Synopsis of the Ph.D. thesis entitled

"FORMULATION, IN VITRO-IN SILICO EVALUATION AND DEVELOPMENT OF PHARMACOKINETIC MODELS FOR SUSTAINED RELEASE FORMULATIONS OF ANTIEMETIC DRUGS"

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By

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1. Introduction:

1.1 Chemotherapy-induced nausea and vomiting (CINV):

Chemotherapy-induced nausea and vomiting (CINV) can be detrimental to the quality of life of cancer patients.[1, 2] CINV occurs in two phases; acute phase and delayed phase. Acute CINV occurs within 1–2 h of chemotherapy administration and can last for up to 24 h while delayed CINV occurs more than 24 h after chemotherapy administration.

Current prophylaxis for delayed CINV include prescribing a NK-1 receptor antagonist (Aprepitant, Fosaprepitant, Casopitant, Rolapitant etc) along with a 5-HT3 receptor antagonists (Granisetron, Ondansetron, Dolasetron etc) and corticosteroids such as dexamethasone for prevention of CINV in patients receiving highly emetogenic chemotherapy (HEC), and a 5-HT3 receptor antagonist and dexamethasone in patients receiving moderately emetogenic chemotherapy (MEC). [3,4,5]

However, differences in pharmacokinetics and pharmacodynamics between the available antiemetics can affect their efficacy in different clinical situations. Drugs or formulations with long duration of action and a good safety profile will play important role in such situations. This is important for effective prevention of CINV and simplifying management, especially in patients with comorbidities who are receiving multiple therapies or patients who are older and/or have cognitive impairment.[6]

A new formulation (APF530) of granisetron (5-HT3) receptor antagonist, has been developed as an alternative long-acting agent for treatment of both acute and delayed CINV under the brand name SUSTOL. APF530 utilizes Biochronomer™ technology to formulate a viscous tri (ethylene glycol) poly (orthoester)-based formulation that delivers by single subcutaneous (SC) injection therapeutic granisetron concentrations over 5 days.[7] However, this product is recommended to be administered in combination with other antiemetics and is only for moderately emetogenic chemotherapy.

Another formulation of granisetron in the form of transdermal patch under brand name SANCUSO has been approved. This product is recommended for the prevention of nausea and vomiting in adults receiving moderately and/or highly emetogenic chemotherapy.[8] However, SANCUSO suffers from delayed Tmax effect and has not been effective in treating acute emesis phase.

FDA has approved Amisulpride intravenous injection (5mg/10mg) for treatment of post-operative nausea and vomiting in Feb 2020.[9] Amisulpride is available in Europe and other markets for treatment of psychosis and schizophrenia at high doses (50-1200 mg).[10,11] Recently

clinical trials also have been conducted for use of amisulpride in CINV.[12] However, there is no long acting formulation available for Amisulpride.

Currently these two are the only long acting formulations available in the market to treat both acute and delayed CINV. Hence there is a need and scope in design of sustained release formulations of antiemetic drugs which can deliver the drug for longer duration i.e. over a weeks' time.

1.2 Long acting Formulations (LAFs):

Long-acting formulations (LAFs) are used for pharmacotherapy as sustained-release medications over a period of several days, weeks, or even months. Compared to conventional preparations, LAFs have many distinguished advantages related to its long-lasting curative effect, as well as its reduced toxicity, dosage and frequency of administration.

The principles to produce long-acting therapeutics are based on maintaining the drug activity for longer periods of time and to improve their tolerance in the body via slow and controlled release, delayed clearance, resistance to enzymes, increased stability, extended half-life. [13, 14]

One of the strategies to manipulate the drug release from delivery systems include micro-encapsulation in which solid or liquid drug substances are dispersed or dissolved in polymeric materials to form microspheres, or as a core surrounded by a polymeric shell to form a microcapsule. [15, 16] The polymers used to produce microspheres include natural (*e.g.*, gelatin, alginate chitosan, etc.), seminatural [*e.g.*, cellulose acetate phthalate (CAP), ethyl cellulose (EC), methyl cellulose (MC), etc.] and synthetic materials [*e.g.*, polylactic acid (PLA), PLGA, etc.]. Among these materials, PLGA is the most popularly used biodegradable material to prepare microspheres, accounting for 46% of all markets. The process of drug release can be adjusted by controlling the PLGA molecular weight, the ratio of drug to polymer, the size of microspheres, the ratio of glycolic acid to lactic acid, the end terminus of polymer and the properties of excipients.[17,18] The major mechanism of drug release from biodegradable microspheres includes diffusion, dissolution as well as polymer erosion and degradation. The possible mechanisms of drug release are following five pathways.[19] The first is initial release from the surface of microspheres; the second is release through the pores in microspheres; the third is diffusion through the intact polymer barrier; the fourth is diffusion through a water swollen barrier; the last is polymer erosion and degradation. All above mechanisms together play a part to achieve sustained drug release.[20,21]

Janus particles are named after the two-faced Roman god Janus because they exhibit distinct properties on different sides. These particles consist of at least two distinct materials or

compartments, each with unique properties (e.g., chemical composition, surface charge, or functionality). The combination of these distinct properties within a single particle allows for versatile applications. Janus particles have been used for dual drug delivery. By incorporating two different drugs into separate compartments, they enable synergistic effects and reduce side effects.[22] Janus particles can carry therapeutic agents (e.g., drugs) and imaging or sensing modalities (e.g., contrast agents). This spatially controlled incorporation allows for combined therapies not achievable with isotropic systems.[23] Due to their asymmetric structure, Janus particles can target specific cells or tissues more effectively. For instance, one side may be functionalized for cellular targeting while the other side carries therapeutic cargo.

In summary, Janus particles offer exciting opportunities for drug delivery, imaging, and personalized medicine. Their unique design allows for tailored approaches to address complex biomedical challenges.

1.3 In-silico modeling:

Cost- and time-effective formulation design can be achieved by adapting new tools such as Quality by Design and *in-silico* modeling & simulation. Model informed drug development utilize quantitative models in formulation development to facilitate decision making processes. First one is Physiologically-based pharmacokinetic modeling (PBPK) as a tool to mechanistically interpret and predict absorption, distribution, metabolism and excretion (ADME) properties of drugs in the human body[24] and second is Physiologically-based biopharmaceutics modeling (PBBM) which establish link between *in-vitro* data and *in-vivo* performance.[25] In both the models, the data obtained from preclinical and clinical studies are used to predict the *in-vivo* performance of formulation and understand the underlying physiological processes. Developing such models will help in robust product development and maximize *in-vivo* success.

Ideally, to assess drug product clinical performance following formulation and manufacturing changes, biopharmaceutics models that capture the interactions between the physiology (i.e., by using physiologically based models) and the pharmaceutical formulation by mechanistic implementation of formulation/manufacturing aspects that are relevant to dissolution/release from the drug product are critical. Such models consider factors beyond physiological and pharmacokinetic (i.e. ADME) components. They should define mechanistic elements of drug dissolution/release relevant to interactions of the pharmaceutical product with physiological conditions and events which can be parameterized to describe the key formulation

characteristics. Once these mechanistic elements are defined, PBBM modelling can be used to predict the impact of variations in the critical material attributes (CMAs) and critical process parameters (CPPs) through the establishment of a safe space via either IVIVCs or *in-vitro in - vivo* relationships (IVIVRs) combined with virtual BE simulations. This approach will facilitate the incorporation of clinical relevance in product quality from initial development through marketing approval to lifecycle management and thereby minimize the need to conduct additional *in-vivo* BE studies, leading to reducing cost in product development and supporting regulatory decisions.

Problem statement:

Prevention and control of nausea and vomiting (N&V) are paramount in the treatment of patients with cancer. Chemotherapy-induced N&V is one of the most common and distressing acute side effects of cancer treatment. It occurs in up to 80% of patients and can have a significant impact on a patient's quality of life. [26] N&V can also result in serious metabolic disturbances, nutritional depletion and anorexia, deterioration of the patient's physical and mental status, Esophageal damage and ultimately can result in patient withdrawal from potentially useful and curative antineoplastic treatment.[26]

The treatment options which address both acute and delayed phases of emesis occurring during chemotherapy are very limited and less effective. The marketed formulations available are SUSTOL (Granisetron SC Injection) and SACUSO (Granisetron transdermal patch) for once a weekly treatment. Currently no generic is available for both the drugs due to patent protection and proprietary polymer technology. However, these individual formulations still require combination with other antiemetics for effective treatment.

The available anti-emetic dosage regimen recommends combination of two or three antiemetic agents to control the delayed emesis in moderate and high emetogenic chemotherapy treatment.

There is need of formulations which provide effective treatment and patient compliance. This can be achieved by designing long acting dosage forms with combination of two drugs, which provide sustained drug release up to one week.

Comparative *in-vivo* studies in human are required for approval of generic or branded product which are costly and time consuming. Prediction of pharmacokinetics in human from *in-vitro* and *in-silico* studies is facilitated by regulatory agencies through modelling and simulation approach to reduce cost and create platform for future research.

Currently there is a need of *in-silico* pharmacokinetic models which can mechanistically link *in-vitro* and pharmacokinetic properties to predict *in-vivo* performance of antiemetic drugs.

Choice of drugs:

Clinical trials suggest that granisetron it is more effective than other 5-HT3 antagonists in preventing delayed nausea and vomiting that occur more than 24 h after the first dose of chemotherapy. Its main effect is to reduce the activity of the vagus nerve which activates the vomiting centre in the medulla oblongata.

Amisulpride is an antagonist of dopamine D2 and D3 receptors, approved since the 1980s as treatment for psychosis, with a favourable safety profile, even when used at doses of 400– 800 mg/day[27]. In a pilot study, a combination of ondansetron and a single 20 mg intravenous (i.v.) dose of amisulpride protected 83% of patients from vomiting and use of rescue medication in the acute phase following cisplatin chemotherapy[28]. Amisulpride has also been shown to be effective at preventing post-operative nausea and vomiting [29,30]. In a randomized, double-blind trial, oral amisulpride at a dose of 10 mg daily was found to be safe and superior to placebo in preventing delayed nausea and vomiting associated with highly emetogenic chemotherapy[37]. The complete response (CR) rate (defined as no emesis or rescue medication use) in the delayed phase was 46% with 10 mg amisulpride, compared to 20% with placebo. [31]

2. Aim:

The aim was to design and develop once-weekly long acting formulations and pharmacokinetic models using *in-vitro in-silico* tools.

3. Objectives:

The objectives of present work were:

- ✓ To develop *in-silico* pharmacokinetic model which will guide in the design and development of sustained release formulations and predict *in-vivo* performance.
- ✓ Development of formulations which will provide sustained drug release over a period of one week.
- ✓ To predict the *in-vivo* pharmacokinetics in humans using *in-vitro* data using the developed *in-silico* model.

Plan of work:

A) Design stage: Development of *in-silico* models

1. Development and verification of pharmacokinetic models
2. Identification of target plasma concentration using dissolution and PBPK model
3. Development of dissolution model for target dissolution profile
4. Calculation of desired dose

B) Development Stage: Analytical method and formulation development

1. Pre-formulation
2. Analytical method development
3. Formulation development

C) Evaluation Stage: *In-vitro* and *in-silico* evaluation

1. *In-vitro* characterization studies
2. Prediction of *in-vivo* pharmacokinetics using the developed PBPK model
3. Virtual bioequivalence studies
4. Predictive IVIVC for establishment of design space

4. Design stage - Development of *in-silico* models:

4.1 Development and verification of pharmacokinetic models:

4.1.1 Collection of data from literature:

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

Physiochemical properties[32, 33]:

Physicochemical properties such as compound lipophilicity, solubility, molecular weight (MW), and pKa values of a drug are fully independent of organism physiology. But these properties were utilized in calculation of tissue partition coefficients in the model.

Biological Properties[32, 33]:

Drug-biological properties (such as fraction of drug unbound, or tissue-plasma partition coefficient), are drug-specific but also defined by the interaction between the drug and the biological system itself, so they are dependent on both the drug and the organism properties.

Marketed Formulations[34]:

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

***In-vivo* pharmacokinetic data** [35]:

In-vivo pharmacokinetic data collected from literature domain for different species and populations.

4.1.2 Allometric scaling, Wajima and Dedrick approach:

4.1.2.1 Allometric methods:

Allometric scaling is an empirical approach developed based on cross species similarities in anatomy, physiology, and biochemistry with a power function correlating physiological parameters with body weight. This method has been applied to the projection of human pharmacokinetics for small-molecule drugs as well as therapeutic proteins and is widely used in the pharmaceutical industry for early decision making at several stages in drug discovery and development.[36] Using allometric scaling, key pharmacokinetic parameters such as volume of distribution and clearance are calculated.

4.1.2.1.1 Prediction of Human clearance:

There are different methods reported for calculating clearance using interspecies scaling.[37] For convenience these methods are summarized in Table 1 and results in table 2. The results of best suited method are presented here. For amisulpride, methods 1-4 and for granisetron, methods 2-5 provided good prediction of human clearance respectively.

Table 1 Methods for calculation of clearance

Method	Method Name	Description	Requirement	Formula
M1	Simple allometry (SA)	CL process is proportional to the power of body weight	CL and body weight in at least two animal species,	$CL = a(BW)^b$ where a and b are the coefficient and exponent of the allometric equation and BW is body weight
M2	Allometric exponent based			CL = CL of species X (70/BW of species) ^b b= 0.75,0.80,.85,0.9
M3	Multiexponential allometry (MA)	CL process is proportional to the power of body weight	CL and body weight in at least two animal species	$CL = a(BW)^b + \frac{[1-(\frac{3}{2})b]}{[1-(\frac{1}{2})b]} X\ a(BW)^{0.9}$ where a and b are the coefficient and exponent of the allometric equation and BW is body weight
M4	Liver blood-flow method	Animal–human correction for LBF; made for rat, dog, monkey and human	Human and animal liver blood flow, animal CL	CL (human) = CL (animal) × (human/animal) Qliver
M5	Rat	Rat–human proportionality for bound drug	CL in at least one species of rat, dog, or monkey.	CL/kg (human) = CL/kg (rat) × 0.152
	Dog	Dog–human proportionality for bound drug		CL/kg (human) = CL/kg (dog) × 0.410
	Monkey	Monkey–human proportionality for bound drug		CL/kg (human) = CL/kg (monkey) × 0.407

Table 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	0.732	1.2
	2	0.62	0.68	1.1
	3	0.62	0.626	1.0
	4	0.62	0.4486	1.4
	5	0.62	0.18	3.4
Granisetron	1	0.325	2.199	6.8
	2	0.325	0.2943	1.1
	3	0.325	0.2584	1.3
	4	0.325	0.4487	1.4
	5	0.325	0.18	1.8

4.1.2.1.2 Prediction of Human volume of distribution:

There are different methods reported for calculating volume of distribution using interspecies scaling.[38] For convenience these methods are summarized in Table 3 and results in table 4. The results of best suited method are presented here. For amisulpride, all three methods and for granisetron, methods 1-2 provided good prediction of human volume of distribution respectively.

Table 3 Methods for calculation of volume of distribution

Method	Method Name	Description	Requirement	Formula
M1	Simple allometry (SA)	Distribution process is proportional to the power of BW.	Vd and body weight in at least two animal species,	$V_{ss} = a\ (BW)^b$ where a and b are the coefficient and exponent of the allometric equation and BW is body weight
M2	Single species scaling		The proportionality expressed by a linear relationship between human data and the corresponding animal data with or without a correction based on the free fraction in plasma in the single-species scaling (SSS) approaches was used	*VD (human = a * VD (animal)* a = 0.59, 0.72, and 0.79 for rat, dog, and monkey
M3	Two species rat and dog		Two species model. Rat–dog–human proportionality for bound drug.	log Vss (human) = (0.07714 log Vss (rat) × log Vss (dog)) + 0.5147 log Vss (dog) + 0.586

Table 4 Calculated volume of distribution for human

Drug	Method	Observed Vd	Calculated Vd	Fold
Amisulpride	1	3.7	3.25	1.1
	2	3.7	2.66	1.4
	3	3.7	3.95	1.1
Granisetron	1	2.2	3.14	1.4
	2	2.2	3.764	1.7
	3	2.2	5.64	2.6

4.1.2.2 Prediction of Human plasma concentration-time profiles:

The prediction of human intravenous plasma concentration-time profiles was performed by 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.[39] Two to three species data are required to build the correlation and have sufficiently good prediction.

4.1.2.2.1 Wajima (Css-MRT) Approach:

In MRT-based normalization approach, the digitalized clinical and generated preclinical datasets were normalized by dividing the plasma concentration values by Css (= dose/Vss) and time profile by MRT (= Vss/CL) to arrive at the predictions.[39] The Wajima predicted plots are presented in Figure 1 (A and B). Good prediction was observed for both amisulpride and granisetron.

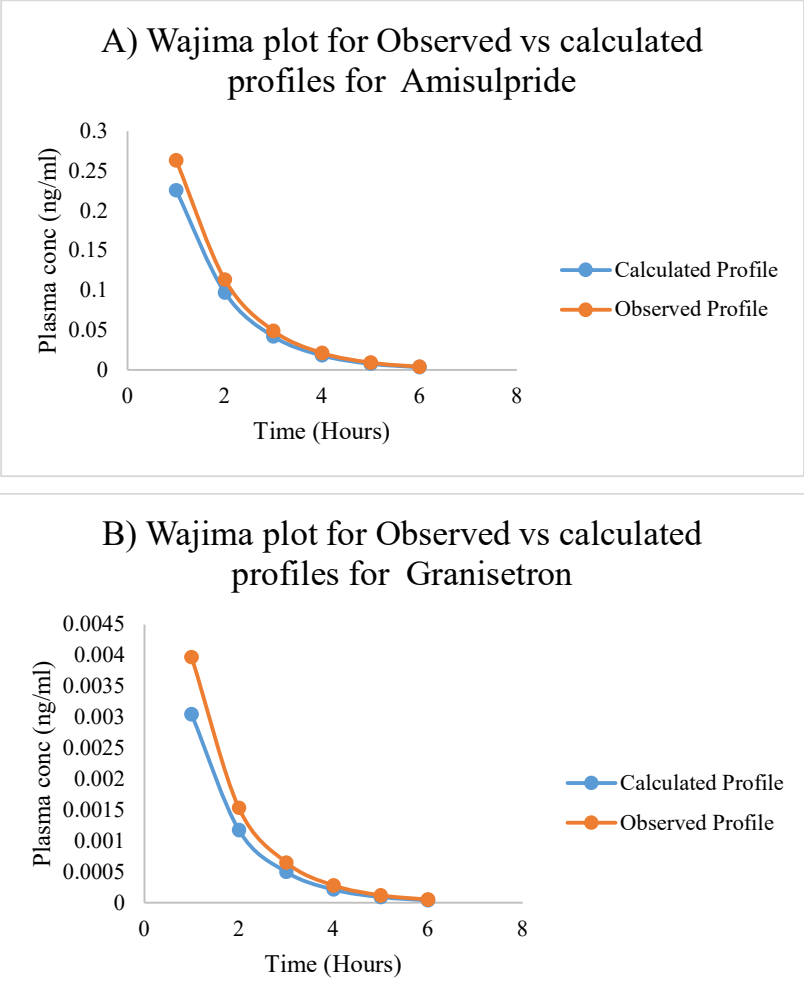

Figure 1: A) Wajima profile for Amisulpride B) Wajima profile for Granisetron

4.1.2.2.2 Dedrick plot:

It is simplest form of calculating the plasma concentration-time profile using following equations.[39]

$$Concentration = \frac{Plasma\ concentration}{Dose/Body\ weight}$$

$$Time = \frac{Time}{Body\ weight^{0.25}}$$

The calculated Dedrick plot for Amisulpride and Granisetron is presented in Figure 2 (A and B). Good correlation was observed for amisulpride, but for granisetron prediction error observed was more.

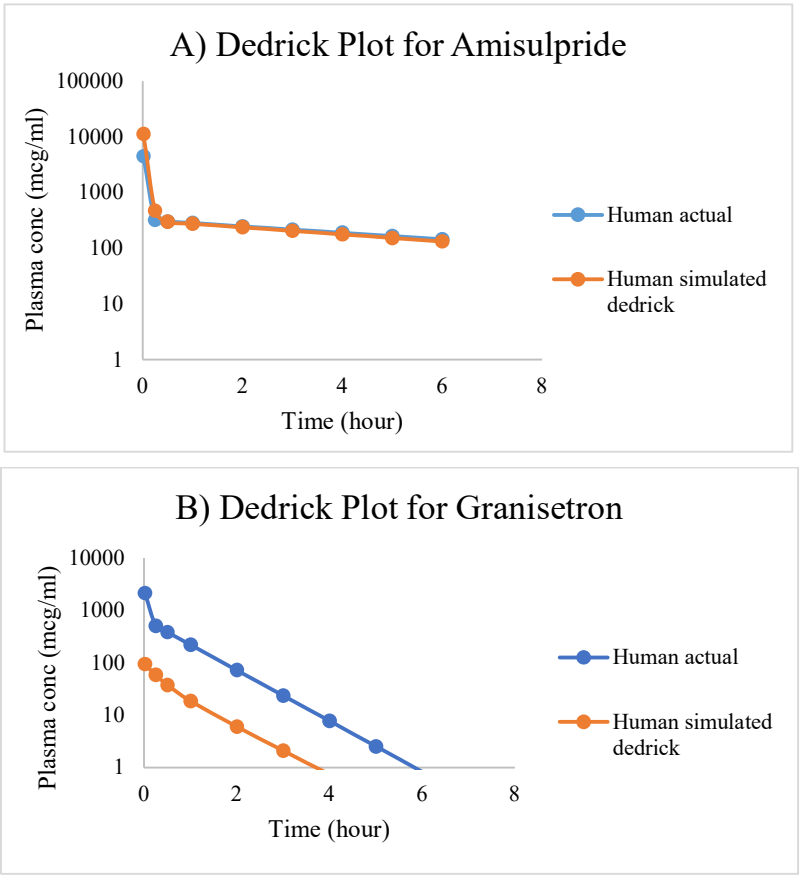

Figure 2: A) Dedrick plot for Amisulpride B) Dedrick plot for Granisetron

From the data analysis, it shows that, Wajima approach was more appropriate for calculation of pharmacokinetic profile in humans from preclinical data for these two molecules. This is in line with literature data, where Wajima approach was found more superior than the dedrick plot approach.

Development and verification of pharmacokinetic models

The modelling has been performed using GastroPlus® (version 9.8.2, Simulations Plus Inc., Lancaster, CA, USA) a commercially available PBPK software. Where demographic data was not reported in the source reference, the default GastroPlus® Population Estimates for Age Related (PEAR) settings have been used. 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. Where measured *in-vitro* data was not available to inform parameter estimates for essential physical chemical properties, ADMET® version 8.2 (Simulations Plus Inc., Lancaster, CA, USA) was used to predict these properties from structure. All tissues within the whole body PBPK model have been specified as permeability limited and tissue partitioning coefficients (Kp's) were calculated using the Lukacova and Rodgers method. The volume of distribution was calculated from physicochemical properties like log P, B/P ratio and % fraction unbound using Lukacova-Rodgers method. Hepatic clearance was calculated from the *in-vitro* Clint obtained from literature and renal clearance was calculated from GFR.

4.1.3 PBPK model of Amisulpride:

Following intravenous infusion, the mean volume of distribution of amisulpride is estimated to be 127 to 144 L in surgical patients and 171 to 218 L in healthy subjects. Amisulpride distributes into erythrocytes. Plasma protein binding is 25% to 30%. Amisulpride undergoes active renal secretion. The mean elimination half-life is approximately 4 to 5 hours and similar between healthy subjects and surgical patients. Population pharmacokinetic analysis estimated that the plasma clearance of amisulpride is 20.4 to 28 L/h in surgical patients and 24.1 to 43.5 L/h in healthy subjects.[40]

After oral administration, absolute bioavailability is 48%. Amisulpride is weakly metabolized in the liver. There is no accumulation of amisulpride and its pharmacokinetics remain unchanged after the administration of repeated doses. The elimination half-life of amisulpride is approximately 12 hours after an oral dose.[41]

The physicochemical properties, pharmacokinetic parameters and selected physiology for development of PBPK model of amisulpride is presented in table 5.[42]

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

Table 5 Amisulpride input data for PBPK modeling

No.	Attribute	Data
Compound tab		
1.	Name	Amisulpride
2.	Molecular Formula 3	C17H27N3O4S
3.	Molecular Weight 3	369.479
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

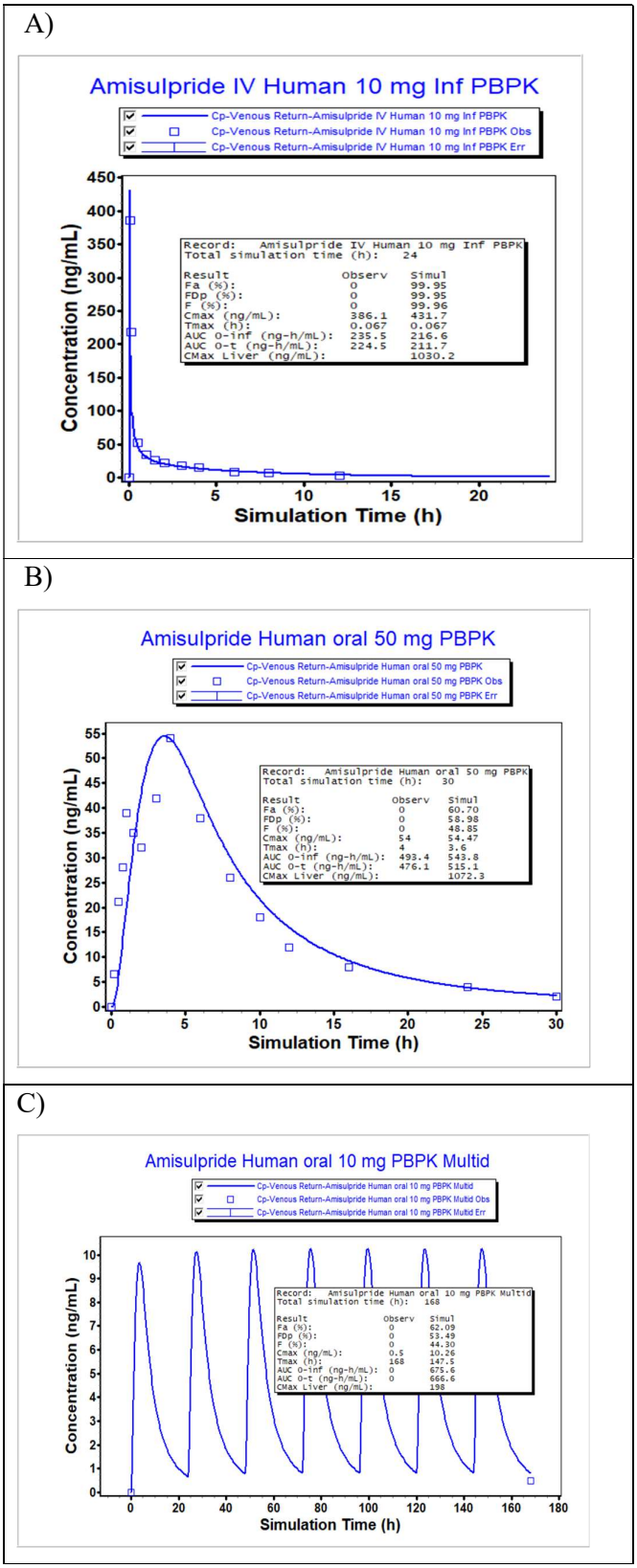

Figure 3: PBPK model of A) IV Amisulpride 10 mg B) Single dose oral Amisulpride 50 mg C) Multiple dose oral Amisulpride 10 mg

4.1.4 PBPK model of Granisetron:

All pharmacokinetic studies on granisetron showed a great intersubject variability of its pharmacokinetic parameters, regardless of formulation administered or population studied. In healthy volunteers after intravenous administration, plasma concentration of granisetron displays multiphasic decline, whereas it becomes log-linear after several hours. The rapid initial decline of plasma concentration implies extensive tissue uptake, which is also depicted on a relatively high apparent volume of distribution ranging from 2.4 to 3.5 L/Kg. Granisetron is known to be primarily metabolized in the liver and approximately 12% of granisetron is excreted unchanged in the urine. Clearance is mainly nonrenal and ranges between 37 and 49.9L/h (approximately 0.6 L/kg/h).[43] Terminal phase t1/2 ranges from 4.2 to 6.1 h.[44] Elderly patients (age>65 years) display a higher volume of distribution (approximately 4 vs. 3 L/kg) which probably results from an increased fat: lean mass ratio. Total plasma clearance is 45% lower, a result of age-related reduction in oxidative metabolism [0.17–1.06 (mean 0.44) L/kg/ h] and elimination t1/2 of granisetron is approximately 7.7 h. In adult patients receiving chemotherapy, granisetron clearance is decreased compared to healthy adults (0.376 L/kg/h). As a result, terminal phase elimination t1/2 was longer (9-12 h).[45,46] Absorption of granisetron after oral administration is complete but bioavailability is about 60% due to first pass metabolism. Granisetron is detectable in plasma after 1 h, and maximal mean concentration is reached 2 h after administration. Oral administration of the drug results in significant heterogeneity in systemic availability which appears to be related to smoking habits of healthy volunteers.[47]

The physicochemical properties, pharmacokinetic parameters and selected physiology for development of PBPK model of amisulpride is presented in table 6.[48]

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

Table 6 Granisetron input data for PBPK modeling

No.	Attribute	Data
Compound tab		
1.	Name	Granisetron
2.	Molecular Formula 3	C18H24N4O
3.	Molecular Weight 3	312.417
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

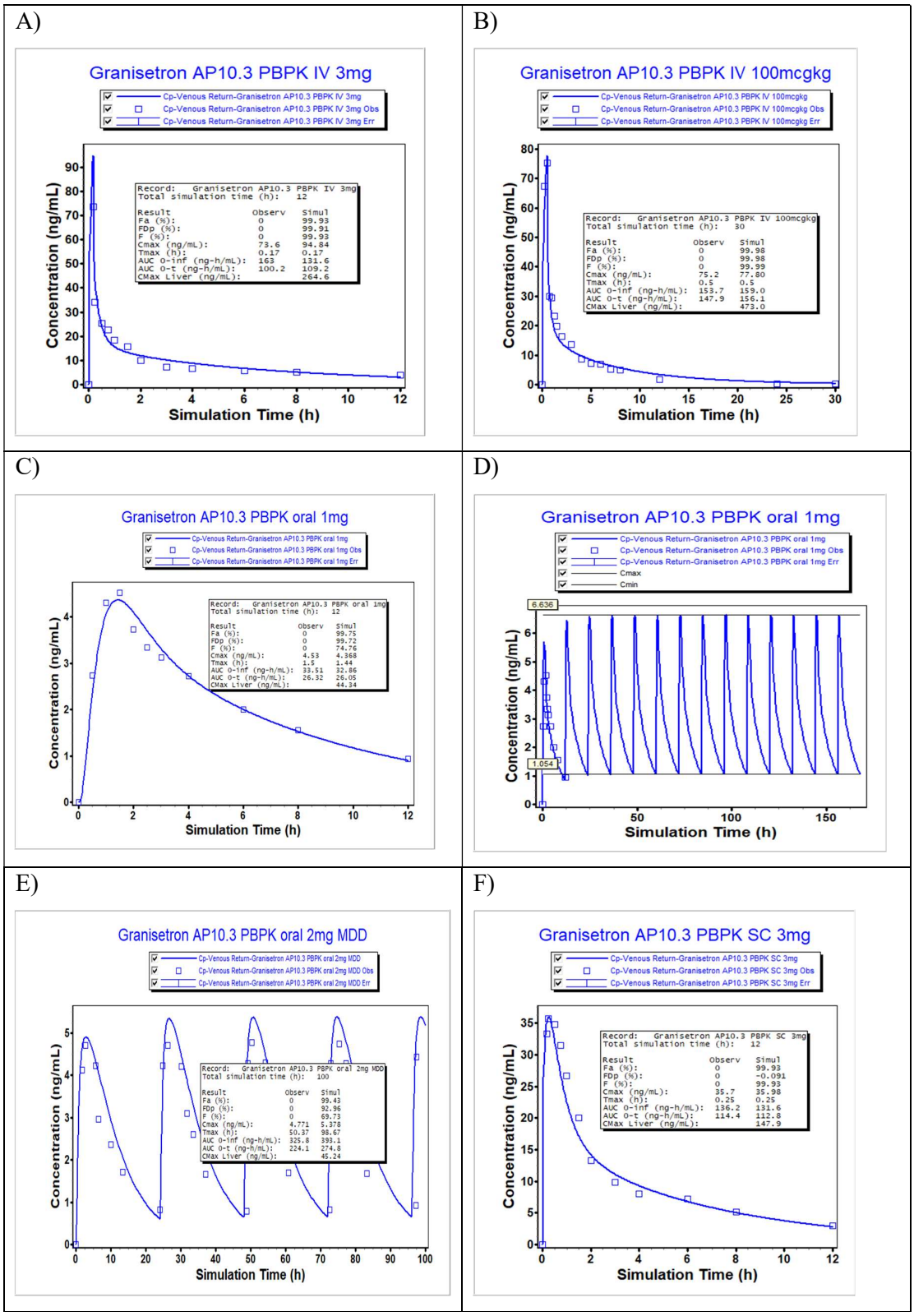

Figure 4: PBPK model of A) IV Granisetron 3 mg B) IV Granisetron 100 mcg/kg C) Single dose oral Granisetron 1 mg D) Multiple dose oral Granisetron 1 mg E) Multiple dose oral Granisetron 2 mg F) Subcutaneous (SC) Injection Granisetron 3 mg

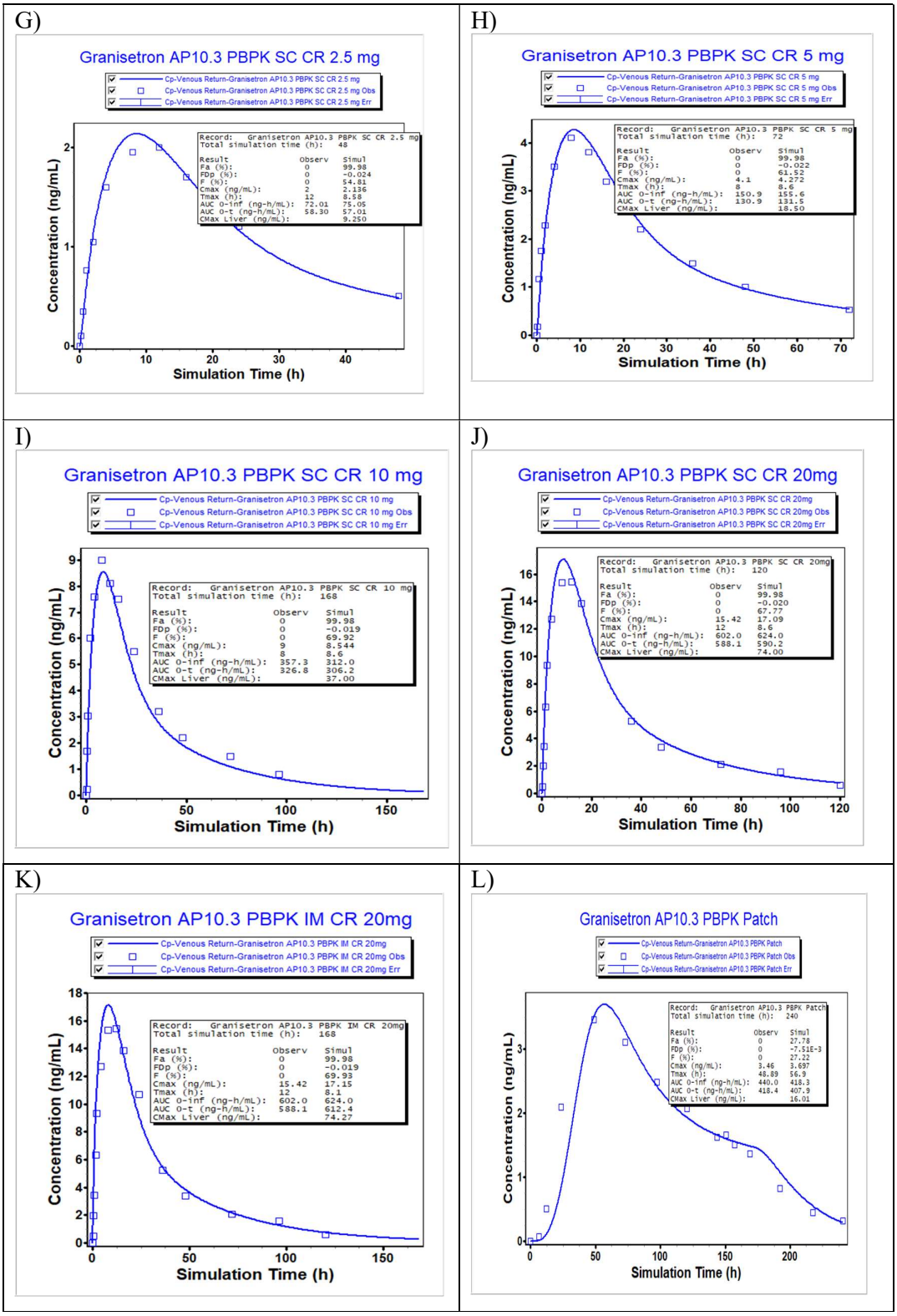

Figure 4: PBPK model of G) SC CR Granisetron 2.5 mg H) SC CR Granisetron 5 mg I) SC CR Granisetron 10 mg J) SC CR Granisetron 20 mg K) Intramuscular CR Granisetron 20 mg L) Transdermal patch Granisetron

4.2 Identification of target steady state plasma concentrations:

The target steady state plasma concentration was calculated using multiple dose PBPK model. The multiple dose simulations provide both Cminss and Cmaxss levels. The intended formulation should fall between these levels.

The details of simulation are presented in Figure 5 (A to D)

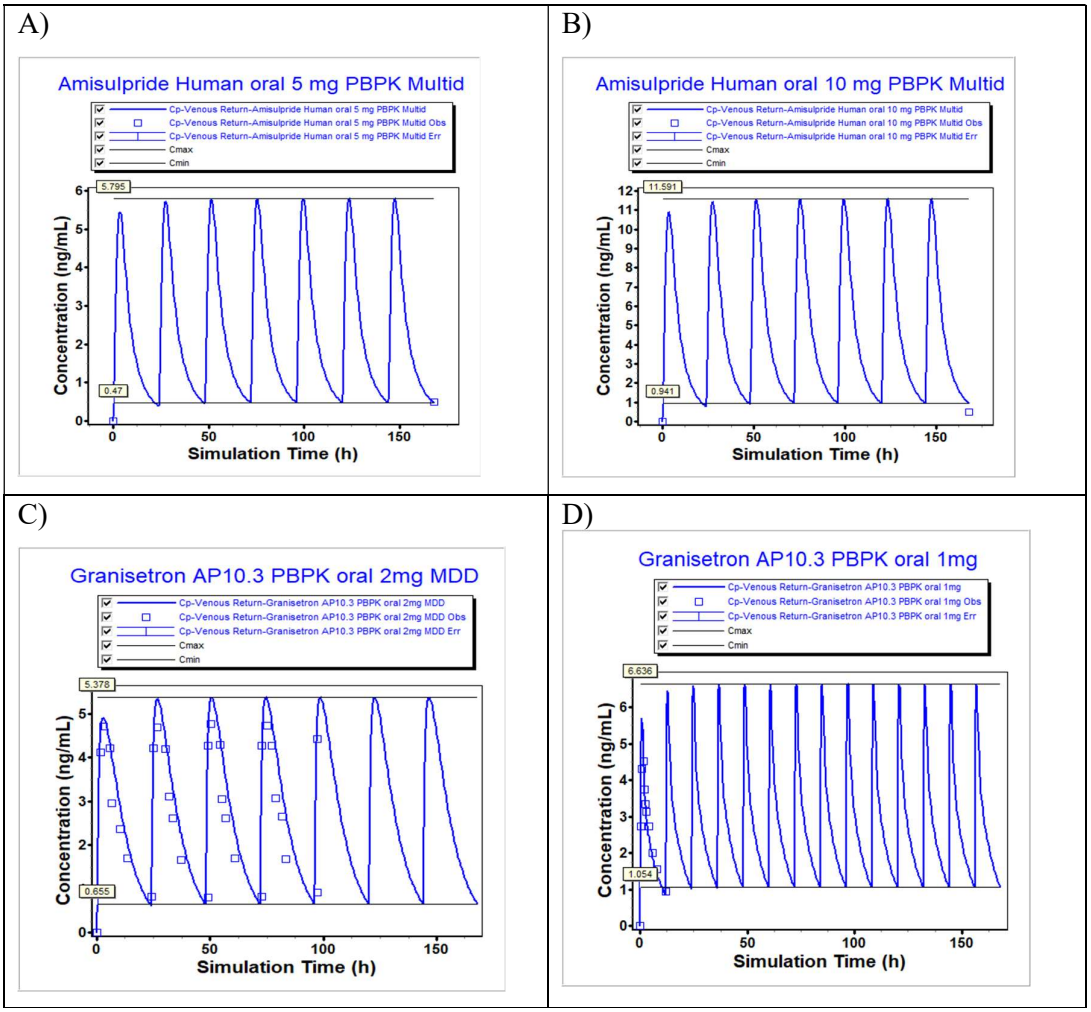

Figure 5: Multiple dose simulations for A) Amisulpride oral 5 mg (Q24hr) B) Amisulpride oral 10 mg (Q24hr) C) Granisetron oral 2 mg (Q24hr) D) Granisetron oral 1 mg (Q12hr)

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 (Sacuso 52 cm^2), Cavass level of 2.2 ng/mL over six days is reported.[49]

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.

4.3 Development of dissolution model for target dissolution profile:

The theoretical dissolution profile was described by two models. First model[50] is relatively simple which follows first order model as follows:

Equation 1 $$\log M_t = logM_0 - 0.43k_1t$$

Where M_0 is initial amount of drug at the beginning
M_t is residual amount of drug at time t
k_1 is first order constant

Second model[51] is complex, where mass of drug at time t (M_t) is calculated by following equation.

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

Where, M_t is mass of drug released Mt at time t
r is Particle radius
C_0 is initial drug loading
C_s is drug solubility
D_t is diffusion coefficient at time t in cm^2/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 1, the calculated drug release is presented in table 7

Table 7 Calculated Drug release as per model 1

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 2, the calculated drug release is presented in table 8.

Table 8 Calculated Drug release as per model 2

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

4.4 Calculation 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[10,28,52] are given in table 9

Table 9 Marketed formulations of Amisulpride and Granisetron

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.

5. Development Stage - Analytical method and formulation development:

5.1 Pre-formulation Studies:

5.1.1 Organoleptic properties:

The organoleptic characteristics of Amisulpride and Granisetron are shown in table 10.

Table 10 Organoleptic characteristics of drugs

Organoleptic characteristics	Drugs	
	Amisulpride	**Granisetron**
Physical state	Solid	Solid
Appearance	Crystalline powder	Crystalline powder
Colour	White	White

5.1.2 FTIR analysis:

The identification of functional groups was performed using Fourier Transform Infrared spectroscopy (FTIR) (Model Cary 620 microscope and Cary 670 FTIR, Agilent, CA, USA) in Attenuated Total Reflectance (ATR) mode with a Germanium crystal in the 400 cm^{-1} to 4000 cm^{-1} wave number range and averaging 16 scans. The FTIR spectra of Amisulpride, Granisetron, Poly(lactic-*co*-glycolic) Acid (PLGA), Polycaprolactone (PCL) and polyvinyl alcohol (PVA) are shown in Figure 6 (A to E).

Figure 6A FTIR spectra of Amisulpride

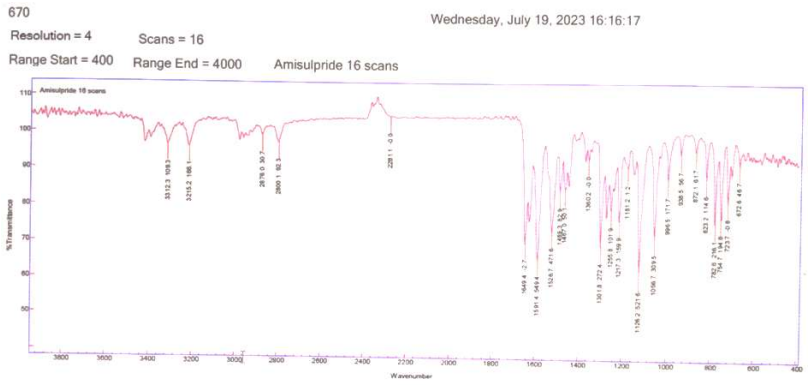

Figure 6B FTIR spectra of Granisetron

Figure 6C FTIR spectra of Poly(lactic-*co*-glycolic) Acid (PLGA)

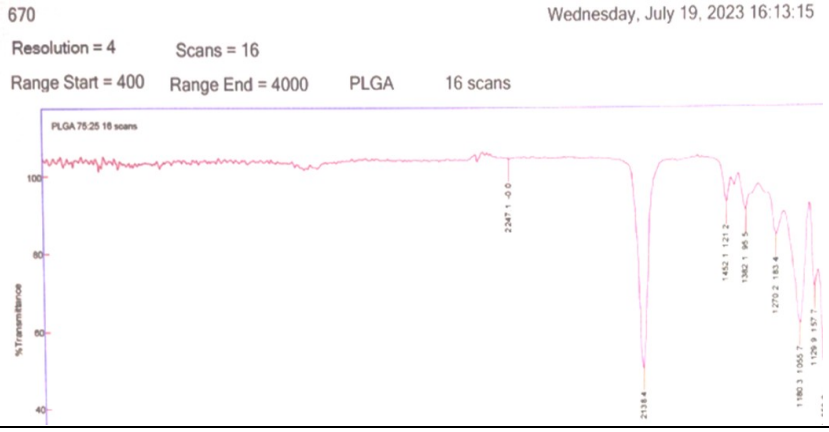

Figure 6D FTIR spectra of Polycaprolactone (PCL)

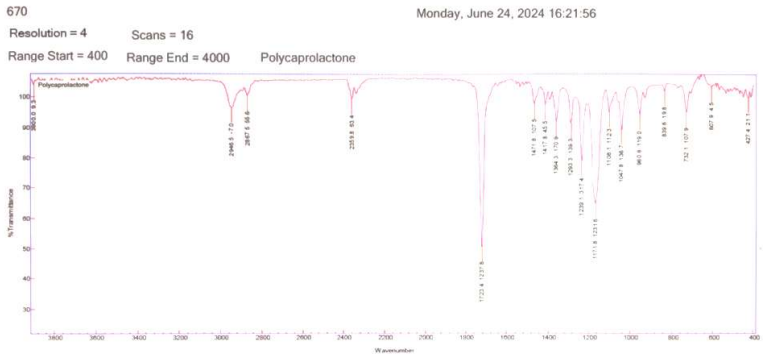

Figure 6E FTIR spectra of Polyvinyl alcohol (PVA)

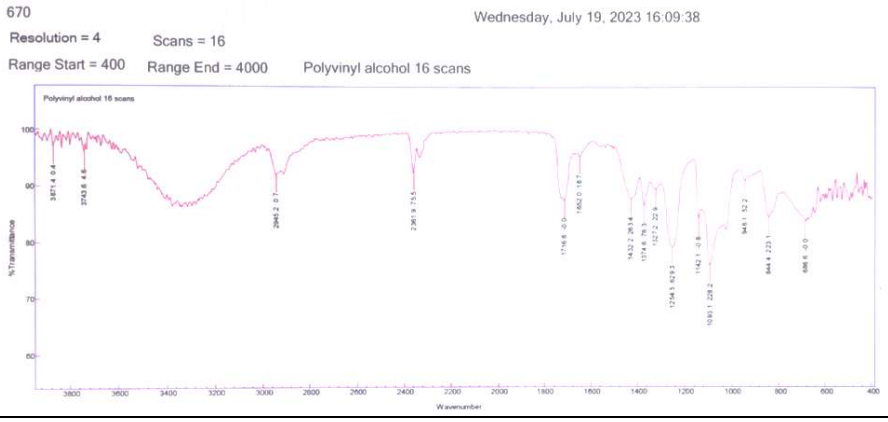

Figure 6 FTIR Spectra of A) Amisulpride B) Granisetron C) PLGA D) PCL E) PVA

Amisulpride shows characteristics peaks at 3312 cm^{-1} and 3215 cm^{-1} corresponding to -NH stretching, at 1649 cm^{-1} corresponding to C=O stretching and at 1056 cm^{-1} corresponding to O=S=O stretching.

Granisetron shows characteristic peaks at 3231 cm^{-1}, 1646 cm^{-1} and 1549 cm^{-1} corresponding to -NH stretching, C=O stretching and -CN stretching respectively.

PLGA shows characteristics peaks at 1749 cm^{-1},1452 cm^{-1},1180 cm^{-1} and 1088 cm^{-1} corresponding to C=O stretching, C-H bends, C-O stretching and C-O-C stretching respectively.

PCL shows characteristics peaks at 1723 cm^{-1},1364 cm^{-1},1293 cm^{-1} and 1239 cm^{-1} corresponding to C=O stretching, O-H stretching, C-O& C-C stretching and C-O-C stretching respectively.

All the observed spectra matched with the literature reported spectra and peak values.[53,54,55,56]

5.1.3 DSC analysis:

The thermal behaviour of amisulpride, granisetron, PLGA and PCL were determined by Differential Scanning Calorimetry (DSC-60, Shimadzu, Japan) using a scan rate of 10 °C/min in the temperature interval of 0 °C at 320°C, with a nitrogen atmosphere. DSC thermograms of Amisulpride, Granisetron, PLGA, PCL are shown in Figure 7 (A to D).

Figure 7A DSC of Amisulpride

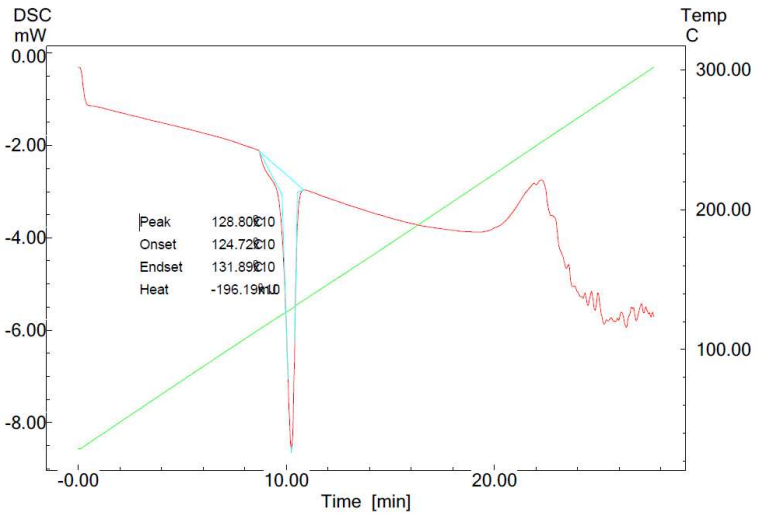

Figure 7B DSC of Granisetron

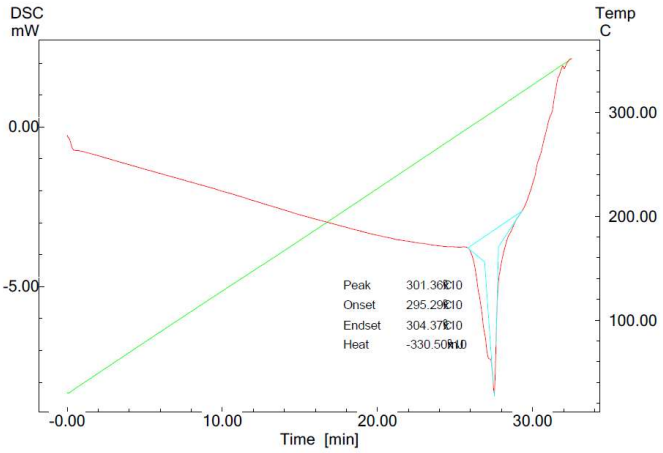

Figure 7C DSC of PLGA

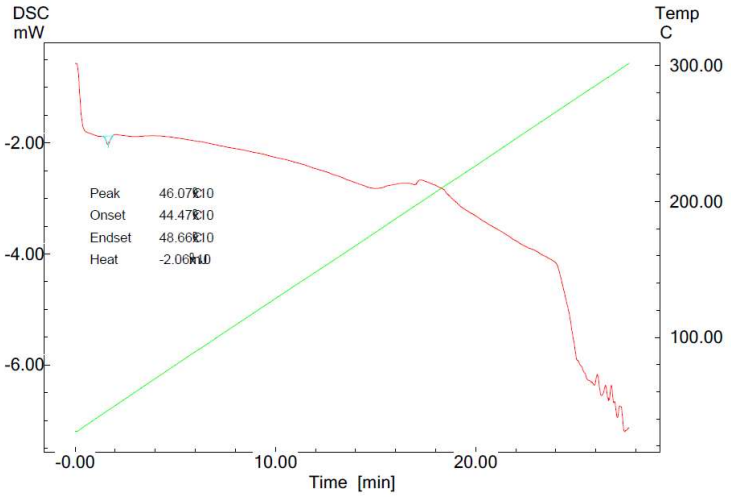

Figure 7D DSC of PCL

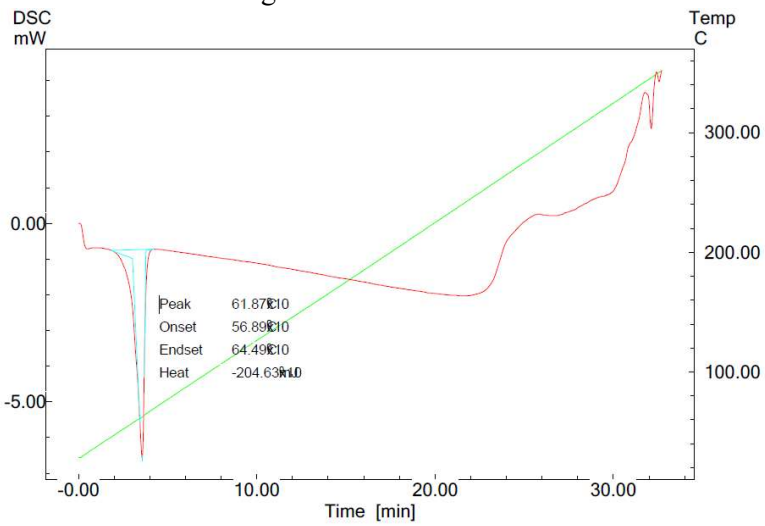

Figure 7 DSC data of A) Amisulpride B) Granisetron C) PLGA D) PCL

Amisulpride shows sharp endothermic peak at 128.8 °C and Granisetron shows endothermic peak at 301.36°C which confirms the identification of both the APIs.[57,58] PLGA being 100% amorphous polymer shows glass transition temperature (Tg) at 46.07°C.[59] PCL is a semi crystalline polymer, so its thermogram presents an endothermic peak at 61.87°C.[60]

5.2 Analytical method development:

5.2.1 Estimation of Amisulpride by UV Visible spectrophotometer:

Aliquots of amisulpride solution (100 µg/mL) ranging from 0.1 to 0.5 ml were transferred into a series of 10 ml volumetric flask and volume was made up to 10 ml 0.1N HCl to get a range from 1 µg/mL to 5 µg/mL. The absorbance of samples was measured at 226.5 nm against reference blank (0.1N HCl) by UV Visible spectrophotometer. The absorption spectra are shown in figure 8, concentrations in table 11 and calibration curve in figure 9.

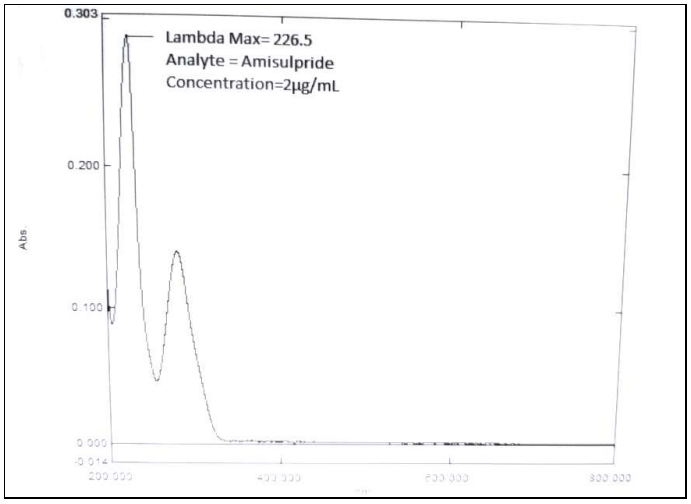

Figure 8 Absorption spectra of Amisulpride

Table 11 Calibration data of Amisulpride in 0.1N HCl

Conc. (µg/mL)	Absorbance ± SD (n=3)	%RSD
1	0.1547 ± 0.0006	0.3733
2	0.2813 ± 0.0006	0.2052
3	0.4210 ± 0.0010	0.2375
4	0.5597 ± 0.0006	0.1032
5	0.7203 ± 0.0006	0.0822

Mean SD	Slope	LOD	LOQ
0.0007	0.1374	0.0159	0.0482

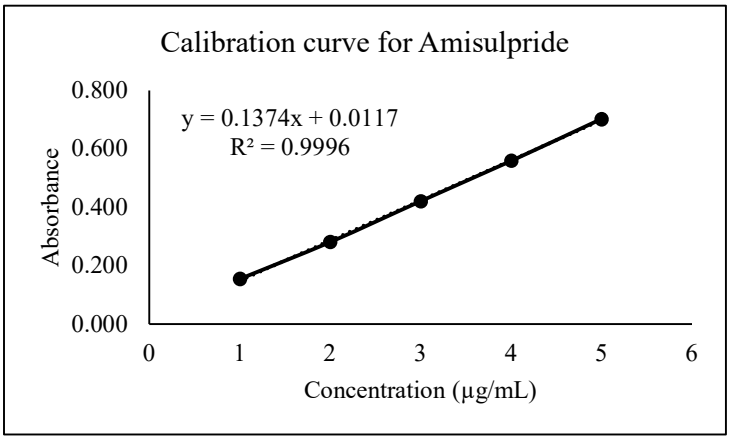

Figure 9 Calibration curve of Amisulpride in 0.1N HCl

5.2.2 Estimation of Granisetron by UV Visible spectrophotometer:

Aliquots of granisetron solution (100 µg/mL) ranging from 0.2 to 1 ml were transferred into a series of 10 ml volumetric flask and volume was made up to 10 ml 0.1N HCl to get a range from 2 µg/mL to 10 µg/mL. The absorbance of samples was measured at 302 nm against reference blank (0.1N HCl) by UV Visible spectrophotometer. The absorption spectra are shown in figure 10, concentrations in table 12 and calibration curve in figure 11.

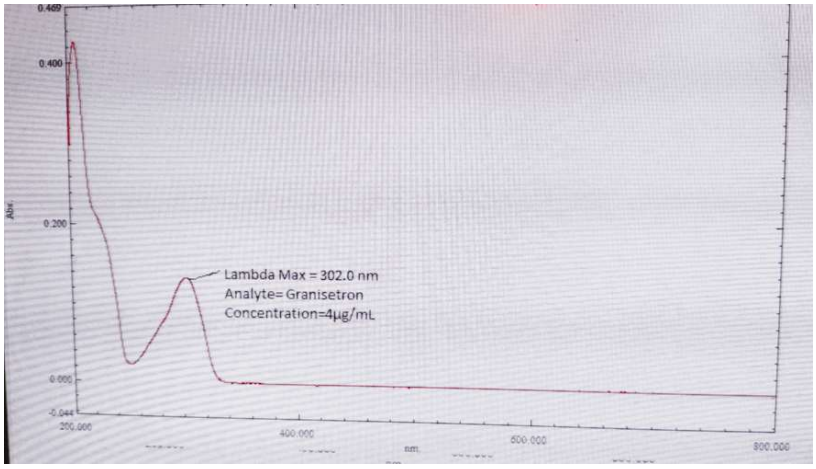

Figure 10 Absorption spectra of Granisetron

Table 12 Calibration data of Granisetron in 0.1N HCl

Conc. (µg/mL)	Absorbance ± SD (n=3)	%RSD
2	0.0730 ± 0.0010	1.3699
4	0.1310 ± 0.0010	0.7634
6	0.1970 ± 0.0010	0.5076
8	0.2690 ± 0.0010	0.3717
10	0.3263 ± 0.0006	0.1769

Mean SD	Slope	LOD	LOQ
0.0009	0.0322	0.0938	0.2843

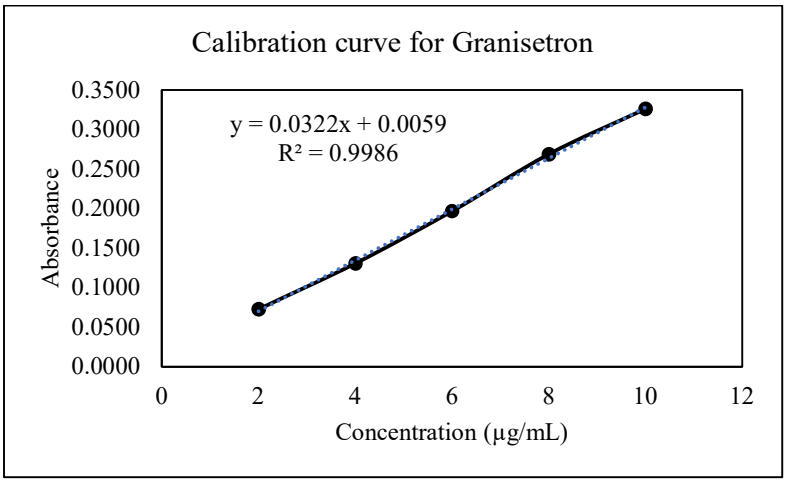

Figure 11 Calibration curve of Granisetron in 0.1N HCl

5.2.3 Simultaneous estimation by UV Visible spectrophotometer:

No previous simultaneous method has been reported to estimate amisulpride and granisetron in combination dosage form. Hence, Simultaneous estimation method was developed for both the drugs in pure and microsphere dosage form.

In brief, standard stock solutions of amisulpride and granisetron were prepared as per individaul methods described earlier. The λmax for amisulpride and granisetron is 226.5 and 302 nm respectively. From overlain spectra shown in figure 12, it is evident that there are two issobestic points (at 220 nm and 290.4 nm). However, the granisetron has shoulder peak at 220 nm isosbestic point which can result in erroraneous results. Hence, estimation was caried out using simultaneous equation method.

The simultaneous equation method of analysis is based on the absorption of the drugs amisulpride and granisetron at their wavelength maximas. Two wavelengths selected for the development of the simultaneous equations are 226.5 nm and 302 nm. The absorptivity values were deterimed for both the drugs at respective wavelengths. The absorbances and absorptivity at these wavelengths were substituted in equations (3) and (4) to obtain the concentration of drugs.

Equation 3 $Cx = (A2*ay1 - A1*ay2)/(ax2*ay1-ax1*ay2)$

Equation 4 $Cy = (A1*ax2 – A2*ax1)/(ax2*ay1-ax1*ay2)$

where A1 and A2 are the absorbance of sample solutions at 226.5 nm and 302 nm, respectively. Cx and Cy are concentrations of amisulpride and granisetron in µg/ml in sample solution. By substituting the values of A1 and A2, the Cx and Cy can be calculated by solving equations (3) and (4).

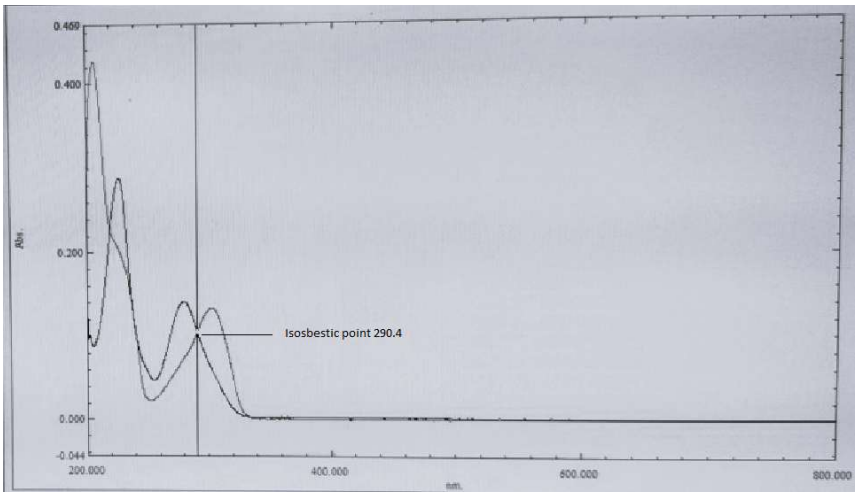

Figure 12 Overlay absorption spectra of Amisulpride and Granisetron

5.2.4 Simultaneous estimation of Amisulpride and granisetron by HPLC:

Preparation process is described as follows. The gradient program is shown in table 13.

Buffer solution

1.0 gm 1-octane sulphonic acid sodium salt was dissolved in 500 ml water and 2.0 ml triethylamine was added. This was further diluted to 1000 ml with water. pH was adjusted to 2.5 ± 0.05 with dilute orthophosphoric acid.

Mobile phase:

Mixture of buffer solution and methanol in the ratio of 600: 400 was prepared and mixed well.

Diluent:

Mixture of buffer solution and methanol in the ratio of 600: 400 was prepared and mixed well. pH of solution was adjusted to 2.5 ± 0.05 with dilute orthophosphoric acid. The diluent was filtered and degassed.

Standard preparation

2 mg of Amisulpride API was accurately weighed and transferred to 100 ml volumetric flask. 30 ml of diluent was added and sonicated to dissolve the API. Volume make up was done using the diluent to obtain concentration of 20 µg/ml.

2 mg of Granisetron API was accurately weighed and transferred to 100 ml volumetric flask. 30 ml of diluent was added and sonicated to dissolve the API. Volume make up was done using the diluent to obtain concentration of 20 µg/ml.

Instrumental conditions:

High-Performance Liquid Chromatograph (HPLC) with the following conditions.

Column	:	Symmetry Shield RP-18 150 X 4.6, 3.5 micron
Flow rate	:	1.0 ml/min.
Detection by UV	:	Detection wavelength 227 nm
Run time	:	About 30 min.
Column temp	:	30°C
Injection volume	:	20 µl
Retention time	:	Amisulpride: 11.5 min Granisetron: 15.4 min
Mode	:	Gradient

Table 13 Gradient HPLC programme details

Time (min)	% Mobile phase A	% Mobile phase B
0	80	20
20	40	60
22	80	20
30	80	20

The chromatogram data is presented in figure 13 (A to C)

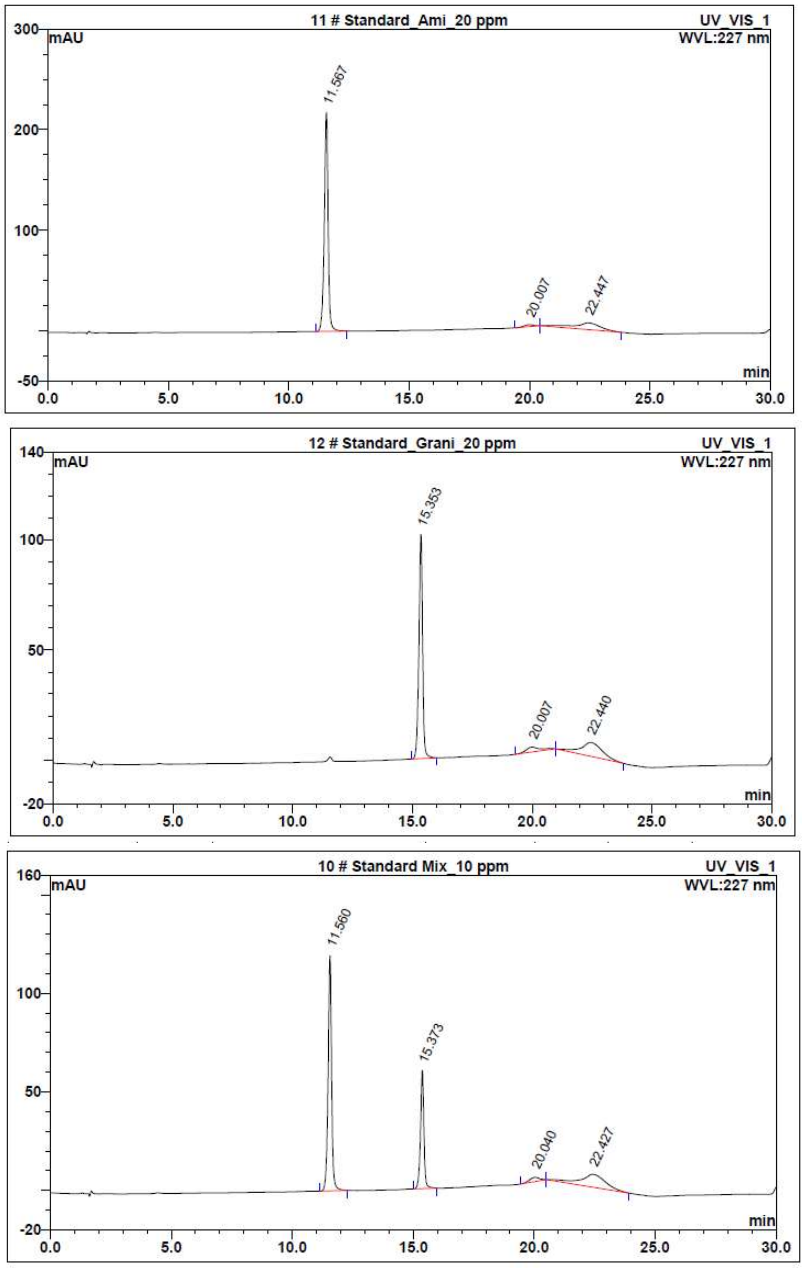

Figure 13 Chromatogram data A) Amisulpride B) Granisetron C) Mixture

The chromatographic analysis shows that the developed method is able to quantify both the drugs individually and simultaneously.

5.3 Formulation development:

5.3.1 Materials:

Granisetron HCl and Amisulpride were obtained from Sun pharmaceutical Industries Ltd (India). PLGA and PCL polymers were obtained as gift samples from Sun pharma and Evonik. All other chemicals and solvents used were of analytical grade.

5.3.2 Equipments:

Digital analytical balance (ATX224 Shimadzu, Japan)

UV-Visible spectrophotometer (1800 Shimadzu, Japan)

High performance liquid chromatograph (Waters)

Differential Scanning Calorimeter (DSC-60-Shimadzu Corporation, Japan)

Mastersizer 3000 (Malvern Panalytical, UK)

Infrared Spectrophotometer (Agilent)

Optical microscope

Scanning electron microscope

Magnetic stirrer (Remi sci. Equipment, India)

Overhead stirrer (Remi)

Heating bath

Analytical ultracentrifuge

5.3.3 Selection of method for preparation of microspheres:

Microspheres were prepared by O/W and W/O/W emulsion-solvent evaporation methods.[61, 62, 63]

5.3.4 Preparation of microspheres for Amisulpride:

Amisulpride being insoluble in water, O/W emulsion method was found to be suitable for preparation of microspheres. PLGA and Amisulpride were dissolved in dichloromethane. This solution was added dropwise in 0.5% - 1% PVA solution to form emulsion under stirring. Stirring was continued for 30 mins and then added to excess water under continuous stirring. Stirring was continued for 8 hours. Emulsion was filtered to obtain microspheres. The obtained microspheres were washed three times with water and dried.[62,64]

5.3.5 Preparation of microspheres for Granisetron:

Granisetron being soluble in water, W/O/W emulsion method was found to be suitable for preparation of microspheres. Granisetron was dissolved in water. PLGA was dissolved in dichloromethane. Granisetron solution was added to PLGA solution to form first W/O emulsion. This emulsion was added dropwise in 0.5% - 1% PVA solution to form W/O/W

emulsion under stirring. Stirring was continued for 30 mins and then added to excess water under continuous stirring. Stirring was continued for 8 hours. Emulsion was filtered to obtain microspheres. The obtained microspheres were washed three times with water and dried.[62,65]

5.3.6 Preparation of microspheres for Amisulpride and Granisetron:

Novel drug loading strategy was used to incorporate hydrophobic (Amisulpride) and hydrophilic (Granisetron HCl) drugs in microsphere formulation using double emulsion-solvent evaporation technique. Two polymers (PLGA and PCL) were selected to enable formation of Janus particles.[61] Granisetron was dissolved in water. PLGA, PCL and Amisulpride were dissolved in dichloromethane. Granisetron solution was added to PLGA-PCL-Amisulpride solution to form first W/O emulsion. This emulsion was added dropwise in 0.5% - 1% PVA solution to form W/O/W emulsion under stirring. Stirring was continued for 30 mins and then added to excess water under continuous stirring. Stirring was continued for 8 hours. Emulsion was filtered to obtain microspheres. The obtained microspheres were washed three times with water and dried.[61,63]

5.3.7 Screening of process and formulation parameters:

The individual drug microspheres were part of feasibility trials. The process and formulation were screened and optimized on the basis of minimum particle size and maximum entrapment efficiency for dual drug loaded formulations.

Initial screening included finalization of stirring speed and stirring time. The results of particle size and entrapment efficiency indicated that stirring speed of 800 rpm during secondary emulsion formation (O/W) and hardening stage was suitable. Stirring time of 8 hours was suitable in the hardening stage.

Further, the selected formulations were screened using different (0.1%, 0.2% 0.5% and 1%) PVA concentrations in the aqueous phase. Based on the quality of microspheres obtained, 0.5% PVA solution provided microspheres with uniform shape and size with high % yield. Hence, 0.5% PVA concentration was finalized for further experiments.

Based on the domain knowledge and calculated doses for both the drugs, it was decided to screen the drug ratio from 1:1 to 1:3 (Granisetron to Amisulpride) and polymer 1 to polymer 2 ratio from 1: 4 to 4:1 (PLGA to PCL). The trials were conducted to find out best suitable drug: drug ratio, polymer: polymer ratio and drug: polymer ratio. The optimization trial summary is presented in table 14.

Table 14 Experiment summary for optimization trials

Trial Nos.	Drug: Polymer ratio	Drug 1 (Granisetron) in mg	Drug 2 (Amisulpride) in mg	Polymer 1 (PLGA) in mg	Polymer 2 (PCL) in mg
1	1:5	50	50	100	400
2		50	50	200	300
3		50	50	250	250
4		50	50	300	200
5		50	50	400	100
6	1:3.3	50	100	100	400
7		50	100	200	300
8		50	100	250	250
9		50	100	300	200
10		50	100	400	100
11	1:2.5	50	150	100	400
12		50	150	200	300
13		50	150	250	250
14		50	150	300	200
15		50	150	400	100

Results and Discussion:

Amisulpride microspheres prepared with O/W method and granisetron microspheres prepared with W/O/W method resulted in uniform microspheres. These initial trials formed basis for incorporation of both soluble and insoluble drugs in single matrix of polymers (PLGA and PCL).

The amount of external phase used for hardening of Janus particles was also found critical to obtain high % yield.

Optimized formulations were chosen based on desired particle size (Below 150 micron) and drug release (best fitting the in-silico model)

7.0 Evaluation Stage - *In-vitro* and *in-silico* evaluation:

7.1 *In-vitro* characterization studies:

7.1.1 Yield:

The production yield of microspheres of various formulations were calculated by dividing the weight of collected microspheres by the total weight of all non-volatile contents of microspheres including drug and polymer used for preparation of microspheres and percent production yield were calculated as per the formula mentioned.

$$\text{Percentage yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

Percentage yield ranged from 60-80% depending on the process variations.

7.1.2 FTIR analysis:

Optimized formulation was analyzed using FTIR method described previously and FTIR spectra is presented in Figure 14.

Figure-. FTIR spectra of Formulation

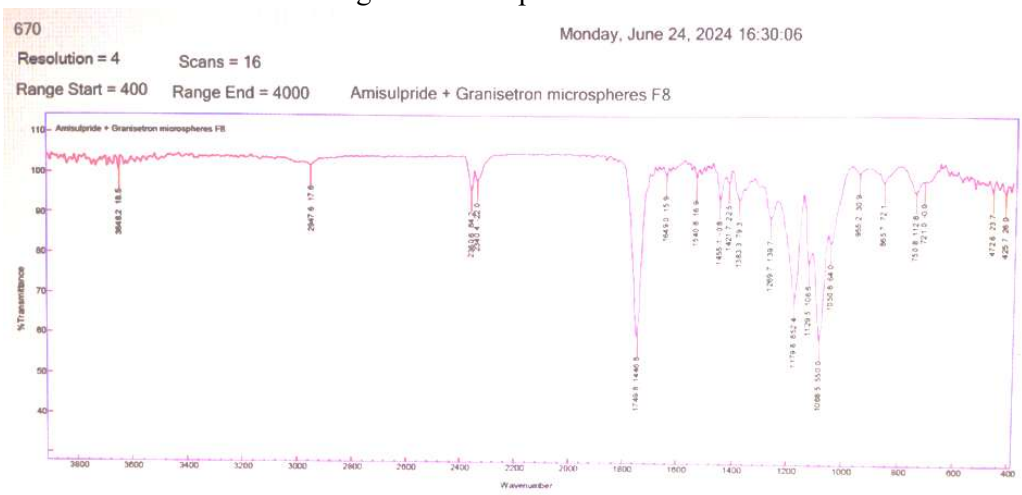

Figure 14 FTIR spectra of Final formulation

Formulation spectra showed the presence of only polymer peaks (1749,1455,1382,1270,1179,1129,1088, 866 and 750 cm^{-1}) suggesting complete encapsulation of drug within the polymer at molecular level.

7.1.3 Thermal analysis:

Optimized formulation was analyzed using DSC method described previously and thermogram is shown in Figure 15.

Figure 16 DSC of Formulation

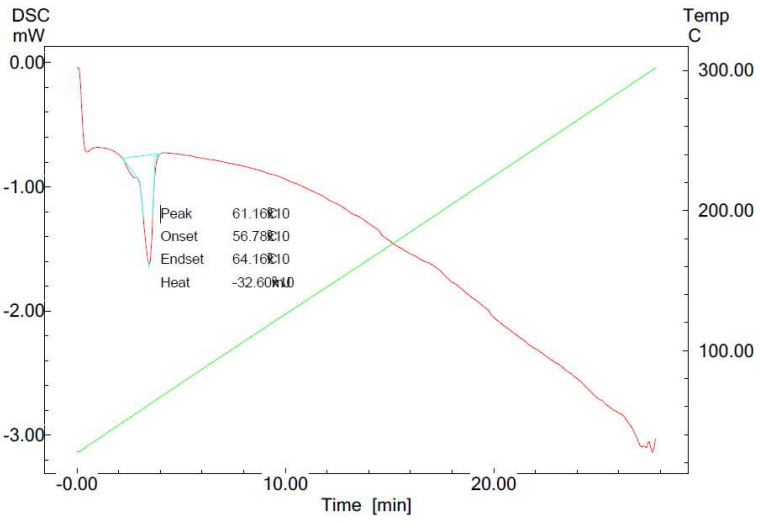

Figure 15 DSC of Final formulation

Figure 17 DSC of Placebo

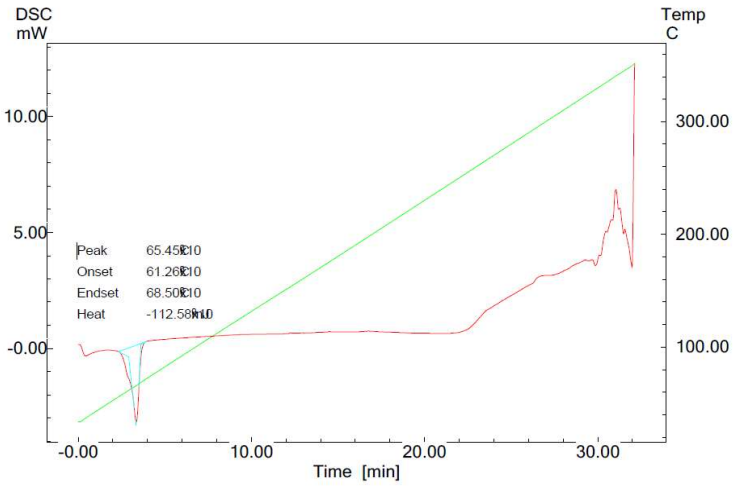

Figure 16 DSC of placebo

The absence of drug peaks (Amisulpride at 128.8°C and Granisetron at 301.36°C) and presence of only polymer peaks confirms the complete encapsulation within the polymer. The formulation thermogram presents the fusion peak of the PCL and PLGA at 61.16°C.[60] The small shoulder peak corresponds to the PLGA glass transition temperature. The same phenomenon is also observed in placebo where the fusion peak and shoulder peak appeared at 65.45°C as shown in figure 16.

7.1.4 Particle size analysis:

The particle size analysis was performed using Malvern 3000 (Malvern Instrument, Worcestershire, United Kingdom). Microspheres were suspended in 0.01% Tween 80 aqueous solution and subjected to ultrasonic treatment for 10 seconds and particle size was measured using laser diffraction. Formulation and process parameters impacted particle size. The particle size was found to be ranged from 60 micron to 200 microns. Optimized formulations showed particle size of around 80-130 microns which are suitable for intramuscular/subcutaneous injection.

7.1.5 Morphology:

The particle size and morphology were studied using optical microscopy and scanning electron microscopy (SEM). It was observed that, dual drug loaded Janus microspheres have typical handbag like structure (observed during process) with both drugs entrapped inside the structures[61] (Figure A to D). After drying, the particles appeared in more spherical shape (Figure E to H). During the manufacturing process, the drugs will be embedded in the polymer phases according to the hydrophilicity.

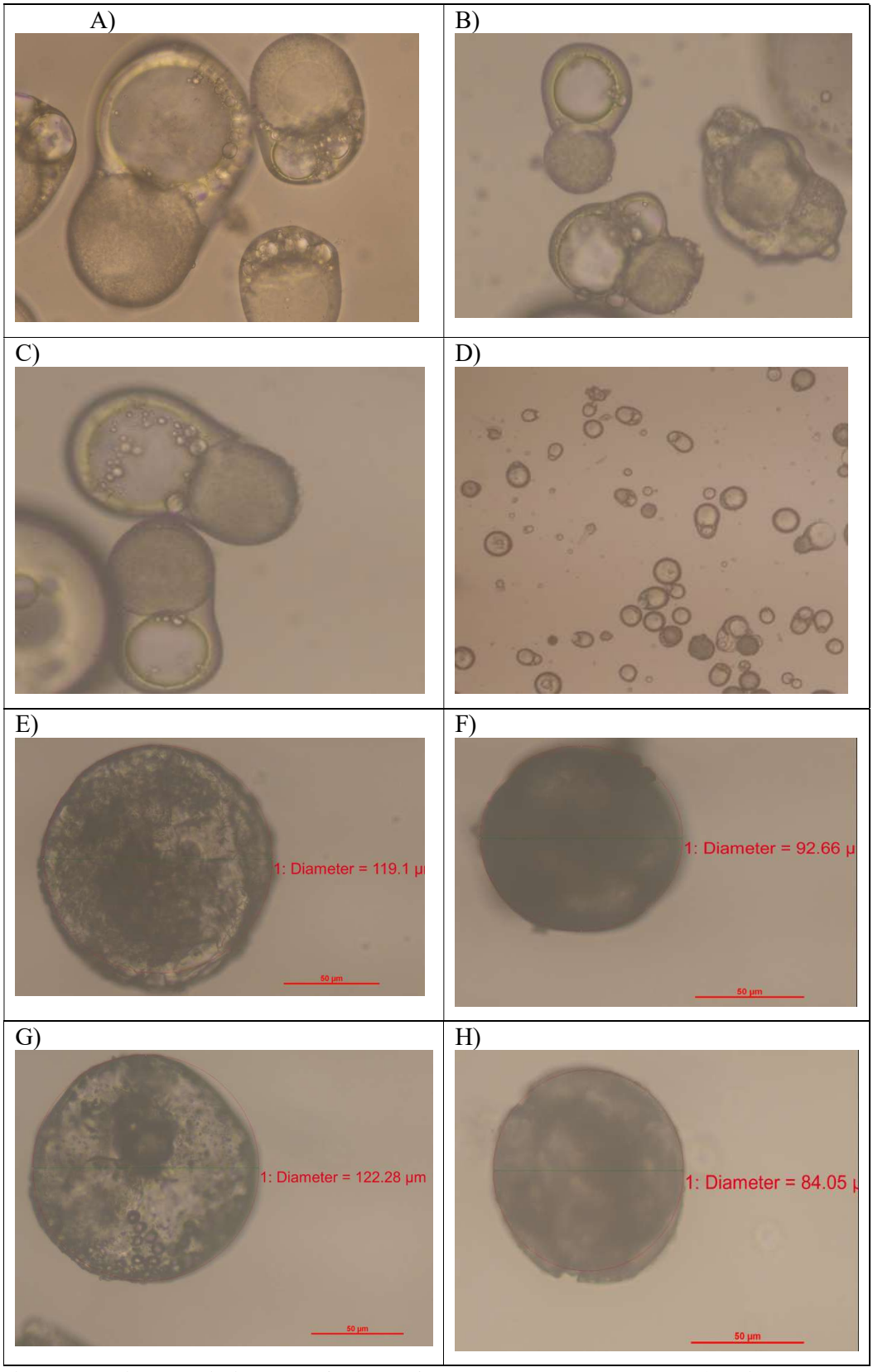

Figure 17 Microscopic images of microspheres A to D: Nascent stage (During process), E to H: After drying (Finished product)

7.1.6 Drug loading and Entrapment efficiency (%):

Methanol was added to weighed quantity of microspheres followed by shaking and centrifugation to collect supernatant to measure amount of free drug. Acetonitrile was added to centrifuged residue. The residue was dissolved by sonication and methanol was added followed by centrifugation to collect supernatant to measure drug loading of microspheres.

$$\%Drug\ loading\ = \frac{(\text{Total drug} - \text{Free drug}) \text{ in mg}}{\text{Weight of microspheres (mg)}} \times 100$$

$$\%EE\ = \frac{\text{Encapsulated drug (mg)}}{\text{Theoroticfal drug (mg)}} \times 100$$

% drug loading ranged from 7-18 % for amisulpride (10-30 mg dose) and 5-7% for granisetron (10 mg dose). The % EE ranged from 50-70%.

7.1.7 Drug release studies:

Drug release studies were carried out on optimized formulations. The drug release methods were selected based on literature and domain knowledge.[62,66,67,68] The Accelerated method help to understand drug release behaviour in short period of time and avoid waiting for results till real time analysis. Correlation between ACC and Real time method accelerate decision making during formulation development and optimization. Pure API were also tested for drug release in same methods.

Accelerated Method:

20 mg microspheres were suspended in 2 mL 0.01 M phosphate-buffered saline (PBS) buffer (pH 7.4) **at 50°C** and shaken at 100 rpm to measure the *in-vitro* release. After centrifugation at 3000 rpm for 5 min, the supernatant was completely withdrawn and replaced by 2 mL fresh release medium at each sampling time. Sampling was done on 1 hr, 4hr, 12hr and 24hr. Drug contents were determined using HPLC. The results of drug release of optimized formulations are shown in table 15 and figure 18. % RSD has been mentioned in bracket.

Table 15 Drug release as per ACC IVRT method

Accelerated *in-vitro* release testing method				
Time (hours)	F6 Ami	F6 Grani	F8 Ami	F8 Grani
0	0	0	0	0
1	25 ± 14.24	21 ± 14.29	22 ± 13.64	16 ± 15.41
4	48 ± 12.50	43 ± 11.63	45 ± 11.11	32 ± 12.50
12	64 ± 9.38	66 ± 9.09	62 ± 9.68	59 ± 7.60
24	98 ± 2.04	98 ± 2.04	99 ± 1.01	95 ± 2.11

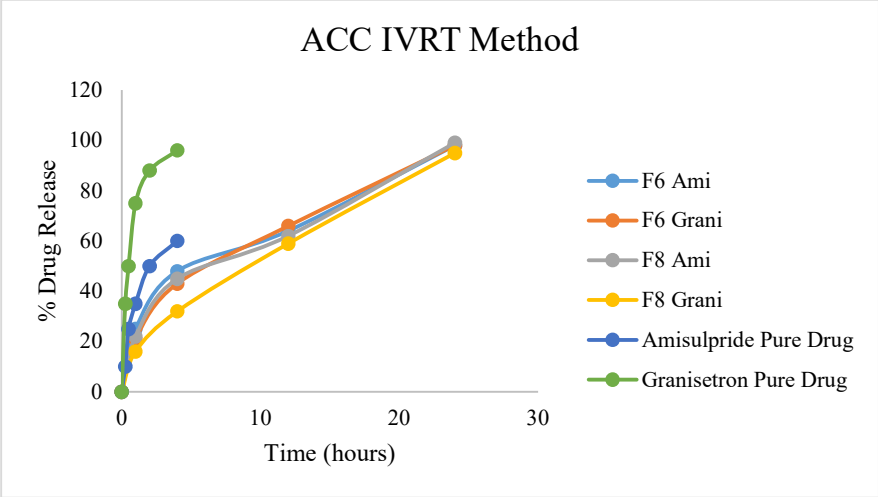

Figure 18 Accelerated *in-vitro* release testing method

Real time method:

20 mg microspheres were suspended in 2 mL 0.01 M phosphate-buffered saline (PBS) buffer (pH 7.4) at **37°C** and shaken at 100 rpm to measure the *in-vitro* release. After centrifugation at 3000 rpm for 5 min, the supernatant was completely withdrawn and replaced by 2 mL fresh release medium at each sampling time. Sampling was done on 1 hr, 4hr, 12hr, 24hr, 48hr, 72hr, 96hr, 120hr, 144hr and 168 hr. Drug contents were determined using HPLC. The results of drug release of optimized formulations are shown in table 16 and figure 19. % RSD has been mentioned in bracket.

Table 16 Drug release as per real time IVRT method

Real time *in-vitro* release testing method				
Time (hours)	F6 Ami	F6 Grani	F8 Ami	F8 Grani
0	0	0	0	0
1	16 ± 16.06	2 ± 50.0	13 ± 12.06	1 ± 43.30
4	19 ± 10.53	8 ± 12.50	16 ± 12.50	4 ± 25.0
12	27 ± 9.44	23 ± 8.70	24 ± 12.50	14 ± 14.29
24	44 ± 9.09	41 ± 7.32	43 ± 12.71	30 ± 13.33
48	63 ± 7.94	66 ± 6.06	62 ± 6.45	57 ± 7.02
72	84 ± 4.76	81 ± 3.70	83 ± 3.61	75 ± 6.67
96	93 ± 2.15	89 ± 3.37	93 ± 2.15	86 ± 4.65
120	93 ± 1.08	94 ± 1.06	93 ± 2.15	92 ± 1.09
144	97 ± 1.03	96 ± 1.04	97 ± 1.03	95 ± 1.05
168	99 ± 0.59	98 ± 1.02	99 ± 0.59	97 ± 1.03

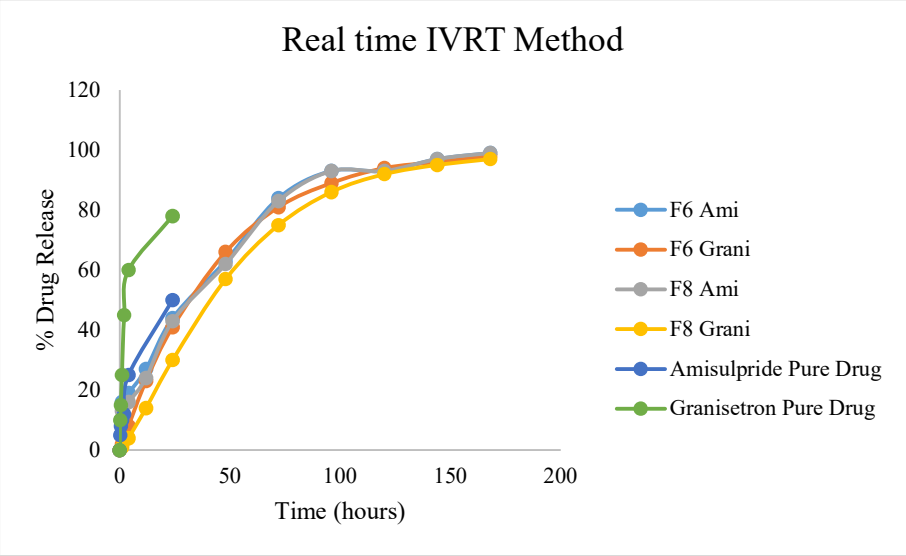

Figure 19 Real time *in-vitro* release testing method

7.1.8 Development of *in-vitro* Gel Diffusion Model:

After intramuscular or subcutaneous injection, the formulation is in close contact with an extracellular matrix consisting of a fibrous polymer framework of collagen and hyaluronic acid components surrounding physiological tissue. This extracellular matrix behaves more like a gel rather than bulk fluid, which results in significant differences in mass transport and diffusion of the drug which can affect its overall absorption profile. In order to understand the bio performance of a formulation administered subcutaneously/intramuscularly, it is critical to recognize the effect this surrounding environment may have. Agarose is a hydrogel consisting of a linear polysaccharide material and has been used for a variety of applications. At relatively low concentrations in aqueous solution, it sets to form a gel at room temperature. Agarose gels form a three-dimensional structure that contains mostly water but have measured pore sizes similar to those encountered in physiological tissue. As a result, diffusion and transport of drugs through agarose gels may provide more realistic conditions for formulations administered via SC and IM injection compared to typical bulk fluid dissolution media. 1% Agarose gel was prepared by the method mentioned by Leung et al.[69] The graphical presentation is shown in figure 20.

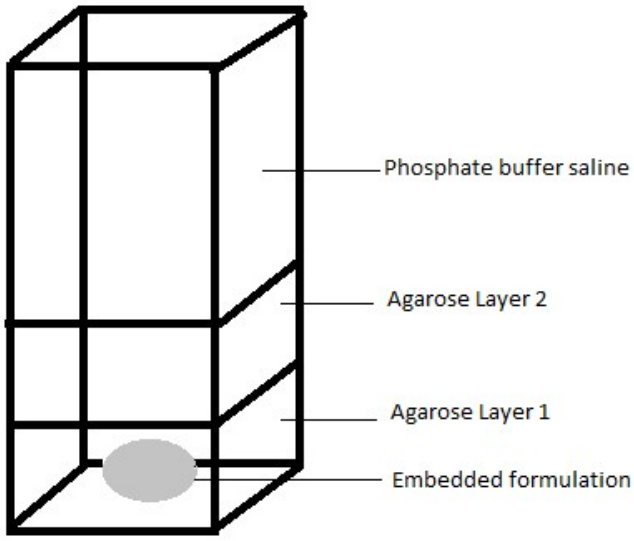

Figure 20 *In-vitro* gel diffusion system setup

Drug released in the PBS was measured periodically up to 24 hours by UV-visible spectrophotometer at respective drug wavelengths. This method simulates *in-vivo* drug release mechanism and conditions after intramuscular/subcutaneous administration.

The results of drug release of optimized formulations are shown in figure 21. % RSD has been mentioned in bracket.

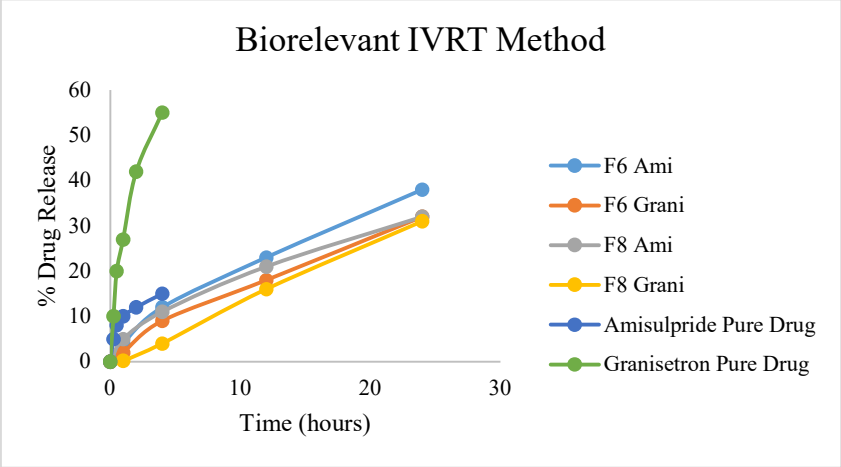

Figure 21 Biorelevant real time *in-vitro* release testing method

Results and Discussion:

Three types of in-vitro drug release methods were developed to gain understanding of mechanism of drug release under different conditions. The extended duration of controlled drug release from polymeric depots have been attributed to delayed degradation of the matrix and has often complicated the IVRT profiling.[68] The use of elevated temperatures has been

associated with free volume diffusion, variations in the polymeric mobility across the matrix along, accelerated mechanisms of release (erosion/ hydration/diffusion/ degradation) with enhanced rates of drug diffusion (especially when performed at temperatures near their second-order phase transition, Tg)[70] It was observed that drug release in the trials with higher PLGA content (5,10,15) was faster than trials with less PLGA content (1,6,11). This was attributed to nature of polymer. The drug release of Amisulpride was best fitted with Korsmeyer-Peppas equation with R^2 close to 0.9. The release of Granisetron was best fitted to first order with R^2 close to 0.9. The optimized trials shown drug release similar to that calculated from dissolution modeling earlier.

7.1.9 Bio-interactions of prepared formulations:

Haemolysis study

For haemolysis study, 1.0 ml blood sample was collected in EDTA solution (30 µl) containing Eppendorf tube from the Sprague Dawley rat by retro-orbital puncture. Blood sample was then centrifuged at 5000 rpm for 10 min at 4 °C to separate the red blood cells (RBCs). The separated RBC pellet was re-suspended in normal saline and plasma components were removed by washing with normal saline (0.9 % w/w Sodium Chloride in water) 3 times before use. Then 0.5 % v/v RBCs were prepared by re-suspending RBC pellet (250 µl) in 50 ml of normal saline. Then 1 ml of RBCs was added to plain drug suspension, formulations containing 1mg equivalent amount of drug were dispersed in 1ml of saline. For positive and negative control, 2.0% Triton-X100 (1ml) and 0.5% DMSO was used respectively. After treatment (with drug suspension, formulations positive control and negative control), RBC dispersion was gently stirred to uniformly disperse RBCs. The treated dispersions were stored at 37°C for 30 min in incubator. After incubation, all the samples were centrifuged at 3000 rpm for 12 min at 4 °C to separate the RBC mass and the solutions were analyzed for UV absorbance at λmax of 540 nm against normal saline as a reference solution.[71,72] Percentage of haemolysis was determined using following equation:

$$\%\ Haemolysis = A540\ of\ sample - A540\ of\ negative\ control\ /A540\ of\ positive\ control - A540\ of\ negative\ control \times 100$$

The absorbance for positive control (Triton X- 100) and negative control (DMSO) was found to be 0.70 and 0.150 respectively. The % haemolysis for optimized formulation was found to be 1.3 % ± 0.05 % corresponding to absorbance value of 0.163. The results demonstrated no significant haemolysis potential of optimized formulation as the % haemolysis was found to be less than 2.

7.2 Prediction of *in-vivo* pharmacokinetics using in-silico PBPK model:

Developed PBPK model using intravenous data and verified with single and multiple dose oral formulations data was used to predict the *in-vivo* performance of sustained release once weekly formulations.

Initially, the drug release data from calculated dissolution models was used to build preliminary PBPK model for sustained release formulations after intramuscular administrations. This was required to set physiological parameters which account for behaviour of drug after intramuscular administration. After initial model refinement, actual drug release data was used to predict the *in-vivo* pharmacokinetics of sustained release formulations. The optimized once a weekly formulation showed comparable *in-vivo* profile to that of multiple dose profile.

The pharmacokinetics of antiemetic drugs is directly predictive of pharmacodynamics. The Cmax and AUC data found to be similar which confirms similar safety and efficacy profile. The details are presented in figure 22 and 23.

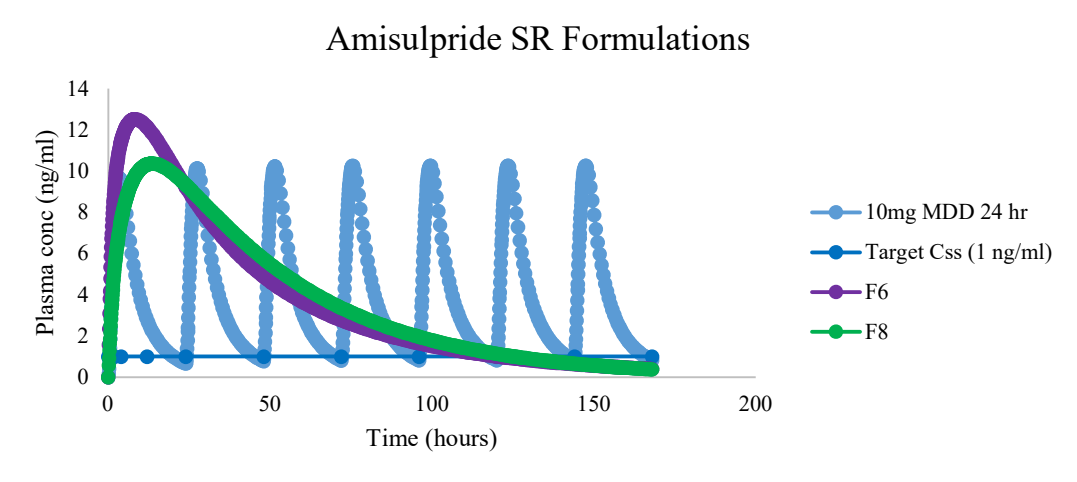

Figure 22 Prediction of *in-vivo* PK for once a weekly formulation of Amisulpride

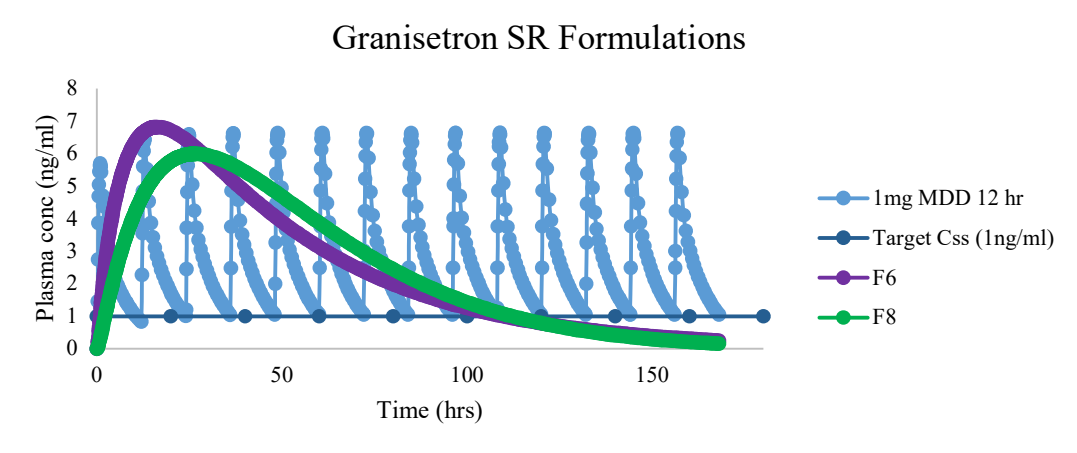

Figure 23 Prediction of *in-vivo* PK for once a weekly formulation of Granisetron

7.3 Virtual bioequivalence studies using in-silico model:

Virtual bioequivalence (VBE) studies were conducted in healthy subjects between optimized test formulation and reference multiple dose formulation to assess the *in-vivo* pharmacokinetic similarity. The VBE results confirmed that the developed once a weekly formulation can achieve similar rate and extent as that of immediate release multiple dose formulations. The summary of results is shown in Figure 24 and Figure 25.

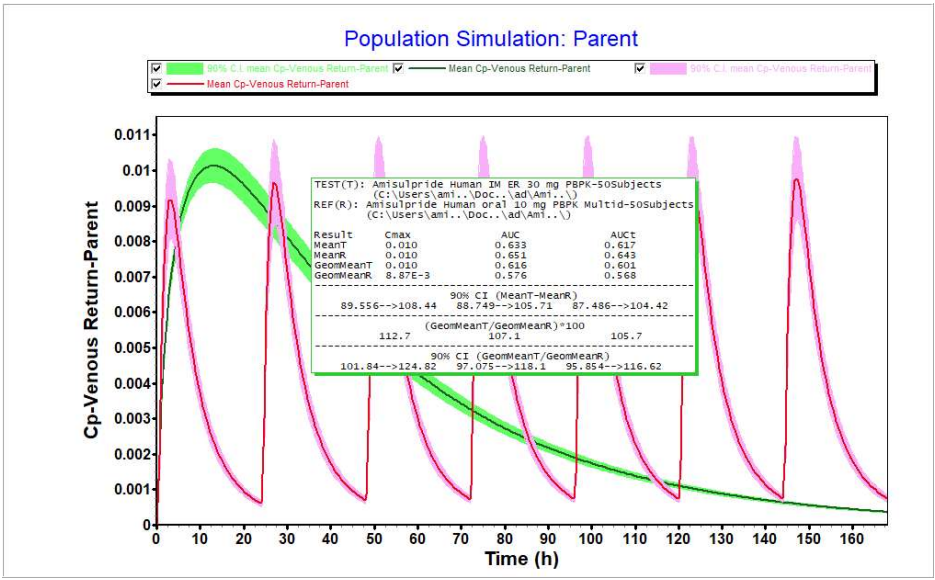

Figure 24 Virtual bioequivalence of once a weekly formulation of Amisulpride (30 mg CR) against oral 10 mg multiple dosing

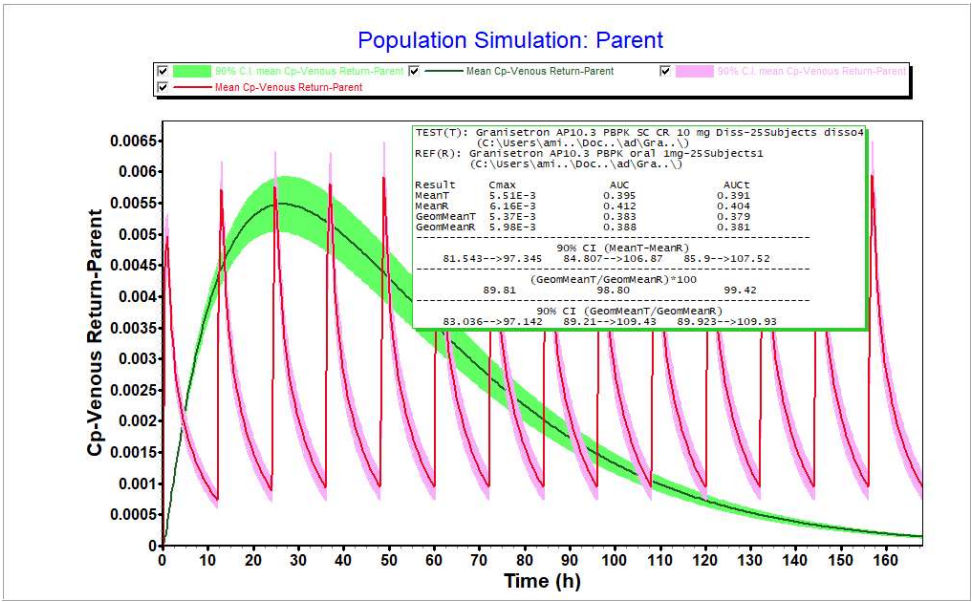

Figure 25 Virtual bioequivalence of once a weekly formulation of Granisetron (10 mg CR) against oral 1 mg multiple dosing

7.4 Predictive IVIVC to stablish design space:

In-vitro in-vivo correlation was developed for slow, medium and fast release formulations. Formulations with different drug: polymer ratio resulted in different release rates, which were used in developing the IVIVC. Advanced compartmental and transit (ACAT) model was used to mechanistically predict the *in-vivo* drug release profiles of three different release rate formulations. The interpolation function was used to build the correlation. Power correlation function was found best suitable with highest R2 value. The convolution results showed, both individual and mean Absolute Percent Prediction Errors well within the limits of NMT 15% and 10% respectively. The details are presented in Table 17 & 18 and Figure 26 & 27 for amisulpride and granisetron respectively.

Table 17 IVIVC Details for Amisulpride

Validation Statistics for Amisulpride IVIVC						
	Cmax (ng/mL)			AUCt (ng/mL*h)		
Drug Record	Obs.	Pred.	% Pred. Error	Obs.	Pred.	% Pred. Error
Amisulpride 30 mg CR Slow	9.11	9.127	-0.187	620.1	632.8	-2.05
Amisulpride 30 mg CR Medium	10.32	10.41	-0.883	626.8	632.3	-0.876
Amisulpride 30 mg CR Fast	13.97	14.37	-2.887	618.5	621.1	-0.411
Mean Absolute Percent Prediction Error	1.319			1.112		
y = 0.997 * (x)^0.992						
where x=Fraction released *in-vitro* and y=Fraction released *in-vivo*						
Correlation = Power Function						
Statistics for Reconstructed Plasma Concentration-Time Profile from Convolution Tab						
Drug Record	R^2	SEP	MAE	AIC		
Amisulpride 30 mg CR Slow	1	0.038	0.02	-43.32		
Amisulpride 30 mg CR Medium	1	0.049	0.026	-37.93		
Amisulpride 30 mg CR Fast	1	0.078	0.039	-27.83		

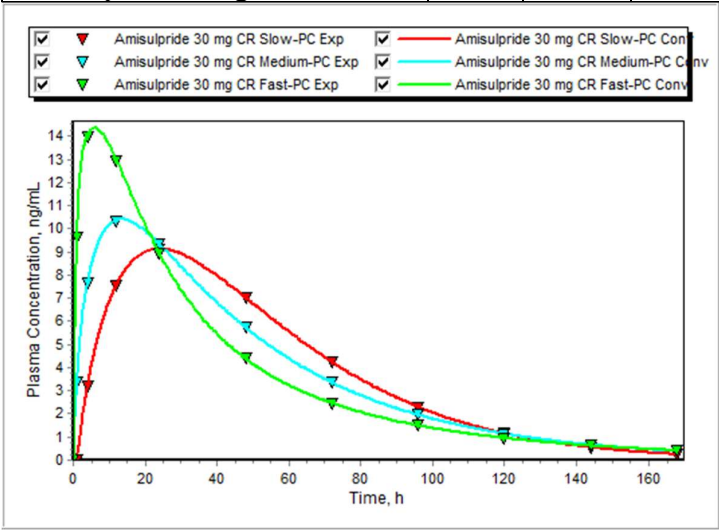

Figure 26 IVIVC predictions for Amisulpride

Table 18 IVIVC Details for Granisetron

Validation Statistics for Granisetron						
	Cmax (ng/mL)			AUCt (ng/mL*h)		
Drug Record	Obs.	Pred.	% Pred. Error	Obs.	Pred.	% Pred. Error
Granisetron CR 10 mg Slow	5.962	5.41	9.25	416.5	410.4	1.468
Granisetron CR 10 mg medium	6.598	6.579	0.286	422.9	416.5	1.519
Granisetron CR 10 mg fast	8.684	8.726	-0.484	423.8	407.8	3.772
Mean Absolute Percent Prediction Error	3.34			2.253		
y = 0.999x^1.033						
where x=Fraction released *in-vitro* and y=Fraction released *in-vivo*						
Correlation = Power Function						
Statistics for Reconstructed Plasma Concentration-Time Profile from Convolution Tab						
Drug Record	R^2	SEP	MAE	AIC		
Granisetron CR 10 mg Slow	0.986	0.241	0.174	-2.934		
Granisetron CR 10 mg medium	0.997	0.116	0.081	-18.95		
Granisetron CR 10 mg fast	0.993	0.247	0.167	-2.381		

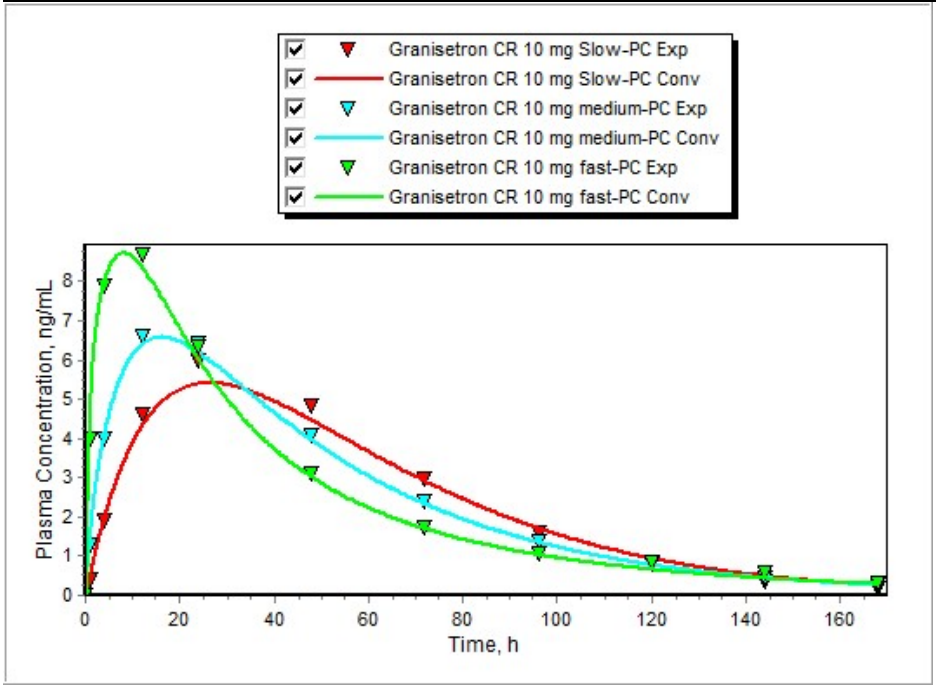

Figure 27 IVIVC predictions for Granisetron

Establishing the safe space for formulations is critical with respect to scale-up and commercialization purpose. The established safe space derived by application of PBBM modeling help to address any process deviations in the large scale and establishing control strategy.

8.0 Summary and conclusion:

The allometric scaling predicted volume of distribution and clearance values for human from preclinical species. The predicted values were within 2-fold error from the observed data for finalized model.

In-vivo pharmacokinetic profiles for human were predicted using Wajima and Dedrick approach. Out of the two approaches, Wajima approach was more suitable in predicting the human PK data from preclinical species. Further, the calculated volume of distribution and clearance values were validated in the developed PBPK model on Gastroplus platform. The simulated PK profiles showed that predicted VD and CL values were also able to predict the human pharmacokinetics similar to observed values.

The dose and target steady state levels were calculated based on pharmacokinetic data and dose ranging studies from literature. The dissolution models were also used to predict the target drug release profile based on first order kinetics and using complex microsphere model in the DDDplus platform.

Dual drug loaded microspheres (Janus particles) were prepared for Amisulpride and Granisetron using W/O/W emulsion-solvent evaporation method. Granisetron being water soluble drug was incorporated in the inner phase and amisulpride being water insoluble drug was incorporated along with oil phase. The microspheres were uniform in size and shape and showed handbag like structure which indicates entrapment of both the drugs. The Janus particles were characterized in terms of FTIR analysis, thermal analysis, % yield, particle size, morphology, % drug loading, % encapsulation efficiency and *in-vitro* drug release testing. The data was satisfactory from CMC perspective of once a weekly sustained release formulation. The IVRT data was further used in the *in-silico* PBPK model to predict the *in-vivo* pharmacokinetics of developed formulations.

The *in-silico* predictions showed that, developed once a weekly formulation is able to provide sustained release profile up-to 5-6 days. This was further proved by running virtual bioequivalence studies between multiple dose formulation and optimized sustained release formulation. The virtual BE Study showed that Cmax and AUC levels were within 80-125% limits and proved the equivalency. Further, IVIVC was developed to establish safe space for bioequivalent formulations.

In conclusion, the present work meets the set objectives and the idea and concept can further be extended to other products with minimal use of *in-vivo* studies.

9.0 Publications:

1. Dabke A, Ghosh S, Dabke P, Sawant K, Khopade A. Revisiting the *in-vitro* and *in-vivo* considerations for *in-silico* modelling of complex injectable drug products. J Control Release. 2023 Jun 24; 360:185-211. doi: 10.1016/j.jconrel.2023.06.029.
2. Saikat Ghosh, Amit Dabke, Ami Patel, Priyansh Pandya, and Krutika K. Sawant "Regulatory perspective and patents for parenteral microemulsion formulation" book chapter in book titled Pharmaceutical microemulsions for parenteral delivery: From Bench to Bedside. Editors: Vivek Chavda, Vandana Patravale. Apple Academic Press, April 2024.

10.0 Poster Presentations:

1. Amit D, Ajay K, Krutika S, **"**Design of Sustained Release Injectable Formulations Using Computational Approach" **Presented** poster in one day seminar on Recent advances in 'Smart Drug Delivery Systems (SDDS)' organized by Chettinad school of Pharmaceutical sciences, Chettinad academy of Research and Education, Chennai on 5th April 2023.
2. Amit D, Ajay K, **"**Artificial Intelligence driven drug design (AIDD)" **Presented** poster in National Science Day workshop organized by Sun Pharmaceutical Industries Ltd. on 24th Feb 2023.

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Appendix 1

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Review article

Revisiting the *in-vitro* and *in-vivo* considerations for in-silico modelling of complex injectable drug products

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ABSTRACT

Complex injectable drug products (CIDPs) have often been developed to modulate the pharmacokinetics along with efficacy for therapeutic agents used for remediation of chronic disorders. The effective development of CIDPs has exhibited complex kinetics associated with multiphasic drug release from the prepared formulations. Consequently, predictability of pharmacokinetic modelling for such CIDPs has been difficult and there is need for advanced complex computational models for the establishment of accurate prediction models for *in-vitro-in-vivo* correlation (IVIVC). The computational modelling aims at supplementing the existing knowledge with mathematical equations to develop formulation strategies for generation of predictable and discriminatory IVIVC. Such an approach would help in reduction of the burden of effect of hidden factors on preclinical to clinical translations. Computational tools like physiologically based pharmacokinetics (PBPK) modelling have combined physicochemical and physiological properties along with IVIVC characteristics of clinically used formulations. Such techniques have helped in prediction and understanding of variability in pharmacodynamic parameters of potential generic products to clinically used formulations like Doxil®, Ambisome®, Abraxane® in healthy and diseased population using mathematical equations. The current review highlights the important formulation characteristics, *in-vitro*, preclinical *in-vivo* aspects which need to be considered while developing a stimulatory predictive PBPK model in establishment of an IVIVC and *in-vitro-in-vivo* relationship (IVIVR).

1. Introduction

Current therapeutic regimens against the major diseases require the administration of multiple agents being dosed multiple times at definite intervals [1]. Such chronic treatments have often presented variable results in terms of safety and efficacy of the intended formulation leading to poor quality-of-life among patients with subsequently reduced adherence to prescribed therapies [2]. Consequently, therapeutic drugs have often been either administered using multiple dosing of the formulations at short intervals or using complex injectable drug products (CIDPs) [3]. CIDPs are long-acting formulations of hydrophobic and amphipathic therapeutic agents which have tendency to form *in-vivo* depots presenting differentiated and modulated delivery of the agents. The CIDPs include micron or nanosized lipid and polymer-based carriers with suitable opportunities to deliver the therapeutic doses of the drug over the desired period of time [4]. Since, all these complex injectable formulations have presented differentiated and modulated drug release profiles as compared to their simple injectable formulations, the entire group of liposomes, nanoparticles, polymeric depots, suspensions and prodrugs have been included in the group of CIDPs [5]. Traditionally, these formulations have been designed to enhance patient compliance, mitigate the issues associated with drug delivery at the intended sites, modulate drug half-life, and improve therapeutic outcomes. Over the last few years, development of effective CIDPs has been a major focus of the researchers to deliver the cargo in naïve state [6]. CIDPs have presented complex combination of release kinetics and have been frequently associated with multiphasic drug release from the prepared formulations. Importantly, the development of generic versions to approved clinical therapeutics would necessitate the development of appropriate discriminatory testing release parameters which would

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