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## A physiologically based model for tramadol pharmacokinetics in horses

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**ABSTRACT:** This work proposes an application of a minimal complexity physiologically based pharmacokinetic model to predict tramadol concentration vs time profiles in horses. Tramadol is an opioid analgesic also used for veterinary treatments. Researchers and medical doctors can profit from the application of mathematical models as supporting tools to optimize the pharmacological treatment of animal species. The proposed model is based on physiology but adopts the minimal compartmental architecture necessary to describe the experimental data. The model features a system of ordinary differential equations, where most of the model parameters are either assigned or individualized for a given horse, using literature data and correlations. Conversely, residual parameters, whose value is unknown, are regressed exploiting experimental data.

The model proved capable of simulating pharmacokinetic profiles with accuracy. In addition, it provides further insights on un-observable tramadol data, as for instance tramadol concentration in the liver or hepatic metabolism and renal excretion extent.

**KEYWORDS:** PBPK model; QSP; Tramadol; Horse; Analgesia.

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# 1 Introduction

The knowledge of pharmacokinetic properties of drugs is fundamental to guarantee an effective and safe therapeutics administration. This is important for every drug and is required information in reports submitted to regulatory agencies for products approval. Analgesic administration is particularly critical because the definition of a correct dosage is not universal but should be chosen for every patient. In addition, a constant, real-time monitoring of sedation state is necessary. This is achieved by controlling some pharmacodynamic parameters and adapting the analgesic administration accordingly, to maintain the subject in the optimal sedation state. Pharmacodynamic parameters are partial indicators of sedation state, allow an indirect and approximated guess of analgesic blood levels, and help anesthetists adapting the dosage based on their experience.

The only possibility to quantify the actual analgesic concentration is blood sampling and the drug concentration assay. Clearly, this comes with several difficulties and limitations, and is not always possible. A valid alternative is the exploitation of mathematical models to predict drug concentration levels. Numerical simulations allow the generation of drugs' blood concentration vs time profiles (model output) given an administration route and dosage (model input).

This work focuses on tramadol, an analgesic drug extensively used for both clinical and non-clinical applications, and proposes a pharmacokinetic (PK) model for the assessment and prediction of tramadol blood concentration in horses. There are two past activities that led to the realization of this work: one has an empirical nature and is linked to studies on tramadol administration in horses (Cagnardi et al., 2014), the other is based on research on PK mathematical models (Abbiati et al., 2016). The joint activity of these research groups is summarized in this paper and is an example of the convergence of different knowledges in the emerging discipline of quantitative systems pharmacology.

A PK model based on a mechanistic approach, named physiologically based pharmacokinetic (PBPK) model, is applied for the *in silico* reproduction (*i.e.* computer simulation) of tramadol concentration vs time profiles in horses. In particular, we propose a minimal complexity PBPK model, this means that the compartmental structure of the model is reduced in accordance with the limited experimental information available. The compartmental formulation of PBPK models is based on the hypothesis of perfectly mixed compartments. Every compartment is assumed as a heterogeneous volume, whose dimensions are comparable to the related body counterpart, see (Abbiati et al., 2016) for further details on mathematical and model-related matters.

## 1.1 Tramadol in horses and PK modeling

In veterinary species, the principles of anesthesia and analgesia are based on different drug administrations. In horse practice, the patient is pre-medicated with sedatives or tranquillizers to decrease the attention level and external stimuli response, induced into an unconsciousness level with hypnotic anesthetic drugs, and then the anesthesia can be maintained with inhalation anesthetics. To prevent nociception during surgery and achieve a stable anesthetic depth, analgesic drugs can be administered before surgery start, as pre-emptive treatment (Miur and Hubbel, 2009).

In veterinary medicine, PBPK models can predict tissue concentrations of active principles and their potential metabolites after complex exposures, and are powerful in extrapolating dosage schemes across species. Thus, PBPK models have been widely used in predictive risk assessment for a variety of environmental contaminants in laboratory-animals and humans, and in estimating tissue residues and withdrawal times for drugs in food animals (Lin et al., 2016).

The use of PBPK models is quite limited in horse. Indeed, two studies were published so far, by the same group of researchers, with the aim of evaluating amiodarone or ketamine pharmacokinetics in ponies (Knobloch et al., 2006; Trachsel et al., 2004).

As far as tramadol is concerned, there are not any PBPK models available in veterinary species. In horse, its PK profiles, following different routes, dosage schemes, and with different formulations was widely studied with classical non-compartmental and compartmental analyses, but at our knowledge it was not simulated with PBPK modeling.

## 1.2 Tramadol

Tramadol, is a synthetic opioid analogue to morphine and codeine. In humans, it is commonly used as an analgesic drug for acute and chronic pain treatment, so as for moderate to severe post-operative pain. It has a low incidence of adverse effects, *i.e.* respiratory depression, constipation and abuse potential, and is not a controlled drug (Grond and Sablotzki, 2004; Scott and Perry, 2000). Tramadol is also used in companion animal veterinary practice for pain management. Since the first report of its analgesic effect in horses after epidural administration by Natalini and Robinson (2000), tramadol has been introduced in equine practice as analgesic drug for moderate to severe pain, and pharmacokinetic and efficacy studies (Dhanjal et al., 2009; Knych et al., 2016; Shilo et al., 2008; Stewart et al., 2011). Tramadol is administered both orally and parenterally. The intravenous dose is extrapolated from human or other species data and ranges from 3 to 5 mg/kg BW, with the consequent uncertainties associated to this practice.

Tramadol undergoes extensive first-pass metabolism in the liver, via two main metabolic pathways involving cytochrome P450 isoenzymes CYP3A and CYP2D6 (Scott and Perry, 2000). The major metabolites present in human plasma are O-desmethyltramadol (M1) and N-desmethyltramadol (M2), and to a minor extent N,N-didesmethyltramadol (M3), N,N,O-tridesmethyltramadol (M4) and N,O-desmethyltramadol (M5) (Grond and Sablotzki 2004). M1 formation is mediated by CYP2D6, whereas M2 is produced by CYP3A4 and CYP2B6. Tramadol and its metabolites are almost completely excreted *via* the kidneys and approximately 10 to 30% of the parent drug is excreted unmetabolised in the urine (Grond and Sablotzki, 2004; Scott and Perry, 2000). These indications are consistent with NIH (2017) data on tramadol elimination, where approximately 30% is eliminated unmodified by kidneys, while 60% is a product of hepatic metabolism. M1 is the main analgesic effective metabolite with a more potent  $\mu$ -receptor effect than the parent compound and its formation occurs at different rates in veterinary species (Cagnardi et al., 2011; Knych et al., 2013; Kukanich and Papich, 2004). In horse, M1 formation seems to be catalyzed by the CYP2D50 enzyme, the equine orthologue to human CYP2D6 (Corado et al., 2016).

## 2 Methods

The experimental data on tramadol PK in horses are available in Cagnardi et al. (2014), with horses data synthetically reported in Table 1.

Table 1 – Horse ID, Breed, and Body Weight summary (Cagnardi et al., 2014). The original study reported 8 horses but one was later excluded due to issues related to blood withdrawals (see missing Horse ID = 4).

Patient	Horse ID	Breed	Body Weight (kg)
1	1	Arabian	292
2	2	Arabian	336

3	3	Arabian	324
4	5	Arabian	303
5	6	Arabian	323
6	7	Thoroughbred	431
7	8	Quarter Horse	490

Eight horses undergoing orchiectomy received, as a pre-emptive analgesic, an intravenous (IV) injection of 4 mg/kg tramadol administered in 60 s, 15 min before surgery, which lasted 20 min. The anesthetic protocol for each horse included: intramuscular acepromazine maleate (0.05 mg/kg BW) and detomidine (range 0.01-0.02 mg/kg BW), as pre-anesthetic medications; IV ketamine (2.2 mg/kg BW) and diazepam (0.05 mg/kg BW) for general anesthesia induction; isoflurane in oxygen (100%) for maintenance. Only the pharmacokinetics of tramadol and its metabolites was monitored in blood and urine. Eleven blood samples were collected at 0.08, 0.16, 0.33, 0.5, 0.75, 1, 1.5, 2, 4, 8, and 10 h post administration.

The adopted PBPK model is the one from (Abbiati et al., 2016), which is a lumped model based on the approach of minimal complexity (Cao and Jusko, 2012). This model was further lumped due to the limitation of experimental data: the original model considers two distribution compartments, the former for the highly perfused organs and the latter for the poorly perfused tissues. Despite this assumption can be important in case of availability of experimental drug concentration in tissues, as proved in Abbiati et al. (2015), we considered inappropriate such a differentiation for the present case as only plasma PK is known. For this simulation, a single compartment for general site of drug distribution was used and named "Tissues". Figure 1 shows the actual model compartmental structure, which features four compartments.

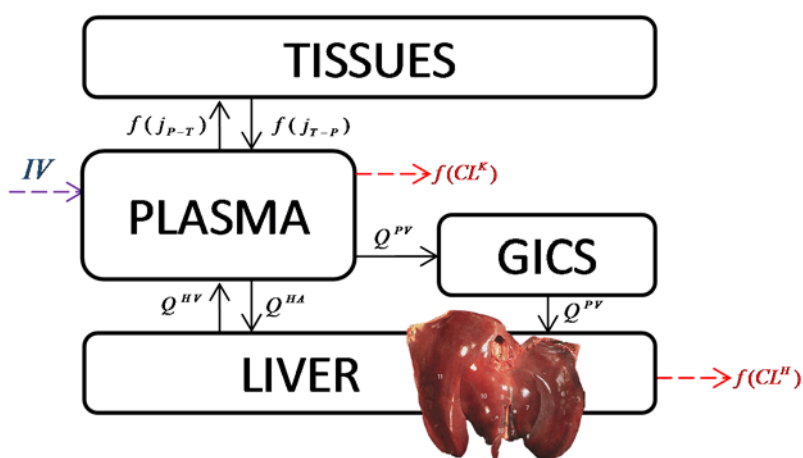


Figure 1: Structure of the lumped PBPK model. Tramadol is administered endovenous (IV) to Plasma compartment. Systemic circulation delivers the drug to the Tissues compartment, which represents a general distribution volume, and to the Liver. Tramadol reaches the Liver directly via the hepatic artery and GICS, which is the vasculature located in the mesentery. Drug is eliminated at different extents via liver metabolism ( $CL^H$ ) and by the kidneys ( $CL^K$ ).

The model eq.s (1 - 4) are:

$$\frac{dC^P(t)}{dt} = -C^P(t) \left( j_{p-t}(1-R) + \frac{Q^{HA}}{V^P} + \frac{Q^{PV}}{V^P} \right) + C^T(t) j_{t-p} \frac{V^T}{V^P} + C^L(t) \frac{Q^{HV}}{V^P} +$$

$$-C^P(t) \frac{CL^K}{V^P} + \frac{IV(t)}{V^P} \quad (1)$$

$$\frac{dC^T(t)}{dt} = -C^T(t) (j_{t-p}) + C^P(t) (1-R) j_{p-t} \frac{V^P}{V^T} \quad (2)$$

$$\frac{dC^{GICS}(t)}{dt} = C^P(t) \frac{Q^{PV}}{V^{GICS}} - C^{GICS}(t) \frac{Q^{PV}}{V^{GICS}} \quad (3)$$

$$\frac{dC^L(t)}{dt} = -C^L(t) \left( \frac{Q^{HV}}{V^L} + \frac{CL^H}{V^L} \right) + C^P(t) \frac{Q^{HA}}{V^L} + C^{GICS}(t) \frac{Q^{PV}}{V^L} \quad (4)$$

Eq. (1) refers to the Plasma compartment (integration variable:  $C^P$ ), eq. (2) to the Tissues compartment (integration variable:  $C^T$ ), eq. (3) to the Gastro Intestinal Circulatory System or GICS (integration variable:  $C^{GICS}$ ), and eq. (4) to the Liver compartment (integration variable:  $C^L$ ). Here,  $j_{P-T}$  and  $j_{T-P}$  are respectively the drug mass transfer coefficients in and out of the Tissues compartment, whilst  $R$  is the fraction of the drug which is bound to plasma proteins.

Clearance terms (eq.s (5) and (6)) are calculated as the product of organ's plasma flow multiplied by the clearance efficiency, whose values are reported respectively in Table 2 and Table 3.

$$CL^H = Q^{PV} \cdot Eff^H \quad (5)$$

$$CL^K = Q^K \cdot Eff^K \quad (6)$$

The model features a system of ordinary differential equations, which are integrated with the *ode45* solver of Matlab R2015a, *i.e.* a single step solver based on an explicit Runge-Kutta (4<sup>th</sup>-5<sup>th</sup> order) formula (Shampine and Reichelt, 1997).

Model parameters assignment follows the methodology detailed in Abbiati et al. (2016). There are three major categories of model parameters: Individualized, Assigned, and Unknown.

The Individualized parameters are determined with literature correlations based on known physical properties. Here parameters as blood flow rate ( $Q$ ) and compartment volumes ( $V$ ) are calculated as a function of individual horse body weight, as shown in Table 2.

Table 2 – Individualized parameters, the "Value" column is provided as an example and refers to horse ID 1 of Table 1 ( $BW = 292$  kg). Density is in  $[g/cm^3]$ .

Parameter	Description	Formula	Value	Units	Reference		
$CO$	Cardiac Output †	$74 \cdot BW$	21,608	$cm^3/min$	(Fisher and Dalton, 1959)		
$Q^{HA}$	Hepatic artery blood flow	$30\% \cdot 25\% \cdot CO$	972	$cm^3/min$	(Drivers, 2015)		
$Q^{PV}$	Portal vein blood flow	$70\% \cdot 25\% \cdot CO$	2,268	$cm^3/min$	(Drivers, 2015)		
$Q^{HV}$	Hepatic vein blood flow	$Q^{HA} + Q^{PV}$	3,241	$cm^3/min$			
$Q^K$	Kidneys blood flow	$17.5\% \cdot CO$	2,268	$cm^3/min$	(Toribio, 2007)		
$V^{GICS}$	GICS volume	$1.7725 \cdot BW$	517	$cm^3$	Patton et al. (2006) † †		
$V^L$	Liver volume	†††	3,527	$cm^3$	Staddon et al. (1984)		
						$BW\%$	Density
		Liver				1.3	1.076
$V^T$	Tissues volume	††† $V_{Fat} + V_{Bones} + V_{Heart} +$ $+ V_{Skin} + V_{Muscles}$	171,110	$cm^3$	Staddon et al. (1984)		
						$BW\%$	Density
		Fat				5.06	0.929
		Bones				10.3	1.68

		Heart	0.66	1.06			
		Skin	7.41	1.09			
		Muscles	40.14	1.065			
$V^p$	Plasma Volume	†††† 60 % · $V^{Blood}$			14,350	mL	Staddon et al. (1984)
			BW %	Density			
		Blood	8.6	1.05			

† CO varies dramatically with horse physical activity. We assumed a horse in rest status as expected in case of tramadol administration for surgery intervention.

††  $V^{GICS}$  was not found in the literature. We propose to calculate it according to geometrical considerations. Length and diameter of the portal vein of a horse are approximately 23 cm and 4 cm (Patton et al., 2006), to account for the complete mesenteric circulatory system we increased these value by 35% and, assuming the portal vein as a cylinder, its volume would be 710 cm<sup>3</sup>. Dividing it by the average weight of a horse (400 kg), we obtain the portal vein volume per kilogram of body weight ( $\omega = 1.77 \text{ cm}^3/\text{kg}$ ).

††† The volume of the organs is calculated with eq. (7):

$$V_{organ} = \frac{Weight_{organ} \% \cdot BW}{Specific\ Weight_{organ}} [cm^3] \quad (7)$$

where  $Specific\ Weight_{organ}$  is  $Density_{organ}/1000$ .

†††† Plasma/serum was assumed as 60% of blood volume. (Tranquilli et al., 2007)

The category "Assigned parameters" refers to known values that cannot be individualized for a specific horse; instead, their values are considered constant for any subject. Tramadol, as many other drugs, has tendency to bind plasma proteins. This has profound and direct consequences on drug distribution extent. Our model accounts for this aspect with  $R$  parameter, which quantifies tramadol fraction bound to plasma proteins, being 19.5 - 20% (i.e.  $R = 0.2$  in eq.s (1-4)) as in (Cagnardi et al., 2014) [ENREF 4](#), (Lee et al., 1993) [ENREF 17](#) and (Abbiati et al., 2016) [ENREF 3](#).

Finally, this model considers four Unknown parameters (i.e.  $j_{p-T}$ ,  $j_{T-p}$ ,  $E_{ff}^H$ ,  $E_{ff}^K$ ). These parameters cannot be found in the literature, in part because they are very specific tissue properties and in part because they are mathematical terms without a direct physical counterpart. For example, the  $j_{p-T}$  term represents the mass transport coefficient of tramadol from vasculature to the generic Tissues compartment. It depends on a number of biological and physical tissue properties, as vasculature fenestration size, blood flow velocity at the various capillary diameters, interstitial liquid and extracellular matrix properties, just to cite a few. In addition, since Tissues is a lumped compartment, it accounts for a number of tissues with very different properties. Consequently, a direct assessment of these parameter values is unfeasible, and we decided to regress them by using a suitable set of experimental data of tramadol PK in horses.

The mathematical model for the regression procedure is the one defined in eq.s (1-4), Individualized and Assigned parameters were given as calculated above, while the Unknown parameters were initialized with first-guess values. The experimental data for the regression procedure are the ones from Table 1 for ID 6, 7, and 8 horses.

The non-linear regression minimizes an objective function defined as the sum of residuals (SR) between experimental measures and model predictions. The SR definition is a fundamental prerequisite for a successful regression. We adopted a logarithmic formulation in order to equalize the contributions of SR. In addition, we added two terms to account for the clearance pathways. In Section 1.2 "Tramadol", we discussed tramadol PK and highlighted that drug elimination occurs for about 60% of the dose as hepatic metabolism, for 30% as renal excretion, while the residual 10% can be ascribed to other delocalized tissues metabolism. At the purpose of parameters regression and as a function of the lumped PBPK model, to efficiently determine parameters such as  $CL^H$  and  $CL^K$ , we imposed that tramadol eliminated via liver metabolism ( $CL^H$ ) accounts for 66% (i.e.  $EL_{MAX}^H$ ), while tramadol eliminated via kidneys ( $CL^K$ ) accounts for



34% (*i.e.*  $EL_{MAX}^K$ ). By doing so, we arbitrarily neglected the previously cited 10% of tissues metabolism at the purpose of keeping the number of model parameters limited. Indeed, we redistributed this contribution between liver and kidneys. The last two terms of SR in eq. (8) enhance the physiological attributes of the mathematical model.

$$SR = \sum_i^{NP} \left( \left( \sum_j^{NS} \left| \log_{10}(C_{exp,j,i}) - \log_{10}(C_{model,j,i}) \right| \right) + \left| \log_{10}(Dose_i \cdot EL_{MAX}^H) - \log_{10}(Emass_{H,i}(t_{fin})) \right| + \left| \log_{10}(Dose_i \cdot EL_{MAX}^K) - \log_{10}(Emass_{K,i}(t_{fin})) \right| \right) \quad (8)$$

Here,  $NP$  is the number of patients with index  $i$ , and  $NS$  is the number of blood samples collected with index  $j$ .  $C_{exp}$  is the experimental measure of tramadol concentration,  $C_{model}$  is the corresponding model calculated one.  $Dose$  is the total administered dose,  $Emass_H(t_{fin})$  is the cumulated value of the liver-metabolized mass at the final time of simulation  $t_{fin}$ ,  $Emass_K(t_{fin})$  is the cumulated value of the kidneys-eliminated mass at  $t_{fin}$ . The regression was performed using the *fminsearch* routine of Matlab R2015a, based on the modified simplex method of Lagarias et al. (1998).

Table 3 – Unknown parameter values, determined via the non-linear regression.

Parameter	Description	Value	Units	Standard Deviation	Confidence Interval (95%)
$j_{P-T}$	Plasma to Tissues mass transfer coefficient	0.108	min <sup>-1</sup>	0.0027	$3.73 \cdot 10^{-4}$
$j_{T-P}$	Tissues to Plasma mass transfer coefficient	0.0125	min <sup>-1</sup>	0.0003	$4.24 \cdot 10^{-5}$
$Eff^H$	Hepatic efficiency of elimination	0.618	-	0.0161	$2.24 \cdot 10^{-3}$
$Eff^K$	Kidneys efficiency of elimination	0.225	-	0.0059	$8.02 \cdot 10^{-4}$

Once the whole set of model parameters is determined, the model was validated by running distinct *in silico* simulations of the remaining subjects (*i.e.* horse IDs 1, 2, 3, and 5). Input data were the administered dose and the horse BW. The Individualized parameters were calculated according to the previous described correlations of Table 2, while the Assigned and Unknown parameters were kept the same for every horse. Eventually, simulation results were compared to experimental data.

### 3 Results and Discussion

The PBPK model was simulated and compared to the experimental measures. Figure 2 shows the tramadol PK simulation for the representative horse ID 5 as all the other simulations show comparable simulated PK profiles. The concentration vs time drug profile displays a rapid increase of tramadol concentration, which reaches the peak value at the end of injection (1 min post administration start). Tramadol goes through a rather rapid distribution process from the central compartment (*i.e.* Plasma) to the Tissues compartment, which acts as a reservoir site. From the peripheral compartment (Tissues) the drug returns to the central one, where it is eliminated via renal excretion. This produces the two-phase decline, which is evident in the semi-log plots of Figure 2. The first phase lasts 25 min post administration with a rapid tramadol distribution, followed by a second elimination phase with slower kinetics.

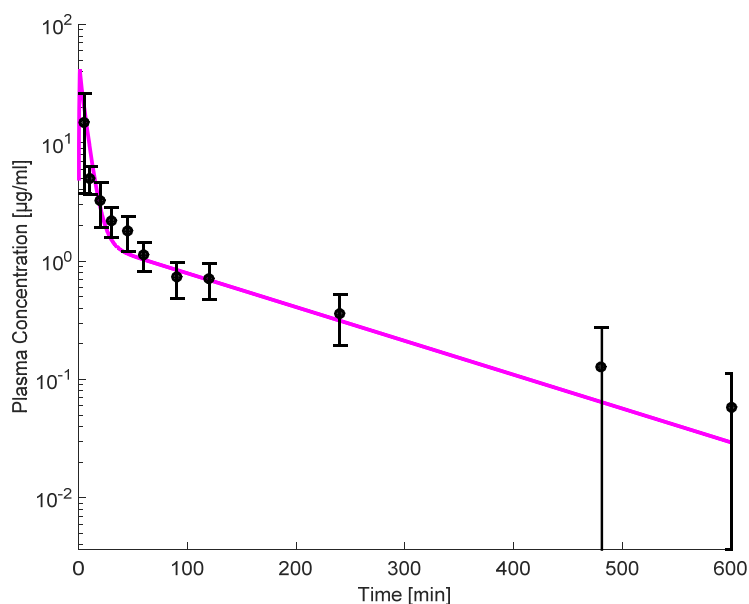


Figure 2: Model validation by comparison with the experimental data (ID 5 horse).

This lumped PBPK model shows a general behavior of a bi-compartmental model, which is the two-phase decline, as expected from the presence of two major compartments (*i.e.* Plasma and Tissues) that are in direct relation.

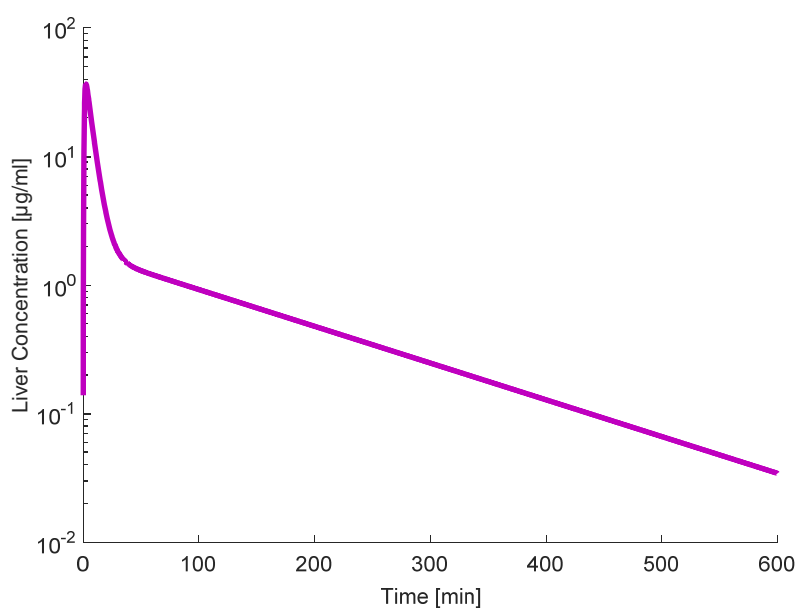


Figure 3: Model simulation of tramadol concentration in the Liver compartment. This is an un-observable compartment from an experimental viewpoint, unless tissue samples are dynamically collected. This practice is complex and usually limited by sensible and ethical constraints. In this perspective, modeling is a necessary instrument for quantitative systems pharmacology.

In addition, it introduces other advantages: it allows the simulation of tramadol PK in sites other than plasma (Figure 3) and it clearly differentiates the elimination process in terms of hepatic metabolism and renal excretion (Figure 4). For the sake of precision, the elimination term for liver metabolism refers to the disappearance of tramadol molecules to give metabolites. The simulated profiles of Figure 4 are important because of the extensive P450 cytochrome metabolic activity. The accurate formulation of the SR objective



function (eq. (8)) allows quantifying each of these elimination routes. Figure 4 evidences that nearly the entire tramadol dose is eliminated in 10 h and is found in the urine, 2/3 as metabolites and the remaining excreted unmodified.

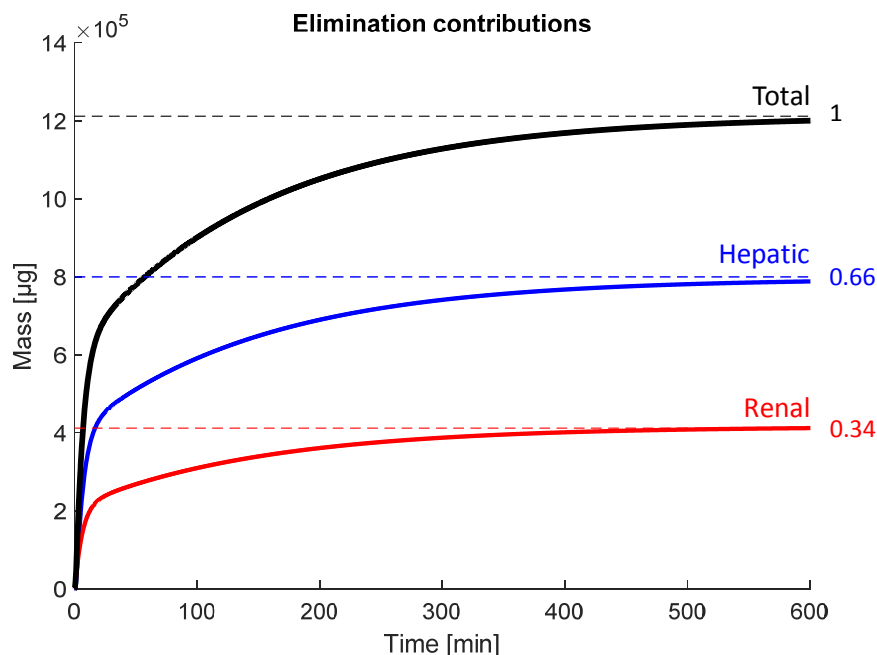


Figure 4: Tramadol elimination by the two physiological routes: hepatic and renal. Left vertical axis reports tramadol mass [µg], right vertical values are the normalized mass fractions respect to the administered dose. Dashed lines represent the expected eliminations by the respective mechanisms: blue (0.66) for liver metabolism and red (0.34) for kidneys elimination. Continuous lines are the model simulated profiles of cumulated eliminated mass: blue for liver metabolism and red for kidneys elimination.

Figure 2 shows that the model prediction is in good agreement with the experimental data, as the concentration vs time curve of tramadol falls within the standard deviation bars for most of the experimental data. To provide a numerical quantification of the pharmacokinetic curve quality we adopted the prediction error coefficient ( $PE$  in eq. (9) and Table 4). This parameter quantifies the normalized difference of model concentration respect to the experimental one, divided by model concentration at the  $j$ -th sampling time.

$$PE_j = \frac{c_j^{model} - c_j^{exp}}{c_j^{model}} \quad (9)$$

Table 4 –  $PE$  values at each blood withdrawal time. The time is exactly the one of the withdrawal. That is why it might slightly be different from the declared ones. Data are reported for horse ID 5, others show similar  $PE$  median values.

Time [min]	5	10	20	30	45	60	85	116	245	480	600
$PE_j$	0.254	0.465	0.1663	0.448	0.560	0.102	0.161	0.002	0.183	0.984	0.991

The reference parameter is the median of this  $PE$ , which is equal to 0.25. This value is strongly and negatively affected by the last two samples (at 480 and 600 min). Here the tramadol concentration is practically negligible, but since  $PE$  is based on a normalized value, it produces errors close to 100%. If these two data were neglected the median  $PE$  would drop to 0.18.

## 4 Conclusions

Analgesic therapies can gain profit from the use of PBPK models since numerical predictions allow forecasting un-observable drug concentration levels. Additionally, since dosage can be varied due to patient response or physician necessity, the use of a robust model allows the instantaneous re-evaluation of the expected drug concentration-time profile. The predictive capability of the PBPK model paves the way to a model-based approach to anesthesia control (e.g., Model Predictive Control), as the individualized patient's response can be evaluated *in silico* and tuned to meet the optimal trajectory of anesthesia treatment as a function of the (dynamically) administered dose. The patient's response to the administered treatment is a feedback signal (input) for the on-line control unit, which determines any necessary corrections to the administered dose via a dynamical model adaptation (Abbiati and Manca, 2016).

The minimal-PBPK model presented in this work has the advantage to preserve a physiological correspondence with the administration of tramadol in horse (see the set of individualized parameters in Table 2, or the structure of Figure 1 where the Liver compartment and metabolism are explicitly considered). The physiological correspondence allows identifying specific processes of drug distribution and elimination. Furthermore, only PBPK models can be adapted to predict PK of other species.

Since experimental data of drug concentration were measured just in serum, tissues concentration were not available. Therefore, complex full-body PBPK model, which are commonly used, do not permit a robust identification of regressed (Unknown) model parameters. This is why we promote the application of models whose complexity is balanced on experimental data availability.

Finally, this model was applied to run tramadol simulations in horses and showed good consistency with experimental data. Consequently, this model with its parameters set (Table 2 and Table 3) is suitable for further simulations of tramadol in horse. Veterinary practice can profit from the use of this model by reducing operative times and costs.

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## References

- Abbiati, R. A., Manca, D., 2016. A modeling tool for the personalization of pharmacokinetic predictions. *Computers & Chemical Engineering* 91, 28-37, doi:<http://dx.doi.org/10.1016/j.compchemeng.2016.03.008>.
- Abbiati, R. A., Cavallaro, G., Craparo, E. F., Manca, D., 2015. Sorafenib in Mice – A Pharmacokinetic Study. *Chemical Engineering Transaction* 43, 283-288, doi:10.3303/CET1543048.
- Abbiati, R. A., Lamberti, G., Grassi, M., Trotta, F., Manca, D., 2016. Definition and validation of a patient-individualized physiologically-based pharmacokinetic model. *Computers and Chemical Engineering* 84, 394-408, doi:10.1016/j.compchemeng.2015.09.018.

- Cagnardi, P., Ferraresi, C., Zonca, A., Pecile, A., Ravasio, G., Zani, D. D., Villa, R., 2014. Clinical pharmacokinetics of tramadol and main metabolites in horses undergoing orchiectomy. *Vet Q* 34, 143-51, doi:10.1080/01652176.2014.963208.
- Cagnardi, P., Villa, R., Zonca, A., Gallo, M., Beccaglia, M., Luvoni, G. C., Vettorato, E., Carli, S., Fonda, D., Ravasio, G., 2011. Pharmacokinetics, intraoperative effect and postoperative analgesia of tramadol in cats. *Research in Veterinary Science* 90, 503-509, doi:10.1016/j.rvsc.2010.07.015.
- Cao, Y., Jusko, W. J., 2012. Applications of minimal physiologically-based pharmacokinetic models. *J Pharmacokinet Pharmacodyn* 39, 711-23, doi:10.1007/s10928-012-9280-2.
- Corado, C. R., McKemie, D. S., Young, A., Knych, H. K., 2016. Evidence for polymorphism in the cytochrome P450 2D50 gene in horses. *J Vet Pharmacol Ther* 39, 245-54, doi:10.1111/jvp.12269.
- Dhanjal, J. K., Wilson, D. V., Robinson, E., Tobin, T. T., Dirokulu, L., 2009. Intravenous tramadol: Effects, nociceptive properties, and pharmacokinetics in horses. *Veterinary Anaesthesia and Analgesia* 36, 581-590, doi:10.1111/j.1467-2995.2009.00492.x.
- Drivers, T. J., 2015. The equine liver in health and disease. *AAEP Proceedings* 61, 66-103.
- Fisher, E. W., Dalton, R. G., 1959. Cardiac Output in Horses. *Nature* 184, 2020-2021.
- Grond, S., Sablotzki, A., 2004. Clinical pharmacology of tramadol. *Clin Pharmacokinet* 43, 879-923.
- Knobloch, M., Portier, C. J., Levison, O. L., Theurillat, R., Thormann, W., Spadavecchia, C., Mevissen, M., 2006. Antinociceptive effects, metabolism and disposition of ketamine in ponies under target-controlled drug infusion. *Toxicol Appl Pharmacol* 216, 373-86, doi:10.1016/j.taap.2006.06.011.
- Knych, H. K., Steffey, E. P., White, A. M., McKemie, D. S., 2016. Effects of age on the pharmacokinetics of tramadol and its active metabolite, O-desmethyltramadol following intravenous administration to foals. *Equine Veterinary Journal* 48, 65-71, doi:10.1111/evj.12364.
- Knych, H. K., Corado, C. R., McKemie, D. S., Scholtz, E., Sams, R., 2013. Pharmacokinetics and pharmacodynamics of tramadol in horses following oral administration. *Journal of Veterinary Pharmacology and Therapeutics* 36, 389-398, doi:10.1111/jvp.12009.
- Kukanich, B., Papich, M. G., 2004. Pharmacokinetics of tramadol and the metabolite O-desmethyltramadol in dogs. *Journal of Veterinary Pharmacology and Therapeutics* 27, 239-246, doi:10.1111/j.1365-2885.2004.00578.x.
- Lagarias, J. C., Reeds, J. A., Wright, M. H., Wright, P. E., 1998. Convergence Properties of the Nelder--Mead Simplex Method in Low Dimensions. *SIAM Journal on Optimization* 9, 112-147, doi:doi:10.1137/S1052623496303470.
- Lee, C. R., McTavish, D., Sorokin, E. M., 1993. Tramadol. A preliminary review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in acute and chronic pain states. *Drugs* 46, 313-40.
- Lin, Z., Gehring, R., Mochel, J. P., Lave, T., Riviere, J. E., 2016. Mathematical modeling and simulation in animal health - Part II: principles, methods, applications, and value of physiologically based pharmacokinetic modeling in veterinary medicine and food safety assessment. *J Vet Pharmacol Ther* 39, 421-38, doi:10.1111/jvp.12311.
- Miur, W. W., Hubbel, J. A. E., 2009. *Equine Anesthesia: Monitoring and Emergency Therapy*. Saunders Elsevier, St. Louis MO, USA.
- Natalini, C. C., Robinson, E. P., 2000. Evaluation of the analgesic effects of epidurally administered morphine, alfentanil, butorphanol, tramadol, and U50488H in horses. *Am J Vet Res* 61, 1579-86.
- NIH, 2017. National Center for Biotechnology Information. PubChem Compound Database; CID=33741 - Tramadol.
- Patton, K. M., Peek, S. F., Valentine, B. A., 2006. Gastric adenocarcinoma in a horse with portal vein metastasis and thrombosis: a novel cause of hepatic encephalopathy. *Vet Pathol* 43, 565-9, doi:10.1354/vp.43-4-565.
- Scott, L. J., Perry, C. M., 2000. Tramadol: a review of its use in perioperative pain. *Drugs* 60, 139-76.
- Shampine, L. F., Reichelt, M. W., 1997. The MATLAB ODE Suite. *SIAM Journal on Optimization* 18, 1-22.
- Shilo, Y., Britzi, M., Eytan, B., Lifschitz, T., Soback, S., Steinman, A., 2008. Pharmacokinetics of tramadol in horses after intravenous, intramuscular and oral administration. *Journal of Veterinary Pharmacology and Therapeutics* 31, 60-65, doi:10.1111/j.1365-2885.2007.00929.x.
- Staddon, G. E., Weaver, B. M., Lunn, C. E., 1984. A "standard horse" for use in physiologically based mathematical modelling. *Equine Vet J* 16, 189-91.
- Stewart, A. J., Boothe, D. M., Cruz-Espindola, C., Mitchum, E. J., Springfield, J., 2011. Pharmacokinetics of tramadol and metabolites O-desmethyltramadol and N-desmethyltramadol in adult horses. *American Journal of Veterinary Research* 72, 967-974, doi:10.2460/ajvr.72.7.967.

- Toribio, R. E., 2007. Essentials of equine renal and urinary tract physiology. *Vet Clin North Am Equine Pract* 23, 533-61, v, doi:10.1016/j.cveq.2007.09.006.
- Trachsel, D., Tschudi, P., Portier, C. J., Kuhn, M., Thormann, W., Scholtysik, G., Mevissen, M., 2004. Pharmacokinetics and pharmacodynamic effects of amiodarone in plasma of ponies after single intravenous administration. *Toxicol Appl Pharmacol* 195, 113-25, doi:10.1016/j.taap.2003.11.009.
- Tranquilli, W. J., Thurmon, J. C., Grimm, K. A., 2007. *Lumb & Jones' Veterinary Anesthesia and Analgesia*. Blackwell Publishing Professional.