CHAPTER - 6

PHARMACOKINETICS

6.1 INTRODUCTION

For evaluation of drug delivery formulation and to ascertain its safety and efficacy, it is essential to conduct pharmacokinetic studies along with repeat dose toxicity studies to establish no observed adverse effect level (NOAEL) in a clinically relevant pre-clinical species. NZ rabbit has been used over the years and is a validated pre-clinical model for intra-nasal formulation evaluation, hence it was selected in the present study to ascertain both nasal irritation potential as well as pharmacokinetics.

6.2 NASAL IRRITATION POTENTIAL

Nasal mucosa has an important protective function by preventing entry of microorganisms and noxious substances from the environment into the body (1). The ocular, nasal, oral, gastrointestinal, vaginal and rectal mucosa can be exposed accidentally or intentionally to pharmaceuticals, personal care products, cosmetics, and chemicals. However, many compounds can irritate the mucosa. Mucosal irritation and damage might increase the susceptibility to pathogens (2) and decrease the patient compliance. Because pharmaceuticals may be applied frequently over a period of months or years, it is extremely important to evaluate the mucosal irritation potential or mucosal tolerance of pharmaceutical formulations.

Pre-clinical studies to evaluate the ocular, nasal, oral, vaginal, and rectal tolerance of both the active agent and the clinical formulation are carried out in vertebrates in compliance as per EU directives and guidelines (3).

While there is significant knowledge about formulation variables to facilitate nasal absorption of both big and small molecules, the knowledge of physicochemical factors of nasal formulations on the potential to cause nasal irritation is limited (4). Studies have shown that various buffers, solution pH, osmolarity and absorption enhancers cause the release of total proteins, phospholipids, membrane-specific proteins and cholesterol which are used as biomarkers representative of nasal damage. Instillation of any molecule into the nasal cavity may provoke a biochemical response in a preclinical model, and this is important to understand by evaluation of magnitude and extent of the release of biomarkers. These conditions are then replicated in clinical nasal lavage studies in humans. Excipients need to be adequately assessed of irritation potential. In the present study, we compared the total protein & lactate dehydrogenase (LDH), and the

rate of release of biomarkers from the rabbit nasal cavity under realistic dosing and bolus wash conditions that can be replicated in clinical nasal lavage studies. The solutions were repeated in a small volume (0.025 ml) into the rabbit nasal cavity and 20 min after the dose, the well was washed with normal bolus saline solution, collected and analyzed for total protein and lactate dehydrogenase (LDH). These analytes were selected to shed light on the potential mechanism of acute damage, namely, mucosal damage, vascular leakage or onset of inflammation. LDH (a cytosolic enzyme) reflects mucosal damage attributed to the rupture of the membranes of epithelial cells releasing cytosolic contents; the secretion of total proteins representing leached mucosal proteins and vascular leakage of damaged blood vessels.

6.2.1 Materials and Methods

Buffers, benzalkonium chloride (BKC) were obtained from Spectrum Chemical Co, USA. Normal saline (0.9% NS) was obtained from Baxter. IH Tapentadol nasal solution (100 mg/ml) solution was manufactured and filled in 0.025ml nasal pumps to provide 5 mg/rabbit dose.

In this study, 12 healthy male New Zealand white rabbits (2.18 ± 0.24 kg) of approximately 18 months of age were used. All animal experiments met the requirements of the institutional ethics committee on the use of experimental animals. Rabbits were divided into three groups comprising 4 animals each. All animals were kept to fastovernight but allowed free access to water

6.2.2 Standardization of Nasal irritation potential method

For the standardization of method, BKC 1% and 4 % solution was prepared in purified water and administered to Rabbits nostril up to 7 days for 1 % and 4 days for 4% solution as a positive control.

This was subjected as BKC is known tocause mucosal irritation and stimulate the release of nasal irritation and inflammation biomarkers such as Total protein (TP) and Lactose dehydrogenase (LDH).

For the standardization following Protocol and procedure was followed:

Rabbits were divided into 3 groups and were given 0.025 ml spray in each nostril as per protocol.

Group	Dose Level	No of Rabbits
I	NS Control	4
II	Positive Control (BKC) (1%) (Total 0.5 mg/ Rabbit / day) up to 7 days	4
III	Positive Control (BKC) (4%) (Total 2.0 mg/ Rabbit/ day) up to 4 days	4

Nasal lavage was taken 30 min prior to dosing and 1 hour after dosing and analyzed for LDH and total protein, lavage was centrifuged at 500 G and stored at -70^0 C until analyzed daily clinical signs, body weight, food consumption was noted in all group of animals.

To wash the nasal cavity, the rabbits were held supine and face down on their back; the rear was raised at an angle of 45 degrees while the head remained horizontal. In this way, drainage of the NS from the nasal cavity was avoided. Washing was immediately collected from the nostrils after washing by returning the rabbit to its normal position.

The reported inflammation cut-off values LDH in nasal lavage increases more than 2/3 of plasma value / ~ 3 fold indicates inflammatory condition in nasal cavity.

In the case of protein if increases more than 2.9 g/dl (>10 fold than control) will indicate inflammatory condition in the nasal cavity. The estimated TP & LDH values from the study conducted is presented below in the Table number 6.1.

Table 6.1: Total protein value for method standardization

Total Protein activity (BCA assay)			
Solution	**Sampling schedule**	**value μg/ml**	**SD**
NS	0'HOUR-DAY1	430.0	148.10
	1'HOUR-DAY1	430.0	252.70
	1'HOUR-DAY3	305.3	132.11
	0'HOUR-DAY5	378.9	109.59
	1'HOUR-DAY5	378.9	62.95
	0'HOUR-DAY7	756.9	204.3
	1'HOUR-DAY7	541.7	127.1
	0'HOUR-DAY10	409.3	93.9
1% BKC	0'HOUR-DAY1	318.2	40.08
	1'HOUR-DAY1	682.9	164.04
	1'HOUR-DAY3	735.9	311.89
	0'HOUR-DAY5	606.5	190.68
	1'HOUR-DAY5	928.1	102.43
	0'HOUR-DAY7	661.0	115.9
	1'HOUR-DAY7	661.7	152.7
	0'HOUR-DAY10	448.2	122.4
4% BKC	0'HOUR-DAY1	482.3	111.77
	1'HOUR-DAY1	437.0	236.15
	1'HOUR-DAY3	1456.8	546.09
	0'HOUR-DAY5	1080.7	433.66
	1'HOUR-DAY5	1378.0	474.64
	0'HOUR-DAY7	936.8	185.2
	1'HOUR-DAY7	1188.1	382.3
	0'HOUR-DAY10	699.5	288.9

Total Protien Value (µg/ml)

Figure 6.1: Total protein value in comparison to normal salineand 1%, 4% BKC administration

Table 6.2: Total LDH value for method standardization

Total LDH activity			
Solution	**Sampling schedule**	**Value IU/ml**	**SD**
NS	0'HOUR-DAY1	0.35125	0.252
	1'HOUR-DAY1	0.215	0.083
	1'HOUR-DAY3	0.161	0.025
	0'HOUR-DAY5	0.2	0.06
	1'HOUR-DAY5	0.1	0.02
1% BKC	0'HOUR-DAY1	0.17625	0.033
	1'HOUR-DAY1	0.858	0.503
	1'HOUR-DAY3	0.97775	0.552
	0'HOUR-DAY5	0.496	0.40
	1'HOUR-DAY5	0.78725	0.40
	1'HOUR-DAY7	1.91	0.89
	1'HOUR-DAY10	0.47	0.15
4% BKC	0'HOUR-DAY1	0.6375	0.384
	1'HOUR-DAY1	0.497	0.331
	1'HOUR-DAY3	1.92975	1.032
	0'HOUR-DAY5	1.7495	0.36
	1'HOUR-DAY5	1.594	0.70
	1'HOUR-DAY7	2.55	0.93
	1'HOUR-DAY10	1.27	1.02

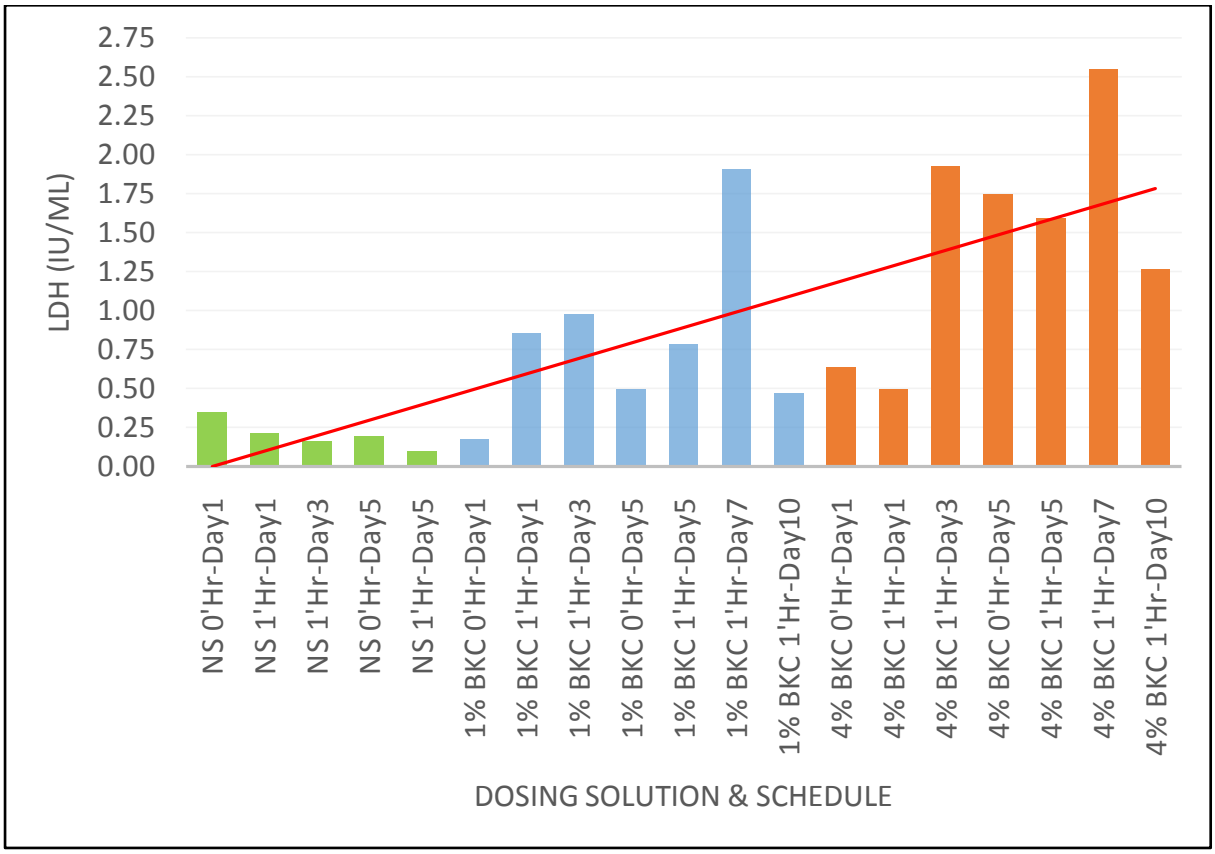

Figure 6.2: Total LDH value in comparison to normal saline and 1%, 4% BKC administration

Based on the study results it was observed that 1% BKC cause overt Clinical Signs (Mild Watery Nasal Discharge). Further, Day-3 onwards marginal increase in TP value was observed and 3 fold increase in LDH was observed which is in line with literature findings.

4% BKC produced overt clinical signs (watery nasal discharge) and more than 3-fold increase in both the inflammatory biomarkers (LDH and TP) on day three.

Hence, the study demonstrated that the experimental methodology can be adopted to ascertain the nasal irritation potential of any chemical entity or excipient. Clinical manifestation and biomarker analysis were used to ascertain the nasal irritation potential of Tapentadol in the present study.

Tapentadol was instilled in rabbits by intra-nasal route to evaluate the irritation potential both single and multiple dosesrepeatedly,the findings indicate that Tapentadol can be administered safely as there was no significant change in the biomarkers trend when

compared to placebo control group.The results are enumerated in the complete patent specification.

PHARMACOKINETICS

A pharmacokinetic study was planned to evaluate selected formulations for systemic availability through the intranasal route in NZ rabbits. Preliminary pharmacokinetic study of Tapentadol aqueous solution in Rabbits showed the highly promising result for systemic availability. As per company's internal policy, the formulation was taken for Phase 1/3 clinical trial for commercialization & provisional patent has been filed for the human pharmacokinetic data. Data from pre-clinical species was used to design Phase 1 studies using allometric scaling (5). The selection of an appropriate site of drug administration is one of the most important factors for optimizing its therapeutic effects (6) Selected formulations were designed using aqueous Gellan gum as a mucoadhesive agent to enhance the nasal retention time and drug encapsulated in SLN preparations has been administered by intranasal route for the present PK study. Pharmacokinetic studies of designed formulations were conducted as per GLP principles.

In the present study, intranasal solid lipid nanoparticles, as well as drug dispersed in-situ Gellan gum based drug delivery system, was formulated and PK study was conducted in NZ rabbits(7).

As discussed in Aims and Objectives, for the analgesic drug, early and higher C_{oax} and shorter $T_{max}$is desired to produce the onset of pharmacological action.

Intranasal administration being a non-invasive method can be an advantageous strategy for delivering Tapentadol into the brain (8). In the current scope, PK study was conducted using TapentadolHCloral solution as a standard dosage form and was compared with two intranasal formulations namely Tapentadol in Gellan gum in-situ gel and SLN particles suspension at an equivalent dose.

Tapentadol free drug concentrations were used to estimate the absorption kinetics and ultimately for the overall assessment of nasal drug delivery system. Direct Brain estimation was not done as it is well reported in the literature (8) in distribution studies conducted in Pre-clinical studies that radioactivity in the target tissues (brain and spinal cord) was 2-fold and 1.4-fold higher than the blood, respectively, indicating good absorption by the CNS.

6.4 MATERIALS AND METHODS

Pure Tapentadol base has been prepared in the laboratory and was used for formulation preparation. The first formulation selected was wherein the drug is dispersed in Gellan gum aqueous solution. In the second formulation, the drug was encapsulated in SLN matrix as per method enumerated in Chapter 5 of this thesis. Oral solution was prepared by dissolving the drug in 1% NaCl solution. Surgical instruments scissors, forceps, glass hypodermic syringes, etc. were used in-house.

6.5 DOSING OF ANIMALS AND SAMPLE COLLECTION

In this study,twelve healthy male New Zealand white rabbits (2.18 ± 0.24 kg) of approximately 18 months of age were used. All animal experiments met the requirements of the institutional ethics committee on the use of experimental animals. Rabbits were divided into three groups comprising four animals each. All animals were kept to fastovernight but allowed free access to water. Prior to nasal administration, the restriction devices were placed in the marginal vein of each animal. Blood samples (0.5 ml) were collected from the restriction devices for control (at 0 min), and then two intranasal formulations were instilled (0.05 ml in both nostrils) using supine micropipette. The oral solution was instilled using gavage. Blood samples were drawn from restriction devices from each rabbit of the marginal ear vein and collected in tubes containing heparin as an anticoagulant at 0.125, 0.25, 0.51, 2, 4, 6, 8, 12 and 24 h after administration. Samples were centrifuged within 0.5 h after collection and plasma samples were stored at -20 ° C until analysis.

The pharmacokinetic evaluation of Tapentadol base in Gellan gum and Tapentadolloaded SLN were performed in white New Zealand rabbits and PK profiles were compared with the oral solution of TapentadolHCl in 0.1%NaCl at equivalent doses.

The study protocol was approved by the Institute's Animal Ethics Committee (Protocol No. TRC / IAEC / 2014 / 085_E1), Ahmedabad, India. Rabbits were acclimatized for at least one week prior to the experiment. Throughout the experiment, the animals were housed in spotless cages maintained at 22 ± 2 ° C and 50-60% RH, under a 12:12 light-dark cycle. The animals were separated according to body weight and labelled with picric acid in the body.

The first rabbit group was treated with an oral solution, the second group with tapentadol base in aqueous Gellan gum solution intranasally. The third group of rabbits was treated with drug encapsulated in solid lipid nanoparticles and dispersed in an aqueous vehicle.

6.6 DOSAGE DELIVERY

For the oral group, the drug was dissolved in 1% NaCl aqueous solution and was administered using oral gavage at 5.1mg/kg of body weight of rabbit. For the second group, Gellan gum aqueous formulation was prepared (Gellan gum concentration of 0.4% w/w along with 0.2% HPMC 5cps in water for injection). The drug was then dispersed in the Gellan gum system to make a concentration of 129mg/gm of Tapentadol formulation. For the third group, a Lyophilized vial of Tapentadol encapsulated in SLN as enumerated in Chapter 5 was reconstituted with 3 ml of Gellan gum solution as used in group 2 to get a concentration of 129 mg/gm suspension.

6.6.1 Dose calculation and study groups:

Study groups: All the study groups were given a Human equivalent dose of 100mg using allometric scaling.

Human IR dose	100 mg
Human dose (mg/kg)	1.66 mg/kg
Rabbit factor (SA)	3.1
Rabbit dose	5.146 mg/kg
Considering 2.5 kg rabbit	12.86 mg/rabbit

6.6.2 Pharmacokinetic data analysis

Pharmacokinetic profile in administration of free Tapentadol base and SLN particles suspension compared to an oral solution is presented in Figure 6.3 and the pharmacokinetic parameters calculated using Winnolin is presented in Table 6.3 and Table 6.4

Mean Plasma Conc. Profile of Tapentadol in NZW Rabbits Male

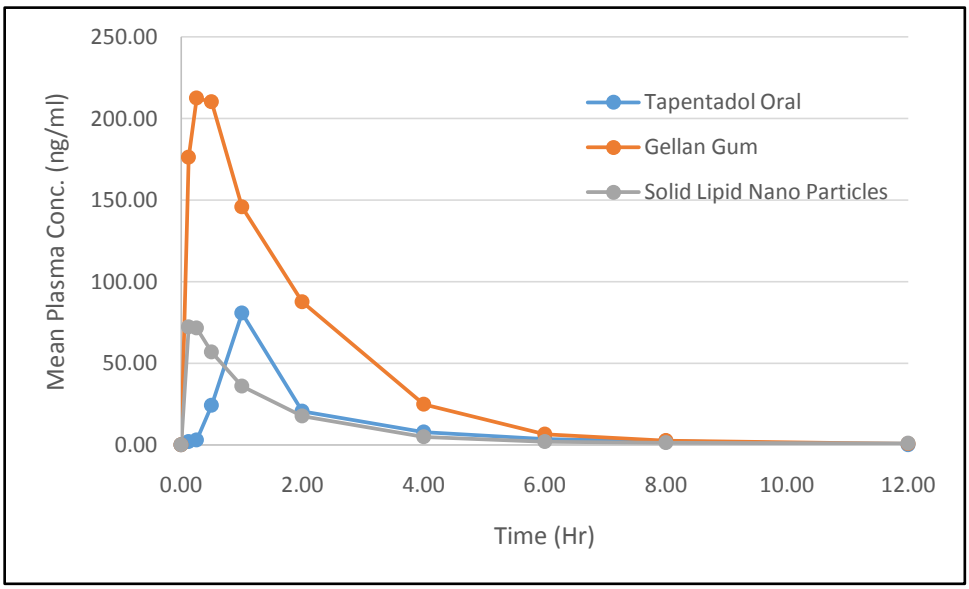

Figure 6.3: **Plasma Concentration versus time profile oral route versus two intranasal formulations**

Table 6.3 : **Mean plasma concentration versus time profile of oral versus intranasal Tapentadol base and SLN suspended particles**

Plasma concentration versus time profile						
Time (hr)	**Tapentadol Oral**		**Gellan gum formulation**		**Solid Lipid Nano particles**	
	Mean (ng/ml)	**SD**	**Mean (ng/ml)**	**SD**	**Mean (ng/ml)**	**SD**
0	0	0	0	0	0	0
0.125	2	0.5	176.143	40.978	72.478	32.242
0.25	3.15	0.95	212.591	28.274	71.682	31.144
0.5	24.44	9.6	210.228	52.781	56.899	22.244
1	80.87	30.5	145.811	33.575	35.985	15.558
2	20.68	8.6	87.569	37.394	17.738	7.344
4	7.82	2.5	24.961	15.29	5.11	0.745
6	3.64	1.3	6.594	2.973	2.167	0.433
8	2.5	0.8	2.578	0.908	1.494	0.581
12	0.15	0.1	0.785	0.61	0.952	0.293
24	0	0	0.561	0.394	1.154	0.529

Table 6.4: Summary of Pharmacokinetic parameters OralTapentadol versus Intranasal Tapentadol in Gellan gum versus Intranasal Solid Lipid Nanoparticles

Summary of PK Parameters							
PK Parameters	**Units**	**TapentadolOral**		**Gellan gum**		**SLN**	
		Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
Kel	hr^{-1}	0.17	0.16	0.19	0.24	**0.14**	**0.14**
$T_{1/2}$	hr	6.65	4.90	9.67	8.02	11.58	10.05
C_{max}	**ng/ml**	**80.87**	**56.55**	**212.59**	**39.45**	**72.47**	**27.80**
T_{max}	**hr**	**1.63**	**0.50**	**0.37**	**0.144**	**0.19**	**0.07**
AUC	**hr*ng/ml**	**160.73**	**60.25**	**442.32**	**102.50**	**124.08**	**35.50**
T_{last}	Hr	8.00	2.05	21.00	6.00	**21.00**	6.00

Using Outlier testing, data has been eliminated

6.7 RESULTS AND DISCUSSION

The TAP plasma concentration-time curves after single oral solution and two intranasal formulations namely Gellan gum and SLN are shown Fig 6.3. The various pharmacokinetic parameters calculated using Winnolin software are presented in Table 6.4. Pharmacokinetic profile of oral solution showed a slow absorptive phase up two hours and biphasic elimination with no detectable concentrations beyond 12 hours. The non-compartmental data analysis of Oral solution indicated an AUC to 160.73 ng.hr/ml and C_{max}80.87 ng/ml at T_{max} of 1.63 hours. As compared to Oral solution, the Gellan gum preparation of TAP resulted in significant increase in AUC to 442 ng.hr/ml with more than 2.5 fold enhancement and $C_{max}$of 212.59 ng/ml, greater than 1.5 fold enhancement, at T_{max} at less than 25 min, 4 fold shorter time. The bioavailability may be attributed to the change in route of administration which by passes the first- pass effect as well increase of nasal residence time owing to the muco-adhesive properties of Gellan gum which can overcome mucociliary clearance. On the contrary to the findings of Gellan gumformulation, SLN formulation when compared with oral, AUC and C_{max} were comparable but a significant reduction of T_{max} achieved in less than 15 mins which are almost 8 times faster. The shortest time to T_{max} has high clinical relevance for management of acute or breakthrough cancer pain by not an invasive route. This may be

attributed probably to rapid absorption of the SLN particles to brain with relatively higher mucociliary clearance as compared to Gellan gum, hence no bioavailability improvement is observed with SLN particles. The elimination rates are also significantly decreased in case of both Gellan gumas well as SLN when compared to oral solution indicating enhanced residence time of the formulations in the body. The decrease in elimination rates for Gellan gum dosage form may be attributed to a longer residence in the nasal mucosa owing to bioadhesive. The decrease in elimination rates in case of SLN particles may be attributed slow release of the drug from the lipidic matrix. The decrease in elimination rates indicates the potential to decrease the frequency of dosing.

Summarizing the findings of the three formulations dosed, shortest T_{max} was observed with Solid lipid nanoparticles which is less than 15 mins when compared to Gellan gum which is close to 25 mins and the oral solution took the longest time to reach T_{max} about1.5 hours. Gellan gum formulations exhibited significantly greater than2.5fold enhancement in exposure as compared to Oral as well as solid Lipid nanoparticles. This may be attributed to highest localized concentration gradient in the nasal mucosa with higher surface area. Elimination rates were slowest with Solid lipid nanoparticles due to encapsulation of the drug in lipidic matrix followed by Gellan gum formulation when compared to oral. Thus, the dosage form designed met the expectations of the hypothesis postulated

Further, multiple dose simulations were carried out using Pharmacokinetics Winnolin software version 7.1 (Phoenix) to assess the steady state Pharmacokinetics using Non-parametric superimposition technique. Multiple dose pharmacokinetics was assessed up to 24 hrs with two different frequency of administration (4 hrs and 6 hrs) with a similar dose which was given in NZ rabbits (~3.7 mg/kg every four or 6 hours). The non-parametric super-imposition technique is employed with loading calculated as 11.1mg/kg using the standard formula and maintenance dose as 3.7mg/kg for assessment of Multiple dose pharmacokinetics.

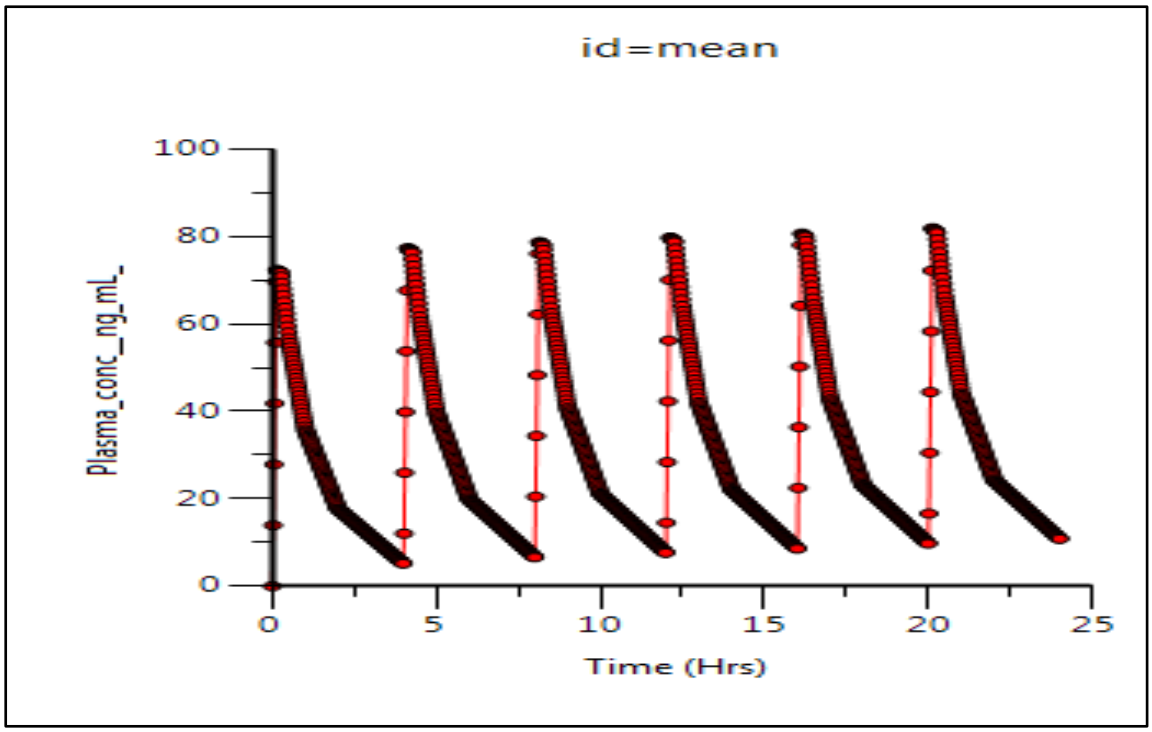

Figure 6.4: Repeat Dose Pharmacokinetics Simulation with every 4 hrs administration

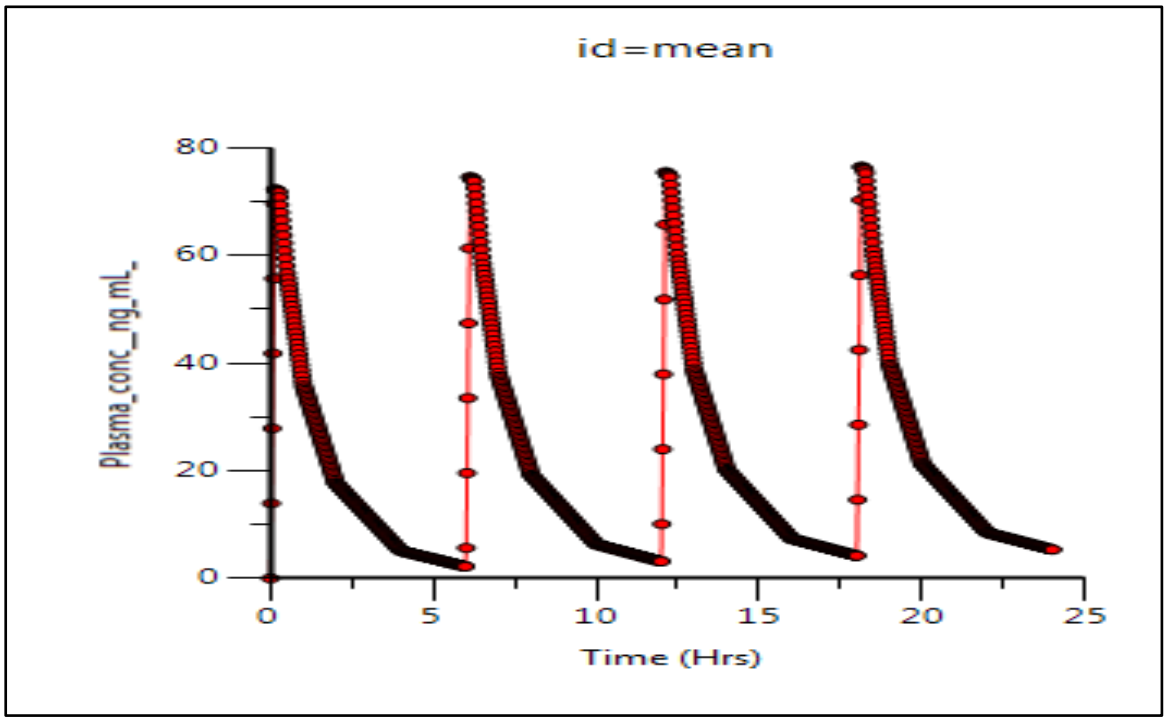

Figure 6.5: Repeat Dose Pharmacokinetics Simulation with every 6 hrs administration.

No dose accumulation was observed with repeated dose administration either with 4 hrs and 6 hrs dose administration and steady state can be achieved immediately from the very first dose.

Extrapolation to obtain brain concentrations

Additionally, an indirect methodology was adopted to estimate probable brain concentrations based on plasma concentration profile and data, as the sacrifice of rabbits, guinea pigs are prohibited in Torrent Research Center, direct estimation of brain concentration was not possible.

As described and reported in the literature that target tissue (Brain and spinal cord) is having more concentration than blood, therefore current data is extrapolated to 2X times and clearance was calculated with Non-compartmental analysis(9). Direct extrapolation is done as Tapentadol follows Linear Pharmacokinetics.These extrapolation is made based on plasma TAP concentration at different time points.

Table 6.5: Extrapolated brain values from Tapentadolplasma concentration

Tapentadol oral versus intranasal instillations extrapolated brain concentrations						
	Oral		**Gellan gum**		**SLN**	
Time in Hours	**Plasma conc ng/ml**	**Brain conc in ng/gm**	**Plasma conc ng/ml**	**Brain conc in ng/gm**	**Plasma conc ng/ml**	**Brain conc in ng/gm**
0	0.00	0.00	0.00	0.00	0.00	0.00
0.125	3.15	6.29	176.15	352.29	72.48	144.96
0.25	24.44	48.88	212.59	425.18	71.68	143.36
0.50	80.87	161.73	210.23	420.46	56.90	113.80
1	20.68	41.35	145.81	291.62	35.99	71.97
2	7.82	15.63	87.57	175.14	17.79	35.48
4	3.64	7.27	24.96	49.92	5.11	10.22
6	2.50	5.00	6.59	13.19	2.167	4.33
8	0.15	0.30	2.58	5.16	1.50	2.99
12	0.00	0.00	0.79	1.57	0.95	1.90
24	0.00	0.00	0.56	1.12	1.16	2.31

Linear Trapezoidal Linear Interpolation was used to calculate the PK parameters

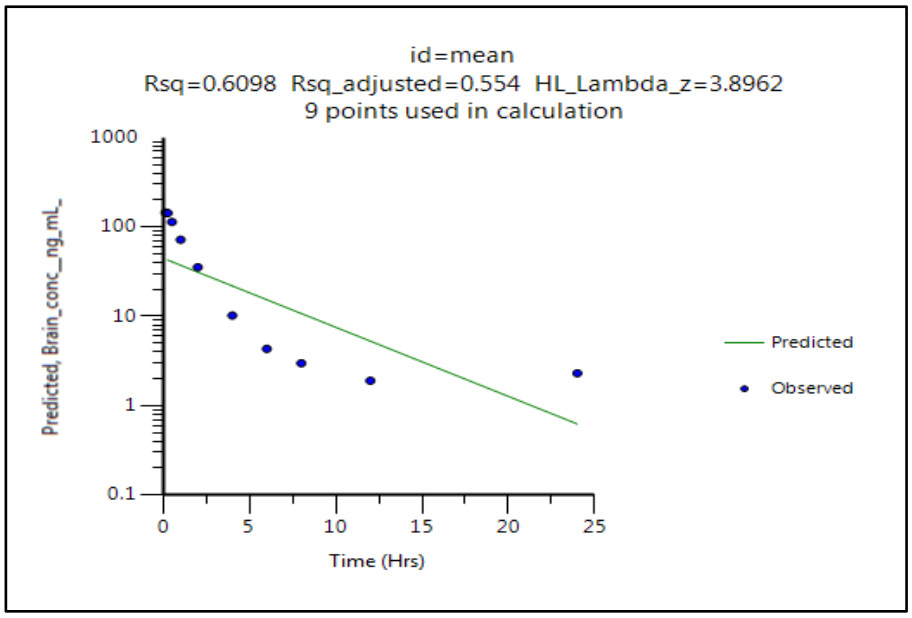

Figure 6.6: Log Transformed Brain concentration of Tapentadol SLN

(Predicted Vs Observed)

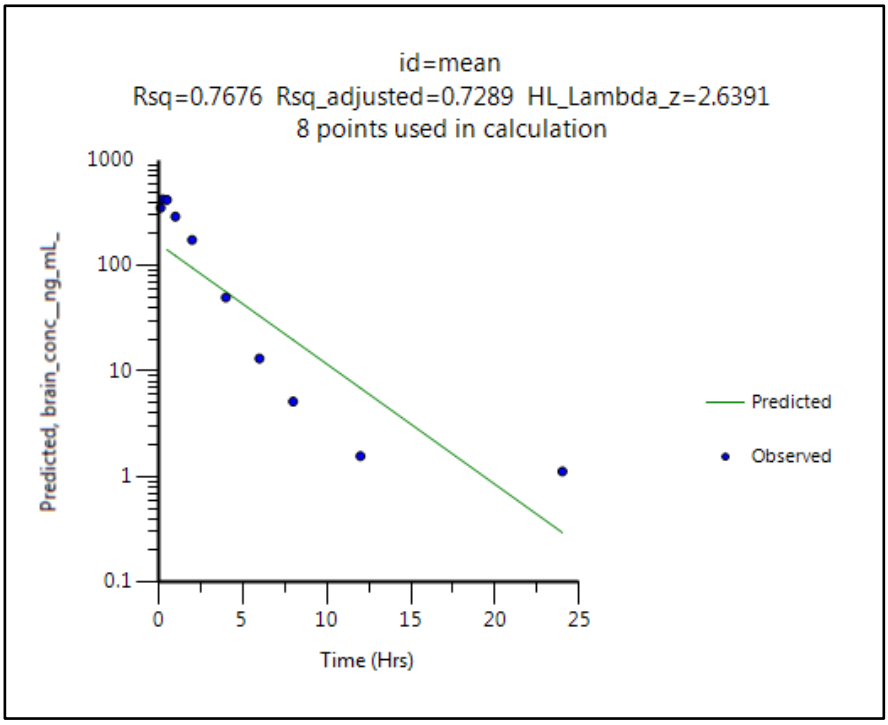

Figure 6.7: Log Transformed Brain concentration of Tapentadolgel(Predicted Vs Observed)Phoenix modeling software was used to calculate PK parameters

Table 6.6: Extrapolated brain PK Parameters

Estimated PK Parameters from extrapolated brain concentrations				
Parameter	Units	Brain Oral	Brain -Gellan gum	Brain_SLN
$T_{1/2}$	hr	2.5	2.639	3.896
T_{lag}	hr	0	0	0
T_{max}	hr	1	0.25	0.125
C_{max}	ng/gm	161.73	425.182	144.956
T_{last}	hr	8	24	24
C_{last}	ng/gm	0.25	1.122	2.308
AUC	ng*hr/gm	326	923.83	262.01
K_{el}	hr^{-1}	0.21	0.262	0.177

Brain extrapolation data indicates sufficient and sustained exposure of Tap in both Gellan gum and SLN formulations when compared to oral. Shortest T_{max} was achieved with the SLN particles & highest C_{max} with Gellan gum formulation but it would be prudent to explore SLN formulations in Human PK studies as it scores over the balance of efficacy versus adverse drug reactions. However, in case of SLN brain concentration is expected to be much higher as TPA might have transported directly to the brain from nasal route as found in earlier research work from the same group.(Ph.D. thesis of VibhuNagpal titled "Studies on Liposomal Delivery of Dopamine to brain via nasal route" 168, 2015, BITS, Pilani)

6.8 CONCLUSION

When compared to an Oral solution both Gellan gum as well SLN by intranasal route reduced the time to T_{max} significantly.

$T_{1/2}$ was highest with SLN formulation in comparison to Gellan gum and oral solution which indicates the slow rate of absorption and improvement of mean residence time hence sustained effect can be achieved with SLN based formulation.

From the estimated Brain PK parameters, it is clearly indicating that elimination rate (K_{el}) is very slow in SLN with respect to Gellan gum formulation and oral formulation. Also, half-life in the target tissue i.e. brain is highest with SLN and

corresponds to early T_{max} as compared to Gellan gum and oral. The concentration at the last time point (C_{last}) also indicates sustained release of the drug thus prolonged action in the brain using SLN formulation.

Pharmacokinetics studies confirmed that effective delivery of Tapentadolis achieved by altering the route of administration from oral to nasal.

Solid lipid nanoparticles by i.n. the route can produce the shortest onset of action as well as longer duration of action. Actual concentration in brainfor SLN TAP might be higher. Thus, the dosage form designed met the expectations of the hypothesis postulated. The pharmacokinetic and extrapolated brain concentrations indicate both the formulation designed Gellan gum and SLN are highly promising and would offer significant clinical advantages over the current marketed oral formulation with respect to early onset of action

Thus, the dosage form designed met the expectations of the hypothesis postulated.