International Journal of Pharmaceutics 473 (2014) 170–178

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Population pharmacokinetic modelling of tramadol using inverse Gaussian function for the assessment of drug absorption from prolonged and immediate release formulations

Nina Brvar [a], Tatjana Mateović-Rojnik [a], Iztok Grabnar [b,*]

[a] Krka, d.d., Novo mesto, Šmarješka cesta 6, 8501 Novo Mesto, Slovenia
[b] University of Ljubljana, Faculty of Pharmacy, Aškerčeva cesta 7, 1000 Ljubljana, Slovenia

ARTICLE INFO

Article history:
Received 7 May 2014
Received in revised form 3 July 2014
Accepted 5 July 2014
Available online 8 July 2014

Keywords:
Tramadol
Population pharmacokinetics
Absorption
Inverse Gaussian function
Steady state

ABSTRACT

This study aimed to develop a population pharmacokinetic model for tramadol that combines different input rates with disposition characteristics. Data used for the analysis were pooled from two phase I bioavailability studies with immediate (IR) and prolonged release (PR) formulations in healthy volunteers. Tramadol plasma concentration–time data were described by an inverse Gaussian function to model the complete input process linked to a two-compartment disposition model with first-order elimination. Although polymorphic CYP2D6 appears to be a major enzyme involved in the metabolism of tramadol, application of a mixture model to test the assumption of two and three subpopulations did not reveal any improvement of the model. The final model estimated parameters with reasonable precision and was able to estimate the interindividual variability of all parameters except for the relative bioavailability of PR vs. IR formulation. Validity of the model was further tested using the nonparametric bootstrap approach. Finally, the model was applied to assess absorption kinetics of tramadol and predict steady-state pharmacokinetics following administration of both types of formulations. For both formulations, the final model yielded a stable estimate of the absorption time profiles. Steady-state simulation supports switching of patients from IR to PR formulation.

1. Introduction

Tramadol hydrochloride is a centrally acting analgesic that is widely used in the treatment of moderate to severe chronic and acute pain (Grond and Sablotzki, 2004). Its analgesic efficacy ranges between that of weak opioids and morphine (Raffa et al., 1995). Analgesic action of tramadol involves two complementary and synergistic mechanisms: opioid activity through activating the μ-opioid receptor by the parent drug and its principal metabolite, *O*-desmethyltramadol (M1), and a separate non-opioid mechanism through inhibiting neuronal noradrenaline and serotonin reuptake by the parent drug (Grond and Sablotzki, 2004). This dual mode of action of tramadol results in the 'atypical' nature of the clinical analgesic efficacy and favourable side-effect profile, with respect to other opioids of comparable efficacy (Grond and Sablotzki, 2004; Raffa et al., 1995).

Following oral administration, tramadol is rapidly and almost completely absorbed (Lintz et al., 1981, 1986, 1998a). While the extent of oral absorption amounts to about 90% of the dose (Lintz et al., 1981, 1986, 1998a), the absolute bioavailability of tramadol is only about 70% (reported mean values range from 64 to 86%) (Lintz, 1980; Lintz et al., 1986, 1998a, 2000; Pedersen et al., 2006). The difference between absorption and bioavailability is attributed to the first-pass metabolism (Lintz, 1980; Lintz et al., 1986, 1998a; Pedersen et al., 2006).

In addition to immediate release (IR) formulations, which normally require oral administration four to six times daily (capsules, drops, dispersible and orodispersible tablets), tramadol is available in various prolonged release (PR) formulations (capsules and tablets), allowing once- or twice-daily dosing (Summaries of Product Characteristics, 2014). Systemic exposure after administration of various PR formulations in terms of AUC is comparable with IR formulations (Bodalia et al., 2003; Eradiri et al., 2006; Grond and Sablotzki, 2004; Karhu et al., 2010; Lai et al.,

Abbreviations: AIC, Akaike information criteria; BCS, Biopharmaceutics Classification System; BLQ, below the limit of quantification; CI, confidence interval; CYP2D6, cytochrome P450 2D6; GC–MS, gas chromatography coupled to mass spectrometry; IIV, interindividual variability; IR, immediate release; IVIVC, *in vitro–in vivo* correlation; LC–MS/MS, liquid chromatography coupled to mass spectrometry; M1, *O*-desmethyltramadol; OFV, objective function value; PR, prolonged release; VPC, visual predictive check.

* Corresponding author. Tel.: +386 1 4769 543; fax: +386 1 425 80 31.
E-mail address: iztok.grabnar@ffa.uni-lj.si (I. Grabnar).

http://dx.doi.org/10.1016/j.ijpharm.2014.07.013
0378-5173/

2003; Malonne et al., 2004; Raber et al., 1999a,b) following single- and multiple-dose administration.

Within therapeutic dose range, tramadol exhibits linear pharmacokinetic (PK) profile for IR (Grond and Sablotzki, 2004; Raffa et al., 1995) and PR dosage forms (Grond and Sablotzki, 2004; Schulz et al., 1999; Sista et al., 2003) following single and multiple dosing.

Tramadol has a high tissue affinity with a mean volume of distribution of about 260–320 l (Lintz et al., 1986; Murthy et al., 2007; Salman et al., 2011; Skinner-Robertson et al., 2011) and 190–270 l (Allegaert et al., 2005; Lintz, 1980; Lintz et al., 1986, 1998a,b, 1999, 2000) reported after oral and intravenous administration, respectively. Plasma protein binding of tramadol is about 20% (Grond and Sablotzki, 2004).

Tramadol is extensively metabolized in the liver resulting in many phase I and phase II metabolites. The primary metabolic route via *O*-demethylation to produce M1 is catalysed by the polymorphic isoenzyme cytochrome P450 2D6 (CYP2D6) (Lintz et al., 1981; Subrahmanyam et al., 2001; Wu et al., 2002). Tramadol and its metabolites are primarily excreted by the kidneys (90%), with the remaining 10% appearing in the faeces (Lintz et al., 1981).

The mean total clearance of tramadol is reported to be about 24–36 l/h (Allegaert et al., 2005; Lintz, 1980; Lintz et al., 1986, 1998a,b, 1999, 2000) and 25–52 l/h (Ardakani and Rouini, 2009; Lintz et al., 1986, 1998a, 2000; Murthy et al., 2007; Salman et al., 2011; Skinner-Robertson et al., 2011) following intravenous and oral administration, respectively.

Tramadol disposition is most often described by a two-compartment PK model (Allegaert et al., 2005; Lintz, 1980; Lintz et al., 1986, 1998a,b, 2000) with the reported terminal half-life ($t_{1/2,\beta}$) of about 5–7 h (Ardakani and Rouini, 2009; Lintz, 1980; Lintz et al., 1986, 1998a,b, 1999).

Several population PK models of tramadol have been reported in the literature for adults (Allegaert et al., 2005; Gan et al., 2004; Murthy et al., 2007; Skinner-Robertson et al., 2011) as well as children (Allegaert et al., 2005; Bressolle et al., 2009; Garrido et al., 2006; Zwaveling et al., 2004), infants (Allegaert et al., 2005, 2008), neonates (Allegaert et al., 2005), preterm neonates (Allegaert et al., 2008) and lactating women (Salman et al., 2011) following either intravenous, rectal or oral administration.

The population PK analyses focused mainly on assessing tramadol disposition in various populations and on the contribution of various covariates (e.g. age, body weight, *CYP2D6* polymorphism and smoking) to variability in clearance and volume of distribution. However, with the exception of the population PK model developed by Murthy et al. (2007), no reports were found on modelling the absorption and disposition of tramadol following oral administration of different tramadol formulations.

In the present study, a population PK model of tramadol was developed using pooled data from bioavailability studies with IR and PR formulations in healthy volunteers. The combined IR/PR PK model was used to assess absorption kinetics of tramadol and predict steady-state pharmacokinetics following administration of both types of formulations.

To our knowledge, the present study and the study by Murthy et al. (2007) are the only studies to report population PK modelling of tramadol using bioavailability data obtained following administration of IR and PR formulations.

2. Material and methods

2.1. In vivo *studies*

Data were obtained from two phase I comparative bioavailability studies (Study I and Study II). Only the data on the reference formulations were used in the present analysis.

Both studies were conducted with approval of the appropriate local ethics committees in compliance with the guideline of the International Committee on Harmonisation on Good Clinical Practice, local regulatory requirements, the ethical requirements of Directive 2001/20/EC and the principles enunciated in the Declaration of Helsinki and its revisions. Written informed consent was obtained from all subjects before they underwent any study-specific procedures.

2.1.1. Study population

Healthy, Caucasian, non-smoking adult (age 18–42 years) male volunteers with a body mass index (BMI) between 19 and 29 kg/m^2 were enrolled in the studies. All subjects met the inclusion criteria and no exclusion criteria described in the protocol and were judged eligible for enrolment in the studies based on medical and medication histories, demographic data (including gender, age, body weight, height, BMI), vital signs measurements (blood pressure, heart rate), electrocardiogram, physical examination, urine drug screen, alcohol screen and clinical laboratory tests (hematology, biochemistry, urinalysis, human immunodeficiency virus test, hepatitis C antibodies, hepatitis B surface antigen).

A total of 52 subjects were included, 26 in each study.

2.1.2. Study procedures

Both trials were designed as an open-label, randomised, 2-period, 2-sequence cross-over studies under fasting conditions.

2.1.2.1. Study I. Study I was a single-dose study, in which subjects were administered test and reference (Tramal® 50 mg capsules, Grünenthal GmbH, Germany) IR capsule formulation containing 50 mg of tramadol hydrochloride. There was a 7-day washout period between the doses.

Study drugs were administered with 200 ml of water. After an overnight fast of at least 10 h prior to drug administration, standard meals were provided at approximately 6, 10 and 14 h after dosing and were identical for both periods. Water was not permitted from 2 h before dosing until 2 h following dosing.

5 ml blood samples were collected in blood collection tubes containing heparin before dosing and at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 6.0, 8.0, 12.0, 14.0, 16.0, 24.0 and 36.0 h post-dose. After collection blood samples were immediately centrifuged, divided into 2 aliquots and frozen for storage at −20 °C pending sample analysis.

Plasma concentrations of tramadol were determined using LC–MS/MS method with the lower limit of quantification of 4 ng/ml and upper limit of quantification of 256 ng/ml. Analytical method was fully validated and was in accordance with appropriate guidelines.

2.1.2.2. Study II. In Study II, PR tablet formulation containing 200 mg of tramadol hydrochloride was dosed to the subjects every 12 h (a total of 5 doses) as either test or reference (Tramal® 200 mg PR tablets, Grünenthal GmbH, Germany) product. The treatment periods were separated by a washout period of 5 days.

All doses were administered with 200 ml of water. Subjects fasted for 10 h before every morning drug administration. On days 1 and 2, subjects received a standardised breakfast, lunch and snack 1, 6 and 9 h after the morning dose, respectively, and a standardized dinner was provided 1 h after the evening dose. On day 3, standardised meals were provided to trial subjects 4, 7 and 10 h post-dose. Post-dose meals were identical for both periods. With the exception of water administered at the time of each dosing, fluids were not permitted from 1 h before dosing to 1 h after dosing on days 1 and 2 and until 2 h after dosing on day 3.

Blood samples of 6–7 ml volume were collected at the following time points: before morning dosing on days 1, 2 and 3 and 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0, 24.0 and 48.0 h after the morning drug administration on day 3.

The heparin-anticoagulated blood samples were centrifuged within 10 min of collection. Plasma samples were stored at −20 °C until assayed.

Plasma samples were analysed for their concentrations of tramadol by means of a validated GC–MS method. Validation of the method was performed in concentration range 10–800 ng/ml according to the regulatory requirements.

2.2. Population pharmacokinetic analysis

Data from the two studies were pooled to form a single dataset and analysed by a population PK modelling approach using NONMEM® (version 7.3, ICON Development Solutions, Ellicott City, MD, USA) (Beal et al., 1989–2013). Model building steps were managed by PsN® (version 3.5.3, http://psn.sourceforge.net/) and Xpose® (version 4.4.0, http://xpose.sourceforge.net/). Fortran subroutines were compiled with the Intel® Visual Fortran Compiler (version 11.0, Intel, Santa Clara, CA, USA).

The structural models tested were one- and two-compartment models with first-order elimination (Di Muria et al., 2009). The input models investigated were first-order absorption (Eq. (1)) and first-order absorption with lag-time (Eq. (2))

$$f_{\mathrm{i}}(t) = \mathrm{dose}\ k_{\mathrm{a}} e^{-k_{\mathrm{a}} t} \tag{1}$$

$$f_{\mathrm{i}}(t) = \begin{cases} 0 & , t < t_{\mathrm{lag}} \\ \mathrm{dose}\ k_{\mathrm{a}} e^{-k_{\mathrm{a}}(t - t_{\mathrm{lag}})} & , t \geq t_{\mathrm{lag}} \end{cases} \tag{2}$$

where $f_{\mathrm{i}}(t)$ is the input rate into the central compartment as a function of time (t), k_{a} is the absorption rate constant and t_{lag} is the absorption lag-time.

Additional models tested were transit compartment model (Eq. (3)) (Savic et al., 2007) followed by first-order absorption

$$f_{\mathrm{d}}(t) = \mathrm{dose}\ k_{\mathrm{tr}} \frac{(k_{\mathrm{tr}} t)^n e^{-k_{\mathrm{tr}} t}}{\sqrt{2\pi}\, n^{n+0.5} e^{-n}} \tag{3}$$

where $f_{\mathrm{d}}(t)$ is the *in vivo* dissolution rate, i.e. input rate in the absorption compartment, k_{tr} is the transit rate constant from compartment $n-1$ to compartment n, and n is the number of transit compartments; and inverse Gaussian function (Eq. (4)) (Wang et al., 2008) with and without subsequent first-order absorption

$$f_{\mathrm{d}}(t) = \mathrm{dose} \sqrt{\frac{\mathrm{MDT}}{2\pi \mathrm{CV}_{\mathrm{D}}^2 t^3}} \exp\left[-\frac{(t - \mathrm{MDT})^2}{2\mathrm{CV}_{\mathrm{D}}^2 \mathrm{MDT}\, t} \right] \tag{4}$$

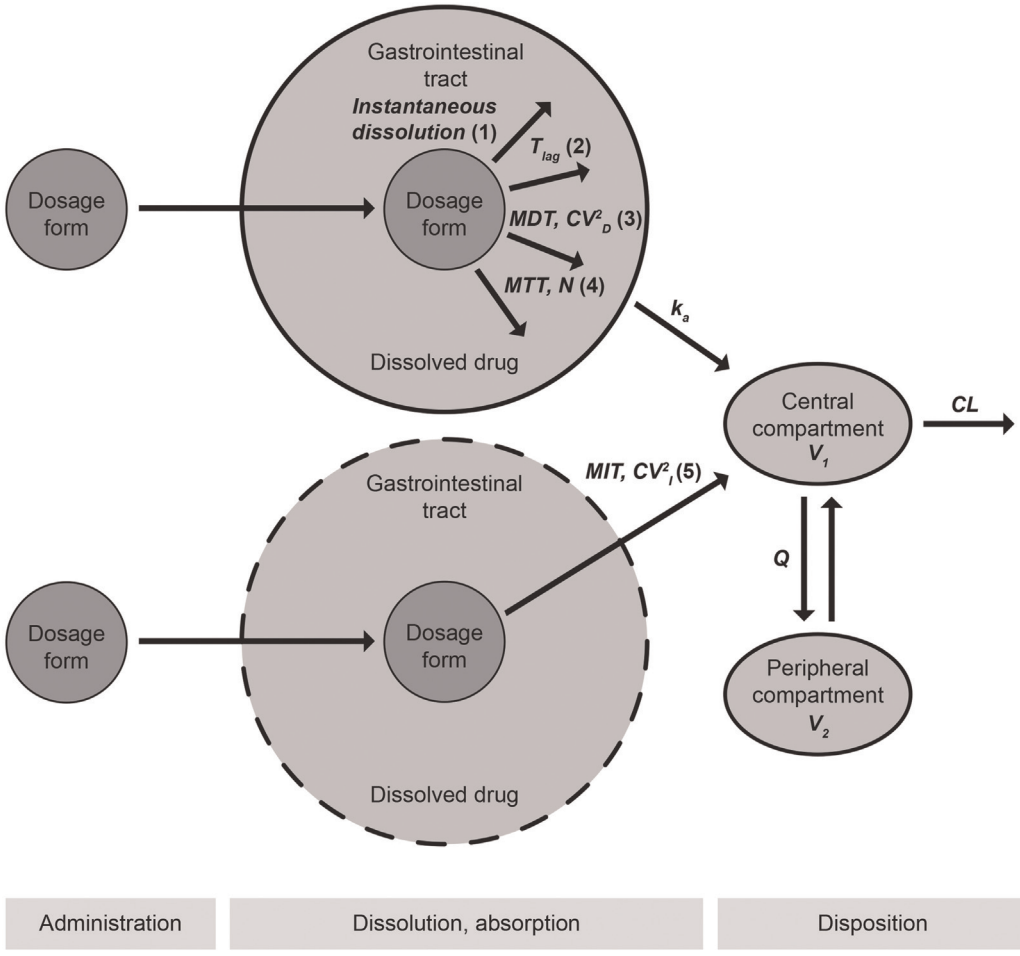

Fig. 1. Schematic representation of the population pharmacokinetic modelling of tramadol following IR and PR formulations. A series of models were evaluated, including one- and two-compartment linear disposition models with first-order elimination and first-order absorption without (1) and with lag-time (2), inverse Gaussian function followed by first-order absorption (3), transit compartment model followed by first-order absorption (4). In the final step the dissolution–absorption processes were combined into a single inverse Gaussian function (5) as an input model. T_{lag}, lag-time; MDT, mean dissolution time; $\mathrm{CV}^2_{\mathrm{D}}$, normalized variance of mean dissolution time; MTT, mean transit time; N, number of transit compartments; k_{a}, absorption rate constant; MIT, mean input time; $\mathrm{CV}^2_{\mathrm{I}}$, normalized variance of mean input time; V_1 and V_2, apparent volumes of distribution for central and peripheral compartments, respectively; CL, apparent total clearance; Q, distributional clearance.

where MDT is mean dissolution time and CV^2_D normalized variance of mean dissolution time. To introduce multiple dosing a method of dose superimposition proposed by Shen et al. (2012) was implemented. The models tested are schematically presented in Fig. 1.

As the absolute bioavailability of tramadol, *F*, could not be estimated from these data, volume of distribution and clearance (i.e. *V* and CL in case of one-compartment model or V_1, V_2, CL and *Q* in case of two-compartment model) could only be estimated as apparent parameters (i.e. *V*/*F* and CL/*F* or V_1/*F*, V_2/*F*, CL/*F* and *Q*/*F*, respectively). A log-normal distribution of individual subjects' parameter values was assumed. Interindividual variability (IIV) was therefore described by exponential random effect model, while additive, proportional and combination (additive + proportional) error models were evaluated to describe residual intra-individual variability of tramadol concentration. The first-order conditional estimation method with interaction option was used for parameter estimation.

Model development was guided by the minimum value of objective function value (OFV), which is approximately equal to −2 times log-likelihood of the parameter values given the data. The difference in OFV between two nested models is approximately χ^2 distributed and a decrease of 3.84 for an extra parameter was considered significant at the 5% significance level. Non-nested models were compared by Akaike information criteria value (AIC) (Ludden et al., 1994) computed as OFV + 2 × N_{par}, where N_{par} is the number of all estimated parameters in the model. Additional guidance in model development was convergence of minimization, number of significant digits more than 3, successful covariance step, gradients in the final iteration between 10^{-3} and 10^2 and parameter shrinkage. The models were also evaluated by standard diagnostic plots and visual predictive check (VPC). With VPC tramadol concentration profiles were simulated with 1000 replications of the original dataset and the 95% confidence intervals (95% CIs) of the simulated 5th, 50th (median) and 95th percentile were compared with the 5th, 50th and 95th percentiles of tramadol concentration observed in the data.

Precision of parameter estimates was derived through bootstrap of 1000 samples and nonparametric 95% CIs were calculated. Bootstrap was stratified on formulation to ensure that subjects were sampled in the same fractions as in the original dataset.

In the final model, covariate effect of weight was included using an allometric relationship by an exponent of 0.75 on all clearance and an exponent of 1 on all volume parameters; centred to a typical body weight of 70 kg. Additionally, in the final model tramadol concentrations below the limit of quantification (BLQ) were handled by M3 method proposed by Beal (2001). With M3 method, continuous and dichotomous (BLQ or not) data are modelled simultaneously. In addition to the likelihood of quantified observations, i.e. concentration measurements above BLQ, the likelihood for observing BLQ data is maximised with respect to model parameters. For this purpose F_FLAG indication variable was used and LAPLACIAN option was added in the estimation block for likelihood calculation (Bergstrand and Karlsson, 2009).

Finally, consistent with the prior knowledge of tramadol pharmacokinetics, genetic polymorphism of *CYP2D6* was considered to contribute to IIV of tramadol clearance. A population of two (slow and rapid) and three (poor, intermediate and extensive) subpopulations of subjects were assumed and tested using a mixture model option in NONMEM® (Beal et al., 1989–2013). The subpopulations were assumed to have different typical values of tramadol clearance, but equal IIV within each subgroup.

The final model has been used for population simulation of the tramadol absorption profiles from both formulations and for simulation of steady-state plasma concentration profiles obtained following 50 mg tramadol hydrochloride q6 h as IR and 100 mg tramadol hydrochloride q12 h as PR formulation. The dose of the PR formulation was changed from 200 mg used in study II to 100 mg for the steady-state simulation to achieve the same total daily dose for both formulations, thus allowing the comparison of tramadol exposure between the PR and the IR formulation. Area under the plasma concentration–time curve during a 12 h interval at steady state (AUC_{ss}), maximum plasma concentration at steady state ($C_{max,ss}$) and minimum plasma concentration at steady state ($C_{min,ss}$) were determined from the simulated steady-state data by non-compartmental analysis using Kinetica™ (Version 5.0, Thermo Fischer Scientific, Waltham, MA, USA). $C_{max,ss}$ and $C_{min,ss}$ were determined directly from the plasma concentration data. AUC_{ss} was calculated using the linear trapezoidal rule. Ln-transformed AUC_{ss}, $C_{max,ss}$ and $C_{min,ss}$ were compared between the PR and IR formulation using two-sample *t*-test. Geometric mean ratios and geometric 90% CIs for the ratios were calculated using IR values as a reference. Statistical evaluation was performed using SAS® TTEST procedure (SAS®, version 9.2, SAS Institute Inc., Cary, NC, USA).

3. Results

Available literature data suggests that pharmacokinetics of tramadol is best described by a two-compartment disposition model. However, graphical analysis of individual plasma concentration–time data revealed that following administration of PR formulation distribution phase was largely overlapped with the absorption process in the majority of individuals. Therefore, model development was started with the data obtained with the IR formulation. Initially, we evaluated one- and two-compartment models with first-order absorption. A two-compartment model provided better fit compared to the one-compartment model (AIC values 2549 vs. 2628, respectively). In addition to the model assuming instantaneous dissolution of tramadol from the dosage form, several other input functions into the absorption compartment were tested (Fig. 1). The AIC values of 2115, 2077 and 2088 were obtained for the lag-time, inverse Gaussian and transit compartment model, respectively. Based on the favourable AIC value, inverse Gaussian model was chosen for further development. It seems interesting to note that the resulting typical *in vivo* dissolution profiles for the inverse Gaussian and transit compartment models were almost identical, indicating that the variability of the *in vivo* dissolution profiles is better described by incorporating random effects on the parameters MDT and CV^2_D of the inverse Gaussian function, than the random effects of the parameters MTT and *N* of the transit compartment model. The estimate of the first-order absorption rate constant (k_a), however was large (6.29 h^{-1}) and imprecise (relative standard error 40.4%). The estimated MDT was 0.512 h, contributing approximately 80% to the mean input time (MIT). Therefore, direct input in the central compartment was tested as suggested by Wang et al. (2008) (Fig. 1). The estimated MIT (0.692 h) obtained with this model was similar to the sum of MDT and mean absorption time (MAT = 1/k_a) of the previous dissolution–absorption model. This simplification provided the lowest AIC (2075) and an adequate agreement between the observed and predicted tramadol concentrations. Inverse Gaussian input directly into the central compartment was tested again with the one-compartment disposition model, however, higher AIC (2372) confirmed that the two-compartment model better fits the concentration–time data.

Two-compartment model with inverse Gaussian input function directly into the central compartment was subsequently used for the analysis of pooled data from both studies and MIT and CV^2_I for PR and IR formulations were estimated simultaneously. Covariate effects of body weight were included in the model using an allometric scaling approach. Inclusion of the effect of weight on all

clearance and volume parameters decreased OFV from 5182 to 5139 and reduced unexplained IIV of these parameters.

Since most of the measurements 36 h after administration of IR formulation (21 of 26) and 48 h following the last administration of PR formulation (20 of 26) were BLQ, initially treated as 0, in the final step M3 method was applied. Simultaneously, concentration data (continuous) and BLQ data (categorical) were modelled and the likelihood for observation being BLQ was maximised. With the M3 method additive component of the combination residual error model was insignificant and was therefore excluded. Proportional error model adequately described the residual variability in tramadol concentration data. Furthermore, as two different analytical methods were used for measurement of tramadol plasma concentration (due to different analytical laboratories, as well as different time periods, under which the studies were conducted), we tried to estimate two residual error parameters, one for each method, but the estimated error parameters were very similar and there was no improvement in OFV.

Distribution of empirical Bayesian estimates of CL revealed some tendency of bimodality with modes appearing at approximately 37.5 and 47.5 l/h. Although, there was no clear departure from log-normal distribution, possible existence of two and three subpopulations was tested based on known genetic polymorphism of tramadol metabolism. No *CYP2D6* genotype information was collected in these studies, however, NONMEM mixture option allows partition of population into two or more subpopulations without sub-population identity information of an individual, based on probability model, so that each subpopulation has its own submodel. No improvement of the model was obtained with a mixture of two subpopulations (typical CL values of 26.9 and 35.4 l/h with fractions of 5.1% and 94.9%, respectively), nor with a mixture of three subpopulations (typical CL values of 27.6, 35.3 and 44.5 l/h with fractions of 30.9%, 35.2% and 33.9%, respectively).

The results of the final model are summarised in Table 1. Parameters were estimated with reasonable precision and we were able to estimate the IIV of all the parameters, except for the relative bioavailability (F_r). We presume that estimation of IIV of relative bioavailability would be feasible if both formulations would have been tested in the same group of subjects (i.e. in a cross-over design study). Standard diagnostic plots of the final model are presented in Fig. 2. Mass balance equations of the final model and NONMEM control stream are available as Supplementary Material.

Simulation properties of the final model were assessed by VPC (Fig. 3). The results of VPC indicated that the model adequately described the typical plasma concentration–time profiles for both formulations, as well as their variability and fraction BLQ.

The final model was used for simulation of *in vivo* absorption profiles of tramadol from IR and PR formulations and for simulation of steady-state plasma profiles in a typical subject. Typical absorption profiles of tramadol with 95% prediction intervals are presented in Fig. 4. Fig. 5 shows simulated steady-state concentrations with multiple dosing of 50 mg (q6 h) tramadol hydrochloride as IR formulation and 100 mg (q12 h) tramadol hydrochloride as PR formulation.

Table 1
Parameter estimates of the final population pharmacokinetic model of tramadol.

Parameter	Estimate	Bootstrap	
		Mean	95% confidence interval
CL(l/h)	34.7	34.9	32.5–38.3
V_1(l)	204	203	183–217
V_2(l)	76.8	78.6	69.0–98.6
Q(l/h)	44.9	46	35.4–66.1
MIT_{IR}(h)	0.549	0.555	0.486–0.650
$CV^2_{I,IR}$	0.193	0.196	0.127–0.287
MIT_{PR}(l/h)	3.57	3.65	3.22–4.15
$CV^2_{I,PR}$	0.839	0.877	0.692–1.18
F_r	1.01	1.02	0.929–1.13
Interindividual variability			
IIV_{Cl} (CV%)	28	28	23.7–31.3
IIV_{V_1} (CV%)	14	14	9.6–17.6
IIV_{V_2} (CV%)	43.2	41.6	26.1–47.8
IIV_Q (CV%)	16.9	20.2	14.8–45.6
$IIV_{MIT_{IR}}$ (CV%)	44.4	44.6	32.5–58.0
$IIV_{CV^2_{I,IR}}$ (CV%)	112	102	51.4–113
$IIV_{MIT_{PR}}$ (CV%)	13.8	12.7	2.00–15.5
$IIV_{CV^2_{I,PR}}$ (CV%)	65.3	65.7	42.3–89.4
Residual variability			
Proportional (%)	10.5	10.4	9.3–11.3

Absorption was modelled using an inverse Gaussian function where input rate $f_i(t)$ is:

$$f_i(t) = \text{dose}\sqrt{\frac{MIT}{2\pi CV_I^2 t^3}}\exp\left[\frac{-(t-MIT)^2}{2CV_I^2 MIT t}\right]$$

CL, apparent total clearance; V_1 and V_2, apparent volumes of distribution for central and peripheral compartments, respectively; Q, distributional clearance; MIT_{IR}, $CV^2_{I,IR}$, MIT_{PR}, $CV^2_{I,PR}$, mean input time and normalized variance of mean input time, respectively, for the IR formulation and PR formulation, respectively; F_r, relative bioavailability of the PR formulation to the IR formulation; IIV, interindividual variability. Note that as the absolute bioavailability of tramadol, F, could not be determined from the two studies, symbols V_1, V_2, CL and Q in fact represent V_1/F, V_2/F, CL/F and Q/F. All parameter values are scaled to typical subject weight of 70 kg, using allometric relationship.

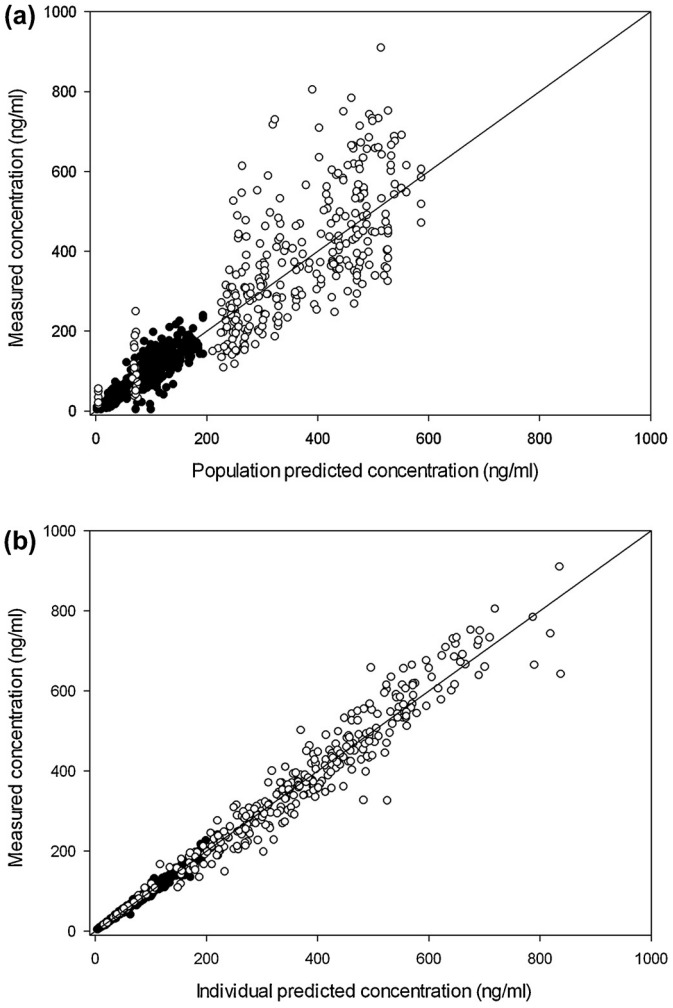

Fig. 2. Goodness of fit plots for the final population pharmacokinetic model. Agreement between the population predicted and measured tramadol concentration (a) and between individual predicted and measured tramadol concentration (b) for the IR (closed circles) and PR (open circles) formulation with lines of identity.

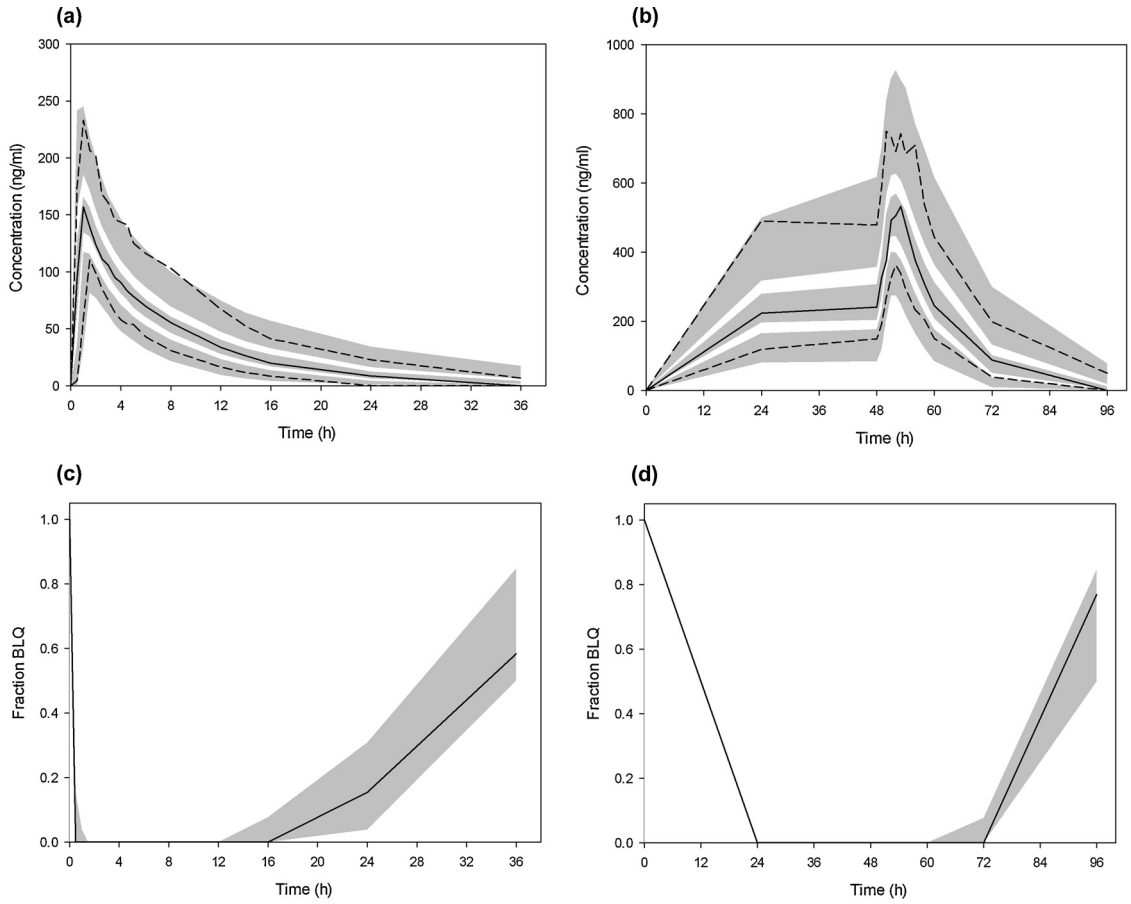

Fig. 3. Visual predictive check of the final model for IR (a) and PR (b) formulation. Median (solid line), 5th and 95th percentile (dashed line) of the observed data and the 95% confidence intervals of the simulated data (shaded area). A visual predictive check for the fraction of data BLQ for IR formulation with LOQ of 4 ng/ml (c) and for PR formulation with LOQ of 10 ng/ml (d). The solid line is the observed fraction BLQ and the shaded area is the 95% confidence interval of the simulated fraction BLQ. Note that for the PR formulation all simulated data were BLQ at time 0 and that there were no BLQ data at 24 h. An appropriate model fit is indicated when lines are within shaded areas.

Geometric mean PR/IR ratios of AUC_{ss}, $C_{max,ss}$ and $C_{min,ss}$ from non-compartmental analysis of the simulated steady-state data were 0.98 (90% CI 0.96, 1.00), 0.91 (90% CI 0.90, 0.93) and 0.93 (90% CI 0.90, 0.95), respectively.

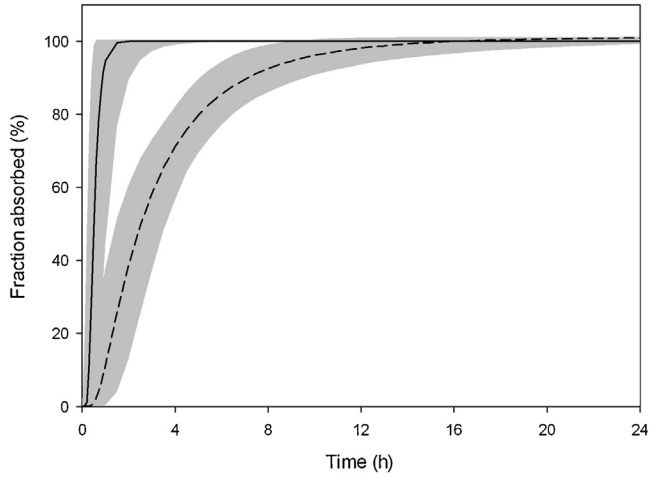

Fig. 4. Typical absorption profiles following administration of the IR (solid line) and PR (dashed line) formulations with 95% prediction intervals (shaded area). Note that fraction absorbed is relative to the cumulative amount of tramadol absorbed following administration of IR formulation. Therefore, an asymptote of 100% does not imply that the bioavailability is 100%.

4. Discussion

A population PK model was developed for tramadol that combines different input rates with disposition characteristics of tramadol. Data used for the analysis were pooled from two

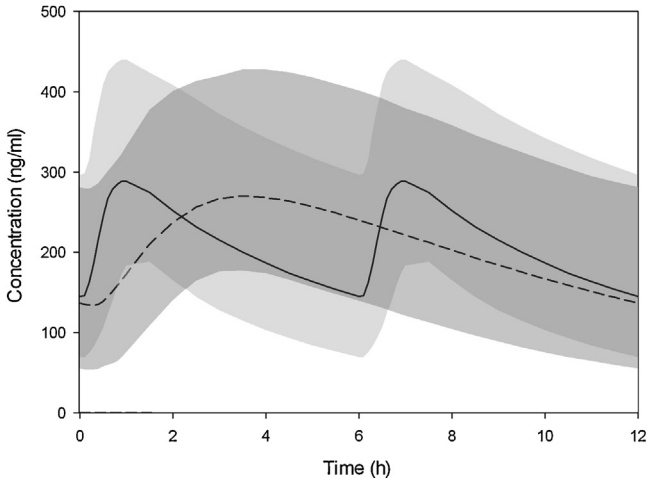

Fig. 5. Simulated steady-state plasma concentration of tramadol in a typical subject (70 kg) obtained with the final model. Geometricmean profiles of the IR (solid line) and PR (dashed line) formulations with 95% prediction intervals (shaded area).

bioavailability studies with IR and PR formulations. Our results show that inverse Gaussian input function directly into the central compartment appropriately describes the absorption of tramadol from both formulations.

Owing to its flexibility at the expense of a modest increase in computational complexity, inverse Gaussian function has been used successfully in the past to model *in vivo* input following oral administration of mostly PR dosage forms. This model has been used to describe a combined dissolution–absorption process (Csajka et al., 2005; Lötsch et al., 1999; Weiss, 1996; Weiss et al., 2012) or only *in vivo* dissolution/gastrointestinal transit, separately from the absorption process (Wang et al., 2008). Although less frequently, it has also been used to describe the *in vitro* release kinetics of oral PR products (Lánský and Weiss, 2003; Weiss et al., 2014). Furthermore, a sum of inverse Gaussian functions has been found as a flexible tool do describe irregular multiple peak concentration profiles (Csajka et al., 2005).

In the present analysis, the input model was at first divided into two separate processes, i.e. dissolution/gastrointestinal transit component, modelled using inverse Gaussian function, followed by a first-order absorption component. Subsequently, it was reduced by removing the first-order absorption process from the model. This model reduction has been justified by the high value of k_a, accompanied by a relatively high standard error of approximately 40%. Additionally, simplification of the input model did not significantly deteriorate the model fit. The background of this simplification is probably the fact that tramadol is a highly permeable drug (Lintz et al., 1981, 1986, 1998a). Based on its permeability characteristics and its high aqueous solubility within the gastrointestinal pH range of 1–6.8, it is classified into class I according to the Biopharmaceutics Classification System (BCS) (Ramirez et al., 2010).

For drugs from BCS class I dissolution from the dosage form is the rate-limiting step which controls the input process following administration of PR dosage forms (Emami, 2006). It can thus be assumed that MIT and CV^2_I estimated for the tramadol PR formulation reflect mainly the *in vivo* dissolution process. Using *in vivo* data of two or more formulations with different dissolution profiles together with the *in vitro* dissolution data, the model could thus be used for establishing an *in vitro–in vivo* correlation (IVIVC). This procedure is referred to as a differential equation-based approach to IVIVC modelling (Buchwald, 2003).

However, in the case of IR tramadol, which can be considered as a very rapidly dissolving product (more than 85% of the labelled amount is dissolved within 15 min), gastric emptying is slower than the product dissolution, suggesting that the derived input process represents the combination of gastric emptying (as the main component of the input process) and passage of the drug through the GI membrane and liver. In this case, when the derived input rate is controlled by the gastric emptying rate, no correlation of *in vivo* data with dissolution rate is expected (Emami, 2006).

As demonstrated by the typical absorption profile, tramadol absorption from the IR formulation is complete within 1–1.5 h, while in the case of PR formulation, absorption takes place over a period of 12 h. The latter is in agreement with the *in vitro* release, reported for this formulation (i.e. 100% release over a 12 h period) (Grond and Sablotzki, 2004).

Although no i.v. data were used in the development of a population pharmacokinetic model for tramadol, which could lead to model identifiability problems, use of the IR data, on which development of the model initiated and subsequent simultaneous fit of the IR and PR data counterbalanced this shortcoming of our analysis. A two-compartment model, used to describe the disposition of tramadol, is consistent with the previous reports on tramadol (Allegaert et al., 2005; Lintz, 1980; Lintz et al., 1986, 1998a,b, 2000). The estimated clearance and distribution volume were consistent with those reported in previous studies (Ardakani and Rouini, 2009; Lintz et al., 1986, 1998a, 2000; Murthy et al., 2007; Salman et al., 2011; Skinner-Robertson et al., 2011). Namely, the total oral clearance of 34.7 l/h, obtained in this analysis, is in the range of values reported in the literature 25–52 l/h. Likewise, the model estimated volumes of distribution of the central and peripheral compartments of 204 and 76.8 l respectively are consistent with the steady-state volume of distribution after oral administration of 260–380 l. Bioavailability of PR relative to IR formulation (F_r) of 1.01, estimated by the final model, is in agreement with the reported similar bioavailability between PR and IR tramadol (Summaries of Product Characteristics, 2014).

An alternative approach to development *in vitro–in vivo* correlation is by introducing *in vitro* dissolution rate directly into the pharmacokinetic model. Di Muria et al. (2010) demonstrated the applicability of this approach in combination with a semi-physiological model. The advantage of this bottom-up approach is that a mathematical description is closer to the actual physical phenomena. However, with this approach one should often face with the identifiability problems.

Population simulation of the steady-state plasma concentration–time profiles of tramadol revealed that tramadol exposure following a 200 mg daily dose of PR (q12 h regimen) is comparable with that of IR formulation (q6 h regimen). These findings are consistent with the results of a cross-over study, comparing PR to IR tramadol (Grond and Sablotzki, 2004) and support switching patients from IR to twice-daily PR tramadol. The latter was similarly demonstrated already by Murthy et al. (2007) for the once-daily PR tramadol. In their study, population PK modelling and Monte Carlo simulations of tramadol steady-state plasma concentration–time profiles were performed with data from three phase I studies with a cross-over design, in which subjects received multiple doses of both IR and PR tramadol. However, although simultaneous fit of PK data from both treatments was performed, their model used different input functions to model the absorption of tramadol from the PR and IR formulation (i.e. two parallel first-order and zero-order input processes with lag-time equal for both input processes for the PR formulation and first-order input process with lag-time for the IR formulation). In addition, a one-compartment model with first-order elimination was used to model the disposition of tramadol and no other disposition models were tested. It is therefore very likely that the derived input is confounded (absorption rate is overestimated) with tramadol distribution. Moreover, unlike in the present analysis, where the relative bioavailability between the PR and IR formulation was estimated, in the analysis by Murthy et al. (2007) relative bioavailability was taken from the literature data.

Although, polymorphic CYP2D6 is involved in the elimination of tramadol (Subrahmanyam et al., 2001), there were no evident subpopulations in the distribution of empirical Bayesian estimates of CL in our study. The possibility of the existence of two and three subpopulations was further tested by the mixture model approach. However, our data did not deviate from a single distribution. This can be explained by a relatively small sample size used in the analysis and a low frequency of the poor metabolizer phenotype, which is about 5–10% in Caucasians (Zhou, 2009). Additionally, this finding is in agreement with the pharmacogenetic studies with tramadol, where a less pronounced effect of *CYP2D6* polymorphism on tramadol plasma concentration profile was observed (extensive/poor metabolizer ratio for AUC of 0.69) compared to M1 (extensive/poor metabolizer ratio for AUC of 2.71) (Pedersen et al., 2006).

In our experience from this analysis there are many advantages with the use of a population PK modelling for the assessment of the input process after oral administration. Namely, whereas traditional deconvolution methods such as Loo–Riegelman or point-area deconvolution require data following administration of the reference

formulation to the same subject in a cross-over study, the assessment of absorption using a population PK approach enabled the analysis of data from two different studies. Furthermore, for both formulations the final model yielded a stable estimate of the absorption time profiles, which is often not the case with the traditional methods.

5. Conclusions

In conclusion, a population PK model was developed to assess the absorption of tramadol from IR and PR formulation. Tramadol plasma concentration-time data were described by an inverse Gaussian function to model the complete input process linked to a two-compartment disposition model with first-order elimination. Using a mixture model approach no evident effect of CYP2D6 polymorphism on plasma pharmacokinetics of parent drug tramadol was observed. The model can be applied to develop *in vitro–in vivo* correlation. Steady-state simulations with the model indicate comparable tramadol exposure with IR formulation given four-times daily and PR formulation twice daily and thus support switching patients from IR to PR tramadol. By our experience, inverse Gaussian functions are a flexible tool for modelling kinetics of drug absorption.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijpharm.2014.07.013.

References

Allegaert, K., Anderson, B.J., Verbesselt, R., Debeer, A., de Hoon, J., Devlieger, H., Van Den Anker, J.N., Tibboel, D., 2005. Tramadol disposition in the very young: an attempt to assess in vivo cytochrome P-450 2D6 activity. Br. J. Anaesth. 95, 231–239 doi:http://dx.doi.org/10.1093/bja/aei170.

Allegaert, K., van den Anker, J.N., de Hoon, J.N., van Schaik, R.H., Debeer, A., Tibboel, D., Naulaers, G., Anderson, B.J., 2008. Covariates of tramadol disposition in the first months of life. Br. J. Anaesth. 100, 525–532 doi:http://dx.doi.org/10.1093/bja/aen019.

Ardakani, Y.H., Rouini, M.R., 2009. Pharmacokinetic study of tramadol and its three metabolites in plasma, saliva and urine. DARU 17, 245–255.

Beal, S.L., Sheiner, L.B., Boeckmann, A.J., Bauer, R.J. (Eds.), 1989–2013. NONMEM 7.3.0 Users Guides. ICON Development Solutions, Hanover, Maryland, USA.

Beal, S.L., 2001. Ways to fit a PK model with some data below the quantification limit. J. Pharmacokinet. Pharmacodyn. 28, 481–504 doi:http://dx.doi.org/10.1023/A:1012299115260.

Bergstrand, M., Karlsson, M.O., 2009. Handling data below the limit of quantification in mixed effect models. AAPS J. 11, 371–380 doi:http://dx.doi.org/10.1208/s12248-009-9112-5.

Bodalia, B., McDonald, C.J., Smith, K.J., O'Brien, C., Cousens, L., 2003. A comparison of the pharmacokinetics, clinical efficacy, and tolerability of once-daily tramadol tablets with normal release tramadol capsules. J. Pain Symptom Manag. 25, 142–149.

Bressolle, F., Rochette, A., Khier, S., Dadure, C., Ouaki, J., Capdevila, X., 2009. Population pharmacokinetics of the two enantiomers of tramadol and *O*-demethyl tramadol after surgery in children. Br. J. Anaesth. 102, 390–399 doi:http://dx.doi.org/10.1093/bja/aen405.

Buchwald, P., 2003. Direct, differential-equation-based in-vitro–in-vivo correlation (IVIVC) method. J. Pharm. Pharmacol. 55, 495–504 doi:http://dx.doi.org/10.1211/002235702847.

Csajka, C., Drover, D., Verotta, D., 2005. The use of a sum of inverse Gaussian functions to describe the absorption profile of drugs exhibiting complex absorption. Pharm. Res. 22, 1227–1235 doi:http://dx.doi.org/10.1007/s11095-005-5266-8.

Di Muria, M., Lamberti, G., Titomanlio, G., 2009. Modeling the pharmacokinetics of extended release pharmaceutical systems. Heat Mass Transfer 45, 579–589 doi:http://dx.doi.org/10.1007/s00231-008-0456-7.

Di Muria, M., Lamberti, G., Titomanlio, G., 2010. Physiologically based pharmacokinetics: a simple, all purpose model. Ind. Eng. Chem. Res. 49, 2969–2978 doi:http://dx.doi.org/10.1021/ie9015717.

Emami, J., 2006. In vitro–in vivo correlation: from theory to applications. J. Pharm. Pharm. Sci. 9, 169–189.

Eradiri, O., Sista, S., Lai, J.C.K., Danyluk, A., Brett, V., 2006. Bioavailability of extended-release and immediate-release formulations of tramadol HCl. J. Clin. Pharmacol. 46, 1091.

Gan, S.H., Ismail, R., Wan Adnan, W.A., Zulmi, W., Jelliffe, R.W., 2004. Population pharmacokinetic modelling of tramadol with application of the NPEM algorithms. J. Clin. Pharm. Ther. 29, 455–463 doi:http://dx.doi.org/10.1111/j.1365-2710.2004.00590.x.

Garrido, M.J., Habre, W., Rombout, F., Trocóniz, I.F., 2006. Population pharmacokinetic/pharmacodynamic modelling of the analgesic effects of tramadol in pediatrics. Pharm. Res. 23, 2014–2023 doi:http://dx.doi.org/10.1007/s11095-006-9049-7.

Grond, S., Sablotzki, A., 2004. Clinical pharmacology of tramadol. Clin. Pharmacokinet. 43, 879–923 doi:http://dx.doi.org/10.2165/00003088-200443130-0000.

Karhu, D., Fradette, C., Potgieter, M.A., Ferreira, M.M., Terblanché, J., 2010. Comparative pharmacokinetics of a once-daily tramadol extended-release tablet and an immediate-release reference product following single-dose and multiple-dose administration. J. Clin. Pharmacol. 50, 544–553 doi:http://dx.doi.org/10.1177/0091270009347673.

Lai, J.C., Sista, S., Eradiri, O., 2003. The pharmacokinetics of novel once daily tramadol hydrochloride extended release tablets in healthy subjects. AAPS PharmSci 5, Abs M1071.

Lánský, P., Weiss, M., 2003. Classification of dissolution profiles in terms of fractional dissolution rate and a novel measure of heterogeneity. J. Pharm. Sci. 92, 1632–1647 doi:http://dx.doi.org/10.1002/jps.10419.

Lintz, W., 1980. Pharmacokinetics of tramadol in man and animals. Naunyn-Schmiedebergs Arch. Pharmacol. 313, R53.

Lintz, W., Erlaçin, S., Frankus, E., Uragg, H., 1981. Metabolismus von tramadol bei mensch und tier. Arzneimittelforschung 31, 1932–1943.

Lintz, W., Barth, H., Osterloh, G., Schmidt-Böthelt, E., 1986. Bioavailability of enteral tramadol formulations. 1st communication: capsules. Arzneimittelforschung 36, 1278–1283.

Lintz, W., Barth, H., Becker, R., Frankus, E., Schmidt-Böthelt, E., 1998a. Pharmacokinetics of tramadol and bioavailability of enteral tramadol formulations. 2nd communication: drops with ethanol. Arzneimittelforschung 48, 436–445.

Lintz, W., Barth, H., Osterloh, G., Schmidt-Böthelt, E., 1998b. Pharmacokinetics of tramadol and bioavailability of enteral tramadol formulations. 3rd communication: suppositories. Arzneimittelforschung 48, 889–899.

Lintz, W., Beier, H., Gerloff, J., 1999. Bioavailability of tramadol after i.m. injection in comparison to i.v. infusion. Int. J. Clin. Pharmacol. Ther. 37, 175–183.

Lintz, W., Becker, R., Gerloff, J., Terlinden, R., 2000. Pharmacokinetics of tramadol and bioavailability of enteral tramadol formulations. 4th communication: drops (without ethanol). Arzneimittelforschung 50, 99–108.

Lötsch, J., Weiss, M., Ahne, G., Kobal, G., Geisslinger, G., 1999. Pharmacokinetic modeling of M6G formation after oral administration of morphine in healthy volunteers. Anesthesiology 90, 1026–1038 doi:http://dx.doi.org/10.1097/00000542-199904000-00016.

Ludden, T.M., Beal, S.L., Sheiner, L.B., 1994. Comparison of the Akaike information criterion, the Schwarz criterion and the *F* test as guides to model selection. J. Pharmacokinet. Biopharm. 22, 431–445 doi:http://dx.doi.org/10.1007/BF02353864.

Malonne, H., Sonet, B., Streel, B., Lebrun, S., De Niet, S., Sereno, A., Vanderbist, F., 2004. Pharmacokinetic evaluation of a new oral sustained release dosage form of tramadol. Br. J. Clin. Pharmacol. 57, 270–278 doi:http://dx.doi.org/10.1046/j.1365-2125.2003.02013.x.

Murthy, B.P., Skee, D.M., Danyluk, A.P., Brett, V., Vorsanger, G.J., Moskovitz, B.L., 2007. Pharmacokinetic model and simulations of dose conversion from immediate- to extended-release tramadol. Curr. Med. Res. Opin. 23, 275–284 doi:http://dx.doi.org/10.1185/030079906X162773.

Pedersen, R.S., Damkier, P., Brøsen, K., 2006. Enantioselective pharmacokinetics of tramadol in CYP2D6 extensive and poor metabolizers. Eur. J. Clin. Pharmacol. 62, 513–521 doi:http://dx.doi.org/10.1007/s00228-006-0135-x.

Raber, M., Schulz, H.U., Schürer, M., Bias-Imhoff, U., Momberger, H., 1999a. Pharmacokinetic properties of tramadol sustained release capsules. 2nd communication: investigation of relative bioavailability and food interaction. Arzneimittelforschung 49, 588–593 doi:http://dx.doi.org/10.1055/s-0031-1300467.

Raber, M., Schulz, H.U., Schürer, M., Krupp, S., Momberger, H., 1999b. Pharmacokinetic properties of tramadol sustained release capsules. 3rd communication: investigation of relative bioavailability under steady state conditions. Arzneimittelforschung 49, 594–598 doi:http://dx.doi.org/10.1055/s-0031-1300468.

Raffa, R.B., Nayak, R.K., Liao, S., Minn, F.L., 1995. The mechanism(s) of action and pharmacokinetics of tramadol hydrochloride. Rev. Contemp. Pharmacother. 6, 485–497.

Ramirez, E., Laosa, O., Guerra, P., Duque, B., Mosquera, B., Borobia, A.M., Lei, S.H., Carcas, A.J., Frias, J., 2010. Acceptability and characteristics of 124 human bioequivalence studies with active substances classified according to the Biopharmaceutic Classification System. Br. J. Clin. Pharmacol. 70, 694–702 doi:http://dx.doi.org/10.1111/j.1365-2125.2010.03757.x.

Salman, S., Sy, S.K., Ilett, K.F., Page-Sharp, M., Paech, M.J., 2011. Population pharmacokinetic modeling of tramadol and its *O*-desmethyl metabolite in plasma and breast milk. Eur. J. Clin. Pharmacol. 67, 899–908 doi:http://dx.doi.org/10.1007/s00228-011-1023-6.

Savic, R.M., Jonker, D.M., Kerbusch, T., Karlsson, M.O., 2007. Implementation of a transit compartment model for describing drug absorption in pharmacokinetic studies. J. Pharmacokinet. Pharmacodyn. 34, 711–726 doi:http://dx.doi.org/10.1007/s10928-007-9066-0.

Schulz, H.U., Raber, M., Schürer, M., Amschler, S., Momberger, H., 1999. Pharmacokinetic properties of tramadol sustained release capsules. 1st communication: investigation of dose linearity. Arzneimittelforschung 49, 582–587 doi:http://dx.doi.org/10.1055/s-0031-1300466.

Shen, J., Boeckmann, A., Vick, A., 2012. Implementation of dose superimposition to introduce multiple doses for a mathematical absorption model (transit

compartment model). J. Pharmacokinet. Pharmacodyn. 39, 251–262 doi:http://dx.doi.org/10.1007/s10928-012-9247-3.

Sista, S., Lai, J.C., Eradiri, O., 2003. Steady state dose proportionality of a novel formulation of tramadol HCl extended release tablets. AAPS PharmSci 5, Abs M1076.

Skinner-Robertson, S., Li, J., Varin, F., 2011. A Population Pharmacokinetic Model of Once Daily Tramadol in Elderly and Healthy Young Volunteers, American Conference on Pharmacometrics (ACoP), San Diego, California Available from: http://www.go-acop.org/sites/default/files/webform/Sybil_Skinner-Robertson_1-1.doc (accessed 02.03.14).

Subrahmanyam, V., Renwick, A.B., Walters, D.G., Young, P.J., Price, R.J., Tonelli, A.P., Lake, B.G., 2001. Identification of cytochrome P-450 isoforms responsible for *cis*-tramadol metabolism in human liver microsomes. Drug. Metab. Dispos. 29, 1146–1155.

Summaries of Product Characteristics for Tramadol, 2014. http://www.medicines.org.uk/emc/searchresults.aspx?term=tramadol&searchtype=QuickSearch (accessed 02.03.14).

Wang, J., Weiss, M., D'Argenio, D.Z., 2008. A note on population analysis of dissolution–absorption models using the inverse Gaussian function. J. Clin. Pharmacol. 48, 719–725 doi:http://dx.doi.org/10.1177/0091270008315956.

Weiss, M., 1996. A novel extravascular input function for the assessment of drug absorption in bioavailability studies. Pharm. Res. 13, 1547–1553 doi:http://dx.doi.org/10.1023/A:1016039931663.

Weiss, M., Sermsappasuk, P., Siegmund, W., 2012. Modeling the kinetics of digoxin absorption: enhancement by *P*-glycoprotein inhibition. J. Clin. Pharmacol. 52, 381–387 doi:http://dx.doi.org/10.1177/0091270010396711.

Weiss, M., Kriangkrai, W., Sungthongjeen, S., 2014. An empirical model for dissolution profile and its application to floating dosage forms. Eur. J. Pharm. Sci. 56, 87–91 doi:http://dx.doi.org/10.1016/j.ejps.2014.02.013.

Wu, W.N., McKown, L.A., Liao, S., 2002. Metabolism of the analgesic drug ULTRAM (tramadol hydrochloride) in humans: API–MS and MS/MS characterization of metabolites. Xenobiotica 32, 411–425 doi:http://dx.doi.org/10.1080/00498250110113230.

Zhou, S.F., 2009. Polymorphism of human cytochrome P450 2D6 and its clinical significance: part I. Clin. Pharmacokinet. 48, 689–723 doi:http://dx.doi.org/10.2165/11318030-000000000-00000.

Zwaveling, J., Bubbers, S., van Meurs, A.H., Schoemaker, R.C., van Heel, I.R., Vermeij, P., Burggraaf, J., 2004. Pharmacokinetics of rectal tramadol in postoperative paediatric patients. Br. J. Anaesth. 93, 224–227 doi:http://dx.doi.org/10.1093/bja/aeh178.