IN-SILICO MODEL DEVELOPMENT

3.1 Development of model from animal data:

Pharmacokinetic models utilize data obtained from preclinical and clinical studies for prediction of the *in-vivo* performance of formulation and understand the underlying physiological processes. Developing such models help in robust product development and maximize *in-vivo* success.

3.1.1 Collection of data from literature:

Rich datasets are required for development, verification and validation of pharmacokinetic models. Extensive data collection was performed to create modelling database.

Physiochemical properties:

Physicochemical characteristics such as lipophilicity, solubility, molecular weight (MW), and pKa values of amisulpride and granisetron were collected from literature [1-5].

Biological Properties:

Biological parameters (such as fraction of drug unbound, or tissue-plasma partition coefficient) of amisulpride and granisetron were collected from literature [1, 4,5].

Marketed Formulations:

Data for marketed formulations was collected to have holistic view on available formulations and strategies [6].

***In-vivo* pharmacokinetic data**:

In-vivo pharmacokinetic data was collected from literature domain for different species and populations [7-18].

3.1.2 Allometric methods:

Allometric scaling uses similarity of anatomy, physiology, and physiological parameters and is the most predominantly used method to calculate the plasma profile in human from animal data [19]. Allometry has been applied to the prediction of human pharmacokinetics for small-large molecule drugs and is widely used by the researchers and companies for early decision making in drug discovery and development [19]. Using allometric scaling, key pharmacokinetic parameters such as volume of distribution and clearance are calculated.

3.1.3 Prediction of Human clearance:

There are different methods reported for calculating clearance using interspecies scaling [20]. For convenience, these methods are summarized in table 3-1 and results in table 3-2. The results of best suited methods are presented here. For amisulpride, methods 1-4 and for granisetron, methods 2-5 provided good prediction of human clearance respectively.

Table 3-1 Methods for calculation of clearance

Method	Method Name	Description	Formula
M1	Simple allometry (SA)	Proportionality of body weight	$CL = a(BW)^b$
M2	Allometric exponent based		$CL = CL\ of\ species\ x\ \left(\frac{70}{BW} of\ species\right)^b$ b = 0.75,0.80, .85,0.9
M3	Multiexponential allometry (MA)		$CL = a(BW)^b + \frac{[1-\left(\frac{3}{2}\right)b]}{[1-\left(\frac{1}{2}\right)b]}\ X\ a(BW)^{0.9}$
M4	Liver blood-flow method	Correction factor for animal-human	CL (human) = CL (animal) × (human/ animal) Qliver
M5	Rat	For bound drug	CL/kg (human) = CL/kg (rat) × 0.152
	Dog	For bound drug	CL/kg (human) = CL/kg (dog) × 0.410
	Monkey	For bound drug	CL/kg (human) = CL/kg (monkey) × 0.407

Table 3-2 Calculated clearance for human

Drug	Method	Observed clearance (L/h/kg) for Human	Calculated Clearance (L/h/kg) for Human	Fold error
Amisulpride	1	0.62	0.73	1.2
	2	0.62	0.68	1.1
	3	0.62	0.63	1.0
	4	0.62	0.45	0.7
	5	0.62	0.18	0.3
Granisetron	1	0.33	2.20	6.7
	2	0.33	0.29	0.9
	3	0.33	0.26	0.8
	4	0.33	0.49	1.4
	5	0.33	0.18	0.6

3.1.4 Prediction of Human volume of distribution:

There are different methods reported for calculating volume of distribution using interspecies scaling [21]. For convenience, these methods are summarized in Table 3-3 and results in table 3-4. The results of best suited methods are presented here. For amisulpride, all three methods and for granisetron, methods 1-2 provided good prediction of human volume of distribution.

Table 3-3 Methods for calculation of volume of distribution

Method	Method Name	Description	Formula
M1	Simple allometry (SA)	Proportionality of body weight	$Vss = a(BW)^b$
M2	Single species scaling	Proportionality factor	$Vdhuman = a\ x\ Vdanimal$ a = 0.59, 0.72, 0.79 for rat, dog and monkey b = 0.75,0.80, .85,0.9
M3	Two species rat and dog		$logVss\ human$ $= (0.07714 logVssrat$ $x\ logVssdog$ $+ 0.5147 \log Vss\ dog + 0.586$

Table 3-4 Calculated volume of distribution for human

Drug	Method	Observed Vd (L/kg)	Calculated Vd (L/kg)	Fold
Amisulpride	1	3.75	3.25	0.9
	2	3.75	2.66	0.7
	3	3.75	3.95	1.1
Granisetron	1	2.23	3.14	1.4
	2	2.23	3.77	1.7
	3	2.23	5.64	2.5

3.1.5 Human *in-vivo* plasma profile prediction:

The estimation of intravenous plasma profiles in humans was done by using Css-MRT approach, also known as Wajima approach and Dedrick plot. These methods provide calculation of human intravenous plasma concentration-profiles using preclinical species data Two to three species data are required to build the correlation and have sufficiently good prediction.

3.1.6 Wajima (Css-MRT) Approach:

In this approach, normalization of plasma profiles was estimated by performing division of the plasma profile by Css and time by MRT from animal data [22]. The Wajima predicted plots are presented in Figure 3-1 (A and B). Good prediction was observed for both amisulpride and granisetron.

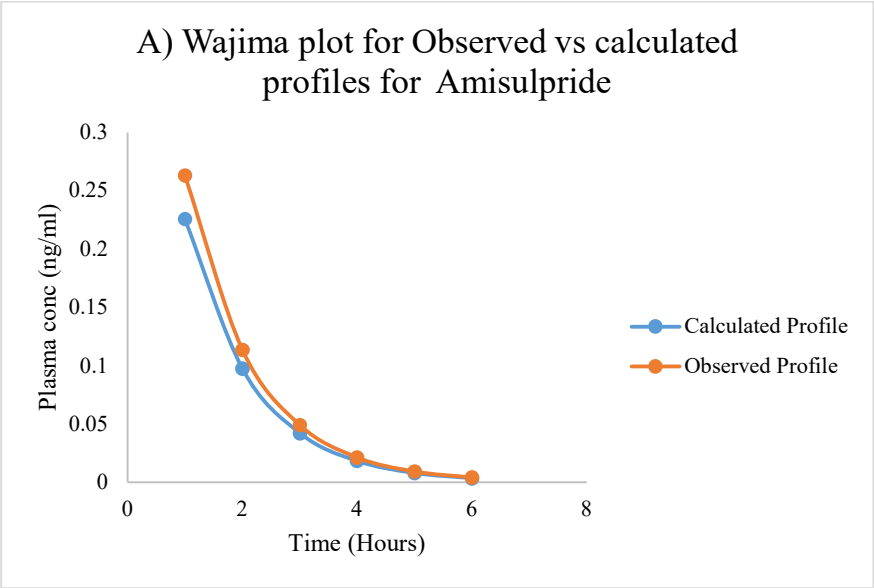


Figure 3-1: Wajima Profiles A) Amisulpride

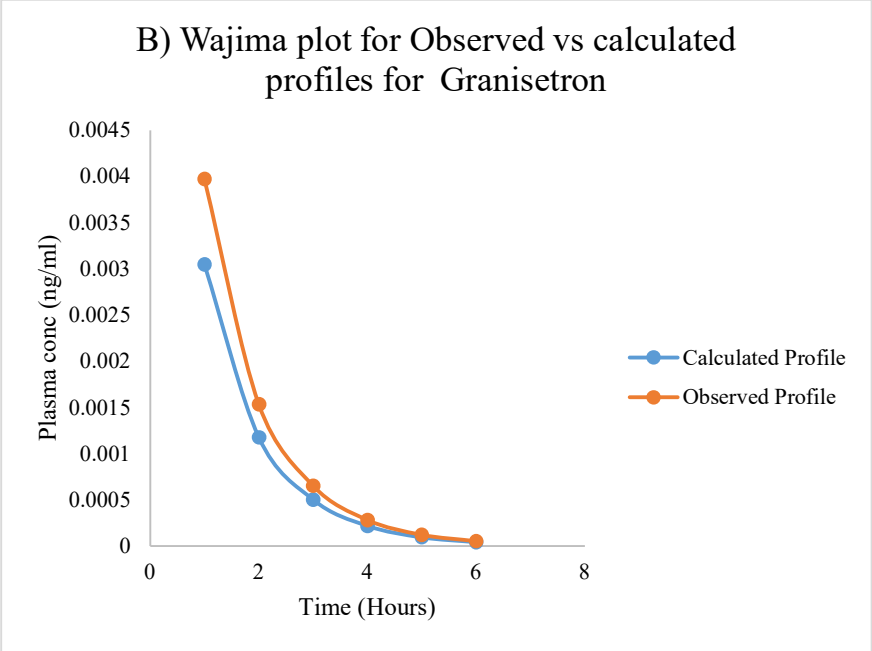


Figure 3-1: Wajima Profiles B) Granisetron

3.1.7 Dedrick plot:

It is the simplest form of calculating the plasma concentration-time profile using following equations 3-1 and 3-2 [22].

$$Calculated\ Concentration = \frac{Plasma\ concentration}{Dose/Body\ wei} \text{----------(3-1)}$$

$$\text{Calculated } Time = \frac{Time}{Body\ weight^{0.25}} \text{----------(3-2)}$$

The calculated Dedrick plot for Amisulpride and Granisetron is presented in Figure 3-2 (A and B). Good correlation was observed for amisulpride, but for granisetron, prediction error observed was more. The correlation depends upon how well the pharmacokinetic parameters in animal are scaled up to human. The variations can come due to study conduct, animal type or inherent molecule properties. The good correlation suggests that the animal species is useful to predict the human pharmacokinetics and poor correlation means one needs to further explore higher animal species to get closer to human physiology in order to have improved predictions.

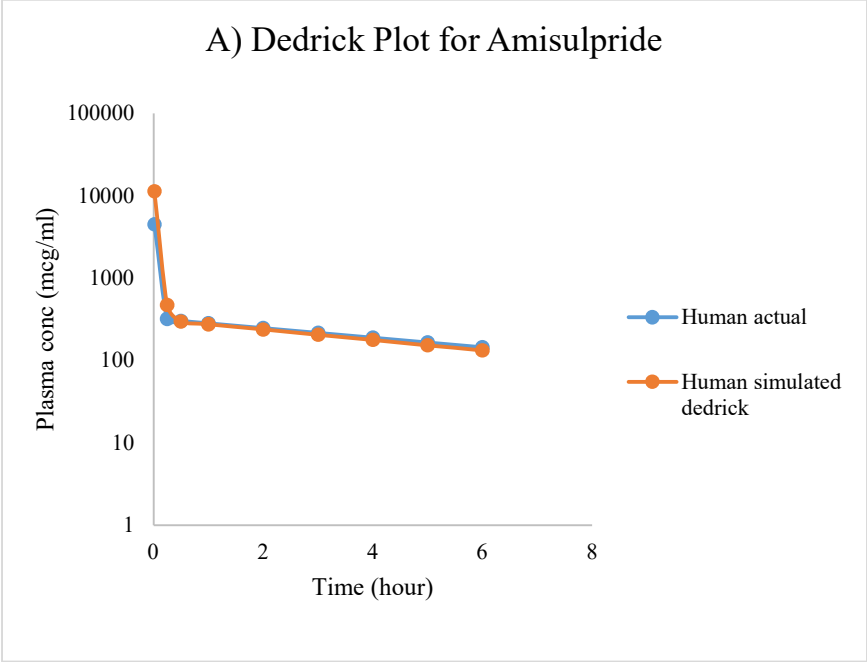


Figure 3-2: Dedrick plots A) Amisulpride

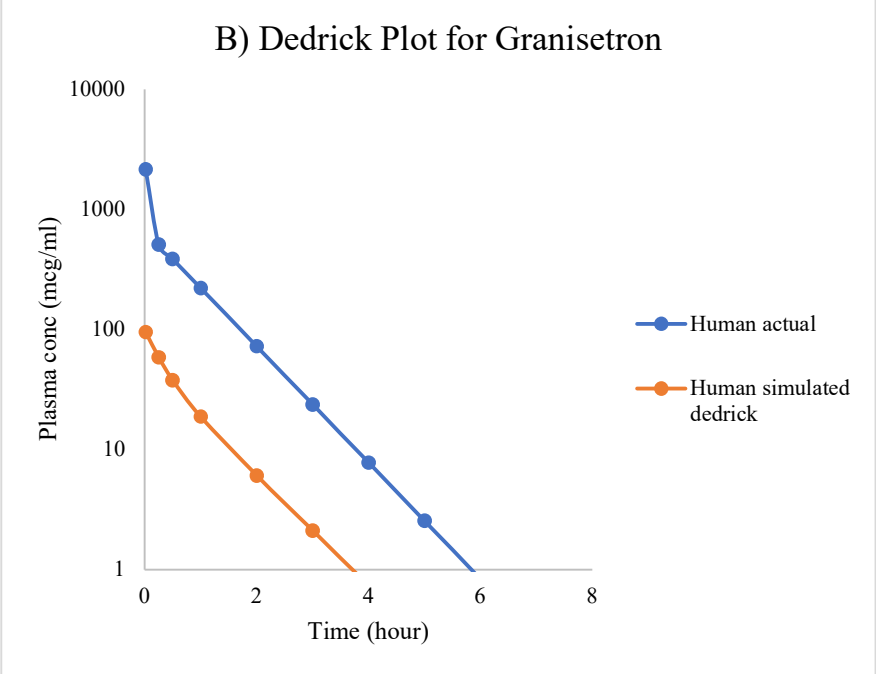


Figure 3-2: Dedrick plots B) Granisetron

Amisulpride has relatively low plasma protein binding (17%), linear plasma profile and renal elimination as major route compared to granisetron plasma protein binding (65%), complex, biphasic plasma profile and hepatic route as major elimination route. These factors contributed to Wajima approach being more superior than the Dedrick plot approach, which was also in-line with literature[22].

3.2 Development and verification of pharmacokinetic models in human

The modelling was performed using PBPK software namely GastroPlus™ (version 9.8.2 from Simulations Plus Inc., Lancaster, CA, USA). Where body weight/age was not available, default weights & age were selected from inbuilt PEAR population of software. The GastroPlus® PEAR physiology settings were used as per the designated population. The model was set to permeability limited and Kp's were calculated using the Lukacova and Rodgers method in the whole body PBPK model have. The volume of distribution was calculated from physicochemical properties like log P, B/P ratio and % fraction unbound using Lukacova-Rodger's method. Hepatic clearance was calculated from the *in-vitro* Clint obtained from literature and renal clearance was calculated from GFR.

3.2.1 PBPK model of Amisulpride:

Volume of distribution of amisulpride was found to be 136 L in surgical patients and 195 L in healthy subjects after intravenous dosing. Amisulpride rapidly distributes into red blood cells. Plasma protein binding was found to be 27.5 %. Amisulpride undergoes active renal secretion. Amisulpride showed elimination half-life of about 4.5 hours and was found to be similar between healthy subjects and surgical patients. Plasma clearance of amisulpride was estimated as 24.2 L/h in surgical patients and 33.8 L/h in healthy subjects using population pharmacokinetic analysis [23].

After oral administration, absolute bioavailability is around 50%. Amisulpride is weakly metabolized in the liver. Amisulpride showed no accumulation and pharmacokinetics was unaltered after the administration of repeated doses [24].

The physicochemical properties, pharmacokinetic parameters and selected physiology for development of PBPK model of amisulpride is presented in table 3-5.

PBPK model was verified using intravenous and oral (single and multiple dose) administrations. The data is presented in Figure 3-3 (A, B & C). This developed model was used to predict *in-vivo* pharmacokinetics of sustained release formulation to be administered once in a week.

Table 3-5 Input data for PBPK modelling of Amisulpride

No.	Attribute	Data
Compound tab		
1.	Name	Amisulpride
2.	Molecular Formula 3	C17H27N3O4S
3.	Molecular Weight 3	369.49
4.	Structure	MOL File
5.	Log P	1.41
6.	pKa (Base)	8.28
7.	Solubility (mg/mL) in water	0.293
8.	Human Permeability (cm/s)	0.45 X 10^4 cm/s
9.	Mean Precipitation Time (sec)	900
10.	Diffusion Coefficient (cm^2/s X 10^5)	0.65
11.	Drug Particle density (g/mL)	1.2
Physiology Tab (In-put)		
1.	Human Fasted Physiology	Selected
Pharmacokinetics Tab		
In-put		
1.	Body weight (kg)	88.5
2.	Blood to Plasma Concentration ratio	0.98
Derived		
3.	Adjusted Percent Unbound in Plasma (Fup) (%)	24.984
4.	Clearance (L/h)	46.151
5.	Volume of Distribution (L)	235.429
6.	Half Life calculated T1/2 (h)	3.535

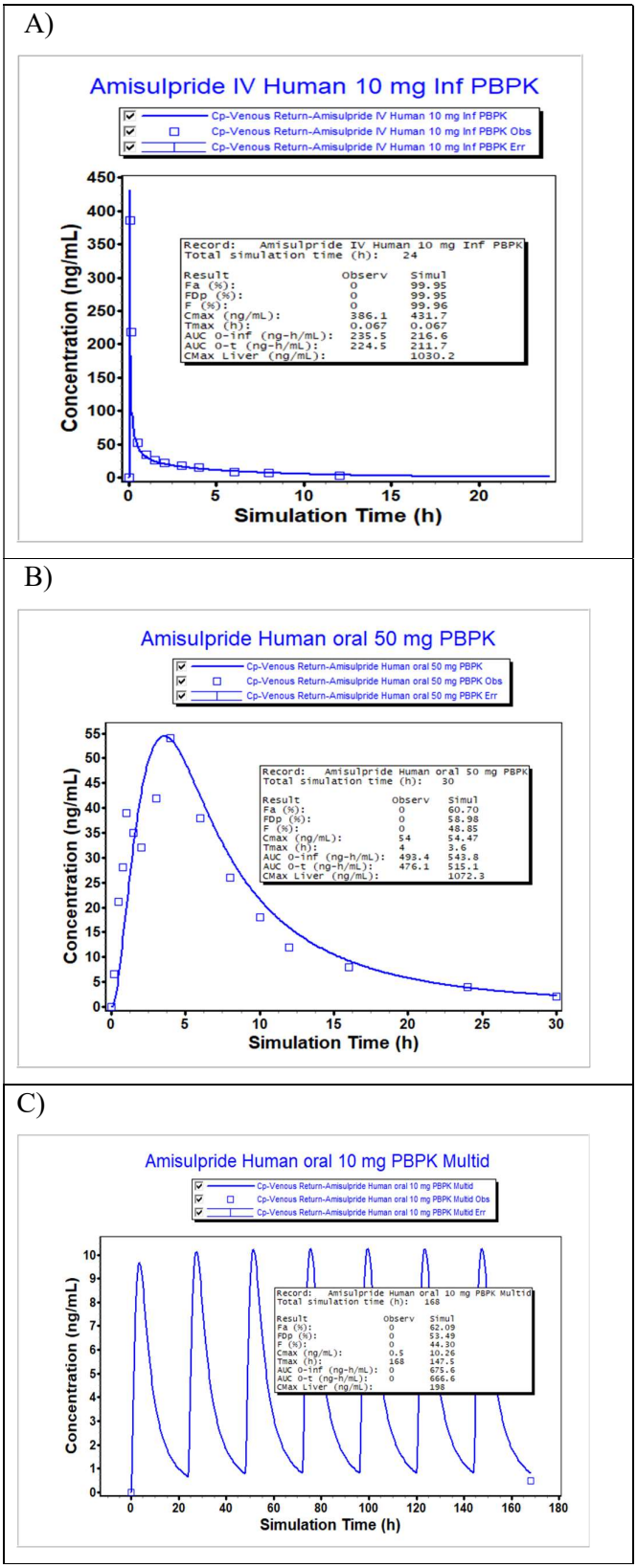


Figure 3-3: PBPK model of Amisulpride A) IV Amisulpride 10 mg B) Single dose oral Amisulpride 50 mg C) Multiple dose oral Amisulpride 10 mg

3.2.2 PBPK model of Granisetron:

Granisetron showed large intersubject variability for all pharmacokinetic parameters irrespective of type of formulation administered or population studied. Granisetron showed multiphasic decline after intravenous administration in healthy subjects. The rapid initial decline in concentration was attributed to extensive tissue uptake, which was also contributed by high apparent volume of distribution (4.2 L/Kg). Granisetron undergoes metabolism mainly in the liver and only around 10% of granisetron got excreted unchanged in the urine. Non-renal clearance ranged between 43 L/h and was found to be major contributing to overall clearance [7] Terminal phase half-life ranged from 5.2 h [25]. Geriatric patients displayed a high volume of distribution (4-5 L/kg) and lower total plasma clearance by around 50% which resulted in higher elimination half-life of 8 h. In chemotherapy receiving adult patients, clearance is decreased to 0.4 L/kg/h, which resulted in increased half-life of 11 h [26,27]. Granisetron was absorbed completely, however due to extensive firs-pass metabolism bioavailability was around 55% after oral administration. Smoking habits of subjects resulted in significant heterogeneity in systemic availability after oral administration [28].

The physicochemical properties, pharmacokinetic parameters and selected physiology for development of PBPK model of Granisetron is presented in table 3-6.

PBPK model was verified after intravenous, oral (single and multiple dose), subcutaneous, intramuscular and transdermal administrations. The data is presented in Figure 3-4 (A to L). This developed model was used to predict *in-vivo* pharmacokinetics of sustained release formulation to be administered once in a week.

Table 3-6 Input data for PBPK modelling of Granisetron

No.	Attribute	Data
Compound tab		
1.	Name	Granisetron
2.	Molecular Formula 3	C18H24N4O
3.	Molecular Weight 3	312.42
4.	Structure	MOL File
5.	Log P	2.2
6.	pKa (Base)	9
7.	Solubility (mg/mL) in water	10 mg/ml
8.	Human Permeability (cm/s)	3.08 X 10^4 cm/s
9.	Mean Precipitation Time (sec)	900
10.	Diffusion Coefficient (cm^2/s X 10^5)	0.65
11.	Drug Particle density (g/mL)	1.2
Physiology Tab (In-put)		
1.	Human Fasted Physiology	Selected
Pharmacokinetics Tab		
In-put		
1.	Body weight (kg)	85.53
2.	Blood to Plasma Concentration ratio	0.86
Derived		
3.	Adjusted Percent Unbound in Plasma (Fup) (%)	33.393
4.	Clearance (L/h)	22.778
5.	Volume of Distribution (L)	145.90
6.	Half Life calculated T1/2 (h)	4.439

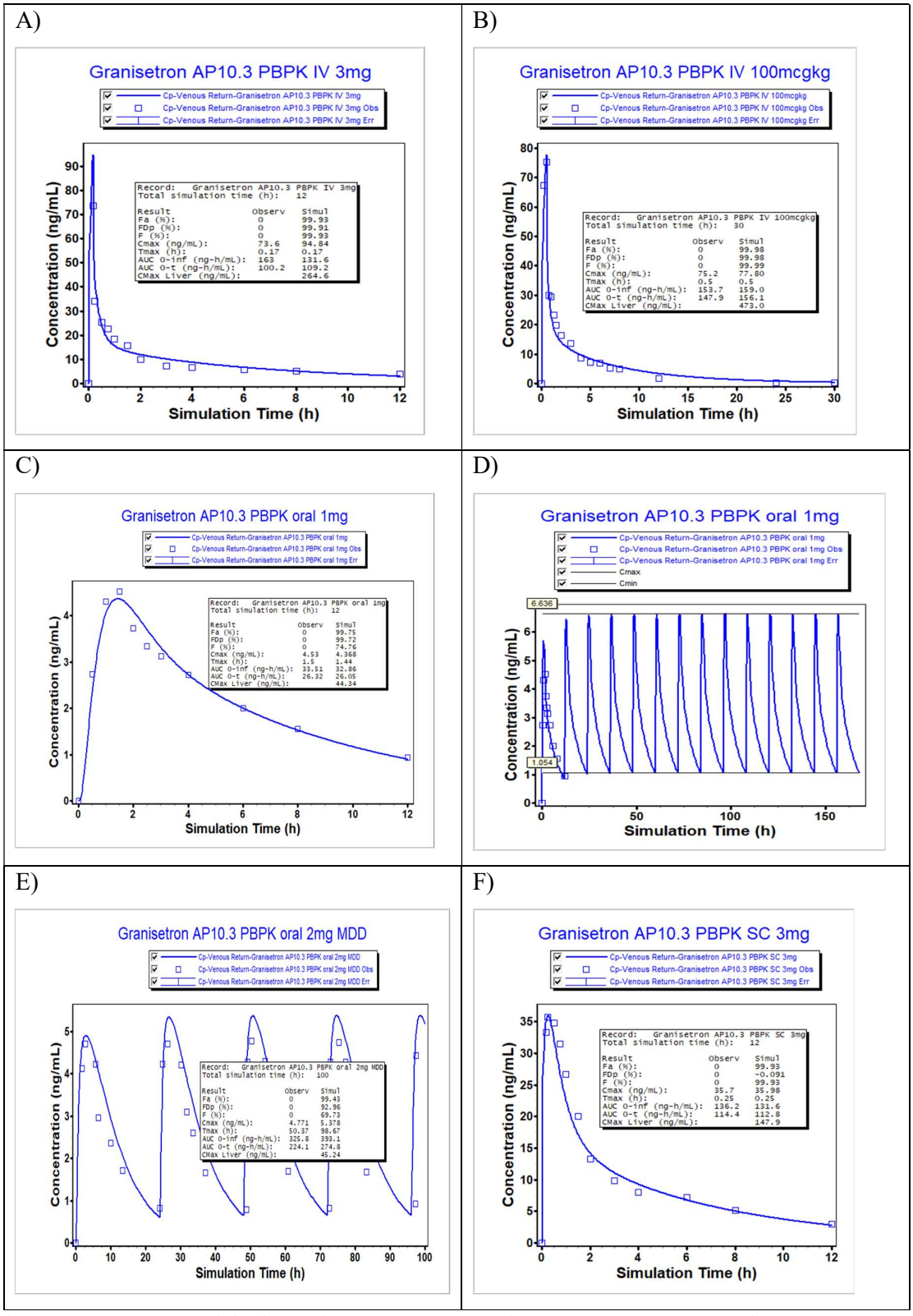


Figure 3-4: PBPK model of Granisetron A) IV Granisetron 3 mg B) IV Granisetron 100 mcg/kg C) Single dose oral Granisetron 1 mg D) Multiple dose oral Granisetron 1 mg E) Multiple dose oral Granisetron 2 mg F) Subcutaneous (SC) Injection Granisetron 3 mg

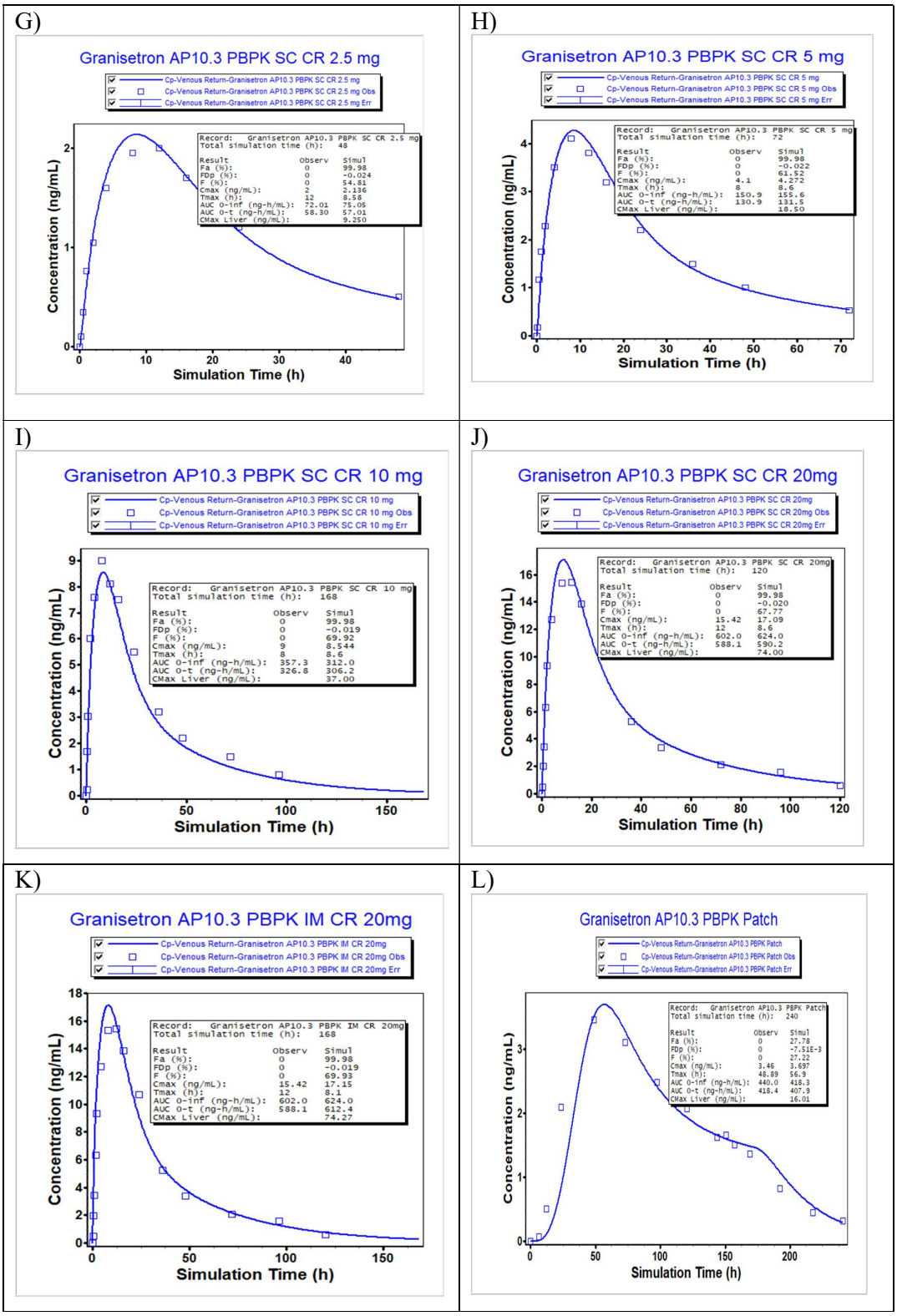


Figure 3-4: PBPK model of G) SC CR Granisetron 2.5 mg H) SC CR Granisetron 5 mg I) SC CR Granisetron 10 mg J) SC CR Granisetron 20 mg K) Intramuscular CR Granisetron 20 mg L) Transdermal patch Granisetron

3.3 Parameter sensitivity analysis (PSA)

GastroPlus includes a powerful Parameter Sensitivity Analysis mode to conveniently assess the importance of a variety of molecular parameters in predicting absorption, pharmacokinetics, and pharmacodynamics. A Parameter Sensitivity Analysis comprises of more than one simulation at a time, unless a 3D Parameter Sensitivity Analysis is desired in which case a grid of simulations is run varying each of the two parameters to produce 3D plots of output variables vs the two parameters.

The results of a Parameter Sensitivity Analysis can be viewed in the form of spider plot as shown below in the Figure Nos 3-5 and 3-6.

Impact of critical parameters on pharmacokinetics of amisulpride and granisetron was checked in terms of maximum plasma concentration (Cmax) and area under the curve (AUC). The selected parameters are mentioned in table 3-7

Table 3-7 Parameters selected for Parameter sensitivity analysis

Sr. No.	Parameter
1.	Log P (Log D)
2.	Fraction unbound (Fup)
3.	Blood to plasma ratio (BP)
4.	Drug release parameters in terms of Weibull function (scale and shift)
5.	Permeability (Peff)

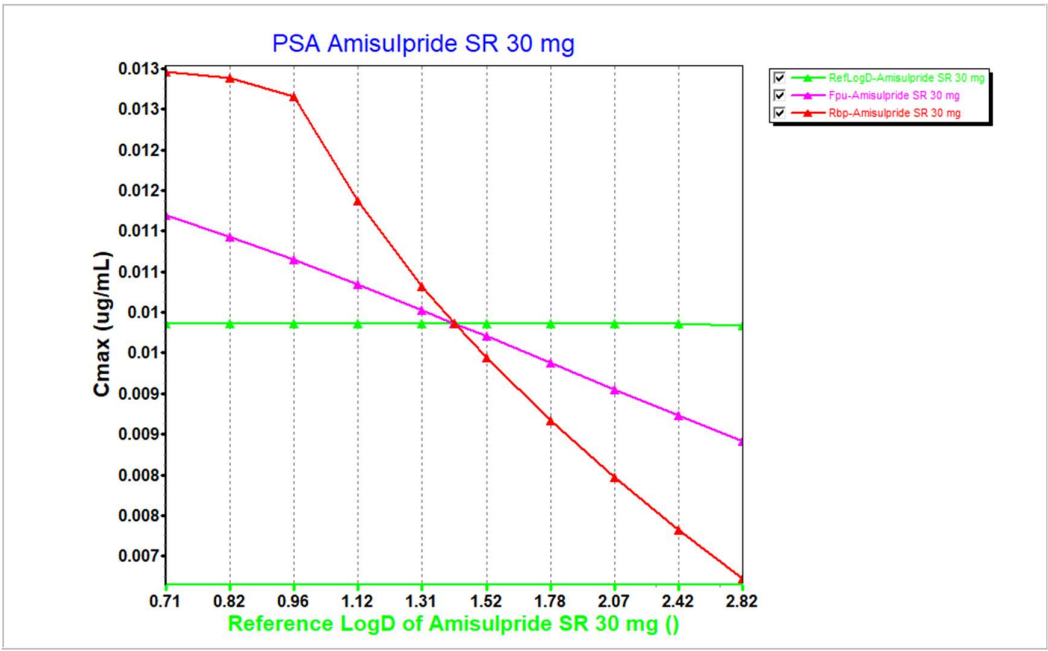


Figure 3-5 Parameter sensitivity analysis A) Amisulpride for Cmax using logP, Fup and BP

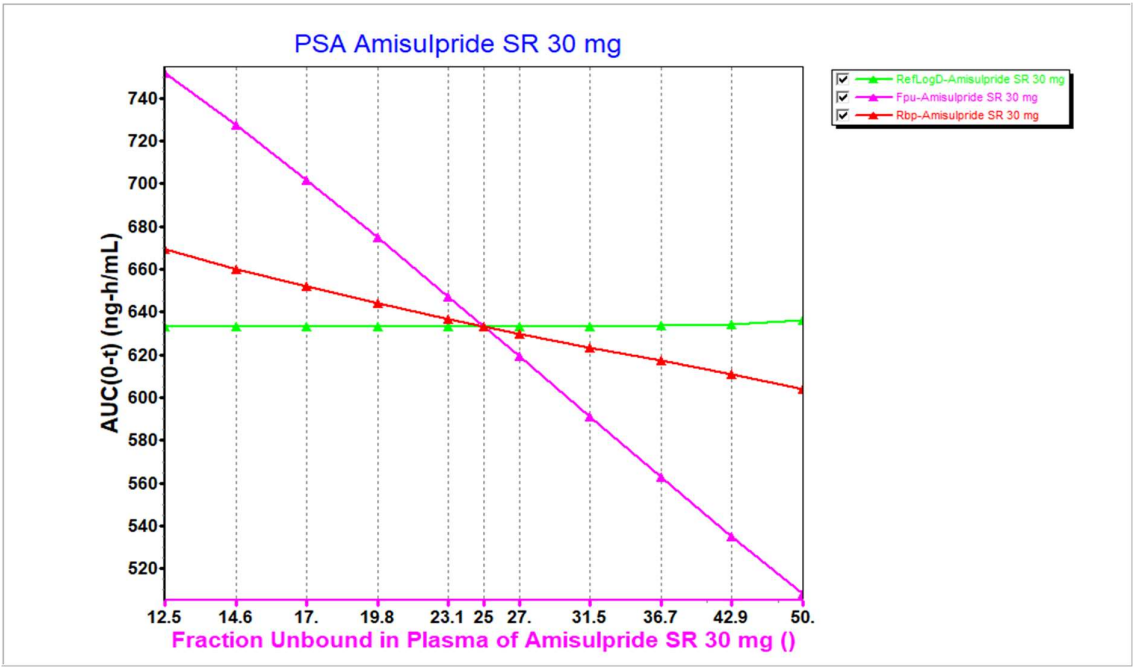


Figure 3-5 Parameter sensitivity analysis B) Amisulpride for AUC using logP, Fup and BP

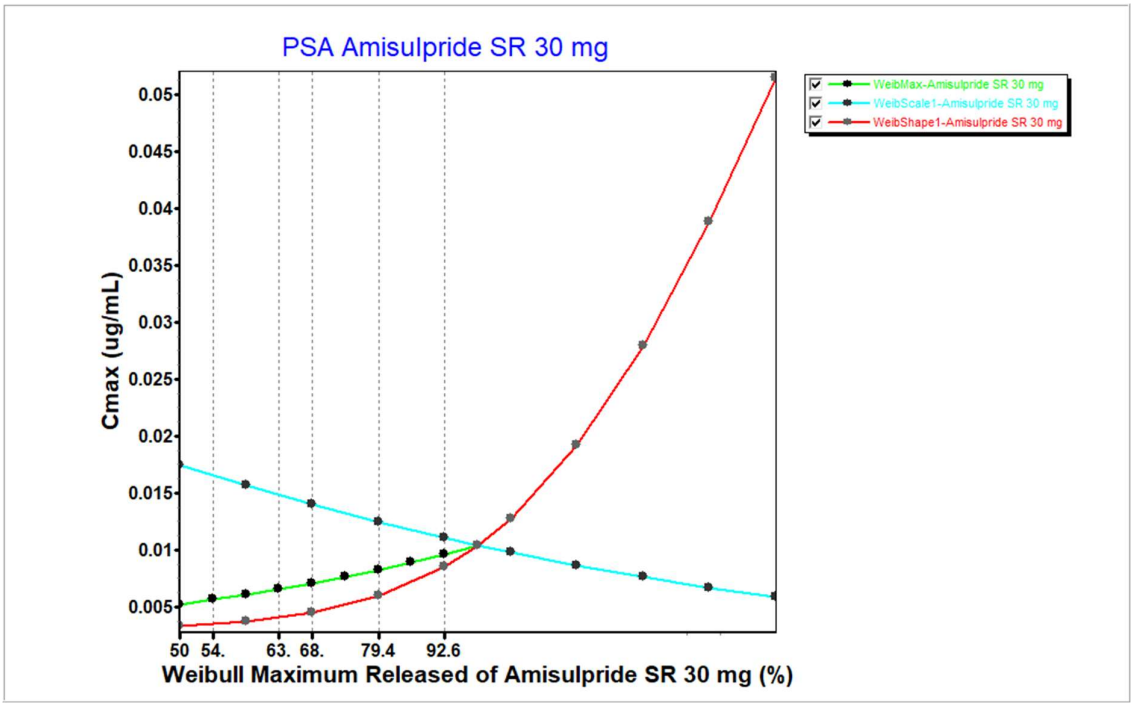


Figure 3-5 Parameter sensitivity analysis C) Amisulpride for Cmax using Weibull parameters for drug release

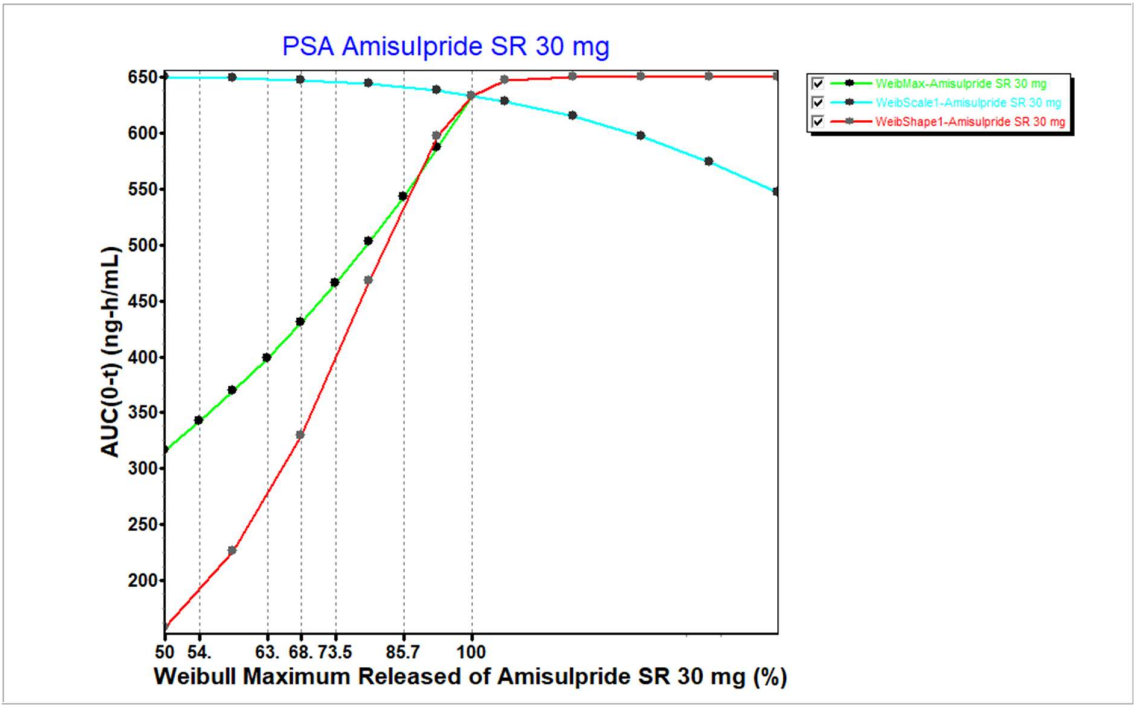


Figure 3-5 Parameter sensitivity analysis D) Amisulpride for AUC using Weibull parameters for drug release

Parameter sensitivity analysis for amisulpride in Fig 3-5 (A & B) showed that fraction unbound and blood to plasma ratio inversely impacted both Cmax and AUC. However, log D value did not show any impact on both Cmax and AUC value.

Drug release of amisulpride was modelled using Weibull function. Weibull parameters such as weibull shape and weibull maximum release have direct relationship with both Cmax and AUC, while Weibull scale showed inverse relationship for both Cmax and AUC as shown in in Fig 3-5 (C & D).

Modelling the drug release provide interpolation of time points that cannot be analysed in reality. This exercise helped to optimize the parameters to achieve desired sustained release profile for amisulpride.

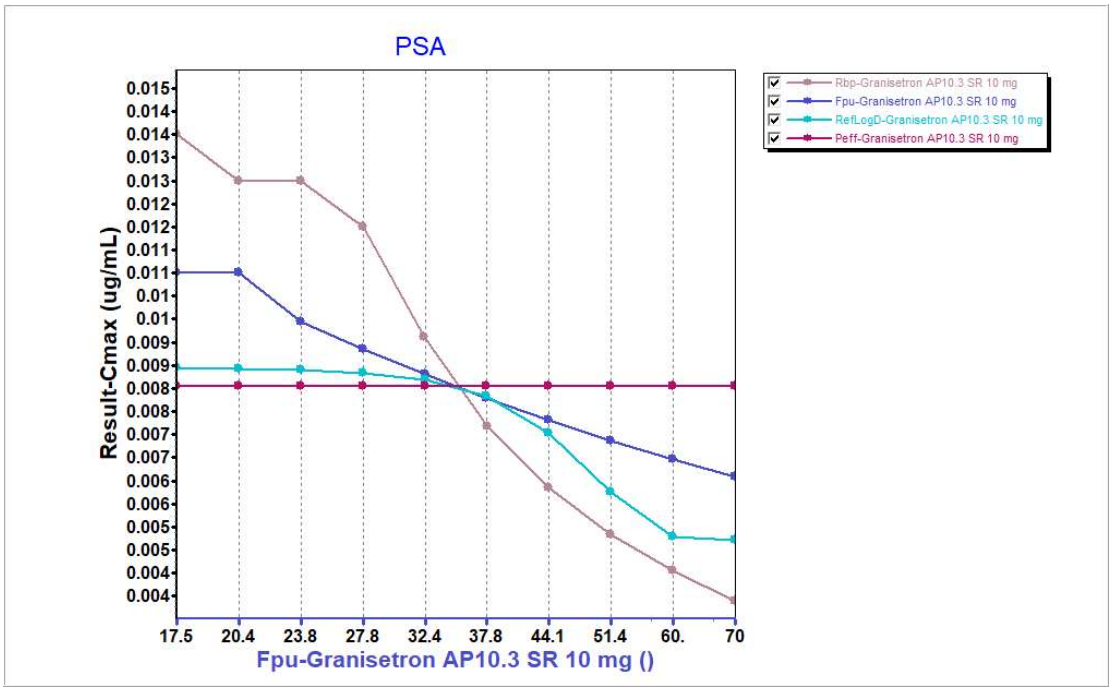


Figure 3-6 Parameter sensitivity analysis A) Granisetron for Cmax using logP, Fup, BP and Peff

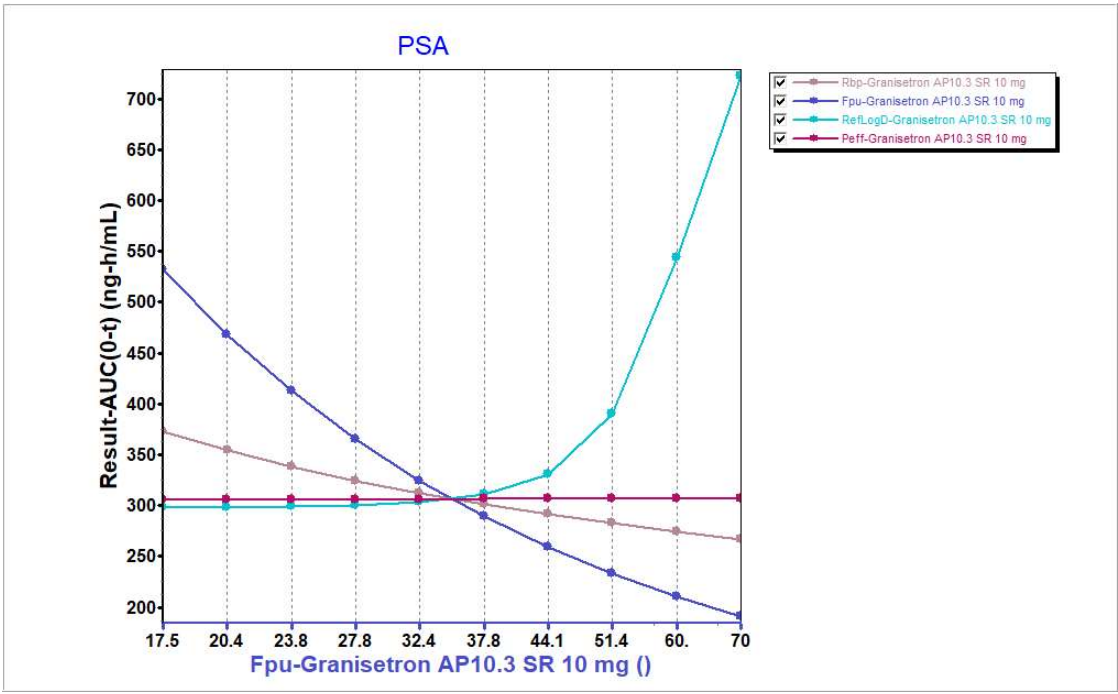


Figure 3-6 Parameter sensitivity analysis B) Granisetron for AUC using logP, Fup, BP and Peff

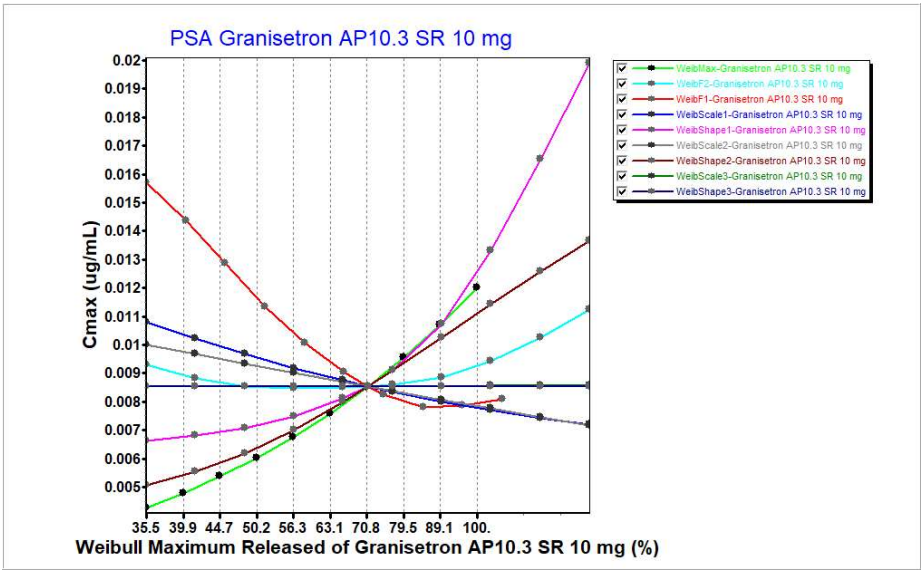


Figure 3-6 Parameter sensitivity analysis C) Granisetron for Cmax using Weibull parameters for drug release

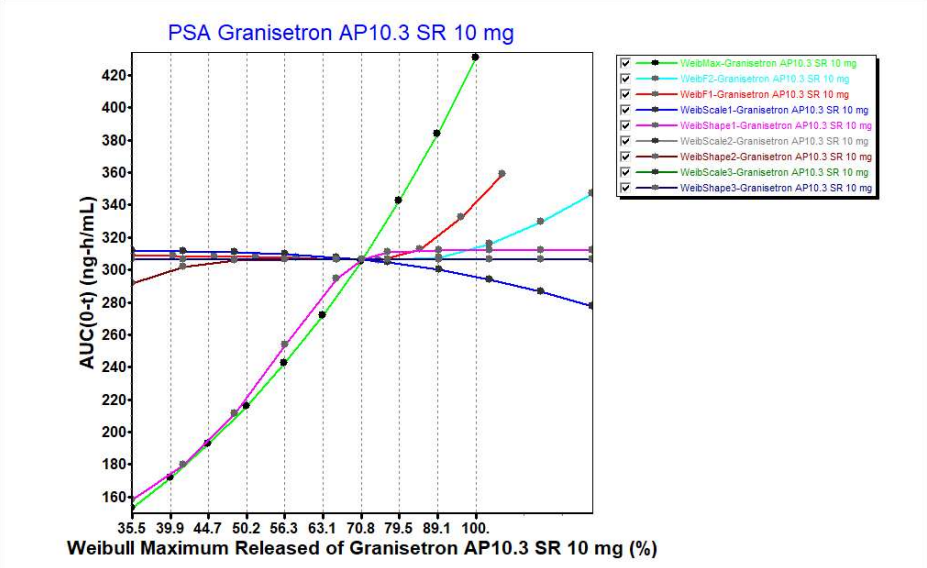


Figure 3-6 Parameter sensitivity analysis D) Granisetron for AUC using Weibull parameters for drug release

Parameter sensitivity analysis for granisetron in Fig 3-6 (A & B) showed that fraction unbound and blood to plasma ratio inversely impacted both Cmax and AUC. Log D value showed inverse impact on Cmax and positive impact on AUC value after around 40% of fraction unbound. No effect of permeability was seen on both parameters.

Drug release of granisetron was modelled using Weibull function. Weibull parameters such as Weibull shape 1 and 2 and Weibull maximum release have direct relationship with Cmax, while Weibull scale showed inverse relationship for Cmax as shown in in Fig 3-6 (C).

Fraction dissolved in Weibull 1 showed inverse relationship with Cmax, while it showed direct relationship with AUC after 70% release as shown in in Fig 3-6 (D).

Weibull maximum release have direct relationship with AUC, while all other parameters showed no significant impact on both parameters as shown in in Fig 3-6 (D).

This exercise helped to optimize the parameters to achieve desired sustained release profile for Granisetron.

3.4 Model validation

The model performance to predict the data was evaluated by overlaying the calculated plasma profiles with the observed data.

Average-fold error (AFE) and absolute average-fold error (AAFE) were used to calculate the overall predictability of the model in terms of bias and precision. AFE limit is simulation falls within 2-fold error (0.5–2-fold) [29].

The equations 3-3 and 3-4 were used for the calculation of AFE and AAFE:

$$\mathrm{AFE} = 10^{\frac{1\ \sum \log\left(\frac{PRED}{OBS}\right)}{n}} \text{----------(3-3)}$$

$$\mathrm{AAFE} = 10^{\frac{1\ \sum |\log\left(\frac{PRED}{OBS}\right)|}{n}} \text{----------(3-4)}$$

To verify the prediction accuracy of the developed PBPK model, the predicted pharmacokinetic parameters were compared with the *in-vivo* experimental data. The predictive accuracy of the developed models was validation by visual comparison (observed and predicted data were compared visually by overlaying both the profiles) and checking errors in terms of ratios.

Goodness-of-fit plots were used to assess the similarity between observed and simulated profiles [30].

The fold error of each time point (FEi), average fold error (AFE) and absolute average fold error (AAFE) are mostly used evaluation criteria to assess the model accuracy [31,32]. The equations of which are given in 3-5, 3-6 and 3-7 respectively.

$$FE = \frac{\text{Predicted } i}{\text{Observed } i} \text{----------(3-5)}$$

$$AFE = 10^{\frac{1}{n}*\sum log\left(\left|\frac{Predicted\ i}{Observed\ i}\right|\right)} \text{----------(3-6)}$$

$$AAFE = 10^{\frac{1}{n}*\sum\left|log\left(\frac{Predicted\ i}{Observed\ i}\right)\right|} \text{----------(3-7)}$$

Regulatory acceptable limits for Fei are 0.3 to 3 and for AFE and AAFE are 0.5 to 2 [33, 34].

Predicted and observed PK parameters which explain the rate and extent of drug absorption Cmax and AUC0-t respectively. Tmax were compared using the predicted vs. observed fold difference as calculated in equation 3-8.

$$FE = \frac{\text{Predicted value of PK parameter}}{\text{Observed } value\ of\ PK\ parameter} \text{----------(3-8)}$$

If the FE are in the range of 0.5 to 2, it can be considered as a successful prediction [35].
As presented in the table 3-8 and figure 3-7, the predicted plasma profiles after intravenous and oral administration for amisulpride are in close agreement with the observed data. The major pharmacokinetic parameters estimated were CL, Vss, Cmax and AUC 0-t after the intravenous administration and Cmax, AUC 0-t and Tmax after oral administration. The predicted values of all these parameters were found to be very well within the limit of 0.5 to 2-fold error.

As presented in the table 3-9 and figure 3-8, the predicted plasma profile after intravenous, oral, subcutaneous and transdermal administration for granisetron are in close agreement with the observed data. The major pharmacokinetic parameters estimated were CL, Vss, Cmax and AUC 0-t after the intravenous administration and Cmax, AUC 0-t and Tmax after oral administration. The predicted values of all these parameters were found to be very well within the limit of 0.5 to 2-fold error.

The accuracy of PBPK model were validated by FEi, AFE and AAFE for each time point of the plasma concentration profile after intravenous and oral dosing.

All the values were within the regulatory acceptable criteria as shown in table 3-8 and 3-9. The spread of values for FEi can be seen around unity line as shown in Fig 3-7 and 3-8.

These results of model validation indicated that the PBPK models of amisulpride and granisetron are accurate and reliable.

Table 3- 8 Model Validation summary for Amisulpride

Dose (Route of administration)	PK Parameters	Observed	Predicted	FE (Limit 0.3-3)	AFE (Limit 0.5-2)	AAFE (Limit 0.5-2)	Reference No.
10 mg (Intravenous)	Cl (L/h)	43.50	46.15	1.1	0.84	1.19	5
	Vss (L)	171.00	235.43	1.4			
	Cmax (ng/ml)	386.10	431.70	1.1			
	AUC(0–t) (ng•h/ml)	224.50	211.70	0.9			
50 mg (Oral)	Cmax (ng/ml)	54.00	54.47	1.0	0.55	1.82	18
	AUC (0–t) (ng•h/ml)	476.10	515.10	1.1			
	Tmax (h)	4.00	3.60	0.9			

Table 3- 9 Model validation summary for Granisetron

Dose (Route of administration)	PK Parameters	Observed	Predicted	FE (Limit 0.3-3)	AFE (Limit 0.5-2)	AAFE (Limit 0.5-2)	Reference No.
100 mcg/kg (Intravenous)	Cl (L/h)	42 (14-179.2)	22.78	0.5	1.12	0.89	8
	Vss (L)	168 (117.6-429.1)	145.90	0.9			
	Cmax (ng/ml)	75.20	77.80	1.0			
	AUC(0–t) (ng•h/ml)	147.90	156.10	1.1			
3 mg (Intravenous)	Cl (L/h)	42 (14-179.2)	22.78	0.5	1.21	0.82	10
	Vss (L)	168 (117.6-429.1)	145.90	0.9			
	Cmax (ng/ml)	73.60	105.59	1.4			
	AUC(0–t) (ng•h/ml)	100.21	109.36	1.1			
1 mg (Oral single dose)	Cmax (ng/ml)	4.53	4.37	1.0	0.99	1.01	13
	AUC(0–t) (ng•h/ml)	26.32	26.05	1.0			
	Tmax (h)	1.50	1.44	1.0			
2 mg (Oral multiple dose)	Cmax (ng/ml)	4.77	5.37	1.1	1.91	0.52	36
	AUC(0–t) (ng•h/ml)	224.10	274.80	1.2			

Dose (Route of administration)	PK Parameters	Observed	Predicted	FE (Limit 0.3-3)	AFE (Limit 0.5-2)	AAFE (Limit 0.5-2)	Reference No.
3 mg (Subcutaneous injection)	Cmax (ng/ml)	35.70	35.98	1.0	0.94	1.07	10
	AUC(0–t) (ng•h/ml)	114.40	112.80	1.0			
	Tmax (h)	0.25	0.25	1.0			
2.5 mg (Subcutaneous sustained release injection)	Cmax (ng/ml)	2.00	2.14	1.1	0.97	1.03	12
	AUC(0–t) (ng•h/ml)	58.30	57.01	1.0			
	Tmax (h)	12.00	8.58	0.7			
5 mg (Subcutaneous sustained release injection)	Cmax (ng/ml)	4.10	4.27	1.0	0.86	1.16	12
	AUC(0–t) (ng•h/ml)	130.90	131.50	1.0			
	Tmax (h)	8.00	8.60	1.1			
10 mg (Subcutaneous sustained release injection)	Cmax (ng/ml)	9.00	8.54	0.9	0.76	1.32	12
	AUC(0–t) (ng•h/ml)	326.80	306.20	0.9			
	Tmax (h)	8.00	8.60	1.1			
20 mg (Subcutaneous sustained release injection)	Cmax (ng/ml)	15.42	17.09	1.1	1.24	0.81	12
	AUC(0–t) (ng•h/ml)	588.10	590.20	1.0			
	Tmax (h)	12.00	8.60	0.7			
34.3 mg (Transdermal Patch)	Cmax (ng/ml)	3.46	3.70	1.1	0.52	1.92	36
	AUC(0–t) (ng•h/ml)	418.40	407.90	1.0			
	Tmax (h)	48.90	56.90	1.2			

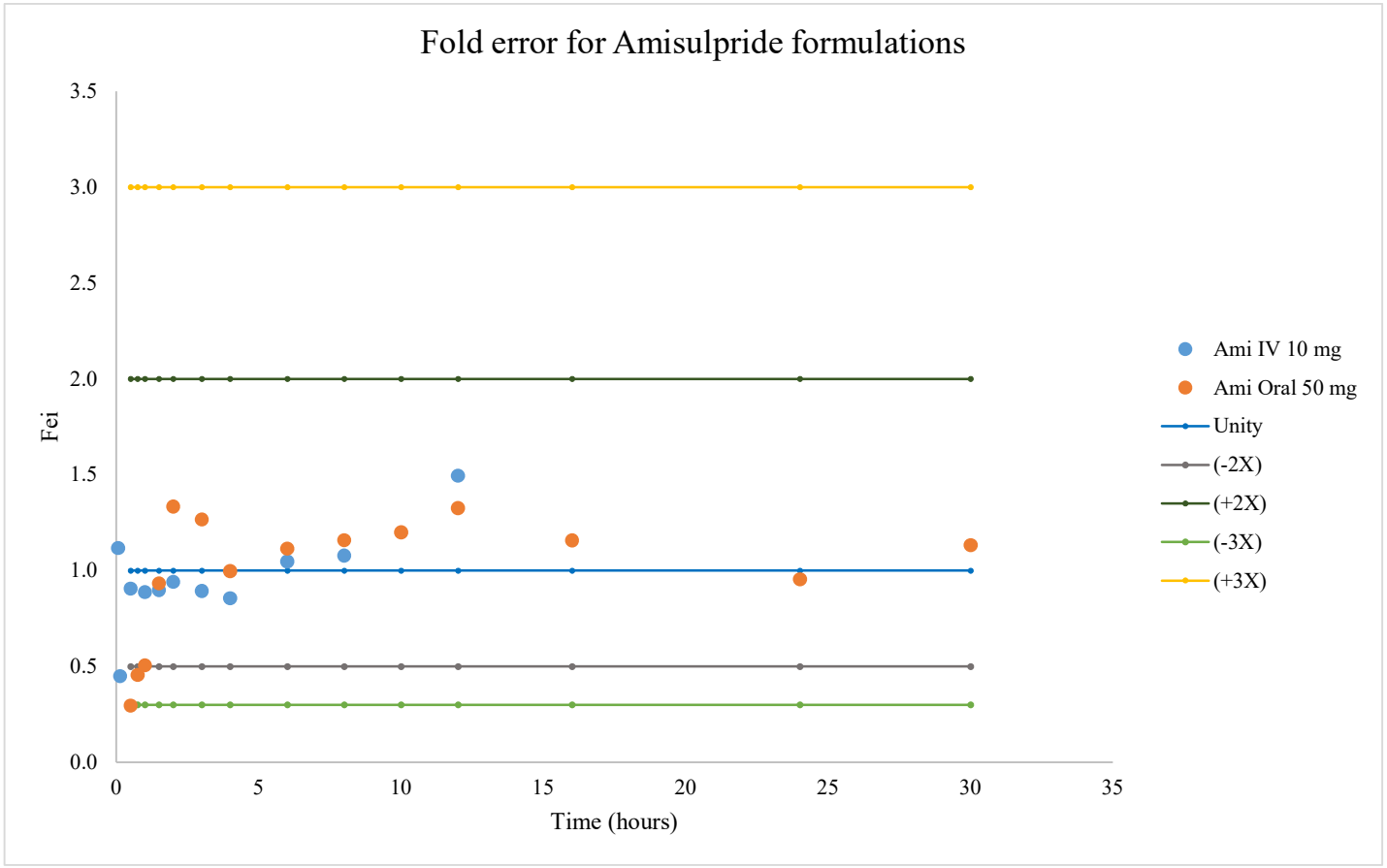


Fig 3-7-Fold error for Amisulpride formulations

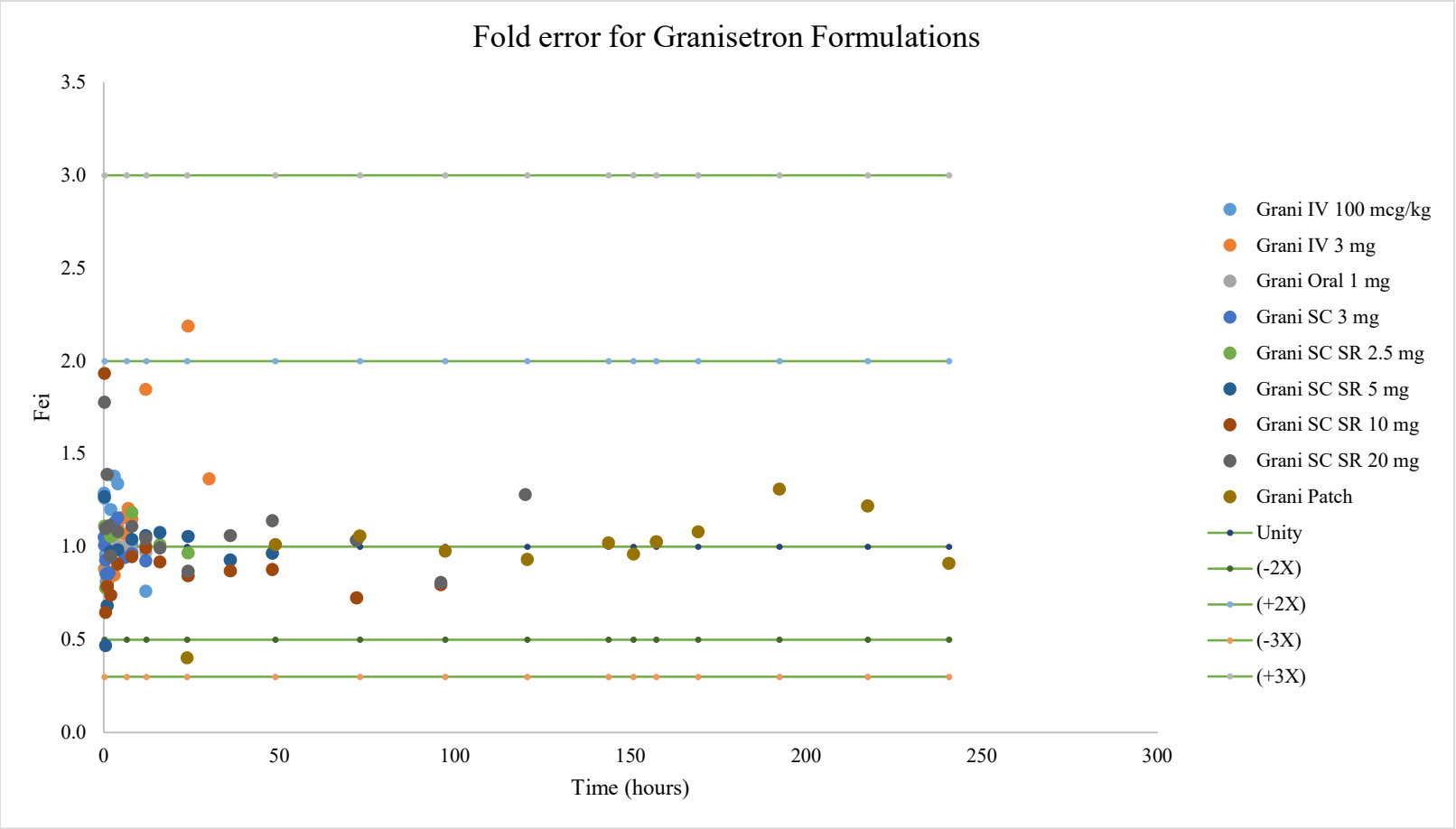


Fig 3-8-Fold error for Granisetron Formulations

3.5 Identification of target plasma concentration levels for sustained release formulations:

The steady state plasma concentration levels were calculated after multiple dosing of immediate release tablet dosage forms for amisulpride and granisetron using the developed PBPK model. The multiple dose simulations provide both Minimum (Cminss) and maximum (Cmaxss) concentration at steady state. The intended formulation should fall between these levels.

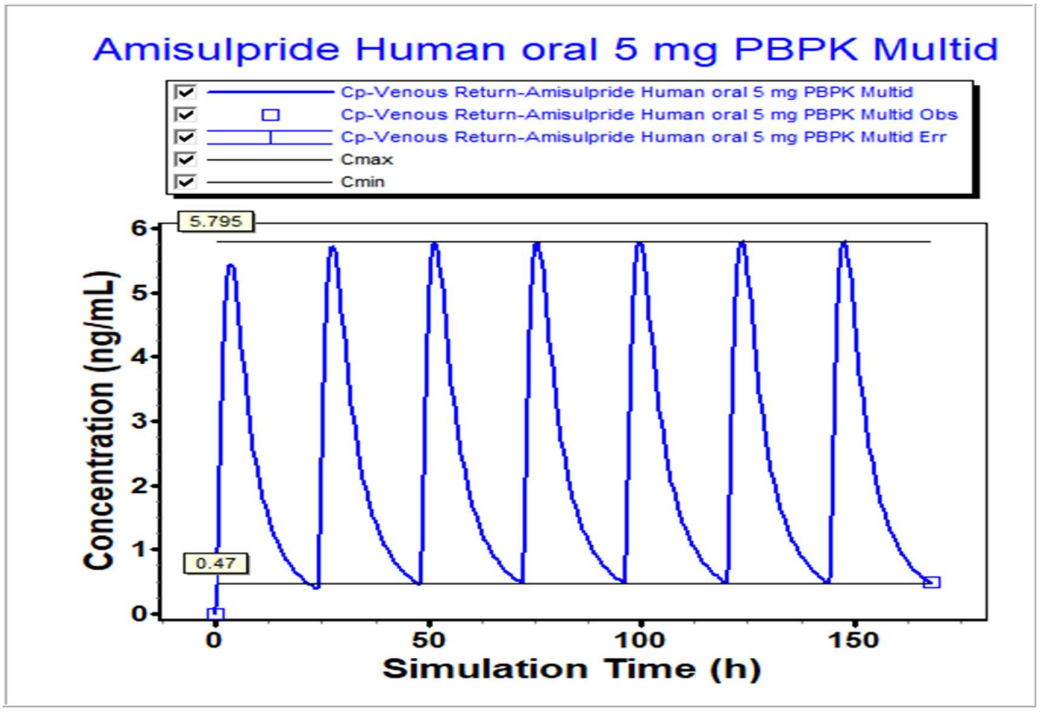
The oral tablets were dosed as per recommended dosing regimens [36-38] mentioned in the table 3-10.

Table 3-10 Dosage regimen for Amisulpride and Granisetron

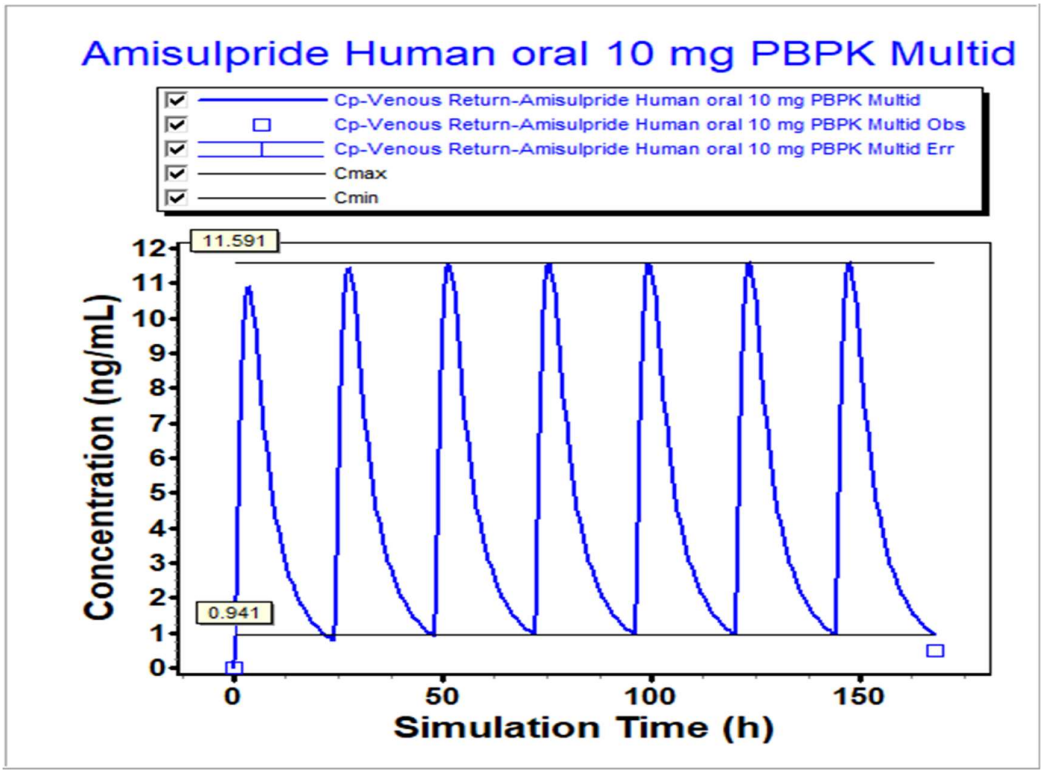
Drug	Dose (mg)	Dosage regimen
Amisulpride	5	Once a day
	10	
Granisetron HCl	1	Twice a day
	2	Once a day

The details of simulation are presented in Figure 3-9 (A to D)

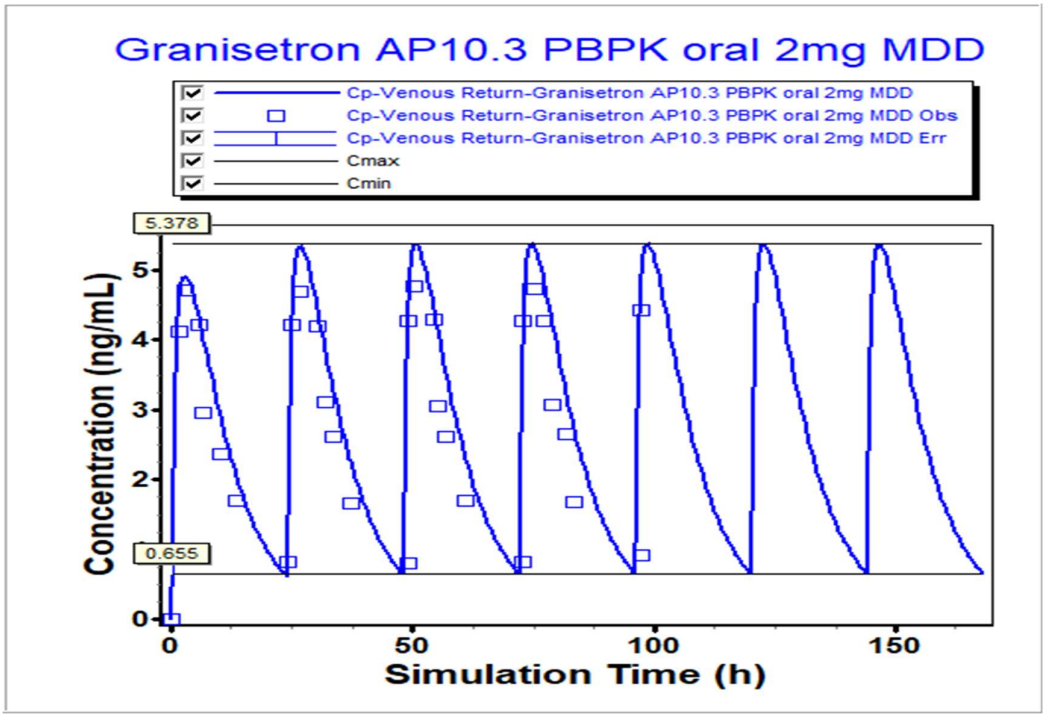
A)



B)


Amisulpride Human oral 10 mg PBPK Multid
Cp-Venous Return-Amisulpride Human oral 10 mg PBPK Multid
Cp-Venous Return-Amisulpride Human oral 10 mg PBPK Multid Obs
Cp-Venous Return-Amisulpride Human oral 10 mg PBPK Multid Err
Cmax
Cmin
11.591
0.941
Concentration (ng/mL)
Simulation Time (h)

C)


Granisetron AP10.3 PBPK oral 2mg MDD
Cp-Venous Return-Granisetron AP10.3 PBPK oral 2mg MDD
Cp-Venous Return-Granisetron AP10.3 PBPK oral 2mg MDD Obs
Cp-Venous Return-Granisetron AP10.3 PBPK oral 2mg MDD Err
Cmax
Cmin
5.378
0.655
Concentration (ng/mL)
Simulation Time (h)

D)

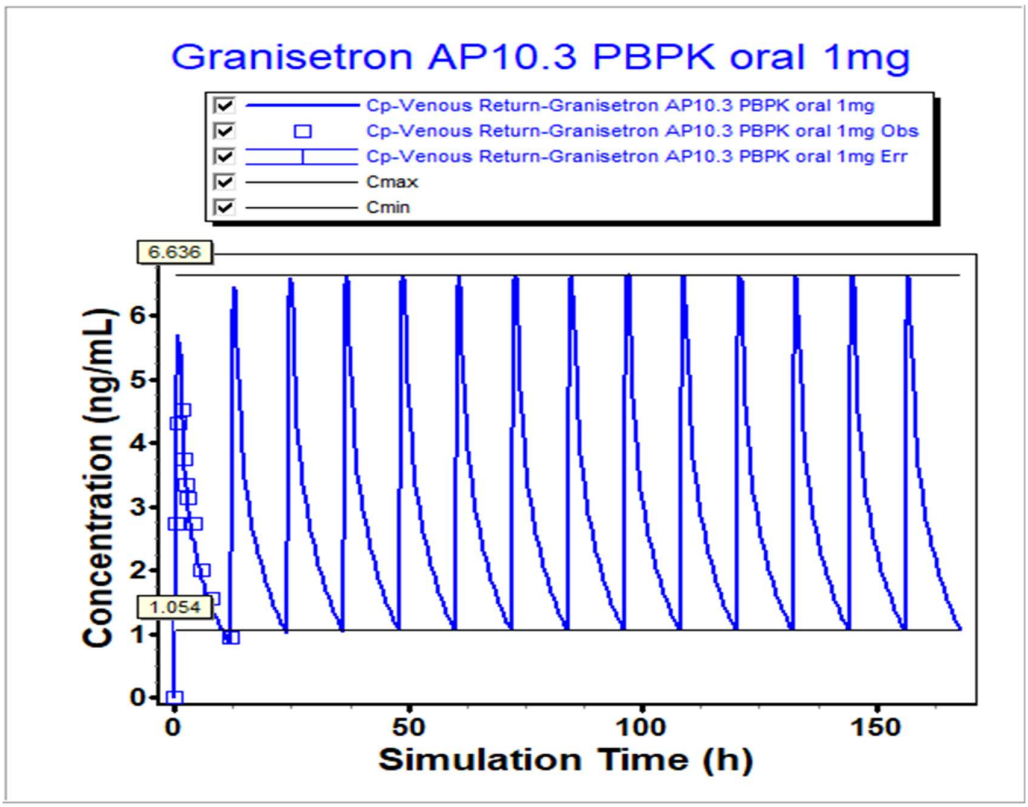


Figure 3-9: Multiple dose simulations for A) Amisulpride oral 5 mg (Q24hr) B) Amisulpride oral 10 mg (Q24hr) C) Granisetron oral 2 mg (Q24hr) D) Granisetron oral 1 mg (Q12hr)

For Amisulpride, the Cminss and Cmaxss levels are 0.47 ng/ml and 5.79 ng/ml after 5 mg (once a day) multiple dosing. The Cminss and Cmaxss levels are 0.941 ng/ml and 11.591 ng/ml after 10 mg (once a day) multiple dosing. In the reported clinical trial, 10 mg dose is selected, hence we have finalized 1ng/ml as target Cminss level and 11 ng/ml as target Cmaxss level for development of sustained release formulations of amisulpride.

For Granisetron, the Cminss and Cmaxss levels are 0.655 ng/ml and 5.378 ng/ml after 1 mg (twice a day) multiple dosing. The Cminss and Cmaxss levels are 1.054 ng/ml and 6.636 ng/ml after 2 mg (once a day) multiple dosing. In the package insert data of Granisetron patch (Sancuso 52 cm^2), Cavass level of 2.2 ng/mL over six days is reported [36].

Considering all the data, we have finalized 1ng/ml as target Cminss level and 6.5 ng/ml as target Cmaxss level for development of sustained release formulations of Granisetron.

3.6 Development of dissolution model for target dissolution profile:

The theoretical dissolution profile was described by two models. First model [39] is relatively simple which follows first order model as per equation 3-9:

$\log M_t = logM_0 - 0.43k_1t$ ----------(3-9)

Where M0 is initial amount of drug ,

Mt is residual amount of drug at time t,

k1 is first order constant

Second model [40] is complex, where mass of drug at time t (M_t) is calculated by following equation 3-10.

$$Mt = 4\pi r^2 \left(\sqrt{2(C_0 - C_S) * C_S D(t) t} + \frac{4C_S D(t) t}{9r} \left(\frac{C_S}{2C_0 - C_S} - 3 \right) \right)$$ ----------(3-10)

Where Mt is drug mass released at time t ,

r is particle radius,

C0 is initial conc. of drug,

Cs is drug solubility,

Dt is diffusion coefficient at time t in $\frac{\text{cm2}}{\text{s}}$,

t is time in seconds

This equation is used to calculate dissolution profile in DDDplus software.

Both the models were used to predict the desired drug release profile.

As per equation 3-9, the calculated drug release is presented in table 3-11

Table 3-11 Calculated Drug release as per model 1 (Equation 3-9)

Drug	Amisulpride (30 mg)		Granisetron (10 mg)	
Time (hours)	Drug released in mg	% Cumulative Drug release	Drug released in mg	% Cumulative Drug release
0	0	0	0	0
1	3.00	10	0.10	1
4	3.90	13	0.43	4
12	6.47	22	1.37	14
24	12.15	40	3.45	35
48	18.20	61	5.67	57
72	24.85	83	8.11	81
96	27.75	92	9.18	92
120	27.75	92	9.18	92
144	29.02	97	9.64	96
168	29.57	99	9.84	98

As per equation 3-10, the calculated drug release is presented in table 3-12.

Table 3-12 Calculated Drug release as per model 2 (Equation 3-10)

Time (hours)	% Cumulative Drug release for Amisulpride	% Cumulative Drug release for Granisetron
0	0	0
1	10	1
4	13	4
12	21	14
24	39	30
48	63	57
72	84	75
96	89	86
120	91	92
144	94	95
168	97	97

3.7 Selection of desired dose:

The estimation of desired dose was defined based on calculated pharmacokinetic constants (VD and CL), dosage regimens, pharmacokinetic data and clinical trials of marketed products. The details of marketed formulations are given in table 3-13.

Table 3-13 Marketed formulations of Amisulpride and Granisetron [41]

Marketed Formulations	**Strengths**	**Dosing regimen**
Drug: Amisulpride		
IV Injection	5mg/2ml, 10 mg/2ml	Once a day
Oral tablets	400 mg	Once to thrice a day as per requirement (For psychosis)
Clinical trial Formulation	10-40 mg	20 mg IV on day 1 and 10-40 mg oral on days 2-4 (For CINV)
Drug: Granisetron		
IV Injection	0.1mg/mL, 1mg/mL, 3mg/mL, 4mg/mL	30 min before chemotherapy and as and when required
Oral solution	2mg/10mL	Once a day
Oral Tablet	1mg 2 mg	1 mg twice day 2 mg once a day
SC ER Injection	10mg/0.4ml	30 min before chemotherapy. Every 7 days
Transdermal patch	3.1mg/24hr	24 hr to 48 hr to 7 days

After considering the reported literature, pharmacokinetic parameters and calculated steady state plasma concentration levels, the desired dose for Amisulpride was found to be 30 mg and for granisetron it was found to be 10 mg. For combination product, the granisetron dose to be kept 10 mg and amisulpride dose can be varied from 10-30 mg based on the clinical response.

3.8 References:

1. Dallmann A, Ince I, Coboeken K, Eissing T, Hempel G. A physiologically based pharmacokinetic model for pregnant women to predict the pharmacokinetics of drugs metabolized via several enzymatic pathways. Clinical pharmacokinetics. 2018 Jun;57:749-68.
2. https://go.drugbank.com/drugs/DB06288
3. https://go.drugbank.com/drugs/DB00889
4. Darakjian LI. Evaluation of the effect of metabolism and transporters on drug pharmacokinetics and pharmacodynamics using PBPK modeling (Doctoral dissertation, Auburn University).
5. https://www.accessdata.fda.gov/drugsatfda_docs/nda/2020/209510Orig1s000MultidisciplineR.pdf
6. https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm
7. Spartinou A, Nyktari V, Papaioannou A. Granisetron: a review of pharmacokinetics and clinical experience in chemotherapy induced-nausea and vomiting. Expert opinion on drug metabolism & toxicology. 2017 Dec 2;13(12):1289-97.
8. Clarke SE, Austin NE, Bloomer JC, Haddock RE, Higham FC, Hollis FJ, Nash M, Shardlow PC, Tasker TC, Woods FR, Allen GD. Metabolism and disposition of 14C-granisetron in rat, dog and man after intravenous and oral dosing. Xenobiotica. 1994 Jan 1;24(11):1119-31.
9. Caritis S, Zhao Y, Chen HJ, Venkataramanan R. Pharmacodynamics of transdermal granisetron in women with nausea and vomiting of pregnancy. American Journal of Obstetrics and Gynecology. 2016 Jul 1;215(1):93-e1.
10. Gurpide A, Sadaba B, Martin-Algarra S, Azanza JR, Lopez-Picazo JM, Campanero MA, Cabello JP, Gil-Aldea I, De La Cruz S, Gallego VF, Reyna C. Randomized crossover pharmacokinetic evaluation of subcutaneous versus intravenous granisetron in cancer patients treated with platinum-based chemotherapy. The Oncologist. 2007 Sep 1;12(9):1151-5.
11. Mason JW, Moon TE, O'Boyle E, Dietz A. A randomized, placebo-controlled, four-period crossover, definitive QT study of the effects of APF530 exposure, high-dose intravenous granisetron, and moxifloxacin on QTc prolongation. Cancer Management and Research. 2014 Mar 26:181-90.
12. https://www.accessdata.fda.gov/drugsatfda_docs/nda/2016/022445Orig1s000ClinPharmR.pdf

13. Zhou Y, Jiang J, Hu P, Wang H. A high-performance liquid chromatography–tandem mass spectrometry method coupled with protein precipitation for determination of granisetron in human plasma and its application to a comparative pharmacokinetic study. Biomedical Chromatography. 2014 Dec;28(12):1597-600.
14. Huang CT, Chen CF, Tsai TH. Pharmacokinetics of granisetron in rat blood and brain by microdialysis. Life sciences. 1999 Apr 16;64(21):1921-31.
15. https://www.accessdata.fda.gov/drugsatfda_docs/nda/2008/022198s000_ClinPharmR.pdf
16. Fox GM, Roffel AF, Hartstra J, Bussian LA, van Marle SP. Metabolism and excretion of intravenous, radio-labeled amisulpride in healthy, adult volunteers. Clinical Pharmacology: Advances and Applications. 2019 Dec 2:161-9.
17. Noh K, Jang YJ, Kwon KI, Kim E, Jeong TC, Yun HY, Kang W. Quantitative determination of amisulpride in rat plasma by HPLC–MS/MS. Archives of pharmacal research. 2015 Jan;38:63-7.
18. Rosenzweig P, Canal M, Patat A, Bergougnan L, Zieleniuk I, Bianchetti G. A review of the pharmacokinetics, tolerability and pharmacodynamics of amisulpride in healthy volunteers. Human Psychopharmacology: Clinical and Experimental. 2002 Jan;17(1):1-3.
19. Jairam RK, Mallurwar SR, Sulochana SP, Chandrasekhar DV, Todmal U, Bhamidipati RK, Richter W, Srinivas NR, Mullangi R. Prediction of Human Pharmacokinetics of Fomepizole from Preclinical Species Pharmacokinetics Based on Normalizing Time Course Profiles. AAPS PharmSciTech. 2019 Jun 18;20(6):221.
20. Ring BJ, Chien JY, Adkison KK, Jones HM, Rowland M, Jones RD, Yates JW, Ku MS, Gibson CR, He H, Vuppugalla R, Marathe P, Fischer V, Dutta S, Sinha VK, Björnsson T, Lavé T, Poulin P. PhRMA CPCDC initiative on predictive models of human pharmacokinetics, part 3: comparative assessement of prediction methods of human clearance. J Pharm Sci. 2011 Oct;100(10):4090-110.
21. Jones RD, Jones HM, Rowland M, Gibson CR, Yates JW, Chien JY, Ring BJ, Adkison KK, Ku MS, He H, Vuppugalla R, Marathe P, Fischer V, Dutta S, Sinha VK, Björnsson T, Lavé T, Poulin P. PhRMA CPCDC initiative on predictive models of human pharmacokinetics, part 2: comparative assessment of prediction methods of human volume of distribution. J Pharm Sci. 2011 Oct;100(10):4074-89.
22. Wajima T, Yano Y, Fukumura K, Oguma T. Prediction of human pharmacokinetic profile in animal scale up based on normalizing time course profiles. Journal of pharmaceutical sciences. 2004 Jul 1;93(7):1890-900.
23. https://www.accessdata.fda.gov/drugsatfda_docs/label/2022/209510s005lbl.pdf

24. https://www.medicines.org.uk/emc/product/3965/smpc#gref
25. Allen A, Asgill CC, Pierce DM, Upward J, Zussman BD. Pharmacokinetics and tolerability of ascending intravenous doses of granisetron, a novel 5-HT 3 antagonist, in healthy human subjects. European journal of clinical pharmacology. 1994 Mar;46:159-62.
26. Cassidy J, Raina V, Lewis C, Adams L, Soukop M, Rapeport WG, Zussman BD, Rankin EM, Kaye SB. Pharmacokinetics and anti-emetic efficacy of BRL43694, a new selective 5HT-3 antagonist. British journal of cancer. 1988 Nov;58(5):651-3.
27. Wada I, Takeda T, Sato M, SAITOH H, NAKABAYASHI T, MINO K, HONMA T, TAKADA M, HIRANO K. Pharmacokinetics of granisetron in adults and children with malignant diseases. Biological and Pharmaceutical Bulletin. 2001;24(4):432-5.
28. Corrigan BW, Nicholls B, Thakrar B, Lam R, Grosse C, Alianti J, Palmer JL. Heterogeneity in systemic availability of ondansetron and granisetron following oral administration. Drug metabolism and disposition. 1999 Jan 1;27(1):110-2.
29. Puttrevu SK, Arora S, Polak S, Patel NK. Physiologically based pharmacokinetic modeling of transdermal selegiline and its metabolites for the evaluation of disposition differences between healthy and special populations. Pharmaceutics. 2020 Sep 30;12(10):942.
30. Frymoyer A, Hersh AL, El-Komy MH, Gaskari S, Su F, Drover DR, Van Meurs K. Association between vancomycin trough concentration and area under the concentration-time curve in neonates. Antimicrobial agents and chemotherapy. 2014 Nov;58(11):6454-61.
31. Tan Z, Zhang Y, Wang C, Sun L. Physiologically based pharmacokinetic modeling of cefadroxil in mouse, rat, and human to predict concentration–time profile at infected tissue. Frontiers in pharmacology. 2021 Dec 23;12:692741.
32. Statelova M, Holm R, Fotaki N, Reppas C, Vertzoni M. Successful extrapolation of paracetamol exposure from adults to infants after oral administration of a pediatric aqueous suspension is highly dependent on the study dosing conditions. The AAPS Journal. 2020 Nov;22:1-7.
33. Rasool MF, Ali S, Khalid S, Khalid R, Majeed A, Imran I, Saeed H, Usman M, Ali M, Alali AS, AlAsmari AF. Development and evaluation of physiologically based pharmacokinetic drug-disease models for predicting captopril pharmacokinetics in chronic diseases. Scientific Reports. 2021 Apr 21;11(1):8589.
34. Li X, Chen C, Ding N, Zhang T, Zheng P, Yang M. Physiologically based pharmacokinetic modelling and simulation to predict the plasma concentration profile of schaftoside after oral administration of total flavonoids of Desmodium styracifolium. Frontiers in Pharmacology. 2022 Dec 14;13:1073535.

35. Ke C, You X, Lin C, Chen J, Guo G, Wu W, Ye L, Huang P. Development of physiologically based pharmacokinetic model for pregabalin to predict the pharmacokinetics in pediatric patients with renal impairment and adjust dosage regimens: PBPK model of pregabalin in pediatric patients with renal impairment. Journal of pharmaceutical sciences. 2022 Feb 1;111(2):542-51.
36. Mason JW, Moon TE. Use and cardiovascular safety of transdermal and other granisetron preparations in cancer management. Cancer Management and Research. 2013 Jul 29:179-85.
37. https://www.accessdata.fda.gov/drugsatfda_docs/label/2009/020239s021,020305s014,021238s007lbl.pdf
38. Herrstedt J, Summers Y, Daugaard G, Christensen TB, Holmskov K, Taylor PD, Fox GM, Molassiotis A (2018) Amisulpride in the prevention of nausea and vomiting induced by cisplatin-based chemotherapy: a dose-escalation study. Support Care Cancer 26:139– 145
39. Pinheiro de Souza F, Sonego Zimmermann E, Tafet Carminato Silva R, Novaes Borges L, Villa Nova M, Miriam de Souza Lima M, Diniz A. Model-Informed drug development of gastroretentive release systems for sildenafil citrate. Eur J Pharm Biopharm. 2023 Jan;182:81-91.
40. https://www.simulations-plus.com/software/dddplus/
https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm