Pharmacokinetics

3. Pharmacokinetic Studies in Rabbits

Bioavailability estimation is the only appropriate tool for verifying the efficacy of formulation. No matter whatever techniques have been utilized for dissolution enhancement of poorly soluble drugs its expression in vivo is one of the important criteria for accepting the dosage form. To understand the achievement of bioavailability enhancement objective in vivo studies in rabbits were performed. Wagner nelson method was used for the calculation of the pharmacokinetic parameters (Wagner and Nelson, **1964,** Wagner, **1970**).

All the developed drug delivery systems showed marked increase in percent drug dissolution of active pharmaceutical ingredient compared to their marketed conventional counterparts. However to minimize the animal trials and give a logical base for *in vivo* studies execution, the drug delivery system showing best *in vitro* performance was selected, which was found to be SEDDS. Self emulsifying drug delivery systems showed the most promising results for *in vitro* dissolution characteristics of CBZ, OCBZ and GPN when compared with their liquisolid, solid dispersion and microcrystals formulation. Hence they were selected for the *in vivo* studies in rabbits.

The protocol was approved by the Institutional ethical committee at the M. S. University of Baroda at Vadodara, India. The experiments were conducted as per CPCSEA (committee for prevention, control and supervision of experimental animals) guidelines.

The developed formulation was administered orally to three adult male rabbits (1800 – 2500 gm) in a dose range of 8 – 10 mg/kg, 5 – 10 mg/kg and 12 – 15 mg/kg of body weight for Carbamazepine, oxcarbamazepine and gabapentin respectively. Blood samples were colleted from the marginal ear vein at 15, 30, 60, 120, 240 min. interval after the drug administration. Plasma was separated by immediate centrifugation and was kept at 20^0C until analyzed.

Pharmacokinetic Steps for Calculating Pharmacokinetic Parameters

(Arima, *et al.*, 2001, De Jaeghere, *et al.*, 2000, Doherty and York, 1989, Khaled, *et al.*, 2001, Zerrouk, *et al.*, 2001)

Pharmacokinetic parameters estimated were as follows

Maximum plasma concentration (C_{max}): It was determined directly from the plasma concentration time profiles.

Time to maximum plasma concentration (T_{max}): It was determined directly from the plasma concentration time profiles.

Area under the plasma concentration-time curve from time '0' to 't' (AUC_{0-t}): It was calculated by using trapezoidal rule. According to trapezoidal rule, the area under the curve from time t_2 to time t_1 is calculated by following equation:

$$AUC_{t_2}^{t_1} = \left(\frac{C_1 + C_2}{2}\right) \times (t_2 - t_1)$$

Where, C_1 and C_2 are concentrations at time t_1 and t_2.

Concentration at zero time (C_0): Time versus $\log_n$ plasma concentration graph was plotted on MS-Excel graph wizard. The terminal linear phase was extrapolated to zero. It gave intercept. The antilog of the intercept was obtained by linear regression wizard, which gave the concentration at zero time (C_0).

Elimination rate constant ($-K_{el}$): The plot of $\log_n$ of plasma concentration vs time was linear for the terminal portion (last three detectable concentrations). Slope of this linear line was calculated. K_{el} = -slope × 2.303

Elimination half life ($t_{1/2}$) : It was determined by ($t_{1/2} = 0.693/K_{el}$)

Area under the plasma concentration-time from time zero to infinity ($AUC_{0-\alpha}$): The trapezoidal rule was used to determine AUC.

Absorption rate constant (K_{ab}): The terminal linear portion of the curve with slope $-K_{el}$ was extrapolated to t=0. The actual plasma levels were subtracted from the corresponding concentrations on the extrapolated linear portions. This gave a series of residual concentration (Cr). The plot of natural log of residual concentration ($\log_n$ Cr) vs time gave a straight line with slope (–Kab).

Volume of distribution (V_d): It is the volume in which drug would have to be distributed to produce the measured plasma concentration.

$$V_d = \frac{F \times G_0}{K_{el} \times AUC_{0-\alpha}}$$

Clearance (CI): It is the total volume of plasma from which the drug have been removed per unit time.

Clearance (CI) = $(V_d \times 0.693)/t_{1/2el}$

Cumulative drug eliminated at t time: It was calculated as,

Drug eliminated = $0.434\ K_{el} * t$

Fraction of drug absorbed at time:

$$t = \frac{C + K_{el} \times AUC_0^t}{K_{el} \times AUC_0^{\alpha}}$$

Area under momentum curve (AUMC): AUMC is the area under the curve of (C_p x t) versus t.

Mean residence time (MRT):

MRT = C_{AUMC}/C_{AUC}

Where, C_{AUMC} = Cumulative AUMC

and C_{AUC} = Cumulative AUC.

3.1. Estimation of drug in plasma

3.1.1. Plasma extraction procedure

A standard or a serum sample was extracted first with an organic solvent. Samples were vortex mixed for 1 min with the solvent, shaken and then centrifuged for 5 min at 2000 rpm. The organic phases were evaporated to dryness in a warm water bath. The residues were dissolved in organic solvent-water and 20/µl were injected into the HPLC column.

3.1.1.1. Extraction efficiency

The extraction efficiency was calculated by adding known amount of CBZ, OCBZ and GPN (0.5, 1 and 1.5 µg/ml; n = 5 per concentration) to 0.5 ml of blank rabbit plasma. The CBZ and OCBZ were extracted into 5 ml of chloroform. The chloroform layer was back extracted into 1 ml 0.1 M hydrochloric acid solution by agitation for 1 minute and centrifuged for 5 minutes. The known amount of aqueous layer was injected into the chromatographic system.

The peak area of sample was compared to those obtained from equivalent volumes of standard solution of drug in 0.1 M hydrochloric acid solution directly injected into the HPLC system. The determination of unextracted samples was performed in triplicate for each concentration.

The developed methods were validated for following parameters.

3.1.1.2. Linearity and range

The linear detector response for the assay was tested as follows. These determination (n=5) from minimum of five concentration levels (100, 200, 300, 500, 700, 1000, 1500 ng/ml) of the analyte were made. Detector response was correlated against analyte concentration by least squares regression.

3.1.1.3. Accuracy and precision

For the determination of intra day and inter day accuracy and precision of the assay, various quantities of CBZ, OCBZ and GPN were added to aliquots of 0.5 ml rabbit plasma to yield 250, 500, 750 and 1000 ng/ml. Accuracy was expressed as Mean percent,

$$Accuracy = \left(\frac{Mean\ measured\ Concentration}{Expected\ Concentration} \right) \times 100$$

Precision was calculated as inter and intra day coefficient of variation

$$\% CV = \left(\frac{SD}{Mean}\right) \times 100$$

3.1.2. HPLC analysis of CBZ

The parameters for HPLC analysis of CBZ from plasma samples of *in vivo* studies are as follows.

System : Chemito LC 6600 Series,

Pump : Knauer's WellChrom isocratic HPLC K – 501.

Double piston operated with 10 mL stainless steel pump head.

Injector : Knauer's manually driven 6 port 3 channel valve with 20 μL fixed loop with 60^0 rotation.

Detector : Chemito LC 6600 Dual wavelength UV Visible detector.

Range of measurement – 0 – 2 AU

Integrator output - ± 1.0 V

Autozero – Fullscale.

Software : Chemitochrom version 1.6

Column : Eurospher 100, 5 μm. ID – 4.8 mm, Column length – 25 cm.

Mobile phase composition : **Acetonitrile 20** mM : KH_2PO_4 (20:80) containing 0.05% of **triethylamine** maintained at (pH 6.30).

Flow rate: 1 mL/min.

UV detection wavelength (λ): 212 nm.

Retention time: 6.2 min.

Table 3.1 gives the values for precision and accuracy of the assay for the estimation of CBZ.

Table 3.1 Precision and accuracy of the assay for the estimation of CBZ.

Concentration					Deviation From nominal conc.
Nominal	Found mean	SD	RSD	CI (p=95%, n=6)	
Intra-day precision and accuracy of CBZ assay					
1.04	0.94	0.04	4.04	0.05	-9.79
10.30	9.76	0.29	3.63	0.26	-5.35
20.49	20.11	0.31	2.22	0.42	-1.92
Inter-day precision and accuracy of CBZ assay					
1.06	0.97	0.06	6.86	0.37	-8.68
10.28	9.94	0.31	5.45	0.34	-3.33
20.29	19.43	0.26	3.13	0.65	-4.24

3.1.3. HPLC analysis of OCBZ

The parameters for HPLC analysis of OCBZ from plasma samples of *in vivo* studies are as follows.

System : Chemito LC 6600 Series,

Pump : Knauer's WellChrom isocratic HPLC K – 501.

Double piston operated with 10 mL stainless steel pump head.

Injector : Knauer's manually driven 6 port 3 channel valve with 20 μL fixed loop with 60^0 rotation.

Detector : Chemito LC 6600 Dual wavelength UV Visible detector.

Range of measurement – 0 – 2 AU

Integrator output - ± 1.0 V

Autozero – Fullscale.

Software : Chemitochrom version 1.6

Column : Eurospher 100, 5 μm. ID – 4.8 mm, Column length – 25 cm.

Mobile phase composition : **Acetonitrile 20** mM : KH_2PO_4 (20:80) containing 0.05% of **triethylamine** maintained at (pH 6.30). (Same mobile phase that was used for estimation of CBZ)

Flow rate: 1 mL/min.

UV detection wavelength (λ): 257 nm.

Retention time: 10.3 min.

Table 3.2 gives the values for precision and accuracy of the assay for the estimation of OCBZ.

Table 3.2 Precision and accuracy of the assay for the estimation of OCBZ.

Concentration					Deviation From nominal conc.
Nominal	**Found mean**	**SD**	**RSD**	**CI (p=95%, n=6)**	
Intra-day precision and accuracy of OCBZ assay					
1.04	0.94	0.04	4.02	0.05	-9.76
10.27	9.73	0.29	3.62	0.26	-5.33
20.43	20.05	0.31	2.21	0.42	-1.91
Inter-day precision and accuracy of OCBZ assay					
1.06	0.97	0.06	6.84	0.37	-8.65
10.25	9.91	0.31	5.43	0.34	-3.32
20.23	19.38	0.26	3.12	0.64	-4.23

3.1.4. HPLC analysis of GPN

Same method was employed for estimation of GPN from solubility, *in vitro* dissolution studies and plasma samples of *in vivo* studies.

The parameters for HPLC analysis of GPN from were as follows.

System : Chemito LC 6600 Series,

Pump : Knauer's WellChrom isocratic HPLC K – 501.

Double piston operated with 10 mL stainless steel pump head.

Injector : Knauer's manually driven 6 port 3 channel valve with 20 □L fixed loop with 60^0 rotation.

Detector : Chemito LC 6600 Dual wavelength UV Visible detector.

Range of measurement – 0 – 2 AU

Integrator output - ± 1.0 V

Autozero – Fullscale.

Software : Chemitochrom version 1.6

Column : Eurospher 100, 5 μm. ID – 4.8 mm, Column length – 25 cm.

For Plasma sample analysis in brief, 0.5 mL of sample was mixed with internal standard (1- (aminomethyl) cycloheptaneacetic acid) and deproteinized with perchloric acid. After derivatization with 2,4,6-trinitrobenzenesulfonic acid at pH 8.5, samples were extracted with toluene. The organic phase was evaporated and the residue dissolved in the mobile phase. 20 μL of sample was injected in column with fixed loop manual injector. Table 3.3 gives the values for precision and accuracy of the assay for the estimation of GPN.

Table 3.3 gives the values for precision and accuracy of the assay for the estimation of GPN.

Mobile phase composition : 5% acetic acid in water : acetonitrile (40:60 v/v)

UV detection wavelength (λ) : 350 nm.

Table 3.3 gives the values for precision and accuracy of the assay for the estimation of GPN.

Table 3.3 Precision and accuracy of the assay for the estimation of GPN.

Concentration					Deviation From nominal conc.
Nominal	**Found mean**	**SD**	**RSD**	**CI (p=95%, n=6)**	
Intra-day precision and accuracy of GPN assay					
1.03	0.93	0.04	4.0	0.05	-9.7
10.21	9.67	0.29	3.6	0.26	-5.3

20.31	19.93	0.31	2.2	0.42	-1.9
Inter-day precision and accuracy of GPN assay					
1.05	0.96	0.06	6.8	0.37	-8.6
10.19	9.85	0.31	5.4	0.34	-3.3
20.11	19.26	0.26	3.1	0.64	-4.2

Table 3.4 tabulates the combined data for limit of detection (LOD), limit of quantification (LOQ) and Recovery studies.

Table 3.4 Combined data for CBZ, OCBZ and GPN validation parameters.

Drug	Range (ng/mL)	Intercept	R^2	Limit of detection (ng/ml; ng) (mean ± S.D., n = 4-5)	Limit of quantification (ng/ml; ng) (mean ± S.D., n = 4-5)	Recovery (%) (mean ± S.D., n = 5)
CBZ	1.0-16.0	0.0209	0.9927	8 ± 3; 0.2	14 ±5; 0.3	108.2 ±13.0
	0.039-0.625	-0.0127	0.9955	8 ± 3; 0.2	22 ±11; 0.4	91.0 ±3.1
OCBZ	1-10.0	0.0316	0.9897	8 ± 3; 0.3	24 ±15:0.5	97.0 + 5.5
	0.039-0.625	-0.0194	0.994	8 ± 3; 0.2	24 ±15; 0.5	98.6 ± 13.3
GPN	1-12.0	0.0136	0.9943	9 ± 2; 0.2	24 ±15; 0.5	98.3 ± 13.3
	0.039-0.625	-0.0345	0.9924	12 ± 2; 0.2	33 ±13; 0.7	96.6 ± 13.3

3.2. Calculation of doses of the drugs in rabbits

The dose of the drug in the rabbits was calculated, depending on the weight of the rabbits in mg/kg using the following formula:

$$\text{HED (Human Equivalent Dose)} = \text{Animal Dose} \times \left(\frac{\text{Animal Weight}}{\text{Human Weight}}\right)^{0.33}$$

The maximum dose of CBZ, OCBZ and GPN that can be given to human in single day is 600 mg. In this study, the dose given to the rabbits was 5 mg/kg which was below the LD_{50} dose.

3.3. Pharmacokinetic study of CBZ

The control (conventional tablets) and SEDDS of CBZ (5 mg/kg) were administered into the oral cavity of each rabbit. Three groups of rabbits were undertaken for study. Each group was contributed for conventional tablets, SEDDS and remaining one as control group. In each group consisted of four rabbits. Blood samples were collected from the marginal ear vein at 0.25, 0.5, 0.75, 1, 2, 4, 6, 12 and 24 hrs after CBZ, OCBZ and GPN. The heparinised blood samples were immediately centrifuged at 4000 rpm for 15 minutes and separated plasma was stored at -4 ^{0}C.

Plasma samples collected from the rabbits were analyzed using developed reverse phase HPLC method (mentioned in earlier part) and the drug plasma concentration values were determined from the calibration curve.

Figure 3.1 shows the pharmacokinetic profile of intravenous (IV) CBZ in rabbits. Figure 3.2 shows the pharmacokinetic profile of CBZ with Vit. E SEDDS whereas Figure 3.3 shows the pharmacokinetic profile of CBZ marketed tablets in rabbits

Figure 3.1 Pharmacokinetic profile of Intra venous (IV) CBZ in rabbits.

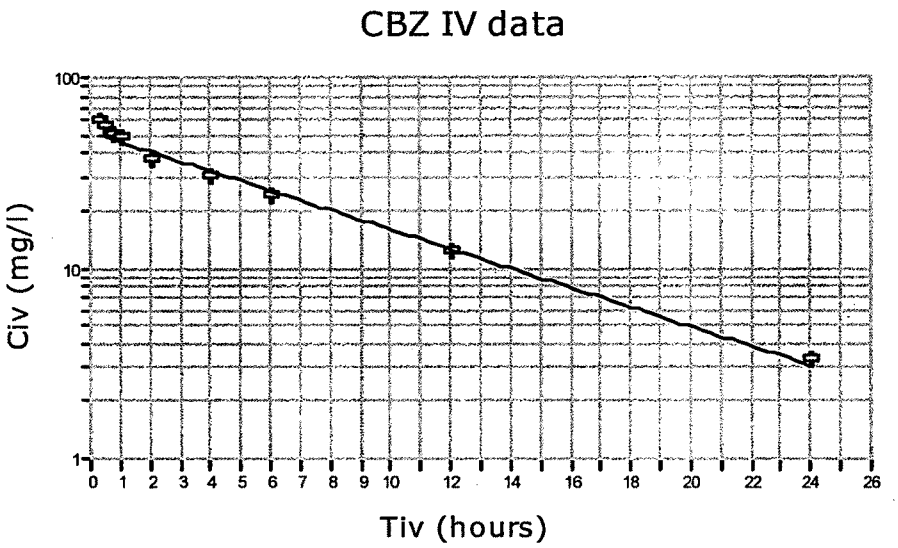

Figure 3.2 Pharmacokinetic profile of CBZ with Vit E SEDDS in rabbits.

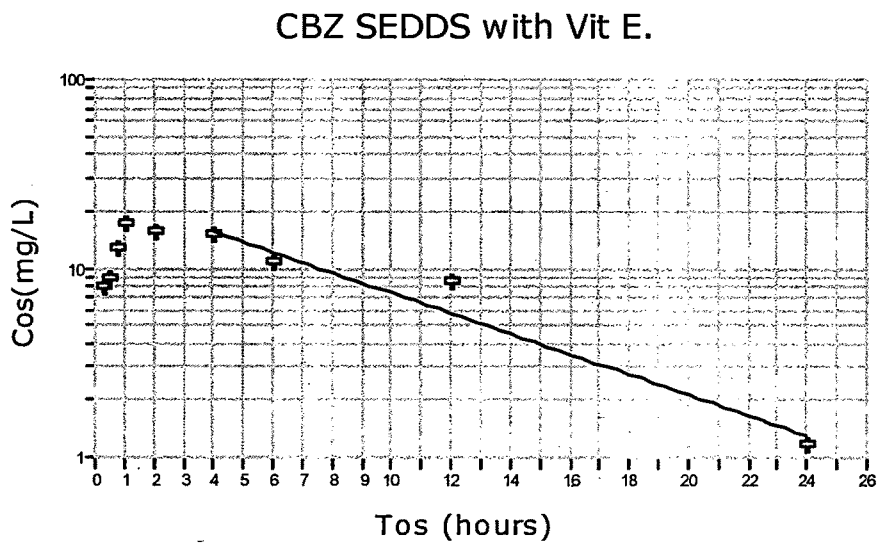

Figure 3.3 Pharmacokinetic profile of CBZ with labrasol SEDDS in rabbits.

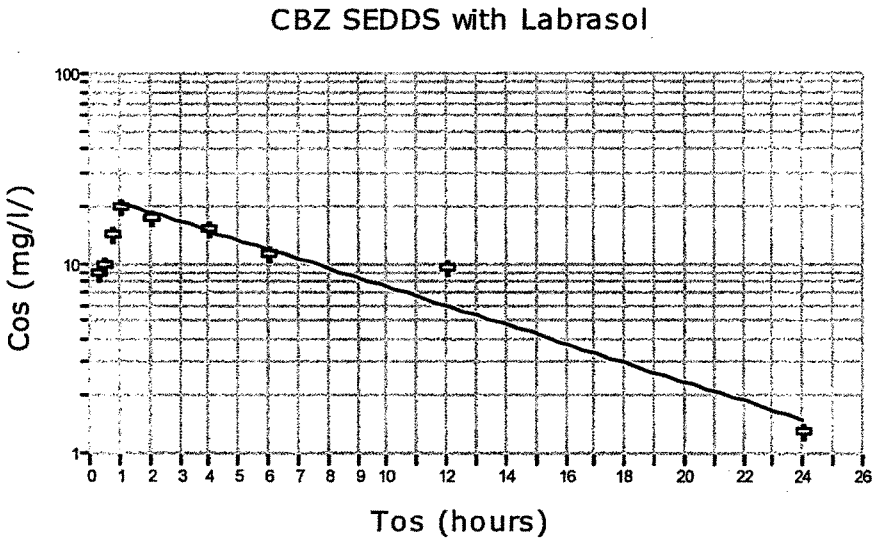

Figure 3.4 Pharmacokinetic profile of CBZ marketed tablets in rabbits.

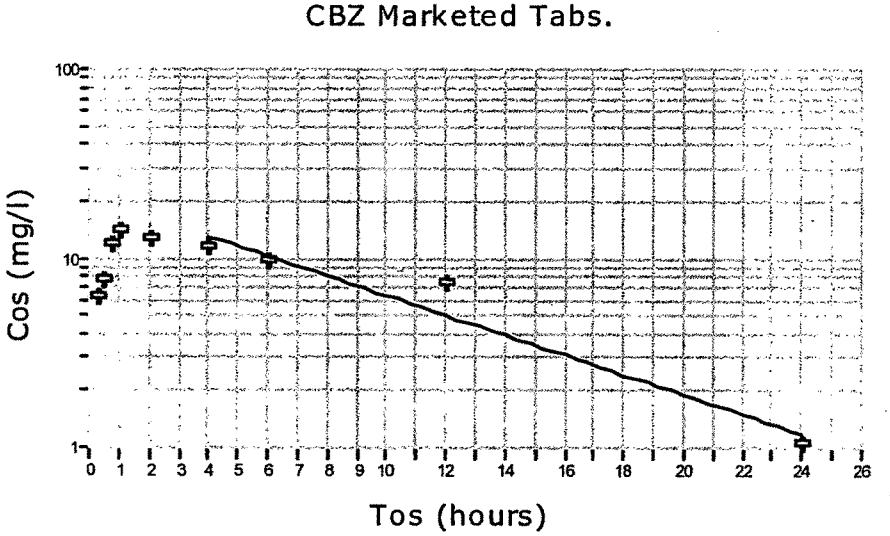

Table 3.5 Comparative profile pharmacokinetic parameters of CBZ SEDDS and Marketed tablets calculated by Wagner-Nelson method.

	CBZ SEDDS with Vit. E	CBZ SEDDS with Labrasol	CBZ Marketed tabs.
Dose (IV)	5.00	5.00	5.00
Dose (os)	5.00	5.00	5.00
A	51.61	51.61	51.61
Alpha	0.12	0.12	0.12
AUC (IV)	438.87	438.87	438.87
AUMC (IV)	3731.99	3731.99	3731.99
MRT	8.50	8.50	8.50
Lz (IV)	0.12	0.12	0.12
Cmax calc (IV)	51.61	51.61	51.61
Tmax calc (IV)	0.00	0.00	0.00
T1/2	5.89	5.89	5.89
Vc	0.10	0.10	0.10
Cmax (os)	17.06	19.58	13.56
Tmax (os)	1.00	1.00	1.00
AUC0n (os)	178.83	193.78	153.26
AUCextra (os)	10.53	13.22	9.81
AUCtot (os)	189.36	206.99	163.07
%AUCextra (os)	5.56	6.38	6.02
Lz (os)	0.12	0.11	0.12
AUMC0n (os)	1433.14	1564.17	1257.11
AUMCextra (os)	337.70	432.89	317.16
AUMCtot (os)	1770.85	1997.06	1574.27
Amax	2.34	2.56	2.01
T50%	0.63	0.62	0.60
T90%	3.20	3.89	6.01
F	0.43	**0.47**	0.37

On observing the different *in vivo* pharmacokinetic parameters tabulated in Table 3.5 it is clear that CBZ SEDDS systems with labrasol showed more bioavailability (F=0.47) when compared with CBZ Vit E SEDDS and marketed conventional tablets (F=0.43 and 0.37 respectively).

Table 3.6 shows in vivo parameters and their values for CBZ Vit E SEDDS, CBZ labrasol and CBZ Marketed tablets formulation.

Table 3.6 *In vivo* parameters for SEDDS of CBZ and Marketed conventional tablets.

CBZ Vit E SEDDS system										
Tos	**Cos**	**AUC**	**AUCcum**	**AUMC**	**AUMCcum**	**A(t)**	**A%(dose)**	**A%(Amax)**	**Unabsorbed%(Amax)**	**dA/dt**
0.25	7.85	0.98	0.98	0.25	0.25	0.77	15.44	32.94	67.06	
0.50	8.69	2.07	3.05	0.79	1.03	0.88	17.53	37.40	62.60	0.98
0.75	12.34	2.63	5.68	1.70	2.73	1.26	25.20	53.76	46.24	1.77
1.00	17.06	3.68	9.35	3.29	6.02	1.76	35.19	75.06	24.94	0.41
2.00	15.34	16.18	25.54	24.13	30.16	1.78	35.54	75.82	24.18	0.09
4.00	14.52	29.85	55.39	89.28	119.44	2.04	40.76	86.94	13.06	0.03
6.00	10.35	24.64	80.03	121.79	241.23	1.91	38.29	81.67	18.33	0.04
12.00	8.25	55.56	135.59	493.76	734.99	2.34	46.88	100.00	0.00	0.01
24.00	1.15	43.24	178.83	698.15	1433.14	2.15	42.98	91.67	8.33	
CBZ with labrasol SEDDS system										
Tos	**Cos**	**AUC**	**AUCcum**	**AUMC**	**AUMCcum**	**A(t)**	**A%(dose)**	**A%(Amax)**	**Unabsorbed%(Amax)**	**dA/dt**
0.25	8.54	1.07	1.07	0.27	0.27	0.84	16.79	32.87	67.13	
0.50	9.61	2.27	3.34	0.87	1.13	0.97	19.38	37.93	62.07	1.09
0.75	13.57	2.90	6.23	1.87	3.01	1.39	27.71	54.23	45.77	2.09
1.00	19.58	4.14	10.38	3.72	6.73	2.02	40.30	78.87	21.13	0.45
2.00	16.74	18.12	28.50	26.95	33.67	1.95	38.93	76.18	23.82	0.03
4.00	14.68	31.37	59.88	93.44	127.11	2.10	42.09	82.36	17.64	0.02
6.00	11.03	25.53	85.41	126.44	253.55	2.04	40.82	79.89	20.11	0.06
12.00	9.21	60.54	145.95	539.44	792.99	2.56	51.10	100.00	0.00	0.02
24.00	1.25	47.83	193.78	771.19	1564.17	2.33	46.58	91.14	8.86	

CBZ Marketed tablets										
Tos	Cos	AUC	AUCcum	AUMC	AUMCcum	A(t)	A%(dose)	A%(Amax)	Unabsorbed%(Amax)	dA/dt
0.25	6.24	0.78	0.78	0.20	0.20	0.61	12.27	30.47	69.53	
0.50	7.42	1.71	2.49	0.66	0.85	0.75	14.95	37.12	62.88	1.11
0.75	11.47	12.36	4.85	1.54	2.39	1.17	23.33	57.93	42.07	1.31
1.00	13.56	3.13	7.98	2.77	5.16	1.40	28.09	69.74	30.26	0.21
2.00	12.35	12.94	20.92	19.32	24.48	1.43	28.70	71.26	28.74	0.06
4.00	11.25	23.59	44.51	70.39	94.87	1.60	31.95	79.32	20.68	0.06
6.00	9.47	20.67	65.18	102.77	197.64	1.66	33.20	82.45	17.55	0.05
12.00	7.25	49.88	115.06	442.27	639.92	2.01	40.27	100.00	0.00	0.01
24.00	1.03	38.20	153.26	617.19	1257.11	1.85	36.91	91.64	8.36	

3.3.1. List of the abbreviations for Variables output obtained by using Wagner-Nelson method for oral administration

A	Coefficient in the exponential
Alpha	Exponent
Weighted Res.	Weighted residuals
AUC	Partial area under the curve
AUMC	Area under the moment curve
MRT	Mean residence time
AUCcum	Accumulated area under the curve
Lz	Total elimination rate constant
T1/2	Half-life
Cmaxcalc	Calculated maximum concentration of IV
Tmaxcalc	Calculated maximum time of IV
Vc	Volume of the plasma compartment
Cmax (os)	Maximum concentration for os
AUCon	AUC from t=0 to tlast
AUCextra	Extrapolated AUC
AUCtot	AUC total
%AUCextra	Percentage of AUC extrapolated with respect to AUC total
Vd	Volume of distribution
A(t)	Amount Of Drug Absorbed
A(t) = Vd.[Cos (t) + Lz.AUC (t)]	
A%(dose)	Amount of absorbed drug compared to the dose
A%(dose)last	The last value of A%(dose)
Amax	Maximal amount of absorbed drug
A%(Amax)	Amount of absorbed drug compared to Amax
A%(Amax)last	The last value of A%(Amax)
Unabs%(Amax)	Amount of unabsorbed drug in %
dA/dt	Rate of absorption

3.4. Pharmacokinetic study of OCBZ

The protocol followed for pharmacokinetic studies of OCBZ SEDDS in rabbits was same as that of CBZ SEDDS.

Figure 3.5 shows the pharmacokinetic profile of intravenous (IV) OCBZ in rabbits. Figure 3.6 shows the pharmacokinetic profile of OCBZ SEDDS whereas Figure 3.7 shows the pharmacokinetic profile of OCBZ marketed tablets in rabbits

Figure 3.5 Pharmacokinetic profile of intravenous (IV) OCBZ SEDDS in rabbits.

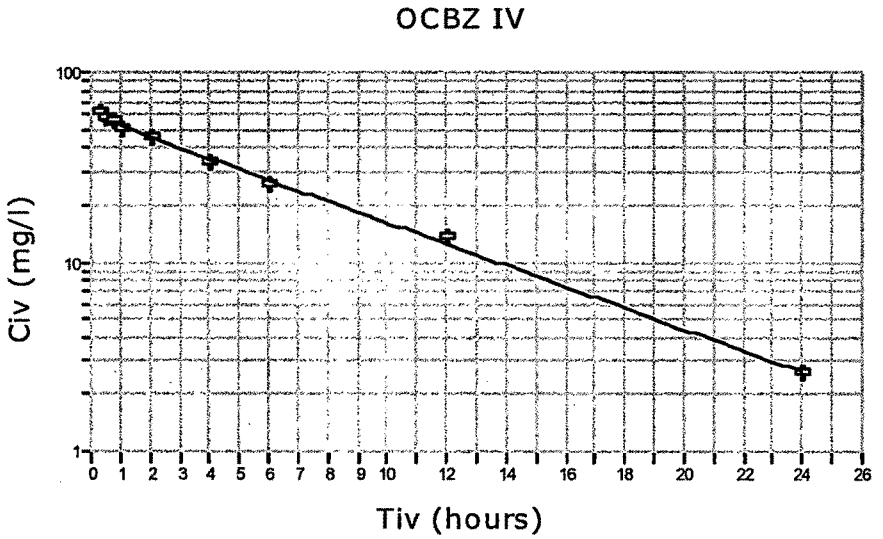

Figure 3.6 Pharmacokinetic profile of OCBZ SEDDS in rabbits.

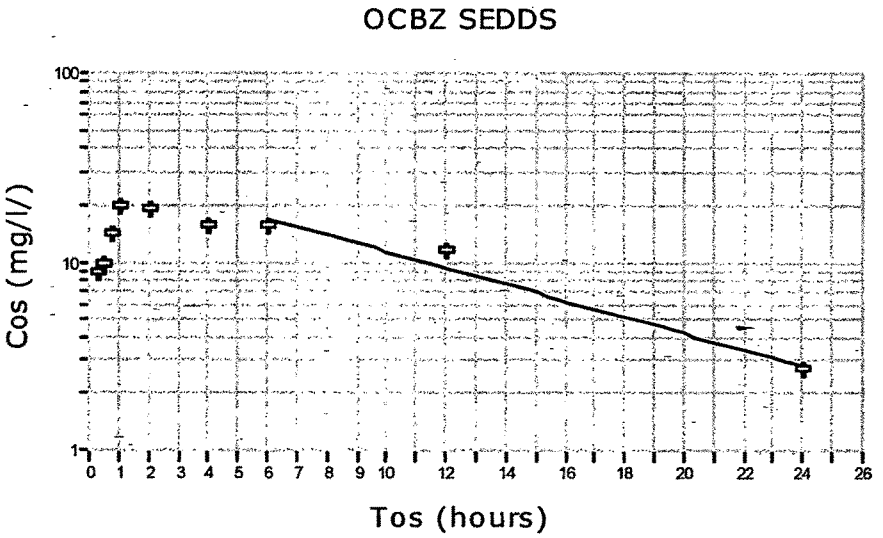

Figure 3.7 Pharmacokinetic profile of OCBZ Marketed tablets in rabbits.

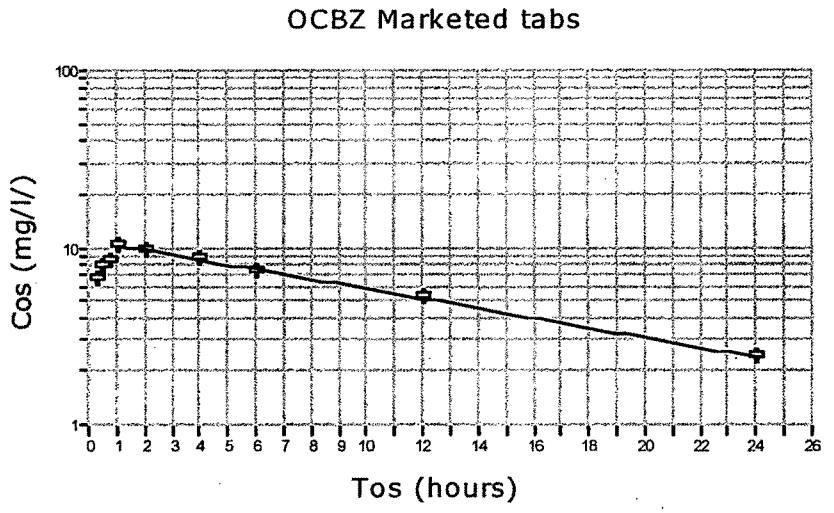

Table 3.7 Comparative profile pharmacokinetic parameters of CBZ SEDDS and Marketed tablets calculated by Wagner-Nelson method.

	OCBZ SEDDS	OCBZ Marketed tabs.
Dose (IV)	5.00	5.00
Dose (os)	5.00	5.00
A	58.92	58.69
Alpha	0.13	0.13
AUC (IV)	454.38	452.38
AUMC (IV)	3503.96	3503.96
MRT	7.71	7.71
Lz (IV)	0.13	0.13
Cmax calc (IV)	58.9228	58.92
Tmax calc (IV)	0	0.00
T1/2	5.35	5.34
Vc	0.08	0.09
Cmax (os)	19.54	19.54
Tmax (os)	1	1.00
AUC0n (os)	243.635	243.64
AUCextra (os)	28.1307	28.13
AUCtot (os)	271.766	271.77
%AUCextra (os)	10.3511	10.35
Lz (os)	0.10	0.06
AUMC0n (os)	2164.85	2164.85
AUMCextra (os)	956.642	956.64
AUMCtot (os)	3121.49	3121.49
Amax	2.90	1.65
T50%	0.86	0.84
T90%	9.39	15.49
F	**0.60**	0.37

From Table 3.7 it is clear that OCBZ SEDDS showed more bioavailability than conventional marketed tablets. The percent bioavailability for developed OCBZ SEDDS formulation was found to be 60 compared to 37 obtained for conventional marketed tablets.

Table 3.8 shows *in vivo* parameters and their values for OCBZ SEDDS and OCBZ Marketed tablets formulation.

Table 3.8 *In vivo* parameters for SEDDS of OCBZ and Marketed conventional tablets.

OCBZ SEDDS										
Tos	**Cos**	**AUC**	**AUCcum**	**AUMC**	**AUMCcum**	**A(t)**	**A%(dose)**	**A%(Amax)**	**Unabsorbed%(Amax)**	**dA/dt**
0.25	8.67	1.08	1.08	0.27	0.27	0.75	14.95	25.74	74.26	
0.50	9.61	2.29	3.37	0.87	1.14	0.85	17.05	29.35	70.65	0.94
0.75	13.54	2.89	6.26	1.87	3.01	1.22	24.36	41.92	58.08	1.84
1.00	19.54	4.14	10.40	3.71	6.72	1.77	35.45	61.02	38.98	0.56
2.00	18.78	19.16	29.56	28.67	35.40	1.92	38.38	66.05	33.95	0.08
4.00	15.47	34.14	63.70	101.33	136.72	2.01	40.27	69.32	30.68	0.10
6.00	14.98	30.45	94.15	152.07	288.80	2.31	46.14	79.42	20.58	0.10
12.00	11.26	78.18	172.33	692.50	981.30	2.85	57.03	98.16	1.84	0.03
24.00	2.64	71.31	243.64	1183.56	2164.85	2.90	58.10	100.00	0.00	
OCBZ MKTD										
Tos	**Cos**	**AUC**	**AUCcum**	**AUMC**	**AUMCcum**	**A(t)**	**A%(dose)**	**A%(Amax)**	**Unabsorbed%(Amax)**	**dA/dt**
0.25	6.54	0.82	0.82	0.20	0.20	0.57	11.32	34.23	65.77	
0.50	7.68	1.78	2.60	0.68	0.89	0.68	13.66	41.30	58.70	0.37
0.75	8.25	1.99	4.59	1.25	2.14	0.75	15.07	45.56	54.44	0.53
1.00	10.25	2.31	6.90	2.05	4.20	0.95	18.99	57.41	42.59	0.20
2.00	9.58	9.91	16.81	14.81	19.01	1.00	20.04	60.58	39.42	0.06
4.00	8.65	18.21	35.02	54.33	73.34	1.12	22.48	67.97	32.03	0.04
6.00	7.25	15.86	50.88	78.83	152.17	1.18	23.60	71.35	28.65	0.04
12.00	5.25	37.18	88.06	328.61	480.78	1.42	28.41	85.90	14.10	0.03
24.00	2.36	43.37	131.43	746.41	1227.19	1.65	33.08	100.00	0.00	

3.5. Pharmacokinetic study of GPN

The protocol followed for pharmacokinetic studies of GPN SEDDS in rabbits was same as that of CBZ SEDDS.

Figure 3.8 shows the pharmacokinetic profile of intravenous (IV) GPN in rabbits. Figure 3.9 . shows the pharmacokinetic profile of GPN SEDDS whereas Figure 3.10 shows the pharmacokinetic profile of GPN marketed tablets in rabbits

Figure 3.8 Pharmacokinetic profile of intravenous (IV) GPN in rabbits.

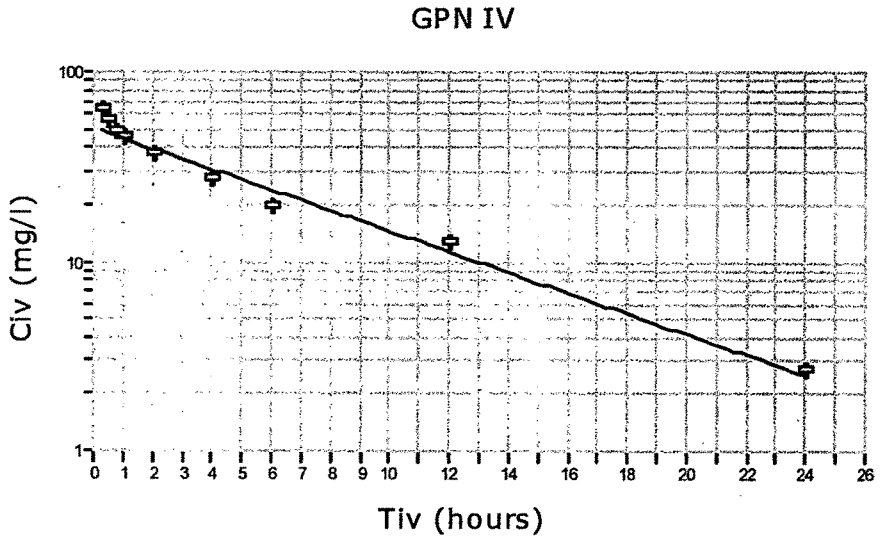

Figure 3.9 Pharmacokinetic profile of GPN SEDDS in rabbits.

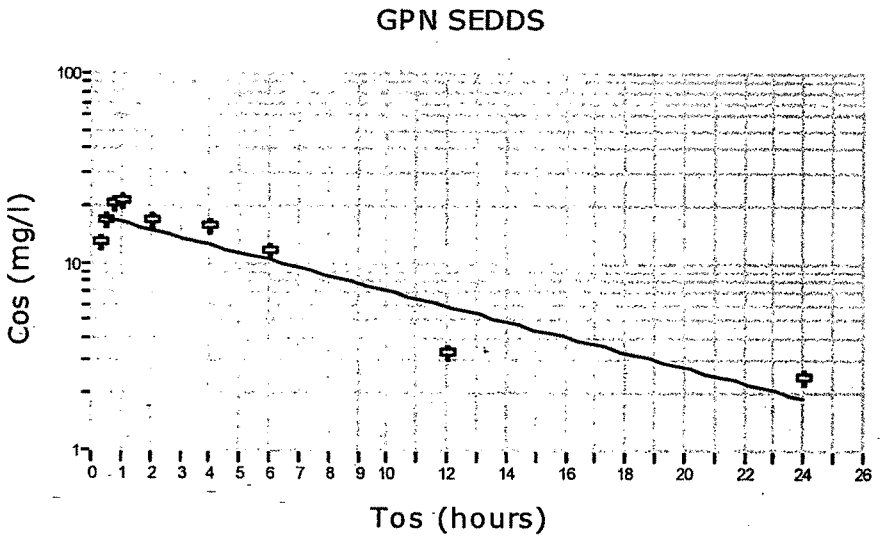

Figure 3.10 Pharmacokinetic profile of GPN Marketed tablets in rabbits.

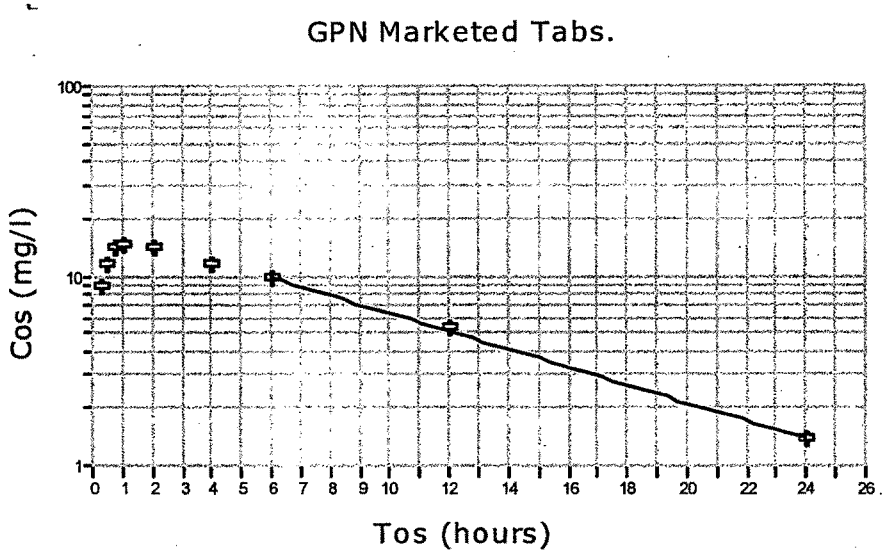

Table 3.9 Comparative profile pharmacokinetic parameters of GPN SEDDS and Marketed tablets calculated by Wagner-Nelson method.

	GPN SEDDS	GPN Marketed Tabs
Dose (IV)	5.00	5.00
Dose (os)	5.00	5.00
A	50.35	50.35
Alpha	0.12	0.12
AUC (IV)	403.84	403.84
AUMC (IV)	3238.78	3238.78
MRT	8.02	8.02
Lz (IV)	0.12	0.12
Cmax calc (IV)	50.3541	50.35
Tmax calc (IV)	0	0.00
T1/2	5.56	5.56
Vc	0.10	0.10
Cmax (os)	20.36	20.36
Tmax (os)	1	1.00
AUC0n (os)	163.461	163.46
AUCextra (os)	20.1605	20.16
AUCtot (os)	183.622	183.62
%AUCextra (os)	10.9794	10.98
Lz (os)	0.09	0.11
AUMC0n (os)	1177.05	1177.05
AUMCextra (os)	698.299	698.30
AUMCtot (os)	1875.35	1875.35
Amax	2.33	1.96
T50%		0.34
T90%	0.72	4.83
F	**0.45**	0.40

GPN SEDDS showed more bioavailability when compared with conventional marketed tablets. However the extent of increase in bioavailability was not in comparison with that observed for CBZ and OCBZ SEDDS systems. The percent bioavailability for developed GPN SEDDS formulation was found to be 45 compared to 40 obtained for conventional marketed tablets.

Table 3.10 shows the *in vivo* parameters for SEDDS of GPN and Marketed conventional tablets

Table 3.10 *In vivo* parameters for SEDDS of GPN and Marketed conventional tablets.

GPN SEDDS										
Tos	**Cos**	**AUC**	**AUCcum**	**AUMC**	**AUMCcum**	**A(t)**	**A%(dose)**	**A%(Amax)**	**Unabsorbed%(Amax)**	**dA/dt**
0.25	12.36	1.54	1.54	0.39	0.39	1.25	24.92	53.41	46.59	
0.50	16.54	3.61	5.16	1.42	1.81	1.71	34.13	73.15	26.85	1.67
0.75	19.74	4.54	9.69	2.88	4.69	2.08	41.60	89.16	10.84	0.99
1.00	20.36	5.01	14.71	4.40	9.09	2.20	44.08	94.46	5.54	-0.02
2.00	16.54	18.38	33.09	27.26	36.34	2.05	41.04	87.96	12.04	0.04
4.00	15.39	31.92	65.01	95.37	131.71	2.33	46.66	100.00	0.00	0.05
6.00	11.25	26.42	91.43	130.74	262.45	2.25	44.98	96.40	3.60	-0.05
12.00	3.25	38.66	130.09	324.50	586.95	1.93	38.67	82.87	17.13	0.00
24.00	2.36	33.38	163.46	590.10	1177.05	2.26	45.16	96.79	3.21	
GPN MKTD										
Tos	**Cos**	**AUC**	**AUCcum**	**AUMC**	**AUMCcum**	**A(t)**	**A%(dose)**	**A%(Amax)**	**Unabsorbed%(Amax)**	**dA/dt**
0.25	8.65	1.08	1.08	0.27	0.27	0.87	17.45	44.43	55.57	
0.50	11.25	2.49	3.57	0.97	1.24	1.16	23.23	59.15	40.85	1.13
0.75	13.65	3.11	6.68	1.98	3.23	1.44	28.77	73.25	26.75	0.76
1.00	14.26	3.49	10.17	3.06	6.29	1.54	30.83	78.48	21.52	0.20
2.00	14.00	14.12	24.30	21.17	27.46	1.69	33.81	86.07	13.93	0.06
4.00	11.27	25.17	49.47	74.61	102.06	1.73	34.64	88.18	11.82	0.03
6.00	9.55	20.77	70.24	103.28	205.35	1.82	36.35	92.53	7.47	0.02
12.00	5.21	42.98	113.22	373.89	579.24	1.92	38.39	97.73	2.27	0.01
24.00	1.37	34.47	147.69	575.55	1154.79	1.96	39.28	100.00	0.00	

3.6. Conclusion

In vivo studies performed in rabbits proved a good tool for assessment of the quality of the formulations. SEDD system is reported as potential formulation technique for dissolution and bioavailability enhancement of various drugs. In present work SEDD systems for CBZ, OCBZ and GPN were designed and developed. Simplex centroid mixture design was used for identifying the critical concentrations of 3 ingredients (drug, surfactant and cosurfactant) used in the formulation. To study the effect of oil (potential permeability enhancer) Vit E was used for preparation of CBZ SEDDS. In another CBZ SEDDS formulation labrasol was used as surfactant. Both formulations when compared with marketed tablets SEDDS with labrasol showed highest *in vitro* drug dissolution as well as *in vivo* plasma drug concentration.

OCBZ SEDDS also when compared with marketed tablets for *in vitro* and *in vivo* behaviour, performed well.

GPN SEDDS also showed improved bioavailability and proportionate rise in plasma drug concentration. However the extent of bioavailability enhancement was not to the extent CBZ and OCBZ SEDDS formulation showed. This may be attribute to physicochemical characters of GPN itself.

3.7. References

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