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IN VITRO AND *IN VIVO* PHARMACOKINETIC ASSESSMENT OF OPTIMIZED PITAVASTATIN SOLID-SNEDDS

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ABSTRACT

In the present research work animal model studies were performed to determine gastrointestinal (GI) absorption and elimination rate of pitavastatin. The *in vitro* evaluation of the prepared solid-snedds release of pitavastatin with the selected oils (labrafac lipophilewl1349, capmul MCM), surfactants (tween 80), co surfactants (egg lecithin**)** confirmed the usefulness compared to the remaining oils, Surfactants and co surfactants for the drug that are selected. From the *in vivo* evaluation studies of optimized formulation, the results are as follows: peak plasma concentration (C_{max}) of the pure pitavastatin and RPTV1 (rabbit dose optimized pitavastatin) were 524 ± 6.49 ng/ml and 469.9 ± 12.09 ng/ml. Time required to extend maximal concentration (T_{max}) in case of formulation (RPTV1) was increased from 1 to 2 h in comparison to pure drug Pitavastatin, AUC_{0-t} was found to be 912.93 ± 1.80 ng.h/ml and 2982.5 ± 0.74 ng.h/ml for pure pitavastatin and formulation RPTV1. For pitavastatin and formulation (RPTV1) elimination rate constant was observed that 0.680 ± 0.001 h^{-1} and 0.560 ± 0.0007 h^{-1}. The $t_{1/2}$ for pure pitavastatin was found to be 1.02 ± 0.007 h and 1.23 ± 0.01 h for RPTV1. Drug releases from the tablet (RPTV1) were increased in comparison with the pure drug in rabbit shows that drug plasma levels maintained up to 12 h. Thus it indicates that improved bioavailability of optimized s-snedds (solid self nano emulsifying drug delivery systems).

Keywords: Pitavastatin, S-snedds, C_{max}. AUC, K_{el}, Bioavailability

1. INTRODUCTION

Pitavastatin well established drug for their pharmacokinetic studies **[1, 2]**. The studies were performed in healthy rabbits (New Zealand, White) **[3]**. Institutional animal ethics committee approved research protocol of animal experimentation, in animal house the animals were housed by providing free access move and laboratory standard diet and water **[4]**. Pharmacokinetic variables were measured by the usage of trial version of Kinetica^{TM} 2000 software **[5]**.

2. MATERIALS AND METHOD

2.1 Subjects

Healthy rabbits were being used of either sex weighing about 2.5-3.5 kg (New Zealand, White) obtained from national institute of nutrition, Secunderabad road, beside railway degree college, Jamia Osmania, Tarnaka, Mettuguda, Telangana 500017 **[6]**.

2.2 Preparation of RPTV1 for rabbits

Powder blends for RPTV1 was prepared and granules are being punched into tablets by the usage of an Elite 10 station minipress having 4 mm diameter flat round punches. Optimized tablets with rabbit dose (RPTV1) were prepared by reducing the dose of optimized formulation of PTV1 and the formulae are given in **Table 1 [7, 8]**.

Table 1: Composition of tablets of RPTV1

Formulation code	Drug	Total weight (mg)
RPTV1	Pitavastatin (0.3mg)	20

2.3 Design

Parallel design method was used in which one group of animals received pure drug suspension in 3 ml of sodium carboxy methyl cellulose 1 % w/v suspension and the second group received the optimized rabbit dose solid snedds tablet by oral gavage. Each group consisted of 6 rabbits belonging to either sex. The tablets were administered by placing in the feeding tube with 3 ml of water to ensure tablet delivery to the rabbit stomach. Pure drug suspension was also administered in the similar way **[9, 10]**.

2.4 Blood samples collection

Blood samples are being withdrawn from marginal ear vein at 0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 12 h after drug administration. At each time interval approximately 500 µl of blood sample was withdrawn and plasma are being filtered by centrifugation at 3500 rpm for 10 min using micro-centrifuge. Until the analysis of all plasma samples were stored at refrigeration condition **[11, 12]**.

2.5 Pharmacokinetic evaluation studies

The optimized formulation PTV1 was

administered after reduced to the rabbit dose as RPTV1. Pharmacokinetic variables are being measured by using non-compartmental model. The peak plasma concentration (C_{max}), time of peak plasma concentration (T_{max}), AUC is the area under the drug plasma level vs time curve from t=0 to t=∞ and is equal to the amount of unchanged drug reaching the general circulation divided by the clearance:

$$AUC_{0-\infty} = \int_0^{\infty} C\,dt$$

$AUC_{0-\infty}$ the area under the plasma drug concentration vs time curve from time zero to infinity was calculated by summing the AUC_{0-t} and the extrapolated area under the curve to time infinity ($AUC_{t-\infty}$) which was equal to the ratio of C_t/K_{el} by the following:

$$AUC_{0-\infty} = AUC_{0-t} + \frac{C_t}{K_{el}}$$

Where C_t is the last assessable concentration at time t and K_{el} is the terminal elimination rate constant. Apparent terminal elimination rate constant K_{el} was calculated from log plasma concentration vs time curve [13]. The half-life is the time taken for the plasma concentration to minimize to half $t_{1/2}$ was calculated by:

$$t_{1/2} = \frac{0.693}{K_{el}}$$

2.6 Evaluation studies of stability

Samples are being loaded in a bottle having screw cap and investigations are being done for 12 months by storing at 30 ± 2 °C/65 ± 5% RH and at 40 ± 2°C/75 ± 5% RH for 12 and 6 months for long term and accelerated studies. Samples are being collected for long term storage condition at zero, three, six and twelve months and for accelerated storage condition at zero, three and six months. Further evaluated for if any modifications observed in post compression studies, drug content and % cumulative drug release **[14, 15]**.

3. RESULTS

3.1 Evaluation parameters of RPTV1

The evaluation parameters of RPTV1 are shown in **Table 2**. The characteristics are estimated in comparison with the PTV1 tablets. *In vitro* dissolution studies are being performed for pure drug pitavastatin and RPTV1 by using pH 6.8 phosphate buffer under similar conditions. Dissolution study profiles of pure pitavastatin and RPTV1 are being displayed in **Table 3** and **Figure 1**.

3.2 Pharmacokinetic evaluation of pitavastatin

The plasma concentration of pitavastatin for individual subject and each mean and standard deviation values at various time intervals is given in **Tables 4, 5**. The kinetic treatment data for pure pitavastatin and RPTV1 are given in **Tables 6, 7**. The plasma concentration-time profile graphs for pure pitavastatin and RPTV1 are shown in **Figures 2, 3**. The comparatively profiles of mean plasma concentration-time were displayed in **Figure 4**.

3. 3 Stability studies of the optimized

formulation

The samples kept for stability studies are being evaluated for Hardness, Disintegration time, and Cumulative % drug released. The results were shown in **Tables 8, 9**. The number of samples estimated was in triplicate for each batch of solid snedds.

Table 2: Characteristics of rabbit dose pitavastatin tablets in comparison with PTV1

Formulation code	Uniformity of weight[a] (mg)	Hardness[b] (N)	Thickness[b] (mm)	Friability (%)	Drug content[d] (%)
PTV1	340.0 ± 1.37	3.45 ± 0.09	2.9 - 3.0	0.32 ± 0.01	98.36 ± 0.057
RPTV1	20.0 ± 2.84	3.5 ± 0.05	2.1 - 2.6	0.25 ± 0.02	98.14 ± 0.04
a: Average weight deviation in mg, n = 20; b: mean ± s.d., n = 3;d: mean ± s.d., n = 3					

Table 3: Cumulative percent drug released vs time of pitavastatin pure drug and RPTV1 in comparison with PTV1

Time (min)	% cumulative drug release (mean ± s.d., n = 3)		
	Pure drug	RPTV1	PTV1
0	0	0	0
5	7.39 ± 0.76	15.24 ± 0.49	17.3 ± 0.33
10	16.6 ± 1.5	39.71 ± 0.54	35.5 ± 0.02
15	19.4 ± 1.2	55.31 ± 0.35	53.2 ± 0.35
20	20.2 ± 2.3	70.11 ± 0.14	69.5 ± 0.49
30	21.4 ± 1.8	81.24 ± 0.63	76.3 ± 0.57
40	23.2 ± 1.1	98.24 ± 0.63	99.9 ± 0.25
50	26.8 ± 0.03	--	--
60	30.2 ± 0.05	--	--

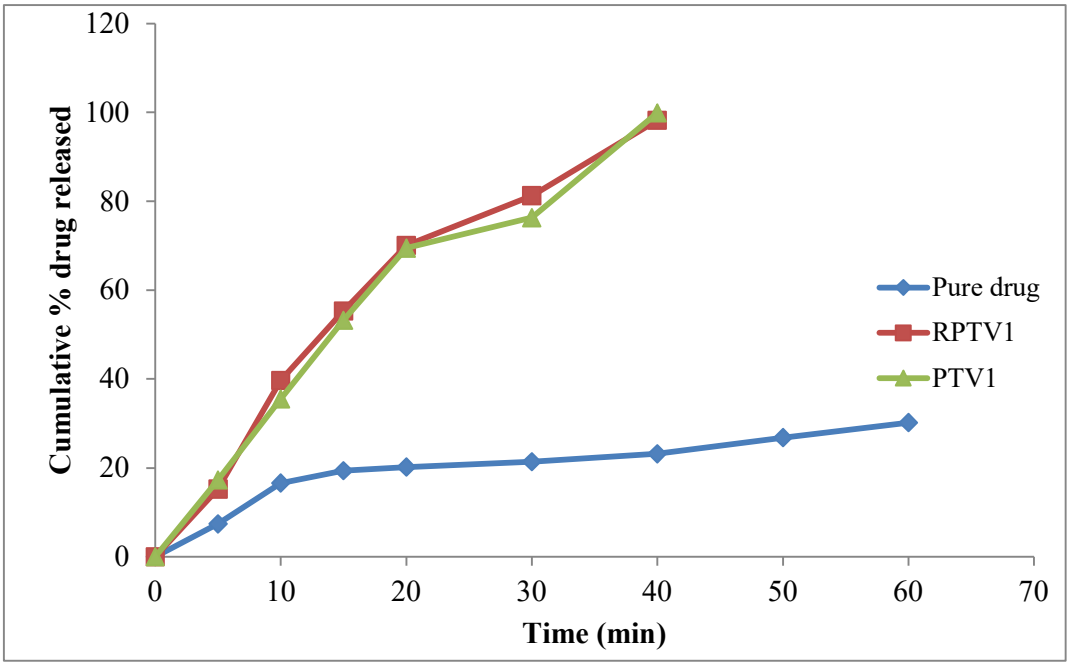

Figure 1: Dissolution profiles of pitavastatin pure drug and RPTV1 in comparison with PTV1

Table 4: Pure drug pitavastatin plasma concentration profiles at different time intervals

Rabbit	Plasma concentration (ng/ml) of pure drug									
	Time (h)									
	0.5	1.0	1.5	2	2.5	3.0	3.5	4	4.5	5
1	201.9	520.3	378.6	233.5	185.6	102.6	89.3	45.6	36.9	10.6
2	199.8	521.4	366.4	244.6	174.5	112.3	95.6	56.8	42.6	12.3
3	189.9	533.2	372.5	250.3	176.8	110.4	101.3	63.4	33.3	9.03
4	183.3	516.7	379.6	249.7	172.5	109.3	99.6	70.2	39.6	12.9
5	182.5	530.9	381.3	253.6	176.5	110.9	95.6	69.3	40.2	11.2
6	179.5	521.8	383.2	247.5	181.6	107.8	94.3	66.7	39.5	10.9
Mean	189.4	524.0	376.9	246.5	177.9	108.8	95.95	62	38.6	11.15
s.d.	9.46	6.49	6.30	7.05	4.83	3.43	4.22	9.38	3.20	1.35

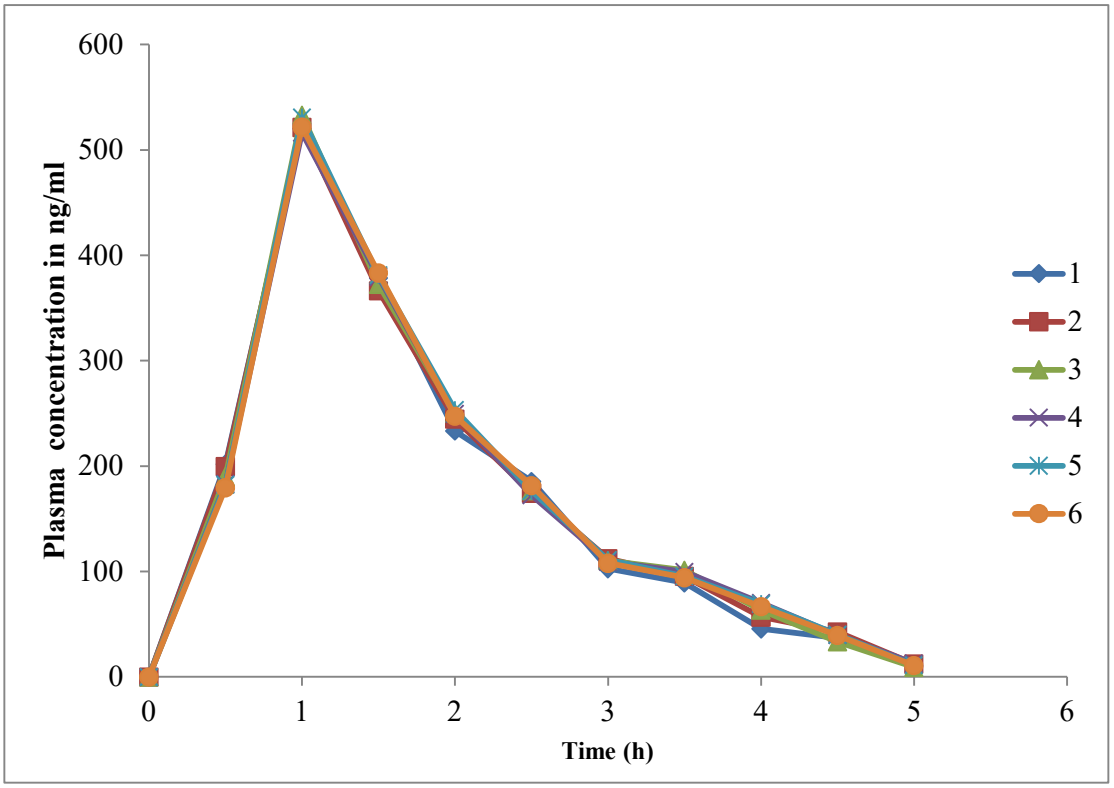

Figure 2: Pitavastatin pure drug in rabbit's plasma concentration vs time profile after oral administration

Table 5: Plasma concentration profiles of pitavastatin RPTV1 at different time intervals

Rabbit	Plasma concentration (ng/ml) of RPTV1											
	Time (h)											
	0.5	1.0	2	3	4	5	6	7	8	9	10	12
1	74.2	410.6	485.3	407.6	365.6	347.5	310.2	220.1	179.5	139.6	112.0	69.3
2	68.5	407.9	476.1	401.9	367.9	341.8	308.6	219.6	181.3	134.4	114.6	60.4
3	62.5	402.9	452	402.1	352.9	333.8	306.9	217.4	165.2	129.6	115.4	66.9
4	66.3	409.2	460	409.2	369.2	333.5	304.1	213.5	172.5	130.9	113.8	69.7
5	67.3	407.8	476	407.8	367.8	340.9	307.2	219.2	179.5	135.7	110.6	62.1
6	66.9	406.3	470	406.8	356.3	339.5	305.9	216.8	170.6	133.1	113.5	69.6
Mean	67.6	407.4	469.9	405.9	363.3	339.5	307.1	217.7	174.7	133.8	113.3	66.36
s.d.	3.81	2.66	12.09	3.12	6.91	5.29	2.11	2.45	6.34	3.58	1.75	4.10

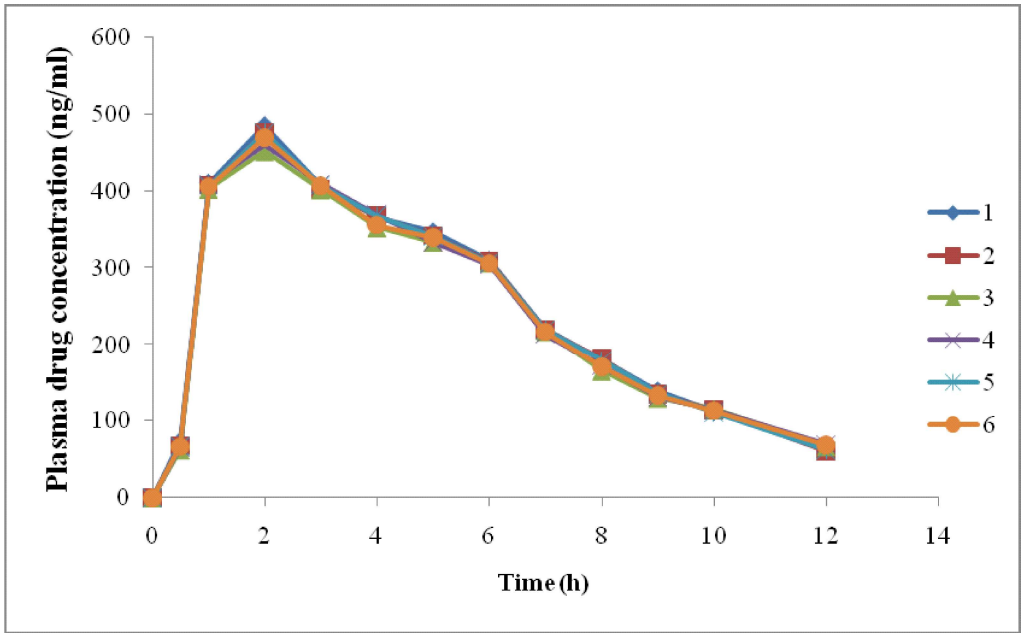

Figure 3: plasma concentration vs time profile of rabbit dose pitavastatin after oral administration

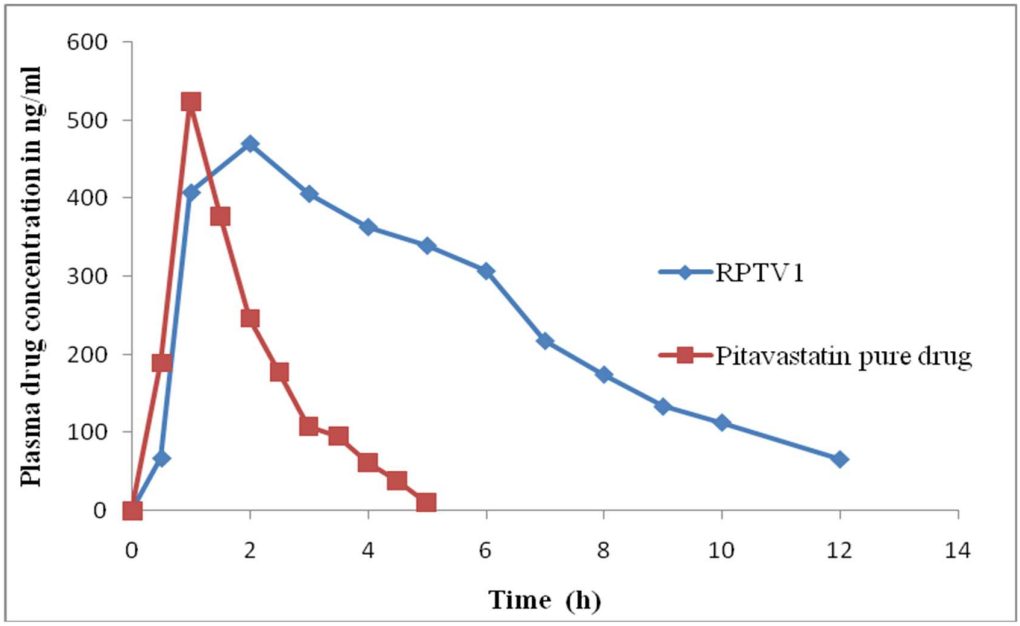

Figure 4: Comparison of pitavastatin pure drug and RPTV1 mean plasma concentration vs time profiles

Table 6: Pharmacokinetic data of pure pitavastatin

Treatment	Subject	T_{max} (hr)	C_{max} (ng/ml)	AUC_{0-t} (h*ng/ml)	$AUC_{0-\infty}$ (h*ng/ml)	K_{el}	$t_{1/2}$ (h)
Reference	1	1.0	520.3	910.5	930.7	0.680	1.01
	2	1.0	521.4	911.4	929.4	0.679	1.02
	3	1.0	533.2	910.6	930.2	0.678	1.03
	4	1.0	516.7	912.4	931.0	0.679	1.02
	5	1.0	530.9	913.5	932.4	0.680	1.01
	6	1.0	521.8	915.1	930.1	0.681	1.02
	N	6	6	6	6	6	6
	Mean	1.0	524.0	912.93	930.6	0.680	1.02
	SD	0	6.49	1.80	1.02	0.001	0.007

Table 7: Pharmacokinetic data of pitavastatin rabbit dose

Treatment	Subject	T_{max} (hr)	C_{max} (ng/ml)	AUC_{0-t} (h*ng/ml)	$AUC_{0-\infty}$ (h*ng/ml)	K_{el}	$t_{1/2}$ (h)
Test	1	2.0	485.3	2982.6	3100.9	0.560	1.23
	2	2.0	476.1	2981.3	3098.3	0.559	1.24
	3	2.0	452	2982.7	3101.2	0.560	1.25
	4	2.0	460	2983.3	3102.1	0.561	1.23
	5	2.0	476	2982.0	3100.4	0.560	1.23
	6	2.0	470	2983.1	3099.2	0.561	1.20
	N	6	6	6	6	6	6
	Mean	2.0	469.9	2982.5	3100.35	0.560	1.23
	SD	0	12.09	0.74	1.38	0.0007	0.01

Table 8: Stability data for long term of PTV1 at 30 ± 2°C/65 ± 5% RH (mean ± s.d., n = 3)

Months	Hardness, N	Disintegration time (sec)	Cumulative % drug released
0	3.45 ± 0.01	80.71 ± 0.04	99.9 ± 0.05
1	3.46 ± 0.01	80.71 ± 0.02	99.8 ± 0.03
2	3.47 ± 0.03	80.61 ± 0.01	99.5 ± 0.02
3	3.49 ± 0.01	80.72 ± 0.05	99.6 ± 0.05
6	3.51 ± 0.02	81.70 ± 0.02	99.3 ± 0.01

Table 9: Accelerated stability data of PTV1 at 40 ± 2°C/75 ± 5% RH (mean ± s.d., n = 3)

Months	Hardness, N	Disintegration time (sec)	Cumulative % drug released
0	3.45 ± 0.04	80.71 ± 0.06	99.9 ± 0.05
1	3.45 ± 0.02	80.72 ± 0.07	99.9 ± 0.02
2	3.53 ± 0.04	81.03 ± 0.06	99.8 ± 0.05
3	3.51 ± 0.02	81.3 ± 0.01	99.6 ± 0.04
6	3.52 ± 0.05	81.4 ± 0.03	99.2 ± 0.03

4. DISCUSSION

Tabletting characteristics of the prepared RPTV1 tablets in comparison with the PTV1 tablets are being within the specifications. Even though the tablet size was being minimized to rabbit dose the *in vitro* dissolution profile was as same as human dose tablets (PTV1).

The highest plasma concentration (C_{max}) of the pitavastatin pure drug and RPTV1 were 524 ± 6.49 ng/ml and 469.9 ± 12.09 ng/ml. The time taken up to reach maximum concentration (T_{max}) in case of formulation (RPTV1) was increased from 1 to 2 h in comparison to pure drug pitavastatin. AUC_{0-t} was found to be 912.93 ± 1.80 ng.h/ml and 2982.5 ± 0.74 ng.h/ml for pure pitavastatin and formulation RPTV1. Pure pitavastatin elimination rate constant and formulation (RPTV1) was observed that 0.680 ± 0.001

and 0.560 ± 0.0007 h^{-1}. The $t_{1/2}$ for pure pitavastatin was found to be 1.02 ± 0.007 h and 1.23 ± 0.01 h for RPTV1. From the above values it was observed that the pure drug showed maximum plasma concentration and formulation (RPTV1) showed maximum area under the curve. The low value of AUC observed with pure drug may be due to their rapid absorption and elimination from the body.

Time taken up to reach maximal concentration was higher in the formulation. Relative bioavailability was enhanced in the pitavastatin. The absorption was rapid in case of pure drug compared to their formulation. The $t_{1/2}$ of formulation (RPTV1) was maintained within the limits as described in drug profiles. All these parameters reveal that the optimized formulation exhibits better release even in rabbits.

No visible changes were observed in all the three different formulations withdrawn from the stability chambers. In accelerated conditions, long term conditions and at refrigerated conditions there were no major differences observed in the studied parameters with respect to the initial values.

5. CONCLUSION

For the optimized formulation (PTV1), *in vitro* drug release studies reveals that the drug release obeys first order kinetics. For *in vivo* studies the tablets size was minimized to rabbit's dose. Drug releases from the tablet (RPTV1) were increased in comparison with the pure drug in rabbit shows that drug plasma levels maintained up to 12 h. There was difference in AUC values for optimized formulation and pure drug indicating significant difference in absorption. Thus, indicates the increased bioavailability for pitavastatin (PTV1). Hence from the stability studies it could be concluded that all the optimized solid-snedds were stable at 30 ± 2 °C/65 ± 5 % RH and 40 ± 2 °C/75 ± 5 % RH.

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Conflict of interest

The authors declare no conflict of interest relevant to this article.

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