed

3.3 Transit rate model

3.3.1 Paracetamol

The mean observed and fitted plasma concentrations of paracetamol using the transit rate model are presented in figure 14. Figure 15 shows the fitted concentration profiles of the individual dogs. The elimination rate constant (k_e) and the volume of distribution (V/F) were estimated by fitting the observed paracetamol concentrations after four treatments in each dog simultaneously. The gastric emptying rate constant (k_S) was estimated for each dog and treatment. In all the dogs except dog 1 (Ior) the fastest gastric emptying occurred after treatment with erythromycin. In dog 1 treatment with morphine and erythromycin resulted in similar gastric emptying rates. The lowest gastric emptying rate constants were obtained in the dogs given atropine treatment (see table 6).

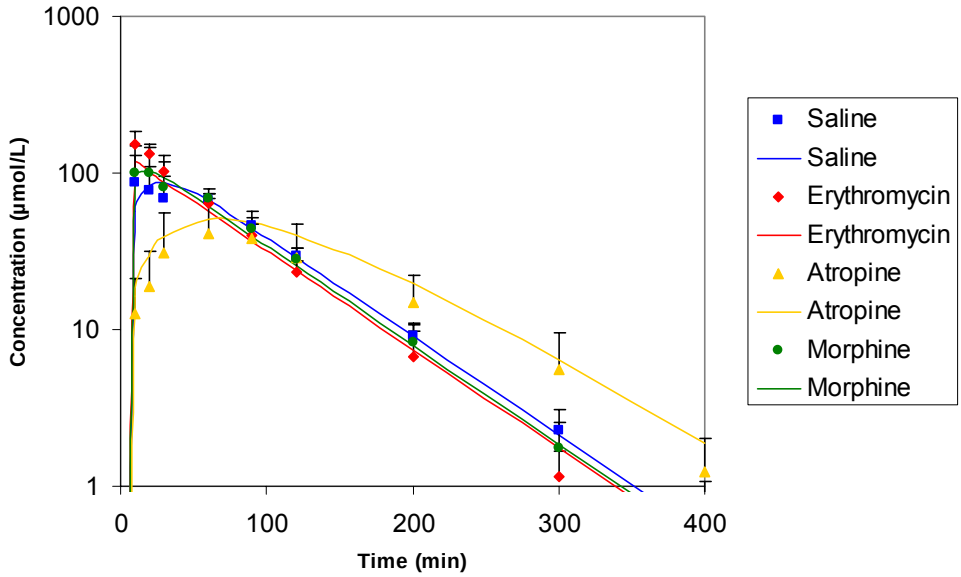


Figure 14. Predicted (lines) and observed (markers) paracetamol concentrations (mean ± SD). The predicted lines were based on the transit rate model.

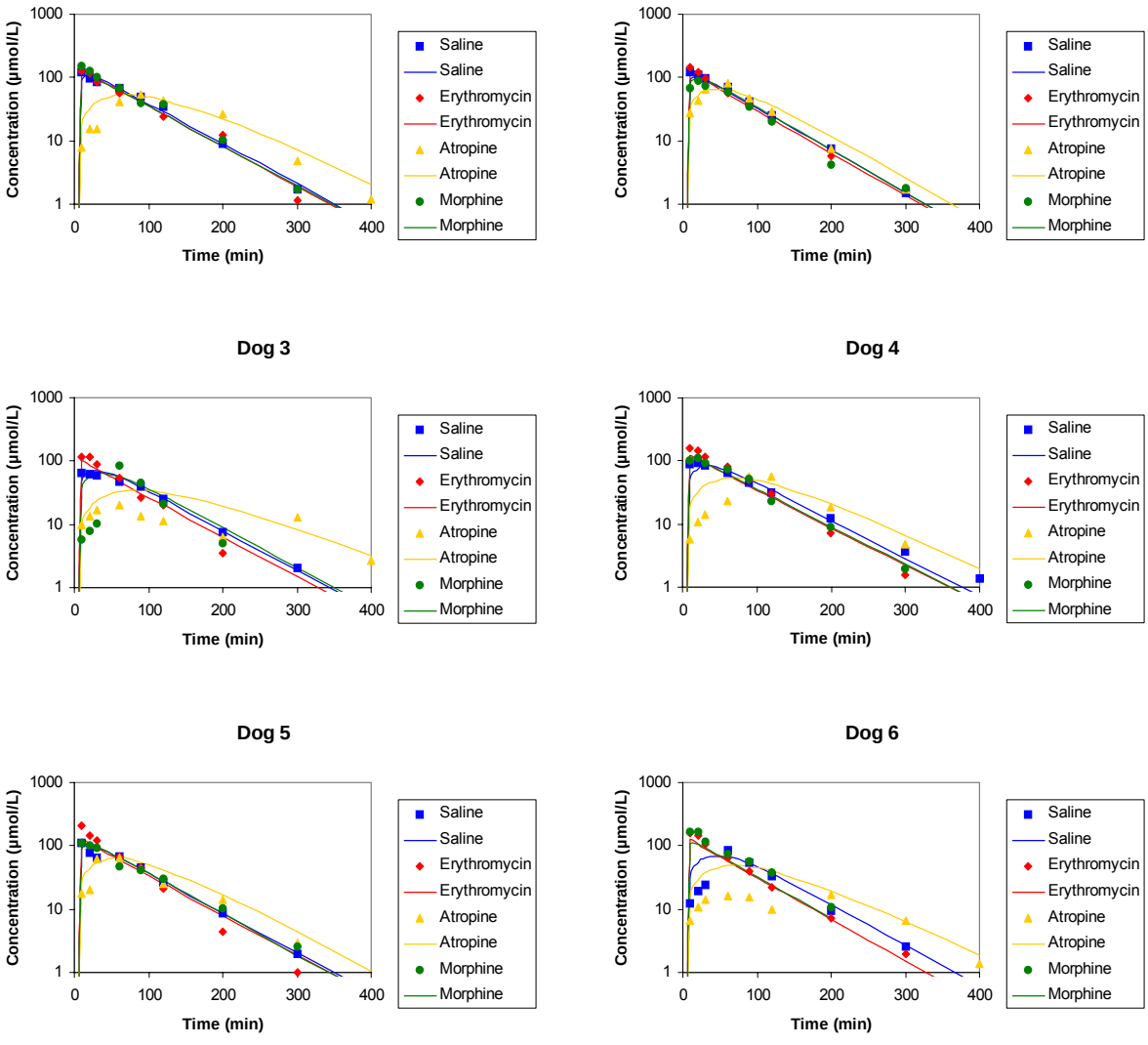


Figure 15. Predicted (lines) and observed (markers) paracetamol concentrations (mean ± SD) in the individual dogs. The predicted lines were achieved using the transit rate model. The parameter estimates are presented in table 6.

3.3.2 Sulfapyridine

The literature data set presented in section 3.2.2 was used to obtain initial estimates for the pharmacokinetic modeling of sulfapyridine. The transit rate model was able to adequately describe the observed concentrations as shown in figure 16. The parameter estimates obtained for the elimination rate constant, the transit rate constant for the small intestine and the volume of distribution were 0.004 1/min, 0.013 1/min and 4.1 L/kg, respectively. The absorption rate constant (k_a), the gastric emptying rate constant (k_s) and the transit rate constant for the colon (k_c) were held constant at 0.1 1/min, 1 1/min and 0.003 1/min, respectively.

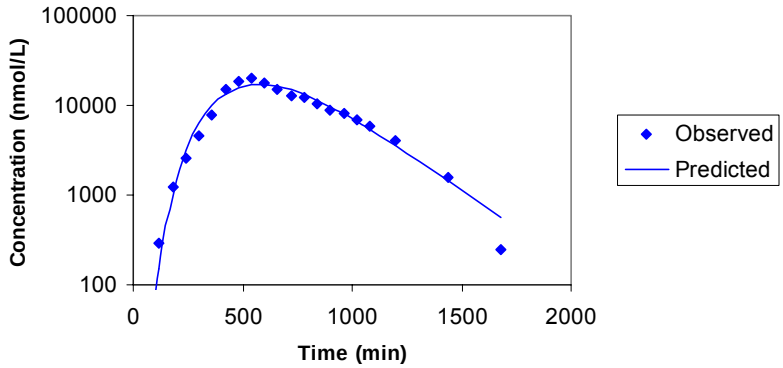


Figure 16. The observed (markers) sulfapyridine concentrations from an article written by Lefebvre et al. (2001) and the predicted (line) concentrations obtained from the transit rate model.

The parameter estimates received above were used as initial estimates and k_a, k_s and k_c were held constant as described above during the modeling procedure of sulfapyridine. In each dog the observed sulfapyridine concentrations after four treatments were fitted simultaneously to estimate the elimination rate constant (k_e) and the volume of distribution (V/F). The transit rate constant for the small intestine (k_i) was estimated for each dog and treatment. The mean observed (± SD) and fitted plasma concentrations of sulfapyridine are shown in figure 17. Figure 18 presents the observed and predicted sulfapyridine concentrations for the individual dogs. The predictions adequately describe the observed concentrations.

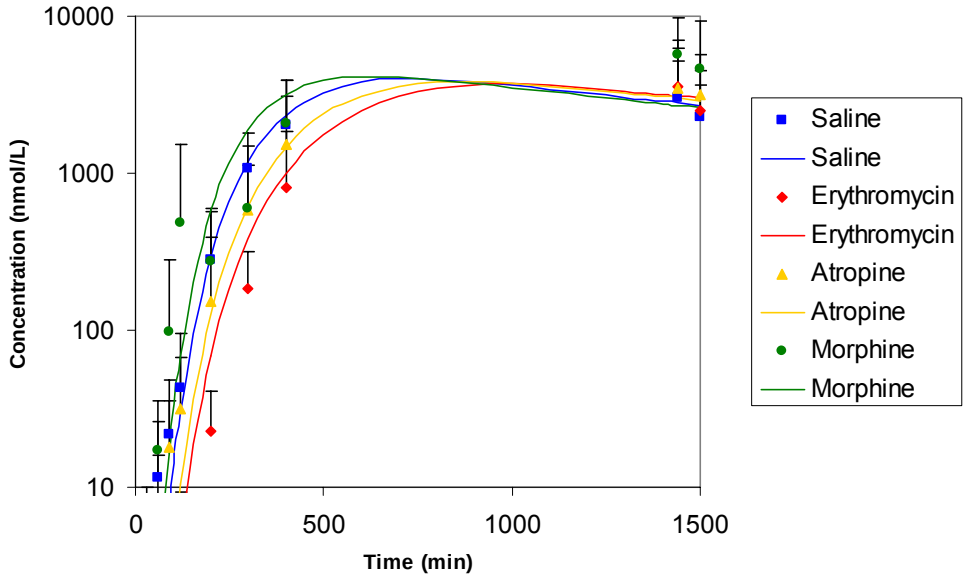


Figure 17. Fitted (lines) and observed (markers) sulfapyridine concentrations (mean ± SD). The predicted lines were obtained using the transit rate model.

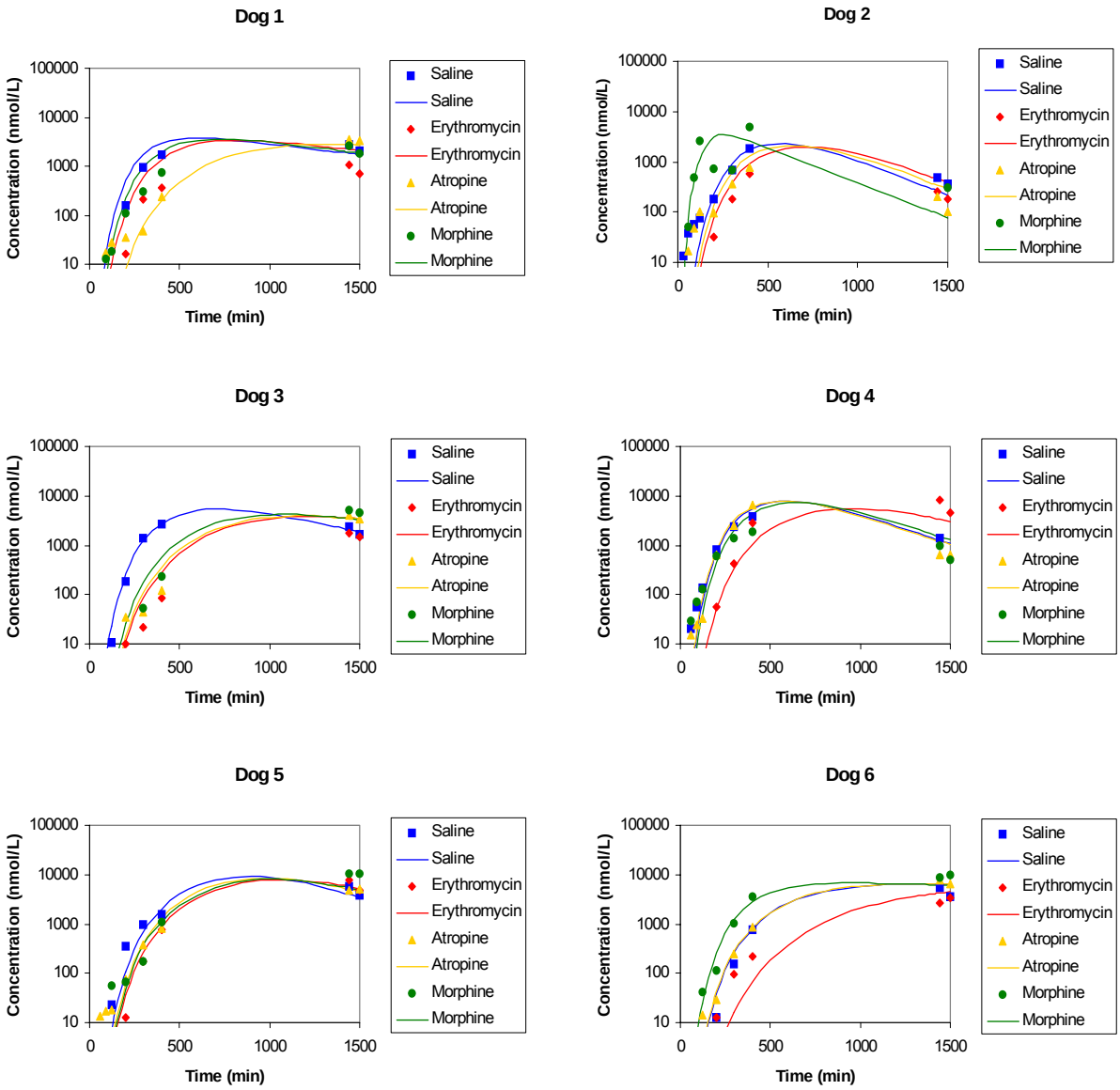


Figure 18. Predicted (lines) and observed (markers) sulfapyridine concentrations (mean ± SD) in the individual dogs. The predicted lines were achieved using the transit rate model. The parameter estimates are shown in table 6.

The different treatments showed no obvious effect on the sulfapyridine profiles in relation to saline. The sulfapyridine profiles after treatment with atropine and morphine, which are expected to inhibit the intestine activity, showed no clear increase in lag time compared to the other profiles. The stimulating effect of erythromycin was not seen in terms of reduced lag time or increased peak. k_i showed no obvious tendencies between the different treatments (se table 6) and even though morphine and saline showed the largest transit rate constants in average, the variation is large.

Table 6. Pharmacokinetic parameters of paracetamol and sulfapyridine obtained using the transit rate model. S, E, A and M represents saline (placebo), erythromycin, atropine and morphine, respectively. k_a denotes the absorption rate constant. k_s, k_i and k_c represent the gastric emptying rate constant, the transit rate constant for the small intestine and the transit rate constant for the colon, respectively. k_e is the elimination rate constant and CL/F and V/F are the apparent clearance and the apparent volume of distribution, respectively.

Parameter	Unit	Dog 1 Ior	Dog 2 Tarzan	Dog 3 Atlas	Dog 4 Basset	Dog 5 Hubert	Dog 6 Måns	Mean	SD
Paracetamol									
k_a [a)]	1/min	1	1	1	1	1	1	1	-
k_{sS}	1/min	0.13	0.14	0.07	0.06	0.13	0.03	0.09	0.05
k_{sE}	1/min	0.36	0.94	0.51	0.42	0.62	0.58	0.57	0.20
k_{sA}	1/min	0.016	0.032	0.011	0.017	0.024	0.015	0.019	0.008
k_{sM}	1/min	0.39	0.11	0.05	0.20	0.14	0.23	0.19	0.12
k_i [a)]	1/min	0.01	0.01	0.01	0.01	0.01	0.01	0.01	-
k_c [a)]	1/min	0.003	0.003	0.003	0.003	0.003	0.003	0.003	-
k_e	1/min	0.015	0.015	0.014	0.014	0.014	0.015	0.015	0.001
CL/F	L/kg/min	0.016	0.018	0.021	0.017	0.017	0.018	0.018	0.002
V/F	L/kg	1.1	1.2	1.4	1.2	1.1	1.2	1.2	0.1
Sulfapyridine									
k_a [a)]	1/min	0.1	0.1	0.1	0.1	0.1	0.1	0.1	-
k_s [a)]	1/min	1	1	1	1	1	1	1	-
k_{iS}	1/min	0.017	0.014	0.012	0.014	0.007	0.008	0.012	0.004
k_{iE}	1/min	0.011	0.010	0.006	0.008	0.006	0.004	0.008	0.003
k_{iA}	1/min	0.006	0.012	0.006	0.014	0.006	0.008	0.009	0.003
k_{iM}	1/min	0.013	0.037	0.007	0.012	0.006	0.013	0.015	0.011
k_c [a)]	1/min	0.003	0.003	0.003	0.003	0.003	0.003	0.003	-
k_e	1/min	0.001	0.003	0.002	0.003	0.006	0.0002	0.003	0.002
CL/F	L/kg/min	0.009	0.030	0.009	0.008	0.006	0.001	0.012	0.010
V/F	L/kg	9.4	9.4	5.1	3.0	1.0	6.3	6	3

[a)] The parameter is fixed

3.4 The lag time model

Ordinary differential equations contain derivatives that depend on function values at the present time. The lag time model also includes delay differential equations that depend on function values in the past. For many software packages, delay differential equations are difficult to implement, and unfortunately the lag time model could not be implemented in WinNonlin. It was, however, possible to implement the lag time model in Berkeley Madonna. The pharmacokinetic profiles shown in figures 19 and 20 and the corresponding parameter estimates in sections 3.4.1 and 3.4.2 were found by simulations in Berkeley Madonna. Consequently, the exact parameter estimates are not as reliable as those for the standard model and the transit rate model.

3.4.1 Paracetamol

The mean observed and simulated plasma concentrations of paracetamol using the lag time model are presented in figure 19. The simulated profiles are based on the following absorption rate constant, elimination rate constant and volume of distribution: 1 1/min, 0.015 1/min and 1.2 L/kg for all four treatments. The gastric emptying rates were 0.09 1/min, 0.57

1/min, 0.015 1/min and 0.19 1/min for treatment with saline, erythromycin, atropine and morphine, respectively. The residence times in the small intestine and the colon were 172 and 300 minutes, respectively.

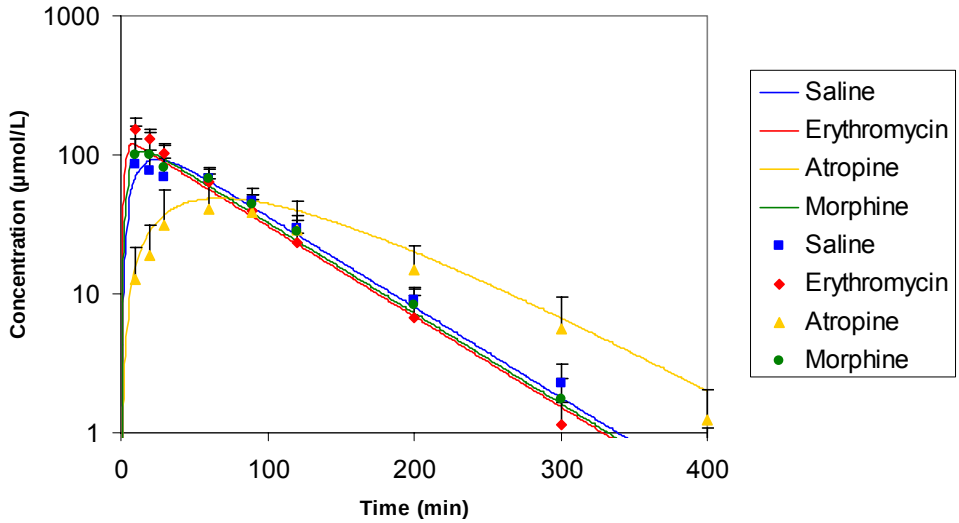


Figure 19. Simulated (lines) and observed (markers) paracetamol concentrations (mean ± SD) based on the lag time model. An oral dose of 159 µmol/kg paracetamol was given to the six dogs after intravenous infusion of different treatments.

3.4.2 Sulfapyridine

The mean observed and simulated plasma concentrations of the sulfapyridine literature data (see section 3.2.2) are presented in figure 20. The simulated profile is based on the following absorption rate constant, elimination rate constant and volume of distribution: 0.01 1/min, 0.003 1/min and 5.2 L/kg, respectively. The gastric emptying rate was 0.007 1/min and the residence times in the small intestine and the colon were 172 and 300 minutes, respectively.

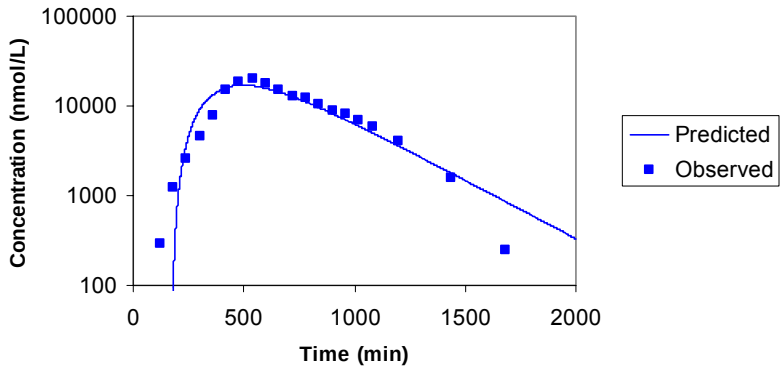


Figure 20. The observed (markers) sulfapyridine concentrations from an article written by Lefebvre et al. (2001) and the simulated (line) concentrations obtained from the lag time model.

3.5 Model and parameter comparison

The standard model and the transit rate model gave similar elimination rate constants for paracetamol (0.015 1/min and 0.015 1/min) and sulfapyridine (0.002 1/min and 0.003 1/min), as indicated in tables 5 and 6. The estimated apparent volume of distribution for paracetamol was also similar in the two different models (estimated values around 1.2 L/kg, as indicated in table 5 and 6). For sulfapyridine, the transit rate model resulted in an average volume of distribution of 6 L/kg compared to a fixed value of 5.2 L/kg in the standard model.

For paracetamol, the standard model and the transit rate model estimated similar values for C_{max} and t_{max}. Treatment with saline resulted in a C_{max} of around 90 µmol/L and t_{max} around 30 minutes. The estimation of C_{max} and t_{max} for sulfapyridine was difficult due to a lack of measurements in the vicinity of t_{max}. The standard model estimated C_{max} around 5500 nmol/L and t_{max} around 770 minutes for sulfapyridine after treatment with saline. Corresponding estimates for the transit rate model were 5660 nmol/L for C_{max} and 785 minutes for t_{max}.

Gastric emptying half-life is defined as the time when 50% of the dose has left the stomach. This is straightforward to estimate in the transit rate model (which has an explicit parameter for the gastric emptying rate) based on equation 5, as shown in figure 21. The standard model does not have a specific compartment for the stomach, but in this model, the gastric emptying rate can be assumed to be correlated with the absorption rate. Thus, gastric emptying profiles can be found based on equation 1, as shown in figure 22. The estimated gastric emptying half-life values obtained from the two models are shown in table 7. Both indicate more rapid gastric emptying after erythromycin treatment and slower gastric emptying after atropine treatment compared to after saline treatment. Figure 23 shows the correlation between the estimates of gastric emptying half-life for the standard model and the transit rate model.

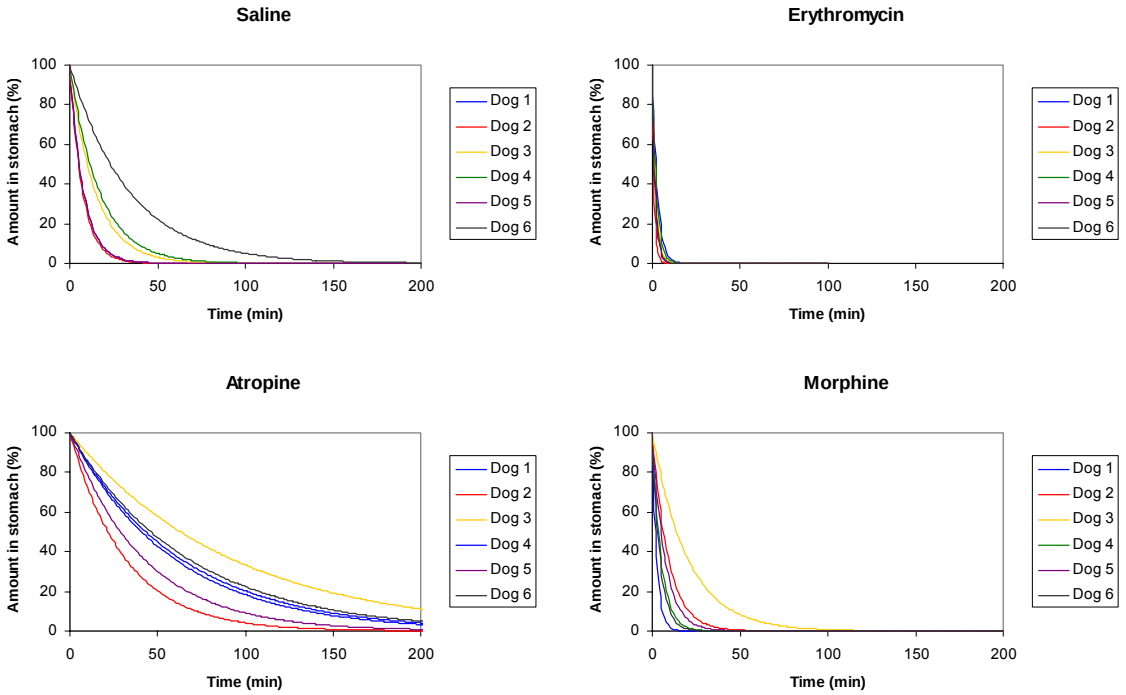


Figure 21. Predicted gastric emptying profiles of paracetamol. An oral dose of 159 µmol/kg paracetamol was given to six dogs after intravenous infusion of different treatments. The profiles are based on equation 5 in the transit rate model.

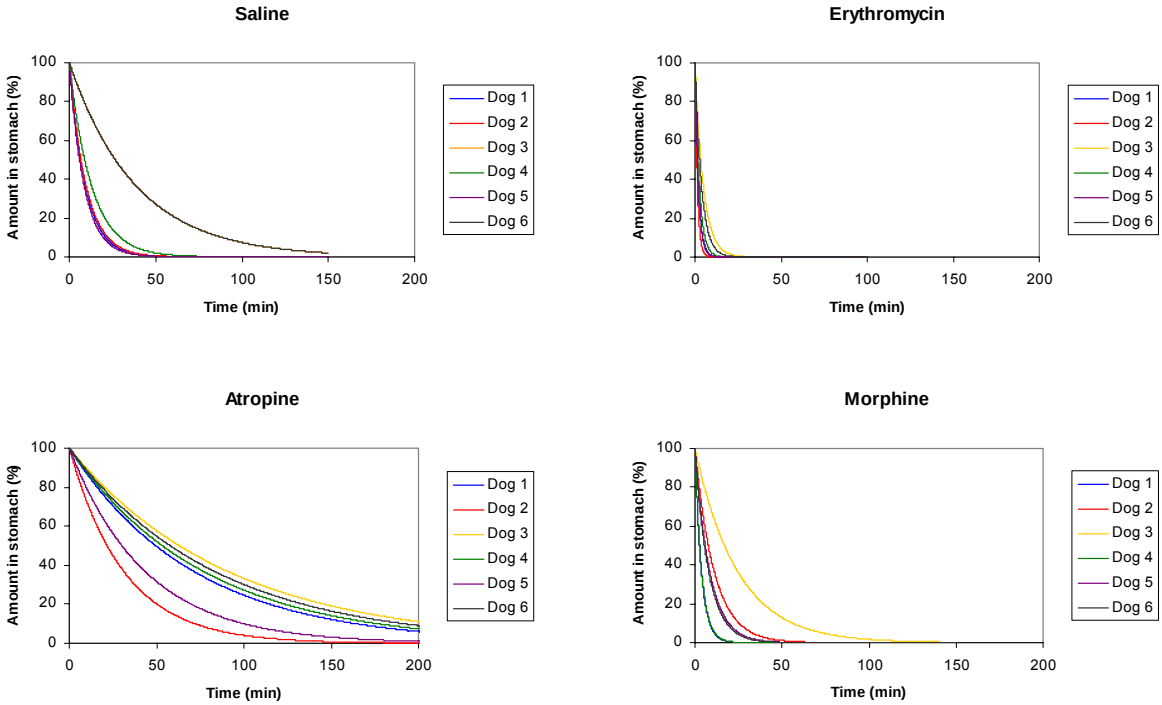


Figure 22. Predicted gastric emptying profiles of paracetamol. An oral dose of 159 µmol/kg paracetamol was given to six dogs after intravenous infusion of different treatments. The profiles are based on equation 1 in the standard model.

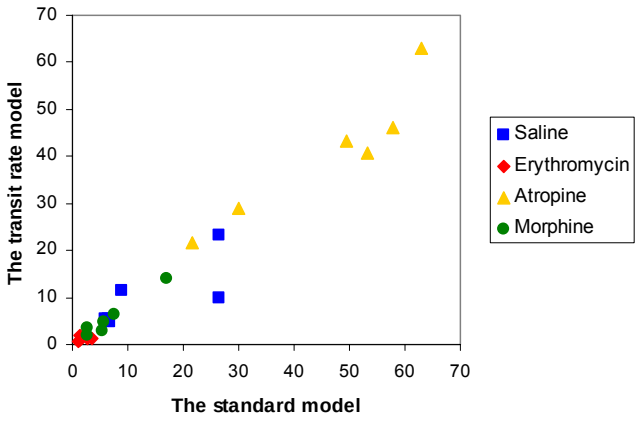


Figure 23. The correlation between the standard model and the transit rate model is shown in their estimates of the gastric emptying half-life for different treatments.

Table 7. The gastric emptying half-life obtained for paracetamol using the standard model and the transit rate model is shown. S, E, A and M represents saline (placebo), erythromycin, atropine and morphine, respectively.

Parameter	Unit	Dog 1 Ior	Dog 2 Tarzan	Dog 3 Atlas	Dog 4 Basset	Dog 5 Hubert	Dog 6 Måns	Mean	SD
The standard model									
t_{50S}	min	6.4	6.7	26.6	8.9	5.9	26.6	13.5	10.2
t_{50E}	min	1.4	1.0	3.4	1.8	1.3	2.6	1.9	0.9
t_{50A}	min	50	22	63	53	30	58	46	16
t_{50M}	min	2.6	7.6	16.9	2.7	5.8	5.5	6.9	5.3
The transit rate model									
t_{50S}	min	5.3	4.9	9.9	11.5	5.3	23.1	10.0	7.0
t_{50E}	min	1.9	0.7	1.4	1.6	1.1	1.2	1.3	0.4
t_{50A}	min	43.4	21.7	63	40.8	28.9	46.2	40.7	14.4
t_{50M}	min	1.8	6.3	13.9	3.5	4.9	3	5.6	4.4

The time of first appearance, which is a measurement of the small intestine transit time, is defined as the time (in whole minutes) to when the level of sulfapyridine first exceeds 100 nmol/L (selected to be well above the experimental detection limit of between 20-40 nmol/L). The agreement between the standard model and the transit rate model for the time of first appearance is not as good as for the gastric emptying half-life above. Not only is the correlation weaker, there is also a systematic difference between the two estimates in that the standard model tends to give higher estimates than the transit rate model (see figure 24). The estimated time of first appearance obtained using the standard model and the transit rate model are shown in table 8.

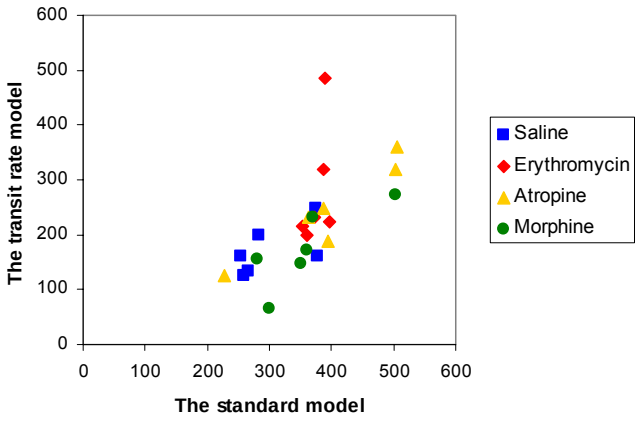


Figure 24. The correlation between the standard model and the transit rate model is shown in their estimates of the time of first appearance for different treatments.

Table 8. The time of first appearance obtained for sulfapyridine using the standard model and the transit rate model is shown. S, E, A and M represents saline (placebo), erythromycin, atropine and morphine, respectively.

Parameter	Unit	Dog 1 Ior	Dog 2 Tarzan	Dog 3 Atlas	Dog 4 Basset	Dog 5 Hubert	Dog 6 Måns	Mean	SD
The standard model									
t_{firstS}	min	267	378	255	259	282	375	303	58
t_{firstE}	min	360	396	387	353	372	390	376	17
t_{firstA}	min	506	394	504	227	364	387	397	103
t_{firstM}	min	360	299	504	351	371	281	361	79
The transit rate model									
t_{firstS}	min	133	161	162	126	200	247	172	45
t_{firstE}	min	200	223	318	216	233	485	279	109
t_{firstA}	min	361	187	318	126	233	247	245	85
t_{firstM}	min	171	66	273	146	233	156	174	72

4 Discussion

In fitting models to observations, there is always a trade-off between model complexity and fit. A model with a large enough number of parameters should be able to fit any data set very well. The reduced error, however, comes at a cost in variation. Complex models are likely to fit well to the data based on which the fit was made, but may not generalize well to other data of the same type (this is referred to as over-fitting). In animal experiments, the amount of data is often limited since the number of samples for ethical reasons must be kept to a minimum.

The advantage of the standard model is that it has few parameters. In general, if two models give similar results, the simpler model should be selected. The disadvantage with the standard model is that is does not take into account the gastric emptying and intestinal transit processes but treats the gastrointestinal tract as a 'black box'. It may also be too simplistic to fit more complicated profiles as we see for the sulfapyridine data.

In the transit rate model the gastrointestinal tract is divided into the stomach, the small intestine and the colon. The small intestine is, in turn, partitioned into 6 segments. The advantage with this model is that it includes gastric emptying and intestinal transit processes and that it is flexible enough to fit more complicated data. Its main disadvantage is that with limited data sets, the number of parameters may be too large for reliable estimation.

The lag time model also divides the gastrointestinal tract into the stomach, the small intestine and the colon. It has the same number of parameters as a transit rate model with 6 small intestine compartments. It too has greater flexibility than the standard model but may be difficult to fit reliably to small data sets. An advantage of this model over the transit rate model is that it is often easier to find information in the literature on how long a substance resides in a compartment than on the corresponding transit rate. The disadvantage with the lag time model is that it seems to be less flexible than the transit rate model with respect to the shape of the pharmacokinetic profile. In order to simulate the lag time model for the literature sulfapyridine data, the gastric emptying and the absorption rates needed to be set to a low value, which contradicts prior knowledge.

For paracetamol, both the standard model and the transit rate model provide good fits to the observed plasma levels. However, in order to fit the transit rate model to the paracetamol data with WinNonlin the absorption rate constant (k_a) was held fixed at 1. This was necessary because based on the available data it was difficult (or impossible) to disentangle the estimates of k_a and k_s and the focus of this study was k_s. Clearly, the choice of k_a will affect the absolute value of k_s but it should not have an impact on the difference in k_s estimates between various treatments. By setting k_a to 1 the transit rate model is in fact almost reduced to the standard model (provided that the small intestine transit rate, as in our case, is small). As expected, the k_a estimates from standard model nearly coincide with the k_s estimates in the transit rate model, as can be seen by comparing table 5 with table 6. In general, the advantage of the transit rate model is that it does allow for separation of the absorption and the gastric emptying processes, so if k_a is known from the literature or from other experiments k_s gives an absolute rather than a relative estimate.

For the sulfapyridine data taken from literature (see section 3.2.2), the transit rate model provided an accurate fit to the observed plasma concentrations. The fit of the standard model was poor, and especially so in the absorption phase. Since the time of first appearance in the plasma is of primary interest, this is a significant weakness of the standard model for this data. For the experimental sulfapyridine data, the results were similar: the transit rate model resulted in good fits while the standard model did not. The experimental data is however more difficult to evaluate due to a lack of measurement points for the central part of the

concentration profiles. If this experiment were to be repeated, additional blood samples should be taken between 400 and 1440 minutes in order to allow for more reliable model fitting and evaluation. Additional measurements should also be made during the elimination phase. To compensate for this increase in the number of samples, some of the initial measurements could be omitted. With pharmacokinetic models they are not as critical as when first appearance is estimated based on the first observation.

The different treatments were selected based on their expected effect on the gastrointestinal tract. For the gastric emptying rate, the observed results are in line with what was expected beforehand. Erythromycin, which was expected to stimulate gastric emptying, indeed resulted in a larger k_a value for the standard model and a larger k_s value for the transit rate model, compared to saline treatment. The expected inhibiting effect of atropine is also clear. For the small intestine transit rate, the results are less clear. The natural variation between experiments seems to be as large as or greater than any treatment effect. This could be due to a reduction in the treatment effect over time, so that by the time that sulfasalazine reaches the colon, natural variation in the transit time for a dog outweighs the effects of the treatments. Another possible explanation is that the data is simply not complete enough to distinguish the true treatment effects. Alternatively, different treatments may affect different dogs in different ways, although this seems less likely as an explanation.

Paracetamol was given in a dose of 24 mg/kg as a solution. Treatment with saline resulted in a C_{max} of around 90 µmol/L and t_{max} around 30 minutes. This can be compared to a C_{max} of 54 µmol/L and a t_{max} of 1.2 hours obtained by Reppas et al. (1998) after giving a gelatin capsule with a dose of around 21 mg/kg on average. Sagara et al. (1995) achieved a C_{max} between 36 µmol/L and 117 µmol/L and a t_{max} between 0.1 hours and 1.1 hours depending on in which gastric contraction phase the dose of 20 mg/kg was given. Lefebvre et al. (2001) gave a dose of 10 mg/kg of paracetamol to Beagle dogs. This resulted in a C_{max} of 27 µmol/L, which appeared at 15 minutes. Thus, our estimates are reasonable compared to those in the literature.

The estimation of C_{max} and t_{max} for sulfapyridine was difficult due to a lack of measurements in the vicinity of t_{max}. The sulfapyridine dose was 20 mg/kg. The dogs treated with saline had average C_{max} values of 5.6 µmol/L and average t_{max} values of around 13 hours. When Takaya et al. (1997) gave an oral dose of 25 mg/kg of sulfasalazine to Beagle dogs, the resulting profile showed a C_{max} of 8 µmol/L and a t_{max} at 11.3 hours. In our experiments, the standard model estimated the time of first appearance (defined as when the level of sulfapyridine first exceeds 100 nmol/L) under saline treatment to 5 hours on average. For the transit rate model the estimate was around 2.9 hours. There are reports in the literature that sulfapyridine first appears in the plasma between 3 and 4 hours after administration (Takaya et al., 1997). Mizuta et al. (1990) and Papasouliotis et al. (1995) report the time of first appearance for sulfapyridine to be 2.9 hours and almost 3 hours, respectively. Consequently, our results based on the transit rate model are more similar to those reported in the literature than are the results based on the standard model.

5 Conclusions

The double marker technique is a useful approach for studying gastric emptying and small intestinal transit. With the help of pharmacokinetic models, the accuracy of the estimated first appearance and gastric emptying rate may be increased – in particular when the amount of available data is limited. For the experiments analyzed in this thesis, the transit rate model seems to be the overall best choice. While both the standard model and the transit rate model fit well to the paracetamol plasma levels, the transit rate model provides considerably better predictions for the sulfapyridine data.

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Appendix 1

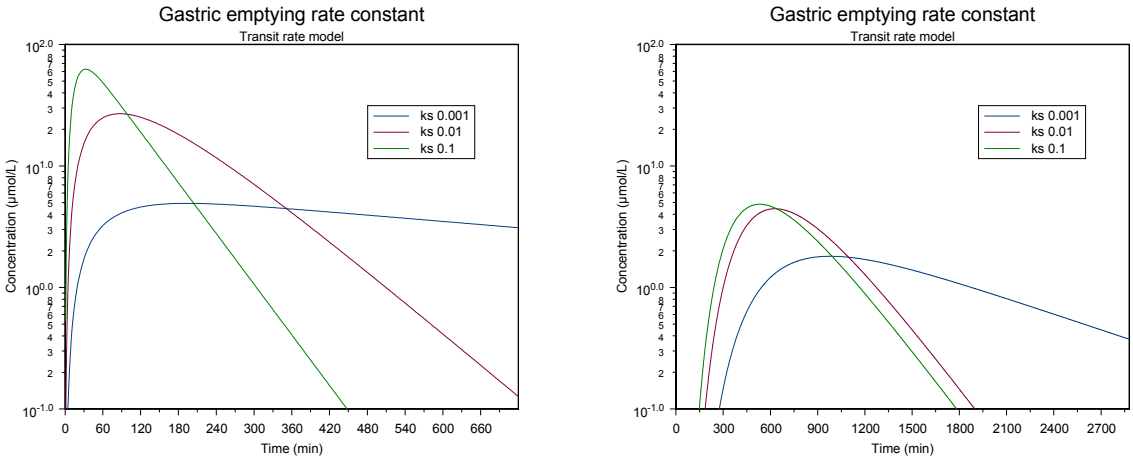

Figure 25. The impact of changing the gastric emptying rate constant (k_s) on the pharmacokinetic profile of paracetamol (left panel) and sulfapyridine (right panel) in the transit rate model.

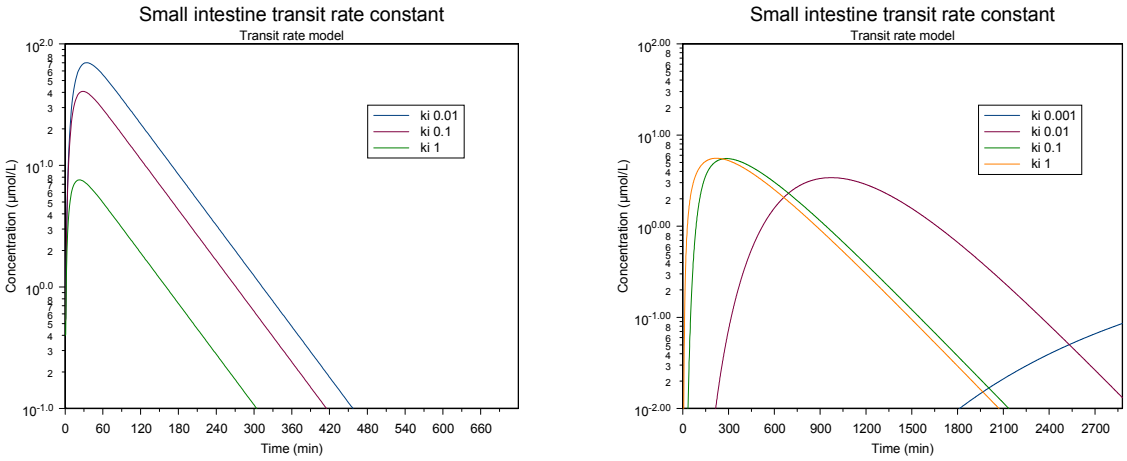

Figure 26. The impact of changing the transit rate constant of the small intestine (k_i) on the pharmacokinetic profile of paracetamol (left panel) and sulfapyridine (right panel) in the transit rate model.

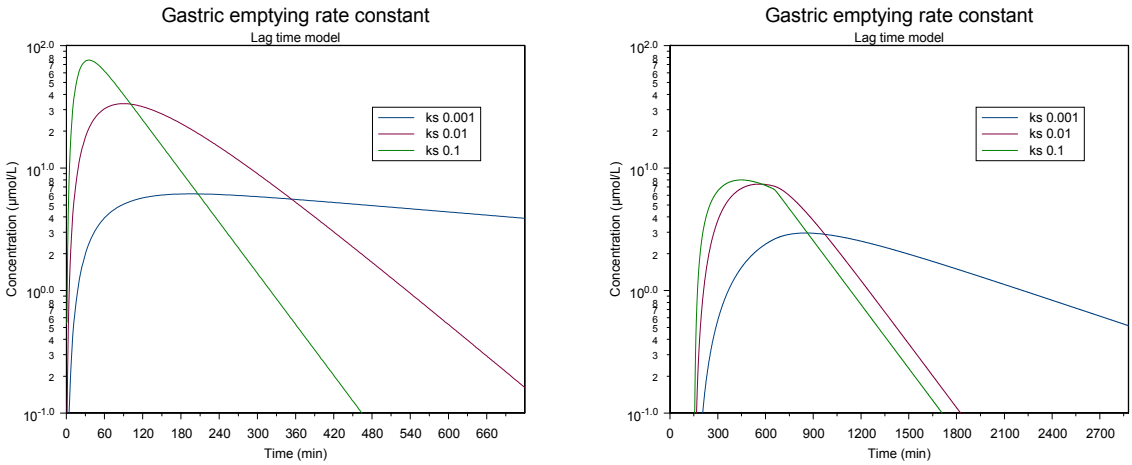

Figure 27. The impact of changing the gastric emptying rate constant (k_s) on the pharmacokinetic profile of paracetamol (left panel) and sulfapyridine (right panel) in the lag time model.

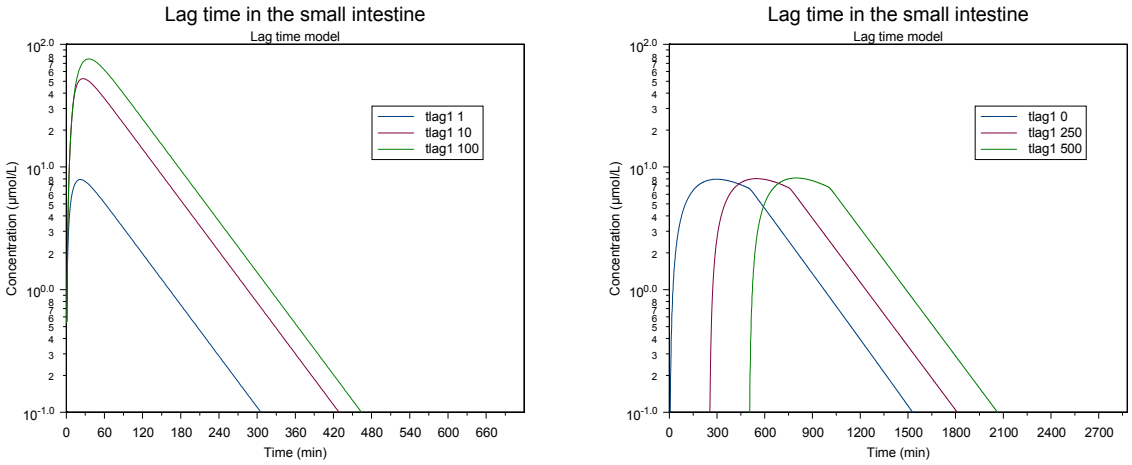

Figure 27. The impact of changing the residence time in the small intestine (t_{lag1}) on the pharmacokinetic profile of paracetamol (left panel) and sulfapyridine (right panel) in the lag time model.

Appendix 2

Berkeley Madonna source code for the standard model

```
{Model: The standard model}
{Marker: Paracetamol}
{Treatment: Saline}
{Dog: 1 (Ior)}

METHOD RK4              {integration method: Runge-Kutta4}

STARTTIME = 0           {initial time of simulation}
STOPTIME = 1440         {final time of simulation}
DT = 0.02               {time step}

dose = 159      {µmol/kg  oral dose}
ka = 0.11       {1/min  absorption rate constant}
ke = 0.014      {1/min  elimination rate constant}
V = 1.1         {L/kg    volume of distribution}


d/dt (A) = -ka * A
init A= dose

d/dt (C) = ka * A/V - ke * C
init C = 0
```

Berkeley Madonna source code for the transit rate model

```
{Model: The transit rate model}
{Marker: Paracetamol}
{Treatment: Saline}
{Dog: 1 (Ior)}

METHOD RK4              {integration method: Runge-Kutta4}

STARTTIME = 0           {initial time of simulation}
STOPTIME = 1440         {final time of simulation}
DT = 0.02               {time step}

dose = 159      {µmol/kg  oral dose}
ks = 0.13       {1/min  gastric emptying rate constant}
ki = 0.01       {1/min  small intestine transit rate constant}
kc = 0.003      {1/min  colon transit rate constant}
ka = 1          {1/min  absorption rate constant}
ke = 0.015      {1/min  elimination rate constant}
V = 1.1         {L/kg    volume of distribution}
n = 6           {number of small intestine compartments}


{Stomach}
d/dt (As) = -ks * As
init As = dose

{Small intestine}
d/dt (Ai[1]) = ks *As - (ki + ka) *Ai[1]
d/dt (Ai[2..n]) = ki *Ai[i -1] - ki *Ai[i]
```

```
init Ai[1..n] = 0

{Colon}
d/dt (Ac) = ki *Ai[n] - kc *Ac
init Ac = 0

{Plasma}
d/dt (C) = ka *Ai[1]/V - ke *C
init C = 0
```

Berkeley Madonna source code for the lag time model

```
{Model: The lag time model}
{Marker: Paracetamol}
{Treatment: Saline}
{Dog: average over six dogs}

METHOD RK4          {integration method: Runge-Kutta4}

STARTTIME = 0       {initial time of simulation}
STOPTIME = 1440     {final time of simulation}
DT = 0.02           {time step}

dose = 159      {µmol/kg  oral dose}
ks = 0.09       {1/min  gastric emptying rate constant}
ka = 1          {1/min  absorption rate constant}
ke = 0.015      {1/min  elimination rate constant}
V = 1.2         {L/kg   volume of distribution}
t1 = 172        {min    residence time in the small intestine}
t2 = 300        {min    residence time in the colon}


{Stomach}
d/dt (As) =  -ks * As
init As = dose

{Intestine}
d/dt (Ai) = ks *As - ka *Ai - delay_i
delay_i = DELAY(ks *As, t1, 0) * EXP(-ka * t1)
init Ai = 0

{Colon}
d/dt (Ac) = delay_i - delay_c
delay_c = DELAY(delay_i, t2, 0)
init Ac = 0

{Plasma}
d/dt (C) = ka * Ai/V - ke * C
init C = 0
```