Contents

9.1 INTRODUCTION

With the aim of addressing poor bioavailability issues associated with currently available marketed formulations of both VPN and NPT, the most preferred alternative to oral route i.e. transdermal route have been explored. As described in previous chapters, various UDLs and PNPs loaded fast dissolving MN patches of both the drugs were successfully developed and evaluated *in vitro* as well as *ex vivo* for characteristics, most suited for transdermal delivery.

Present chapter has been devoted to pharmacokinetic (PK) and pharmacodynamic (PD) evaluation of VPN and NPT via their newly developed formulations. The *in vivo* studies were performed to provide a better insight regarding the potential of these newly developed formulations in solving the aforesaid problem.

9.2 METHODS

9.2.1 Study protocol authorization

The *in vivo* studies were carried out in accordance with the guidelines issued by the Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. The protocols for both

pharmacokinetic and pharmacodynamic studies of VPN (No.: MSU/IAEC/2014-15/1408) and NPT (No.: MSU/IAEC/2016-17/1632) were approved by Institutional Animal Ethics Committee of Faculty of Pharmacy, The Maharaja Sayajirao University of Baroda, Vadodara, India.

9.2.2 Animal procurement

The healthy female Sprague-Dawley (SD) rats weighing 220-240 g were used for *in vivo* studies. The rats were procured from Zydus Research Centre, Ahmedabad and housed in appropriate cages at the animal house facility of Faculty of Pharmacy recognized by CPCSEA (Reg. No.: 404/PO/Re/S/01/CPCSEA; dated 28th October, 2015). The temperature of the animals' room was maintained at 21-23 °C with a 12:12 h light-dark cycle. All animals were maintained on a standard diet with free access to water and allowed one-week acclimatization period before initiating the experiments. Good Laboratory Practice was followed for animal handling routines.

9.2.3 Animal equivalent dose calculation

VPN and NPT at their lowest recommended human dose (15 and 10 mg/day, respectively) were used for both PK and PD studies. The animal equivalent dose (AED) of VPN and NPT were calculated as per USFDA guidelines (1) using **Eq. 9-1**.

$$AED = \frac{\left(\frac{D_H}{W_H}\right) \times K_H}{K_A} \qquad \textbf{Eq. 9-1}$$

where,
AED = animal equivalent dose (in mg/kg);
D_H = Human daily dose (VPN, 15mg; NPT, 10 mg);
W_H = average weight of healthy human adult (60 Kg);
K_H = Human constant (37) and
K_A = Animal constant (6)

The AED thus calculated for VPN (1.54 mg/Kg) and NPT (1.03 mg/Kg) were utilized to estimate accurate dose requirement for individual rats based on their body weight.

9.2.4 Pharmacokinetic study of VPN

After acclimatization, a total of 30 rats were randomly allocated to 5 treatment groups of 6 rats each as summarized in **Table 9-1**. The dorsal surface of rats, assigned to receive transdermal formulations (groups PK-I to PK-IV), were carefully shaved using electrical clipper avoiding any damage to skin surface. Animals of group PK-V were fasted overnight with free access to water before dosing.

Table 9-1. Treatment groups for pharmacokinetic study of VPN

Groups	Treatment
PK-I	Transdermal patch of VPN loaded UDL (VPN UDL TP)
PK-II	Transdermal MN patch of VPN loaded UDL (VPN UDL MNP)
PK-III	Transdermal patch of VPN loaded PNP (VPN PNP TP)
PK-IV	Transdermal MN patch of VPN loaded PNP (VPN PNP MNP)
PK-V	Per oral suspension of VPN (VPN Susp Oral)

The rats of each group were administered with the formulations containing calculated dose of VPN as per the treatment plan (**Table 9-1**). 0.5 ml of blood samples were collected from the lateral tail vein at 0.5, 1, 1.5, 2, 4, 8, 12 and 24 h using 23 G needle in 1.5 ml clean lock-capped micro-centrifuge tubes containing 0.03 ml of 100 IU heparin solution. The blood cells from these heparinized whole blood samples were removed by centrifugation at 1500 g and 4°C for 10 min (Remi Centrifuge, Mumbai, India). The supernatant plasma (0.25 ml) samples were taken in separate ria vials. 2 ml of chilled Acetonitrile (at -20 °C) was added to all samples and vortexed for 5 min for VPN extraction and protein precipitation. The samples were centrifuged at 1700 g at 4 °C for 15 min to allow settling of precipitated proteins. Supernatant layer was carefully transferred to ria vials and evaporated to dryness at 40 °C under the gentle stream of nitrogen. The dried residues were further reconstituted with 25 μl of mobile phase and vortexed for 30 seconds. The VPN present in these samples were quantified using the HPLC method described earlier in chapter 3. These plasma drug concentrations at various time points were utilized to calculate different pharmacokinetic parameters viz., C_{max} (peak plasma concentration), t_{max} (time to reach peak plasma

concentration), AUC_{0-t} (area under the plasma drug concentration versus time curve up to last sampling time point), $AUC_{0-\infty}$ (area under the plasma drug concentration versus time curve up to infinite time), $t_{1/2}$ (half-life), MRT (mean residence time) etc. Thermo Scientific™ Kinetica Software version 5 was used to calculated these parameters. Relative bioavailability for all the transdermal formulations were also calculated with respect to orally administered standard dose using **Eq. 9-2**.

$$F_{rel} = \frac{(AUC_{0-\infty})_{test}}{(AUC_{0-\infty})_{std}} \times 100 \qquad \textbf{Eq. 9-2}$$

where,

$(AUC_{0-\infty})_{test}$ = extent of drug absorption via transdermal formulation
$(AUC_{0-\infty})_{Std}$ = extent of drug absorption via oral formulation

9.2.5 Pharmacokinetic study of NPT

After acclimatization, a total of 30 rats were randomly allocated to five treatment groups of six animals each as summarized in **Table 9-2**. The dorsal surface of rats, assigned to receive transdermal formulations (groups PK-VI to PK-IX), were carefully shaved using electrical clipper avoiding any damage to skin surface. Animals of group PK-V were fasted overnight with free access to water before dosing.

Table 9-2. Treatment groups for pharmacokinetic study of NPT

Groups	Treatment
PK-VI	Transdermal patch of NPT loaded UDL (NPT UDL TP)
PK-VII	Transdermal MN patch of NPT loaded UDL (NPT UDL MNP)
PK-VIII	Transdermal patch of NPT loaded PNP (NPT PNP TP)
PK-IX	Transdermal MN patch of NPT loaded PNP (NPT PNP MNP)
PK-X	Per oral suspension of marketed NPT powder (NPT Susp Oral)

The animals were administered with the formulations containing calculated dose of NPT as per the treatment plan (**Table 9-2**). A similar procedure was followed for blood withdrawal, plasma sample preparation and calculation of pharmacokinetic parameters as discussed above for pharmacokinetic study of VPN. The NPT present in these samples were quantified using the HPLC method described earlier in chapter 3.

9.2.6 Pharmacodynamic study of VPN

The effectiveness of VPN loaded formulations were evaluated against ketamine, an NMDA receptor antagonist, induced amnesia in healthy female Sprague-Dawley rats weighing 220-240 g using morris water maze test. A total of 42 rats were randomly allocated to 7 treatment groups of 6 animals each as summarized in **Table 9-3**.

Table 9-3. Treatment groups for pharmacodynamic study of VPN

Group	Treatment
PD-I: Normal control	Healthy animals without any treatment
PD-II: Model control	Intra-peritoneal ketamine injection (A)
PD-III: Vehicle control 1	Placebo UDL MNP followed by (A)
PD-IV: Test V1	VPN UDL MNP followed by (A)
PD-V: Vehicle control 2	Placebo PNP MNP followed by (A)
PD-VI: Test V2	VPN PNP MNP followed by (A)
PD-VII: Standard control V	VPN Susp Oral followed by (A)

9.2.6.1 Spatial Learning performance using Morris Water Maze test

The Morris water maze test was carried out in a black round water tank having a diameter of 153 cm and a height of 63 cm. The tank was filled to a depth of 33 cm with water and the temperature of water was kept around 26 °C.

☆ Training Procedure: The tank was divided into four imaginary quadrants [8]. The rats were taken from their home cage and placed into the water maze at the designated quadrant facing the wall. They were allowed a maximum of 60 seconds to locate the platform. After 60 seconds in the water maze, the rats were guided to the platform with the use of a wooden plank and later returned to home cage. Each rat was trained to remember the location of the initially visible platform with a diameter of 8 cm that is subsequently submerged in water during acquisition. A 6 minutes of interval was given between each trial and latency to reach the platform with all four paws was recorded for each rat. A reduction in the latency to localize the platform over successive trials was indexed as learning. In order to obtain Spatial Learning performance per rat, mean latencies was calculated across the trials per session for each rat. The Morris water maze training was accomplished

within 5 consecutive days, with 2 sessions (morning, afternoon) per day, each session consisting of 4 trials for a final total of 40 trials (**Fig. 9-1**).

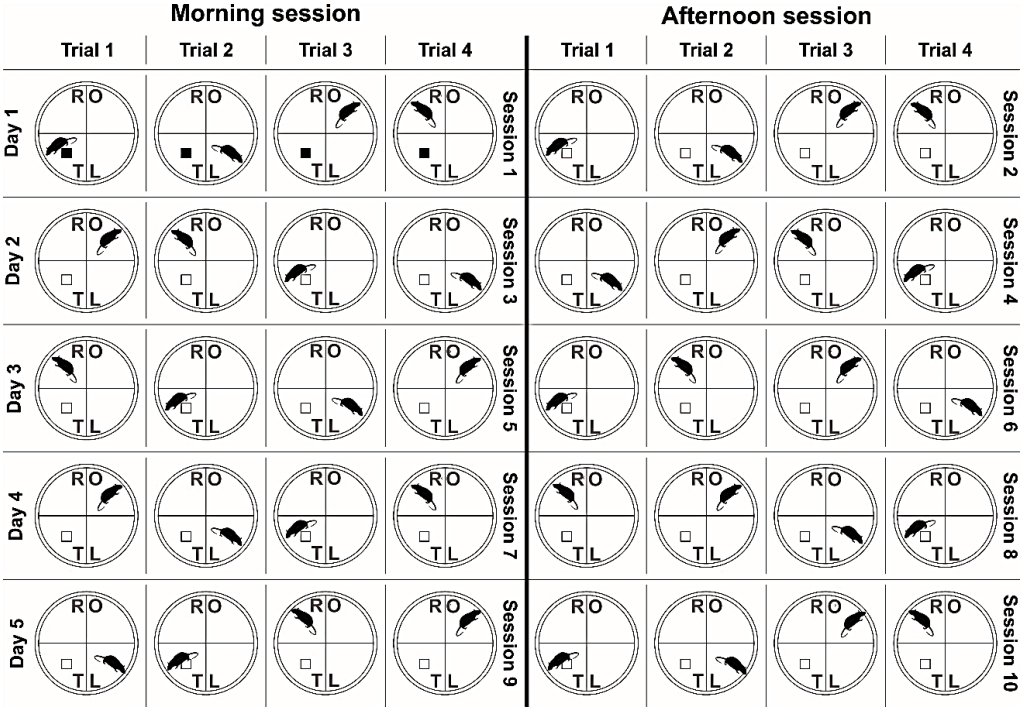

Fig. 9-1. Training schedule for each rat

☆ Test Procedure: On sixth day, the rats of Group-III to Group-VI were shaved on the neck region of their skin by electrical clipper avoiding any damage to skin surface. Transdermal patches were applied to shaved area as per the treatment schedule (**Table 9-3**). Rats of Group-VII were administered with suspension of marketed VPN tablet orally using oral feeding tube. One hour after applying the formulations, an intra-peritoneal injection of ketamine (dose, 12 mg/kg body weight) was given to each rat of Group-II to Group-VII to induce amnesia. After another one hour, the mean escape latency of each animal was recorded using morris water maze.

9.2.7 Pharmacodynamic study of NPT

The effectiveness of NPT loaded formulations were evaluated against ketamine, an NMDA receptor antagonist, induced amnesia in healthy female Sprague-Dawley rats weighing 220-240 g using morris water maze test. A total of 42 rats were randomly allocated to 7 treatment groups of 6 animals each as summarized in **Table 9-4**.

Table 9-4. Treatment groups for pharmacodynamic study of NPT

Group[#]	Treatment
PD-I: Normal control	*Healthy animals without any treatment*
PD-II: Model control	*Intra-peritoneal ketamine injection (A)*
PD-III: Vehicle control 1	*Placebo UDL MNP followed by (A)*
PD-V: Test N1	NPT UDL MNP followed by (A)
PD-VI: Vehicle control 2	*Placebo PNP MNP followed by (A)*
PD-VIII: Test N2	NPT PNP MNP followed by (A)
PD-X: Standard control N	NPT Susp Oral followed by (A)

[#] Groups shared from pharmacodynamic study of VPN are denoted in italics

A similar procedure was followed for training of rats and testing of formulations, as discussed above for pharmacodynamic study of VPN.

9.3 RESULTS & DISCUSSION

The results of pharmacokinetic and pharmacodynamic studies are presented and discussed in this section.

9.3.1 Pharmacokinetic study of VPN

The concentrations of VPN in blood plasma of rats were estimated by HPLC and the data are presented in **Table 9-5**. The data are also represented graphically in **Fig. 9-2** by plotting 'VPN concentration in plasma' on Y-axis and 'Time' on X-axis.

Table 9-5. VPN concentration in plasma at various sampling time points

Time (hr)	Plasma drug concentration (ng/mL)[#]				
	VPN UDL TP	VPN UDL MNP	VPN PNP TP	VPN PNP MNP	VPN Susp Oral
0.5	2.4 ± 0.4	4.4 ± 0.7	BQL	3.7 ± 0.7	13.9 ± 3.5
1	4.2 ± 0.7	8.3 ± 1.5	2.8 ± 0.8	5.4 ± 1.2	32.2 ± 4.9
1.5	7.9 ± 1.9	13.1 ± 3.4	3.5 ± 0.6	8.6 ± 1.3	27.5± 4.6
2	10.8 ± 2.4	17.4 ± 5.1	4.7 ± 1.2	11.2 ± 1.9	20.7 ± 3.4
4	9.7 ± 2.5	15.2 ± 3.9	6.7 ± 1.1	16.3 ± 2.6	11.2 ± 2.5
8	8.2 ± 1.5	12.9 ± 2.3	5.5 ± 0.8	13.4 ± 2.4	4.4 ± 0.7
12	6.8 ± 1.2	10.1 ± 1.9	4.9 ± 0.8	10.9 ± 2.3	BQL
24	4.6 ± 0.8	5.8 ± 1.2	3.6 ± 1.1	6.7 ± 1.2	BQL

[#] Mean ± SD; BQL, Below Quantification Limit

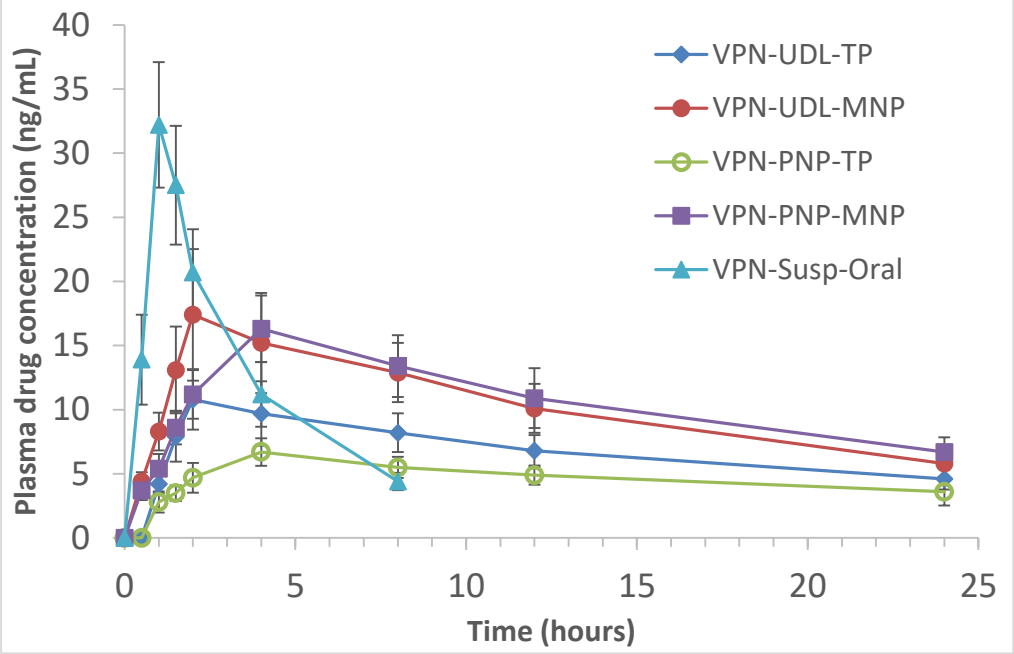

Fig. 9-2. Plasma VPN concentration vs Time profile of various dosage forms in Sprague Dawley rats

These data were utilized to obtain various pharmacokinetic parameters from Thermo Scientific™ Kinetica Software which are presented in **Table 9-6**.

Table 9-6. Pharmacokinetic parameters (VPN) computed using Kinetica Software

Parameters	VPN UDL TP	VPN UDL MNP	VPN PNP TP	VPN PNP MNP	VPN Susp Oral
C_{max} (ng/mL)	10.8	17.4	6.7	16.3	32.2
t_{max} (h)	2.0	2.0	4.0	4.0	1.0
AUC_{0-t} (ng*h/mL)	163.601	244.674	111.423	250.353	101.910
$AUC_{0-\infty}$ (ng*h/mL)	286.197	360.563	136.657	400.316	118.833
$t_{1/2}$ (h)	18.75	13.99	26.35	15.71	2.72
MRT (h)	28.07	21.22	39.36	24.35	4.27
F_{rel}	241	303	115	337	-

Administration of VPN suspension through oral route showed a rapid achievement of higher concentrations (C_{max}, 32.2 ng/mL and t_{max}, 1 hour) but for a shorter duration ($t_{1/2}$, 2.72 hours and MRT, 4.27 hours) as compared to other formulations. This clearly advocates the necessity of frequent dosing of currently marketed tablets. On the contrary, transdermal formulations exhibited $t_{1/2}$ ranging from 14-26 hours and MRT ranging from 21-39 hours signifying a better controlled plasma levels for prolonged duration. Among all transdermal fast dissolving patches, VPN PNP TP showed lowest C_{max} and AUC_{0-inf} indicating poor

penetrability of these carriers through intact skin. This was further supported by significant improvement of C_{max} and $AUC_{0\text{-}inf}$ observed via VPN PNP MNP. However, VPN UDL TP showed a higher C_{max} and $AUC_{0\text{-}inf}$ as compared to VPN PNP TP demonstrating the better penetrability of UDL than PNP across intact skin. Comparison of VPN UDL MNP and VPN PNP MNP showed insignificant difference in C_{max} and $AUC_{0\text{-}inf}$ suggesting comparable permeation via UDL as well as PNP across lower epidermis and dermis region to reach systemic circulation when stratum corneum barrier is physically breached using microneedles. The optimized transdermal formulations showed significantly low C_{max} when compared to orally administered suspension. However, it was found above reported MEC level of 0.8 ng/mL throughout the study duration (2). In addition, a >3-fold rise in relative bioavailability was observed advocating a chance of dose reduction in developed formulations.

9.3.2 Pharmacokinetic study of NPT

The concentrations of NPT in blood plasma of rats were estimated by HPLC and the data are presented in **Table 9-5**. The data are also represented graphically in **Fig. 9-3** by plotting 'NPT concentration in plasma' on Y-axis and 'Time' on X-axis. These data were utilized to obtain various pharmacokinetic parameters from Thermo Scientific™ Kinetica Software which are presented in **Table 9-7**.

Table 9-7. NPT concentration in plasma at various sampling time points

Time (hr)	Plasma drug concentration (ng/mL)#				
	NPT UDL TP	NPT UDL MNP	NPT PNP TP	NPT PNP MNP	NPT Susp Oral
0.5	BQL	2.1 ± 0.3	BQL	1.7 ± 0.2	15.8 ± 2.6
1	1.4 ± 0.2	3.5 ± 0.3	BQL	2.5 ± 0.4	7.4 ± 0.9
1.5	2.4 ± 0.4	4.6 ± 0.4	1.6 ± 0.2	3.8 ± 0.6	3.3 ± 0.4
2	2.7 ± 0.3	4.1 ± 0.7	2.1 ± 0.2	4.3 ± 0.8	BQL
4	2.4 ± 0.3	3.3 ± 0.3	1.7 ± 0.3	3.4 ± 0.4	-
8	1.8 ± 0.3	2.4 ± 0.2	1.5 ± 0.3	2.7 ± 0.2	-
12	1,2 ± 0.2	1.7 ± 0.3	1.0 ± 0.1	1.9 ± 0.3	-
24	BQL	1.0 ± 0.2	BQL	1.1 ± 0.2	-

Mean ± SD; BQL, Below Quantification Limit

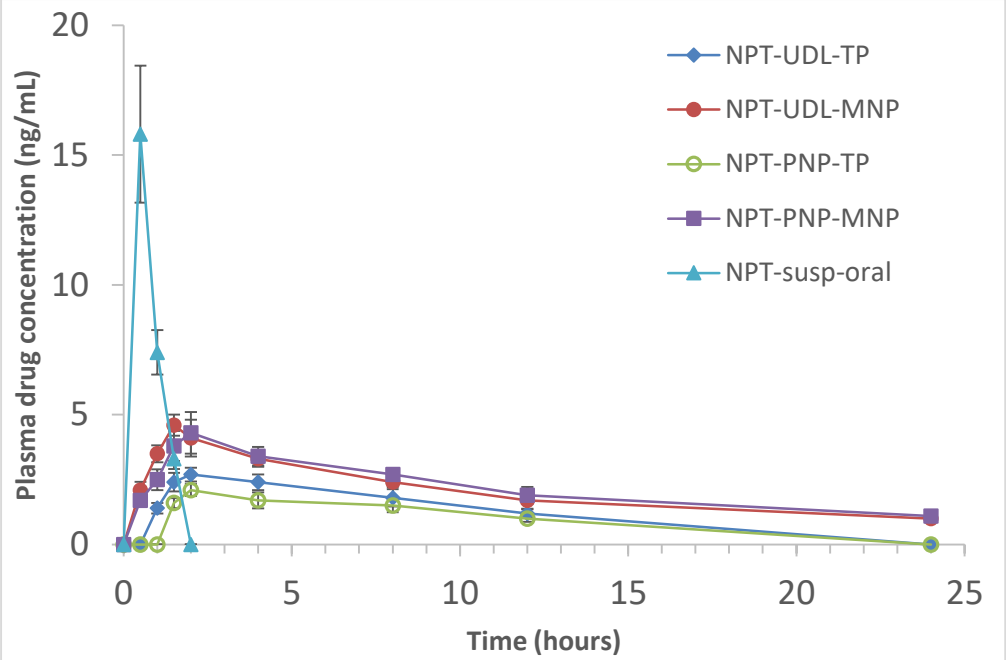

Fig. 9-3. Plasma NPT concentration vs Time profile of various dosage forms in Sprague Dawley rats

Table 9-8. Pharmacokinetic parameters (NPT) computed using Kinetica software

Parameters	NPT UDL TP	NPT UDL MNP	NPT PNP TP	NPT PNP MNP	NPT-Susp-Oral
C_{max} (ng/mL)	2.7	4.6	2.1	4.3	15.8
t_{max} (h)	2	1.5	2	2	0.5
AUC_{0-t} (ng*h/mL)	21.931	48.748	16.435	51.557	12.026
$AUC_{0-\infty}$ (ng*h/mL)	37.249	65.084	31.591	70.453	14.15
$t_{1/2}$ (h)	8.5	11.89	10.08	12.3	0.44
MRT (h)	13.42	17.29	15.91	18.26	0.96
F_{rel}	263	460	223	498	-

Administration of NPT suspension through oral route showed a rapid achievement of higher concentrations (C_{max}, 15.8 ng/mL and t_{max}, 0.5 hour) but for a shorter duration ($t_{1/2}$, 0.44 hours and MRT, 0.96 hours) as compared to other formulations. This clearly advocates the necessity of frequent dosing of its currently marketed tablets. On the contrary, transdermal formulations exhibited $t_{1/2}$ ranging from 8.5-12.3 hours and MRT ranging from 13.4-18.3 hours signifying a better controlled plasma levels for prolonged duration. Among all transdermal fast dissolving patches, NPT PNP TP showed lowest C_{max} and AUC_{0-inf} indicating poor penetrability of these carriers through intact skin. This was further supported by significant improvement of C_{max} and AUC_{0-inf} observed via NPT PNP MNP. However, NPT UDL TP showed a higher

C_{max} and $AUC_{0\text{-}inf}$ as compared to NPT PNP TP demonstrating the better penetrability of UDL than PNP across intact skin. Comparison of NPT UDL MNP and NPT PNP MNP showed insignificant difference in C_{max} and $AUC_{0\text{-}inf}$ suggesting comparable permeation via UDL as well as PNP across lower epidermis and dermis region to reach systemic circulation when stratum corneum barrier is physically breached using microneedles. An almost 5-fold rise in relative bioavailability could owe to avoidance of first-pass metabolism together with better penetrability and controlled drug release behavior of developed formulations.

9.3.3 Pharmacodynamic study of VPN

The results of training sessions are tabulated (**Table 9-9**) and graphically represented (**Fig. 9-4**). A reduction in latency was observed after each training sessions which was declined up to 5-6 seconds indicating the readiness of animals for pharmacodynamic evaluation of both the drugs through developed patches.

Table 9-9. Mean escape latencies (in second) during training sessions

Treatment Group / Training sessions	PD-I: (Cage-1)	PD-II: (Cage-2)	PD-III: (Cage-3)	PD-IV: (Cage-4)	PD-V: (Cage-5)	PD-VI: (Cage-6)	PD-VII: (Cage-7)	PD-VIII: (Cage-8)	PD-IX: (Cage-9)	PD-X: (Cage-10)
Day-1: Morning	40±25	42±23	39±24	41±19	43±23	38±19	43±21	38±22	38±19	42±18
Day-1: Afternoon	30±17	29±15	34±20	38±19	41±22	39±18	40±17	36±22	27±22	31±20
Day-2: Morning	26±17	24±17	27±11	24±14	26±17	27±16	27±14	29±14	25±17	26±16
Day-2: Afternoon	14±10	13±8	14±12	13±9	14±12	16±7	18±8	18±11	15±12	14±10
Day-3: Morning	7±4	8±5	8±9	9±11	10±12	10±8	12±8	11±10	9±12	9±12
Day-3: Afternoon	9±7	11±8	9±7	9±6	10±7	10±6	12±7	10±6	10±5	10±6
Day-4: Morning	8±7	8±8	7±6	7±6	7±6	7±6	8±7	7±5	8±5	9±6
Day-4: Afternoon	5±3	5±2	5±2	5±2	5±2	6±2	6±2	6±2	6±2	6±2
Day-5: Morning	6±2	6±2	6±2	6±2	6±2	5±1	6±2	6±2	6±2	6±1
Day-5: Afternoon	5±2	5±2	5±2	5±2	5±2	5±1	5±2	5±2	5±1	5±1

[#]Mean±SD (n=6)

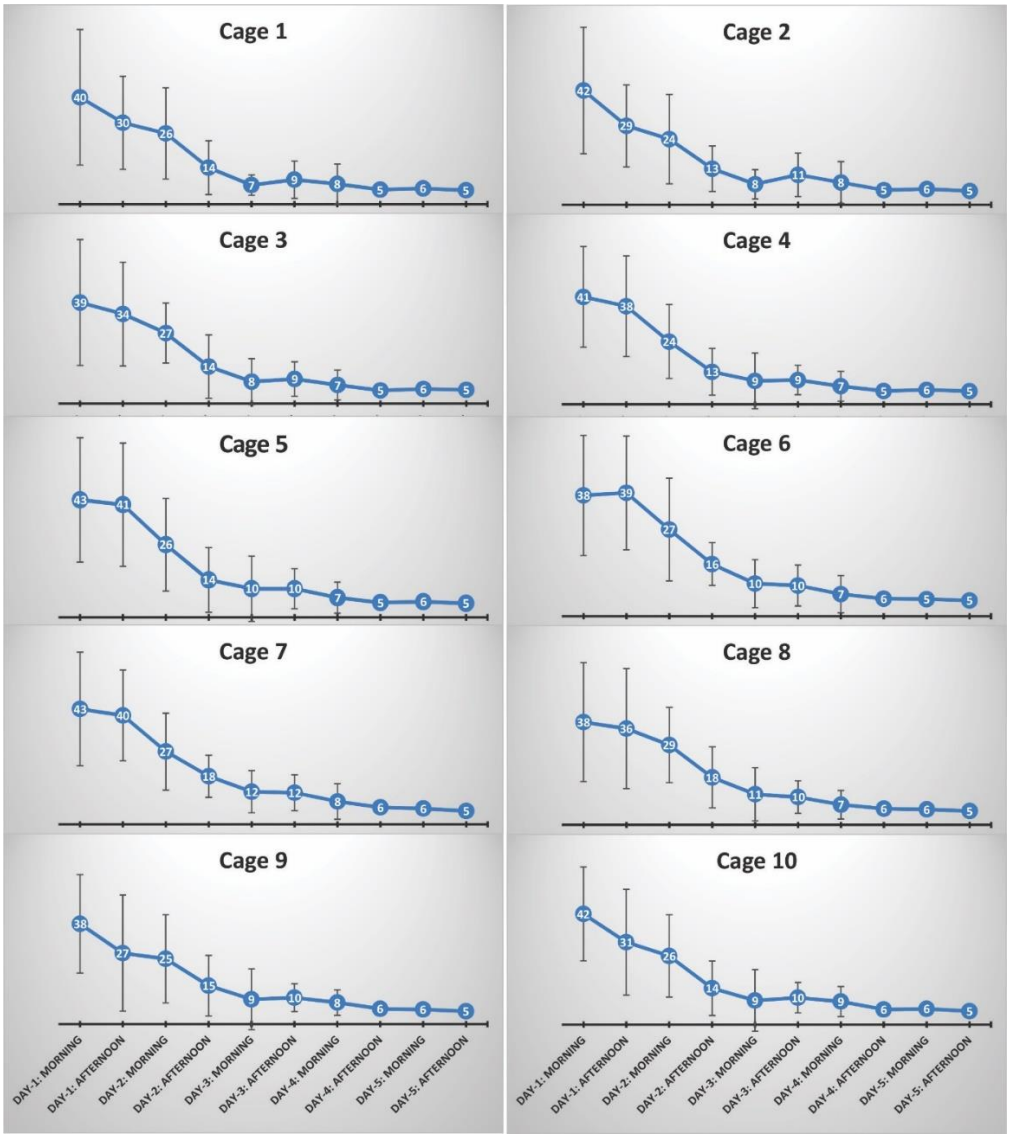

Fig. 9-4. Mean escape latency time (in second) during training sessions

The Mean escape latency time of each treatment group is presented in **Table-9-10** and **Fig. 9-5**. One way ANOVA followed by Tukey's multiple comparison test was performed using Prism software and the data are presented in **Table 9-11** and **Table 9-12**, respectively.

Table 9-10. Escape latencies for each treatment group of VPN

Treatment group	Latency (in second)							
	Rat-1	Rat-2	Rat-3	Rat-4	Rat-5	Rat-6	**Mean**	**SD**
PD-I: Normal control	5	3	7	4	9	8	**6**	**2**
PD-II: Model control	29	41	32	24	36	28	**32**	**6**
PD-III: Vehicle control 1	34	27	30	21	39	31	**30**	**6**
PD-IV: Test V1	12	19	11	8	16	11	**13**	**4**
PD-V: Vehicle control 2	23	24	35	37	28	39	**31**	**7**
PD-VI: Test V2	19	15	12	10	13	21	**15**	**4**
PD-VII: Standard control V	16	10	7	12	9	14	**11**	**3**

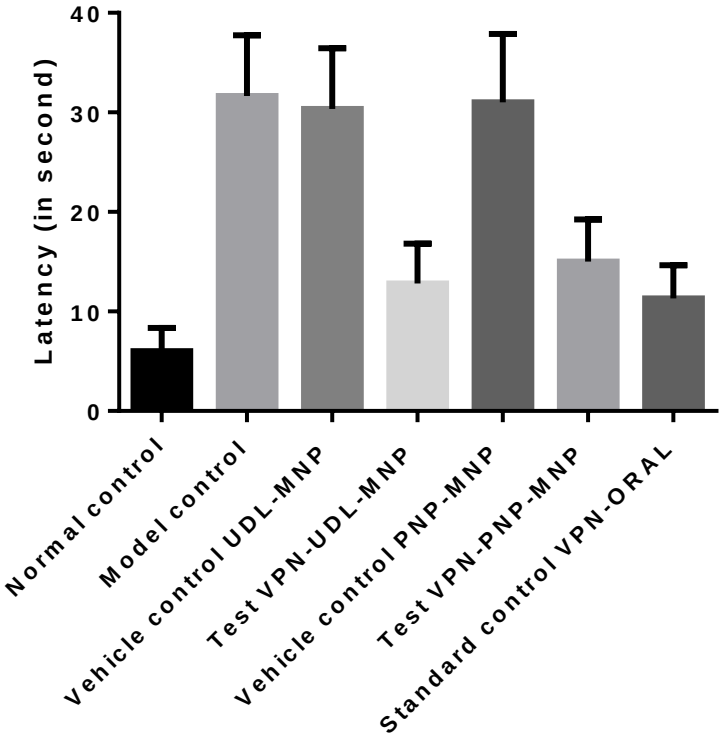

Fig. 9-5. Graphical representation of mean escape latencies for PD study of VPN

Table 9-11. One way ANOVA results for PD study of VPN

ANOVA table	SS	DF	MS	F value	P value
Treatment	4265	6	710.9	28.84	< 0.0001
Residual	862.8	35	24.65		
Total	5128	41			

A significant difference in mean latency of Normal control and model control group animals was observed signifying the validity of selected model where ketamine at selected dose levels produced amnesia in SD rats. An insignificant difference in mean latency of Model control and vehicle control groups further indicated that excipients used in the developed formulations did not have any pharmacological effect on selected disease model. Animals treated with fast dissolving microneedle patches of VPN loaded nanocarriers showed a significant reduction in latency as compared to model control group. The same was found comparable to latency of standard control group animals administered

with oral suspension of VPN. The results showed almost similar response of VPN through UDL MNP and PNP MNP

Table 9-12. Tukey's multiple comparisons test for PD study of VPN

	Mean Diff.	95% CI of diff.	Significant?
Normal control vs. Model control	-25.67	-34.63 to -16.71	Yes (****)
Normal control vs. Standard control VPN ORAL	-5.33	-14.29 to 3.63	No
Model control vs. Vehicle control UDL MNP	1.33	-7.63 to 10.29	No
Model control vs. Test VPN UDL MNP	18.83	9.87 to 27.79	Yes (****)
Model control vs. Vehicle control PNP MNP	0.67	-8.29 to 9.63	No
Model control vs. Test VPN PNP MNP	16.67	7.71 to 25.63	Yes (****)
Model control vs. Standard control VPN ORAL	20.33	11.37 to 29.29	Yes (****)
Test VPN UDL MNP vs. Test VPN PNP MNP	-2.17	-11.13 to 6.79	No
Test VPN UDL MNP vs. Standard control VPN ORAL	1.50	-7.46 to 10.46	No
Test VPN PNP MNP vs. Standard control VPN ORAL	3.67	-5.29 to 12.63	No

9.3.4 Pharmacodynamic study of NPT

The Mean escape latency time of each treatment group is presented in **Table 9-13** and **Fig. 9-6**. One way ANOVA followed by Tukey's multiple comparison test was performed using Prism software and the data are presented in **Table 9-14** and **Table 9-15**, respectively.

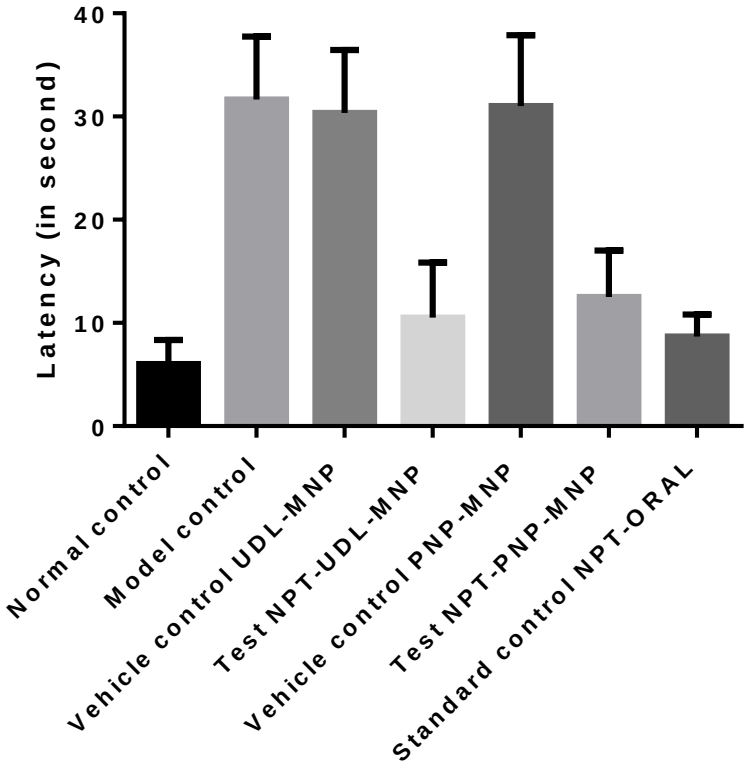

Fig. 9-6. Graphical representation of mean escape latencies for PD study of NPT

Table 9-13. Escape latencies for different treatment groups of NPT

Treatment group	Escape latency (in second)							
	Rat-1	Rat-2	Rat-3	Rat-4	Rat-5	Rat-6	**Mean**	**SD**
PD-I: Normal control	5	3	7	4	9	8	**6**	**2**
PD-II: Model control	29	41	32	24	36	28	**32**	**6**
PD-III: Vehicle control 1	34	27	30	21	39	31	**30**	**6**
PD-VIII: Test N1	6	16	8	4	12	17	**11**	**5**
PD-V: Vehicle control 2	23	24	35	37	28	39	**31**	**7**
PD-IX: Test N2	17	7	18	10	14	9	**13**	**5**
PD-X: Standard control N	9	12	10	7	6	8	**9**	**2**

Table 9-14. One way ANOVA results for PD study of NPT

ANOVA table	SS	DF	MS	F value	P value
Treatment	4934	6	822.4	31.73	< 0.0001
Residual	907.0	35	25.91		
Total	5841	41			

Animals treated with fast dissolving microneedle patches of NPT loaded nanocarriers showed a significant reduction in latency as compared to model control group. The same was found comparable to latency of standard control group animals administered with oral suspension of NPT. The results showed almost similar response of NPT through UDL MNP and PNP MNP.

Table 9- 15. Tukey's multiple comparisons test for PD study of NPT

	Mean Diff.	95% CI of diff.	Significant?
Normal control vs. Model control	-25.67	-34.85 to -16.48	Yes (****)
Normal control vs. Standard control NPT ORAL	-2.67	-11.85 to 6.52	No
Model control vs. Vehicle control UDL MNP	1.33	-7.85 to 10.52	No
Model control vs. Test NPT UDL MNP	21.17	11.98 to 30.35	Yes (****)
Model control vs. Vehicle control PNP MNP	0.67	-8.52 to 9.85	No
Model control vs. Test NPT PNP MNP	19.17	9.98 to 28.35	Yes (****)
Model control vs. Standard control NPT ORAL	23.00	13.81 to 32.19	Yes (****)
Test NPT UDL MNP vs. Test NPT PNP MNP	-2.00	-11.19 to 7.19	No
Test NPT UDL MNP vs. Standard control NPT ORAL	1.83	-7.35 to 11.02	No
Test NPT PNP MNP vs. Standard control NPT ORAL	3.83	-5.35 to 13.02	No

9.4 CONCLUSION

The results of pharmacokinetic and pharmacodynamic studies revealed that UDL and PNP of both VPN and NPT via fast dissolving microneedle patch were able to maintain plasma drug concentration above MEC for prolong period of time and therefore seems suitable for once daily application.

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