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An *in vitro-in vivo* correlation study of modified release formulations of Venlafaxine.HCl

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ABSTRACT

A single dose, randomized, complete and four treatment cross over study was conducted in healthy human subjects for *IVIVC* of venlafaxine.HCl. Plasma concentrations were estimated by a simple, rapid, sensitive and validated LCMS method. Cetirizine was used as the internal standard (IS). The analytes and the IS were extracted from the human plasma by liquid–liquid extraction technique. The reconstituted samples were chromatographed on Kromasil C18 column using an isocratic solvent mixture [acetonitrile–water, 90:10 (v/v)] at a flow rate of 0.5 mL/min. Method validation was performed as per FDA guidelines and the results met the acceptance criteria. USP dissolution apparatus I (Basket) and pH 6.8 at 100 rpm was found to yield acceptable *IVIVC* for the drug. The developed dissolution method would discriminate bioinequivalent batches. A 'Level A' correlation was observed for the selected formulations at the *in vitro* dissolution conditions developed. The dissolution method predicted the best absorption rate for the selected modified release formulations. The validity of the correlation was assessed by determining how well the *IVIVC* model could predict the rate and the extent of absorption as characterized by C_{max} and AUC. A percent prediction error of ≤ 10 % for C_{max} and AUC obtained establishes the predictability of the developed *IVIVC* model. It may, therefore, be concluded that the developed dissolution method can surrogate for human bioequivalence study.
Keywords: *IVIVC*, Bioequivalence. Dissolution. Human plasma. Venlafaxine. HCl.

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INTRODUCTION

The rational development of a controlled release drug delivery system is very expensive because formulation development and optimization involves the use of varying excipient levels, processing methods, identifying discriminating dissolution methods and subsequent scale up of the final product. As quantitative and qualitative changes in the formulation may alter drug release and *in vivo* performance, developing tools that facilitate product development by reducing the necessity of biostudies is desirable (Suvakanta, 2010). In this context, the use of *in vitro* data to predict *in vivo* performance is useful in the development of controlled release formulations.

In recent years a regulatory guidance has, therefore, been developed to minimize the need for additional bioavailability studies as part of formulation design. This guidance referred to as the, *IVIVC* Guidance, was developed by the United States Food and Drug Administration (USFDA) and is based on scientifically sound research (U.S.,1997a; Leeson, 1995; Uppoor, 2001). The ultimate goal of an *IVIVC* is to establish a meaningful relationship between *in vivo* behaviour of a dosage form and its *in vitro* performance, which would allow *in vitro* data to be used as a surrogate for *in vivo* behaviour (Bankim, 2011). A meaningful *IVIVC* for an extended/sustained release dosage form would be of benefit as a surrogate for bioequivalence studies which might typically be required with scale up or minor post-approval changes (SUPACs) in formulation equipment, manufacturing process or in the manufacturing site. Such a study would lead to improved product quality and decreased regulatory burden (Rackley, 1997; U.S., 1997b).

Several *IVIVC* studies of modified release formulations have been reported (Modi, 2000; Lake, 1999; Radovanovic, 1998; Yu, 1998). An *IVIVC* can help avoid bioequivalence studies by using the dissolution profile from the changed formulation and subsequently predicting the *in vivo* concentration-time profile (Hwang, 1995). For orally administered drugs, *IVIVC* is expected

for highly permeable drugs or drugs under dissolution rate limiting conditions, as supported by the Biopharmaceutical classification system (BCS) (Dressman, 1999; U.S., 1999). BCS is a fundamental guideline for determining the conditions under which *IVIVC*s are expected (Strickley, 1999; Amidon, 1995a).

The appropriate dissolution testing conditions should also discriminate between different formulations that possess different release patterns. Common dissolution mediums are water, simulated gastric fluid (pH 1.2), or intestinal fluid (pH 6.8 or 7.4) without enzyme, and buffers with a pH range of 4.5 to 7.5[17(Sievert, 1998)]. For sparingly water soluble drugs, use of surfactants in the dissolution medium is recommended (Shargel, 1993). A simple aqueous dissolution media is also recommended for BCS Class I drugs as this type of drugs exhibit lack of influence of dissolution medium properties (Galia, 1998).

There are no reports, however, of such studies for the selected formulations of Venlafaxine.HCl. According to the Biopharmaceutics classification system, this drug can be classified as a Class 1 drug, namely high solubility and high permeability drug (Amidon, 1995b).

Venlafaxine.HCl is a unique antidepressant and differs structurally from other commercially available antidepressants. Chemically it is a bicyclic phenyl ethylamine derivative (Holliday, 1995; Morton, 1995). Neuronal reuptake of serotonin and norepinephrine inhibition by the administration of Venlafaxine.HCl makes its usefulness in the treatment of depression.

The purpose of this study was, therefore, to develop an *IVIVC* study for selected modified release formulations of Venlafaxine HCl and establish the validity of the correlation through internal and external predictability.

MATERIALS AND METHODS

Acetonitrile, methanol, ortho phosphoric acid, potassium dihydrogen ortho phosphate, disodium hydrogen orthophosphate, ammonium acetate, perchloric acid, disodium tetraborate, ethylacetate, hydrochloric acid, potassium biphthalate, sodium acetate, acetic acid, sodium hydroxide and triethylamine were obtained from Qualigens Fine Chemicals and S.D. Fine chemicals. Water (HPLC grade) was obtained from Milli-Q RO system. All the reagents and chemicals used were of HPLC or Analytical grades.

The Reference product A, RPA (immediate release formulation of Vexor tablet containing 75 mg of Venlafaxine. HCl) and Reference product B, RPB (marketed modified release formulation of Veniz XR capsule containing 75 mg of Venlafaxine.HCl)) used for the study was obtained from Cadila Pharma, India, and Sun Pharmaceuticals industries, Dadra, India, respectively. The test formulations used, namely, fast modified release formulation (Test Product C, TPC) and slow modified release formulation (Test Product D, TPD) were given by Sipali chemicals, Chennai. Working standard of Venlafaxine.HCl was obtained as gift sample from M/s Neo Pharma, Abudhabi.

Instrumentation and chromatographic conditions

Shimadzu 2010A LCMS system used consists of a LC-10 AD-VP solvent delivery system (pump) with SIL 10 AD-vp Auto injector, SPD M-10AVP photo diode array detector, CTO 10 vp column oven, DGU 14AM degasser and LCMS solution data station.

Bioequivalence study design and data handling

A single dose, randomized, complete, four treatments cross over study was conducted in twenty four healthy human subjects for each drug formulation. On the basis of preliminary screening, 24 volunteers were selected and their liver and renal functions and haematological parameters such as hemoglobin content, RBC and WBC counts, blood sugar, cholesterol, bilirubin and ECG were examined by standard clinical and biochemical investigations. All the selected subjects were made to assemble 12 hours prior to dose administration in the Bioequivalence Center, J.S.S. College of Pharmacy, Ootacamund. In each dosing session, volunteers received either RPA or RPB or TPC or TPD. A wash out period of seven days was allowed between dose administrations. The protocol (#CADRAT/BE/076-03) of the study was submitted to the Institutional Human Ethical Committee and the approval for conducting the same was obtained. After overnight fasting, the volunteers were given code numbers and allocated to treatment in accordance with the randomized code. Volunteers received either Test or Reference product according to their code numbers with 240 mL of water. The order of treatment administration was randomized in four sequences (ABCD, BCDA, CDAB and DABC) in blocks of four.

As per the FDA recommendation, the bloods samples were collected up to at least three or more half-life of the drug and the time points were selected based on the pharmacokinetic parameters (T_{max} and $t_{1/2}$) of Venlafaxine. HCl. Blood samples (4 mL) were collected using disposable syringes in pre¬heparinised centrifugal tubes at 0 (before drug administration), 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 12.0, 18.0 and 24.0 h post dosing. The samples were centrifuged at 3500 rpm for 10 minutes to separate plasma. They were transferred into air tight containers and stored at deep freeze condition until starting of the analysis. A similar procedure adopting cross over design in drug treatment was repeated after 7 days of wash out period. The plasma samples were extracted using liquid-liquid extraction (LLE) and their drug levels were quantified using LCMS technique. Pharmacokinetic parameters, namely C_{max}, t_{max}, k_{el}, $t_{1/2}$, AUC_{0-t} and $AUC_{0-\infty}$ were determined for individual drug treatments.

Preparation of standard and sample solution of the drug

Preparation of standard stock solution of the drug

100mL of 1 mg/mL stock solution of the drug was prepared and the volume was made up with acetonitrile and water. This solution was labelled and stored in a refrigerator below 8°C.

Preparation of standard solution of the drug

50 mL each of 5.0, 10.0, 20.0, 25.0, 50.0, 75.0, 100.0, 125.0, 200.0 and 250.0 ng/mL of the drug standard solutions were prepared using the standard stock solution and stored at –20 ± 20 C until analysis.

Preparation of spiking solution

Spiking solutions of 200.0, 400.0, 800.0, 1000.0, 2000.0, 3000.0, 4000.0, 5000.0, 8000.0 and 10000.0 ng/mL of the drug were prepared using the standard stock solution and stored at –20 ± 20 C until analysis.

Preparation of calibration (CC) standards

Calibration (CC) standards of 5.0, 10.0, 25.0, 50.0, 75.0, 125.0, and 250.0 ng/mL of the drug were prepared using 250 µL of spiking solution; the total volume of 10 mL was made with blank plasma. The standards were vortexed and stored at –70 ± 20 C until processing.

Preparation of quality control (QC) samples

Quality control (QC) standards of 20.0, 100.0 and 200.0 ng/mL of the drug were prepared using 250 µL of spiking solution; the total volume of

10 mL was made with blank plasma. The samples were vortexed and stored at –70 ± 20 C until processing.

Sample processing

A volume of 0.5 mL of plasma standard or subject sample was pipetted into 2.0 mL centrifuge tube and to this 0.1 mL of IS solution (2000.0 ng/mL) and 1 mL of precipitating agent were added. The resulting solution was vortexed for 5 minutes and centrifuged at 4000 rpm for 10 min. Supernatants from the above solutions were separated and used for analysis.

Validation of LCMS method

The method developed was validated in terms of accuracy, precision, selectivity, linearity & range, limit of detection (LOD), limit of quantitation (LOQ), ruggedness, robustness, long term stability, short term stability and system suitability of the method as per USFDA guidelines.

Development of in vitro dissolution methods

The release characteristics of Test and Reference products were determined using USP XXIII dissolution apparatus (Type I, Basket), at 75 and 100 rpm. The dissolution medium used were buffers of pH 1.2, 4.5, 6.8, 7.4 and distilled water, maintained at 37±0.5˚C. Dissolution tests were performed on six tablets or capsules. Samples (5 mL) were withdrawn at 0.0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 12.0, 18.0 and 24.0 h time intervals for a period of 24 h. Equal quantity of the dissolution medium was replaced to the dissolution jar after each sampling. The amount of the drug released was estimated by the optimized and validated LCMS method. Percentage drug release at various time intervals were calculated and compared.

In vivo data analysis

The pharmacokinetic parameters, namely Cmax, tmax, AUC_{0-t}, $AUC_{0-\infty}$, $t_{1/2}$ and k_{el} were determined using WinNonlin-Standard edition version 1.5 for individual drug treatments from the observed plasma concentration-time data. The measured plasma concentrations were used to calculate the area under the plasma concentration-time profile from time zero to the last concentration time point ($AUC_{(0-t)}$¬). The AUC ($_{0-t}$) was determined by the trapezoidal method. $AUC_{(0-\infty)}$ was determined by using the equation,

$$AUC_{(0-\infty)} = AUC_{(0-t)} + \frac{C(t)}{kel}$$

k_{el} was estimated by fitting the logarithm of the concentrations versus time to a straight line over the observed exponential decline. The Wagner-Nelson method was used to calculate the percentage of the dose absorbed, F(t), using the following equation,

$$F_{(t)} = C_{(t)} + kel\ AUC_{(0-t)}$$

The percent dose absorbed was determined by dividing the amount absorbed at any time by the plateau value, k_{el}, AUC ($_{0-\infty}$) and multiplying this ratio by 100.

$$\%\ dose\ absorbed = \left[\frac{C_{(t)} + kel\ AUC_{(0-t)}}{kel\ AUC_{(0-\infty)}}\right] X\ 100$$

Statistical analysis of pharmacokinetic data

The statistical analysis using least square means (LSM) was carried out for each component of the Test and Reference products on the pharmacokinetic data obtained from 24 volunteers. The untransformed and log transformed pharmacokinetic parameters (Cmax, AUC0 t, AUC0 ∞) were analyzed by an Analysis of Variance (ANOVA) including the effects for treatments, sequence of dosing, subjects nested within sequences, and period of treatment and drug formulations as factors in the statistical model. The two one sided 'T' tests for bioequivalence, 95% confidence intervals for the difference between treatments and least square means were calculated for ln-transformed Cmax, AUC0 t, AUC0 ∞ parameters. The confidence interval was expressed as a percentage relative to the LSM of the reference treatments.

In vitro dissolution data analysis

Percentage drug released or dissolved at various time intervals were calculated using the formula,

$$Percentage\ release = \frac{Concentration\ (mg/mL)\ X\ bath\ volumes\ (mL)}{Drug\ content\ (mg)} X\ 100$$

The dissolution profiles were determined by plotting the cumulative percent drug dissolved at various time points. The *in vitro* drug release profiles of the slow (TPD) and fast (TPC) modified release formulations were compared using the similarity factor, f2, as given in the following equation;

$$f_2 = 50 \log \left\{ \left[1 + 1/n \sum_{t=1}^{n} (Rt - Tt)^2 \right]^{-0.5} X\ 100 \right\}$$

where R_t and T_t are the percent dissolved at each time point for the Reference product and the Test product, respectively.

In vitro-in vivo correlation (IVIVC)

Linear regression analysis was used to examine the relationship between the percentage of the drug dissolved and the percentage of the drug absorbed. The percentage of the drug unabsorbed was calculated from the percentage absorbed. The slope of the best-fit line for the semi-log treatment of this data was taken as the first order rate constant for absorption. The dissolution rate constants were determined from % released vs the square root of time. Linear regression analysis was applied to the *IVIVC* plots and the coefficient of correlation (r^2), slope and intercept values were calculated. A 'Level A' correlation was estimated by a two-stage procedure, deconvolution followed by comparison of the percentage drug absorbed to the percentage drug dissolved.

IVIVC model validation

Internal validation

The predictability of the *IVIVC* was examined by using the mean *in vitro* dissolution data and the mean *in vivo* pharmacokinetics of the selected modified release formulations. The mean *in vitro* dissolution rate constants was correlated with the mean absorption rate constants for the modified release formulations. These two data points, along with the zero-zero intercept, were used to calculate the expected absorption rate constants. The prediction of plasma concentration was then accomplished.

To further assess the predictability and the validity of the correlations, the observed and *IVIVC* model-predicted C_{max} and AUC values for each formulation were determined. The criteria set in the USFDA guidance on *IVIVC* for 'Level A' correlation are the mean absolute percent prediction error (% PE) should not exceed 10% for C_{max} and AUC, and the prediction error for individual formulations should not exceed 15%.

External validation

For establishing external predictability, the exposure parameters for a new formulation are predicted using its *in vitro* dissolution profile and the *IVIVC* model, and the predicted parameters are compared to the observed parameters. The prediction errors (PE) are computed as for the internal validation. For C_{max} and AUC, the prediction errors for the external validation formulation should not exceed 10%. A prediction error of 10% to 20% indicates inconclusive predictability and illustrates the need for further study using additional data sets. For drugs with narrow therapeutic index, external validation is required despite acceptable internal validation, whereas internal validation is usually sufficient with non-narrow therapeutic index drugs.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

The standard solution of the drug was analyzed by LCMS system using the direct injection probe with ESI and APCI interfaces. The mass spectrum of the drug and the IS are given in Figures 1a and 1b. From the mass spectrum recorded, the detection molecular ion selected was 278 for the drug. To elute the drug from the stationary phase, acetonitrile was selected as the mobile phase because of its favorable UV transmittance, low viscosity and better solubility for the drug. The pH of the initial mobile phase selected was 2.0 because a low pH protonates column silanols (free hydroxyl group in reverse phase column) and reduces their chromatographic activity i.e., it forms hydrogen bonds with the polar groups. Further, a low pH (less than 3) is usually quite different from the pKa value of the weakly acidic drug under study. At low pH, therefore, the retention of the drug will not be affected by slow changes in the pH and the LCMS method will be more rugged. The chromatographic conditions used for analysis are presented in Table 1.

Validation of LCMS method

The method developed was validated as per USFDA guidelines and the results met the acceptance criteria. The summary results of the experimental parameters of the validated LCMS method for the quantification of Venlafaxine.HCl in human plasma are presented in Table 2.

Estimation of the drug in plasma sample

The calibration samples, quality control samples and plasma sample solutions were injected with the optimised and validated chromatographic conditions and the chromatograms were recorded. The retention time of the drug and the IS was 5.90 and 3.12 min, respectively (Figure 2). The quantification of the chromatogram was performed using peak area ratios (response factor) of the drug to the IS. The calibration curve was constructed routinely for spiked plasma containing the drug and the IS during the process of pre-study validation and in study validation. The mobile phase used for the assay provided a well defined separation between the drug, the IS and endogenous components. The zero h (pre dose) samples of all subjects showed no interference on retention times of both the selected drug and the IS.

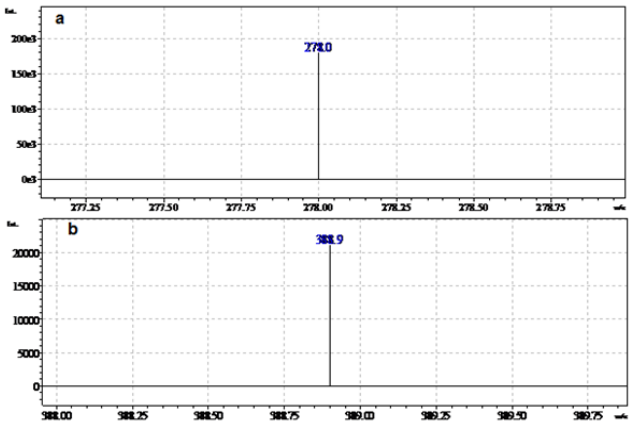

Figure 1. (a) The mass spectrum of the drug venlafaxine. HCl; (b) mass spectrum of the internal standard, Cetrizine.

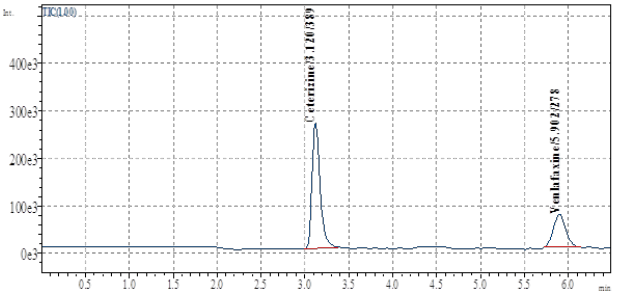

Figure 2. The retention time of the drug and the internal standard.

Table 1: Chromatographic conditions used for analysis of Venlafaxine.HCl

LC Condition	
Stationary phase	Kromasil C18 (100 x 4.6 mm i.d., 5 m)
Mobile phase	Acetonitrile and Water of 90:10 % v/v (Elution mode: isocratic)
Flow rate	0.5 mL/min
Injection volume	10 mL using auto injector
Oven temperature	30oC.
MS Condition	
Interface	APCI
Operation mode	SIM
Polarity	Positive
Probe temperature	Ambient
APCI temperature	400º C
CDL temperature	250º C
Block temperature	200º C
Detector voltage	1.3 kv
Nebulizer gas flow	2.5 L/min
Drying gas	2.0 L/min
Detection	Venlafaxine.HCl – 278
Data station	LC MS solution data station
Internal standard	Cetirizine – 388.9

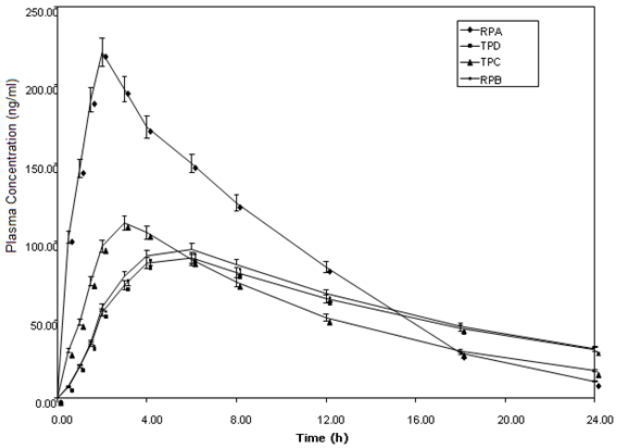

Figure 3. Mean concentration time curve of Venlafaxine.HCl

In vivo data analysis

Pharmacokinetic parameters, namely Cmax, tmax, AUC_{0-t} , $AUC_{0-\infty}$, k_{el} and $t_{1/2}$ were calculated and the blood level data of the selected formulations were compared. The mean pharmacokinetic profile for the selected formulations is presented in Table 3 and Figure 3.The data reveal that the mean pharmacokinetic profile for the TPD and the RPB are almost similar. However, the profile for the TPC shows a faster rate of absorption when compared to TPD and the RPB. There is thus a decrease in the absorption for the TPC and TPD when compared to the RPA. The results were further subjected to statistical analysis. The results of log transformed data of T/R ratio and 90% confidence interval of TPD Vs RPB and TPD Vs TPC are presented in Table 4.

The results of the statistical analysis thus reveal that the TPD is bioequivalent to RPB whereas the TPD is not bioequivalent to the TPC and also the RPA is not bioequivalent to the TPC and TPD.

There are noticeable differences in the plasma level concentrations between TPD, TPC and RPA. Also a rank order of release is observed in the dissolution testing as apparent from the plasma drug concentration profiles. However, the same rank order is not observed in the AUC. There are no noticeable differences in the AUC between the TPD (slow releasing dosage form) and the TPC (fast releasing dosage form) despite the differences in the release rates between the two dosage forms. The AUC of the selected drug is much higher from the extended release forms (RPB, TPC and TPD) than from the RPA. This may possibly be due to changes of drug metabolism as a result of change in the location of drug absorption in the GI tract.

Table 2: Summary of Experimental Parameters & Results of Validated LCMS method for the quantification of Venlafaxine. HCl in K2EDTA Human plasma.

S.No.	Experimental Parameters	Acceptable Range/Criteria (in %)	Results Obtained (in%)
1	Specificity and Selectivity % of passing lots (%CV of Area Ratio)	 >80 ≤20	 100 4.02
2	Carry over test	Analyte ≤20 Internal standard ≤ 5	0.00 0.00
3	Matrix Effect (%)	85 - 115	101.58
4	Intra Batch Accuracy in LOQQC (% Nominal)	80 - 120	84.27 to 98.53
	Intra Batch Accuracy in LQC, MQC & HQC (% Nominal)	85 - 115	87.83 to 100.16
5	Intra Batch Precision in LOQQC (%CV)	≤20	1.23 to 10.18
	Intra Batch Precision in LQC, MQC & HQC (% CV)	≤ 15	0.71 to 6.82
6	Inter Batch Accuracy in LOQQC (% Nominal)	80 - 120	91.33
	Inter Batch Accuracy in LQC, MQC & HQC (% Nominal)	85 - 115	91.77 to 96.08
	Inter Batch Precision in LOQQC (%CV)	≤20	9.73
	Inter Batch Precision in LQC, MQC & HQC (% CV)	≤ 15	3.71 to 6.84
7	Analyte Recovery (%)	<110	66.17
	%CV of mean LQC,MQC & HQC	<15	5.52
	Internal Standard Recovery (%)	<110	78.49
8	Re-injection reproducibility (%Nominal) (%CV) Ratio of means	85 – 115 ≤ 15 0.85 – 1.15	110.98(LQC) & 100.21(HQC) 5.67(LQC) & 1.03(HQC) 1.11(LQC) & 1.02(HQC)
9	DILUTION INTEGRITY		
i	2 Times (%Nominal)	85 – 115	95.83
ii	4 Times (% Nominal)	85 - 115	94.19
10	STABILITY		
i	Freeze-Thaw Stability (03cycles) (<-50°C) % Nominal	85 -115	95.54(LQC) 95.86(HQC)
iii	Bench top stability (10 h) % Nominal	85 -115	92.29(LQC) 96.17(HQC)
iv	Auto sampler stability – Venlafaxine.HCl (43h) % Nominal	85 -115	106.25(LQC) 98.44 (HQC)
v	Auto sampler stability – IS (43h) % Stability of Auto sampler IS stability	85 -115	96.52
ix	Short term stock stability LQC (10h) The % stability of Venlafaxine.HCl	90 -110	97.35
x	Short term stock stability HQC (10h) The % stability of Venlafaxine.HCl	90 -110	100.49
xi	Long term stock stability (14days) The % stability of Venlafaxine.HCl The % stability of IS	90 -110 90 - 110	100.62 98.73
xii	Long term plasma sample stability (54days) % Nominal	90 - 110	102.68 (LQC) 93.89 (HQC)

Table 3: Mean pharmacokinetic parameters of Venlafaxine.HCl (n=24)

Drug name		C_{max}	t_{max}	AUC_{0-t}	k_{el}	$t_{1/2}$	$AUC_{0-\infty}$
Venlafaxine.HCl	RPA	225.31 (1.53)	2.02 (0.35)	2141.23 (80.03)	0.14 (0.01)	5.09 (0.18)	2067.26 (547.68)
	TPD	94.48 (12.55)	5.58 (1.02)	1374.00 (136.92)	0.06 (0.01)	11.92 (2.28)	1921.71 (261.53)
	TPC	131.73 (12.22)	3.46 (1.18)	1311.88 (199.29)	0.09 (0.01)	7.62 (1.08)	1517.77 (203.43)
	RPB	100.89 (10.52)	5.79 (1.25)	1441.00 (114.61)	0.06 (0.01)	11.27 (2.04)	1967.14 (210.77)

RPA- Reference Product A(Immediate release formulation); TPD- Test Product D(Slow modified release formulation); TPC- Test Product C(Fast modified release formulation); RPB- Reference Product B(Marketed modified release formulation).

Table 4: The results of log transformed data of T/R ratio and 90% Confidence Intervals of TPD Vs RPB and TPD Vs TPC

PK Parameter	Ratio T/R (%)	90% Confidence Interval		Power %	ISCV %
		Lower	Upper		
TPD Vs RPB					
C_{max} (ng/ml)	107.1	95.70	104.57	100	15.8
AUC_{0-t}(ng.h/ml)	105.0	96.19	102.85	100	11.0
AUC0-inf (ng.h/ml)	102.6	100.77	106.33	100	11.5
TPD Vs TPC					
C_{max} (ng/ml)	140.0	93.57	146.42	100	15.8
AUC_{0-t} (ng.h/ml)	105.5	96.68	108.71	100	11.0
AUC0-inf (ng.h/ml)	126.7	90.76	118.91	100	11.5

TPD- Test Product D (Slow modified release formulation); TPC- Test Product C (Fast modified release formulation); RPB- Reference Product B(Marketed modified release formulation).

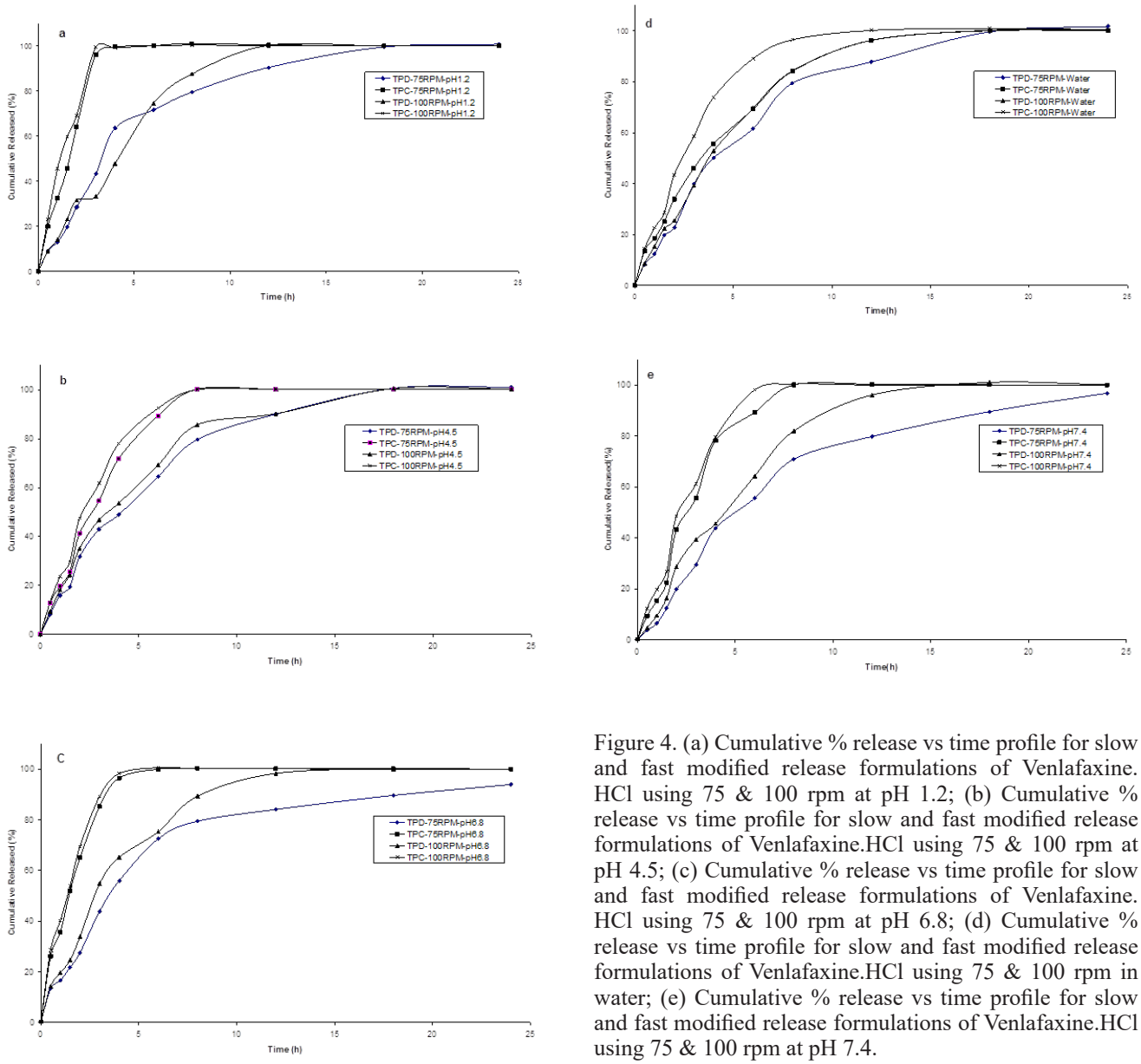

Figure 4. (a) Cumulative % release vs time profile for slow and fast modified release formulations of Venlafaxine. HCl using 75 & 100 rpm at pH 1.2; (b) Cumulative % release vs time profile for slow and fast modified release formulations of Venlafaxine.HCl using 75 & 100 rpm at pH 4.5; (c) Cumulative % release vs time profile for slow and fast modified release formulations of Venlafaxine. HCl using 75 & 100 rpm at pH 6.8; (d) Cumulative % release vs time profile for slow and fast modified release formulations of Venlafaxine.HCl using 75 & 100 rpm in water; (e) Cumulative % release vs time profile for slow and fast modified release formulations of Venlafaxine.HCl using 75 & 100 rpm at pH 7.4.

In vitro-in vivo correlation (IVIVC)

The *in vitro* release characteristics of TPC and TPD were determined. The percentage drug release at various time intervals were calculated and are presented in Figures 4a-e. The data reveal that when dissolution tests are performed at pH 4.5 buffers, pH 7.4 buffers and water at 75 and 100 rpm, the release of the drug is almost indistinguishable between the TPD and TPC.

The f2 equation is a logarithmic transformation of the sum of squares of the difference between Test and Reference profiles. The results are values between 0 and 100. The value of f2 is 100 when the Test and Reference profiles are identical and approaches zero as the dissimilarity increases. An f2 value 50 suggests that the two drug release curves differ by at least 10%. The f2 value greater than 50 (between 50 and 100) ensures sameness or equivalence between the two dissolution profiles. This equation is only applicable for comparing profiles in which the average difference between R and T is less than 100. If this average difference is greater than 100, the equation will yield a negative number.

In the present study the f2 value for pH 4.5 buffer, pH 7.4 buffer and water at 75 rpm is 66.67, 55.53 and 79.93, respectively, whereas at 100 rpm, the f2 value is 65.44, 56.19 and 63.60, respectively. The higher than 50 f2 values confirm that these dissolution mediums are indistinguishable and ensure sameness or equivalence between the two dissolution profiles and hence not considered for the present study. However, the best discrimination is achieved at pH 1.2 buffer and pH 6.8 buffer at 75 rpm as well as 100 rpm. The f2 value for pH 1.2 buffer and pH 7.4 buffer at 75 rpm is 44.96 and 44.84, respectively, whereas at 100 rpm, the f2 value is 47.89 and 47.60, respectively. The lower than 50 f2 values suggest that the two profiles are dissimilar. The dissolution results of pH 1.2 buffer and pH 6.8 buffer at 75 and 100 rpm were found to be the more discriminating dissolution media and hence selected for *IVIVC* model development.

IVIVC model development

A 'Level A' correlation was developed by constructing *IVIVC* plot using the percent dissolved vs. the percent absorbed data for both the TPD and TPC. The slope of the best-fit line was examined for percentage of the drug dissolved at pH 1.2 buffer and pH 6.8 buffer dissolution media at both 75 and 100 rpm and the percentage of drug absorbed. Linear regression analysis was applied to the *IVIVC* plots and the coefficient of correlation (r^2), slope and intercept values calculated are presented in Figures 5a-d. The correlation coefficient (r^2) for pH 1.2 buffer and pH 6.8 buffer at 75 rpm is 0.9579 and 0.9701, respectively, whereas it is 0.9625 and 0.9768 for pH 1.2 buffer and pH 6.8 buffer at 100 rpm, respectively. A good linear regression relationship was thus observed between the dissolution testing at pH 6.8 buffer and 75 and 100 rpm and hence this was selected for further analysis.

The dissolution rate constants were determined from the percentage drug released vs. the square root of time. The slope of the best-fit line for the semi-log treatment of this data was taken as the first order rate constant for absorption. The mean *in vitro* dissolution rate constant was correlated to the mean absorption rate constant for TPD and TPC. These two data points along with the zero-zero intercept were used to calculate the expected absorption rate constants. Linear regression analysis was applied to the *IVIVC* plots and the coefficient of correlation (r 2), slope and intercept values were calculated and are presented in Figures 6a and b. The correlation coefficient (r^2) for pH 6.8 buffer at 75 and 100 rpm is 0.9583 and 0.9981, respectively. A good linear regression relationship was observed when the dissolution studies were carried out in pH 6.8 buffer at 100 rpm and hence this was selected as the dissolution media of choice.

Internal Validation

The observed and the *IVIVC* model predicted C_{max} and AUC values for the drug are presented in Table 5. The percentage prediction errors for C_{max} and AUC were calculated and are presented in Tables 7. The C_{max} and AUC prediction errors values for the TPC and TPD formulations were very close to the observed mean values.

External Validation

% PE of 10% or less for C_{max} and AUC establishes the external predictability of an *IVIVC*. % PE between 10 - 20% indicates inconclusive predictability and the need for further study using additional data sets. Results of estimation of PE from all such data sets should be evaluated for consistency of predictability. % PE greater than 20% generally indicates inadequate predictability, unless otherwise justified.

The *in vitro* release characteristics of TPC, TPD, and RPB were determined. Cumulative percentage drug release at various time intervals were calculated and are presented in Figure 7a. The data reveal that when dissolution tests were performed at pH 6.8 buffer at 100 rpm, the release of the drug was almost indistinguishable between the TPD and RPB. The f2 value for TPD and RPB is 89.48, whereas the f2 value for TPC and RPB is 47.63. The higher f2 values (more than 50) confirm that these dissolution mediums are indistinguishable and ensure sameness or equivalence between the two dissolution profiles.

The *IVIVC* plot was constructed using percentage of drug dissolved at pH 6.8 buffer dissolution media at 100 rpm vs the percentage of drug absorbed. The slope of the best-fit line was examined using linear regression analysis and the coefficient of correlation (r^2), slope and intercept values calculated are presented in Figure 7b. The correlation coefficient (r^2) 0.9608 obtained shows a good linear regression relationship.

The dissolution rate constants were determined from the percentage drug released vs the square root of time. The slope of the best-fit line for the semi-log treatment

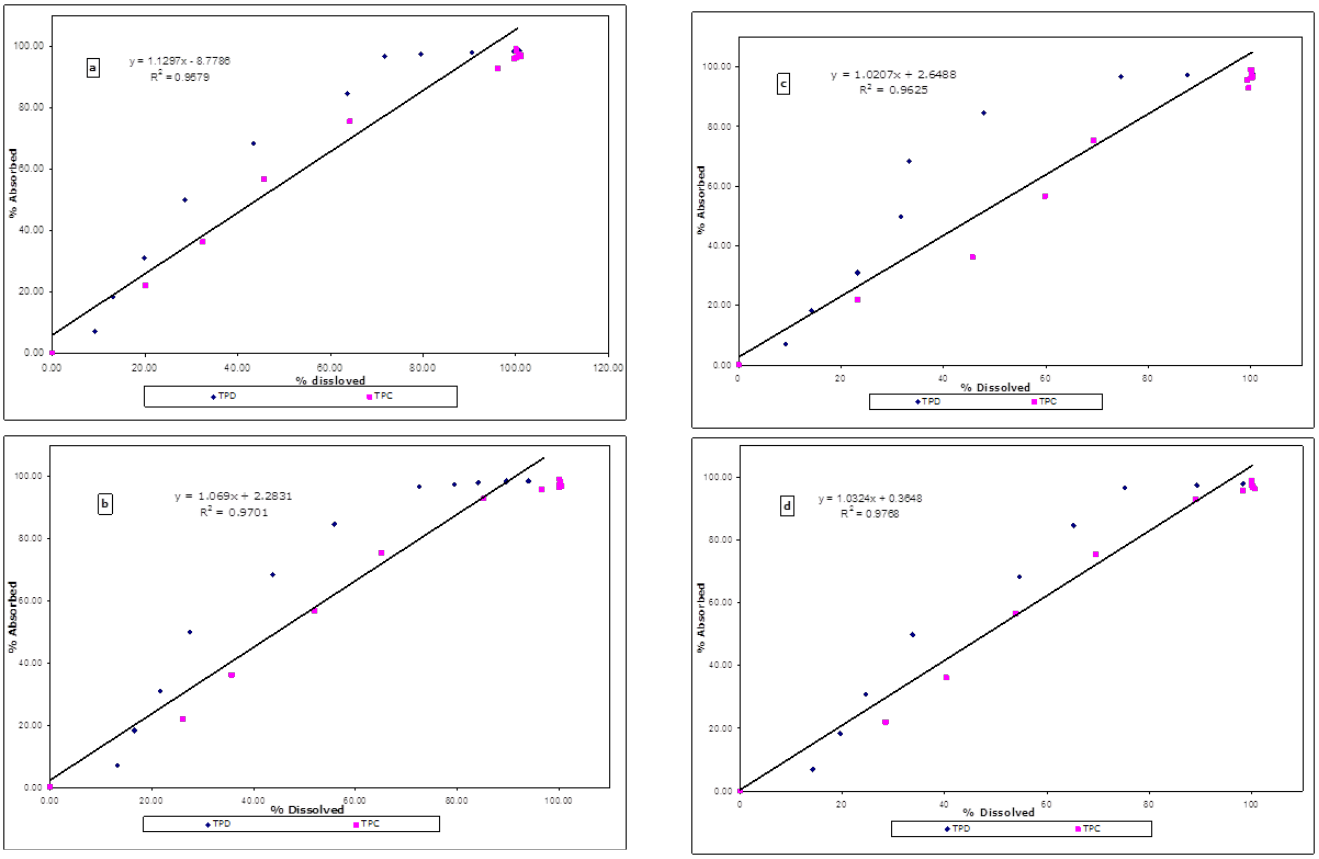

Figure 5. (a) *IVIVC* model linear regression plots of % absorbed vs % dissolved for the slow and fast Venlafaxine.HCl formulations using pH1.2, 75 rpm; (b) *IVIVC* model linear regression plots of % absorbed vs % dissolved for the slow and fast venlafaxine.HCl formulation using pH6.8,75 rpm; (c) *IVIVC* model linear regression plots of % absorbed vs % dissolved for the slow and fast venlafaxine.HCl formulations using pH1.2, 100 rpm; (d) *IVIVC* model linear regression plots of % absorbed vs % dissolved for the slow and fast Venlafaxine.HCl formulations using pH6.8, 100 rpm

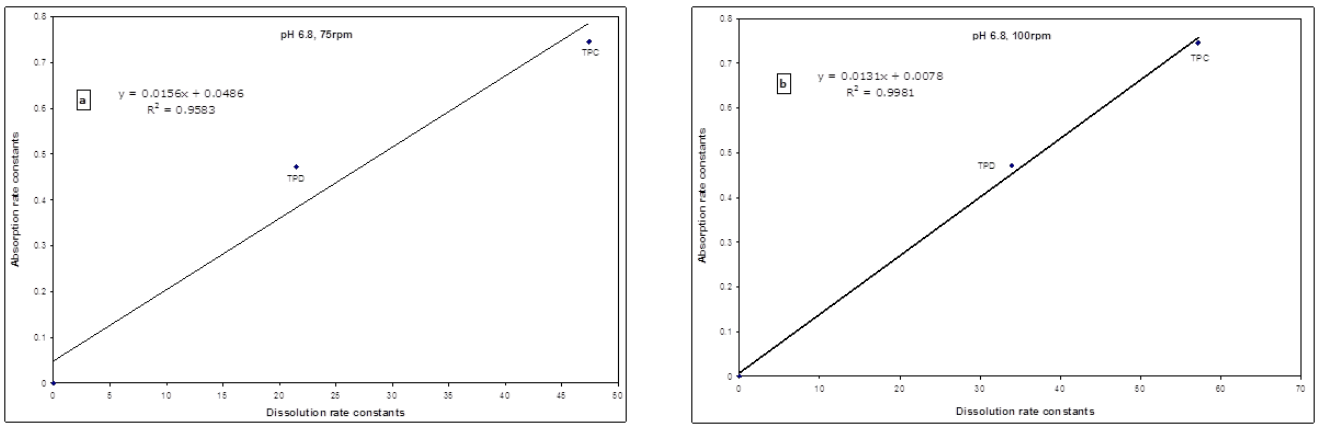

Figure 6. (a and b) Plot of *in vitro* dissolution rate constants vs *in vivo* absorption rate constants for Venlafaxine.HCl (The zero-Zero point is theoretical).

Table 5: Observed and *IVIVC* model predicted C_{max} and AUC values for Venlafaxine.HCl

Time (h)	TPD		TPC	
	Fraction observed	Fraction predicted	Fraction observed	Fraction predicted
0.00	0.00	0.00	0.00	0.00
0.50	7.93	2.24	30.37	12.58
1.00	20.55	10.20	48.49	33.00
1.50	34.35	22.59	74.64	63.15
2.00	54.80	42.60	97.08	91.53
3.00	72.28	66.89	111.99	113.37
4.00	86.28	85.60	105.95	105.42
6.00	89.62	89.43	88.61	77.44
8.00	80.31	74.75	74.19	54.63
12.00	63.68	48.08	51.47	26.26
18.00	44.67	23.69	30.15	8.81
24.00	31.07	11.50	17.78	2.97
Cmax	89.62	89.43	111.99	113.37
AUC	1897.40	1898.97	1510.74	1509.88

TPD- Test Product D (Slow modified release formulation); TPC- Test Product C(Fast modified release formulation)

Table 6: Observed and *IVIVC* model predicted C_{max} and AUC values for Venlafaxine.HCl

Time (h)	RPB		TPD	
	Fraction observed	Fraction predicted	Fraction observed	Fraction predicted
0.00	0.00	0.00	0.00	0.00
0.50	7.20	1.87	7.93	2.24
1.00	20.16	9.29	20.55	10.20
1.50	35.80	22.01	34.35	22.59
2.00	58.20	42.59	54.80	42.60
3.00	77.99	68.73	72.28	66.89
4.00	91.07	86.82	86.28	85.60
6.00	95.06	92.20	89.62	89.43
8.00	85.35	77.56	80.31	74.75
12.00	66.89	49.10	63.68	48.08
18.00	45.93	23.22	44.67	23.69
24.00	31.66	10.91	31.07	11.50
Cmax	95.06	92.20	89.62	89.43
AUC	1950.99	1951.13	1897.40	1898.97

RPB- Reference Product B (Marketed modified release formulation); TPD- Test Product D (Slow modified release formulation).

Table 7: Prediction errors (%) associated with C_{max} and AUC for Venlafaxine.HCl

Formulation	Cmax	AUC
Internal Validation		
TPD	0.22	-0.083
TPC	-1.23	0.057
Average	-0.505	-0.013
External validation		
TPD	0.22	-0.082
RPB	3.02	-0.007
Average	1.62	-0.044

TPD- Test Product D (Slow modified release formulation); TPC- Test Product C(Fast modified release formulation); RPB- Reference Product B(Marketed modified release formulation).

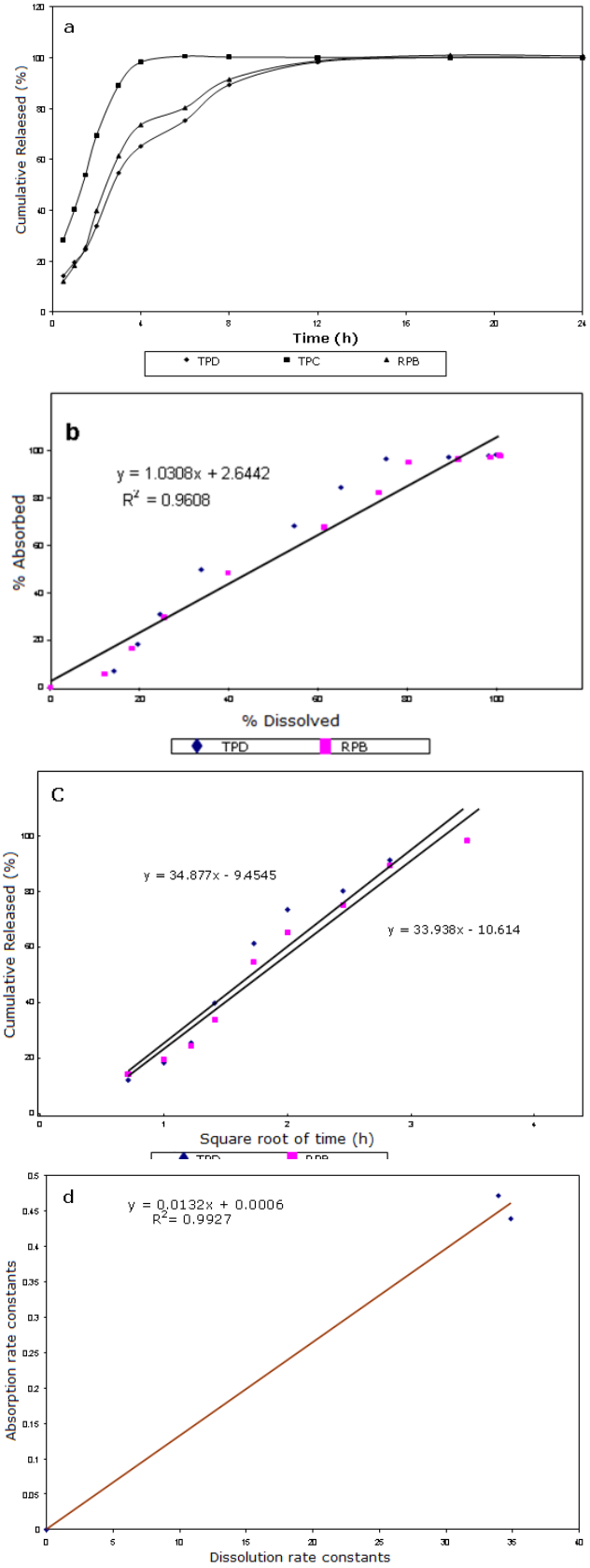

Figure 7. (a) Cumulative Venlafaxine.HCl release vs time profile for the modified release formulations using pH6.8, 100rpm; (b) *IVIVC* model linear regression plots of % absorbed vs % dissolved for the venlafaxine.HCl formulations using pH6.8, 100 rpm; (c) cumulative venlafaxine.HCl release vs square root of time profile for reference and slow modified release formulation using pH6.8, 100 rpm; (d) Plot of *in vitro* dissolution rate constant vs *in vivo* absorption rate constants for venlafaxine.HCl (The zero-zero point is theoretical)

of this data was taken as the first order rate constant for absorption. Linear regression analysis was applied to the *IVIVC* plots and the coefficient of correlation (r^2), slope and intercept values calculated are presented in Figures 7c and 7d. The correlation coefficient (r^2) 0.9927 obtained shows a good linear regression relationship.

The observed and the *IVIVC* model predicted C_{max} and AUC values for the drug are presented in Table 6. The percentage prediction errors for C_{max} and AUC were calculated and are presented in Tables 7. The C_{max} and AUC prediction errors values for the TPD and RPB formulations are very close to the observed mean values.

The C_{max} and AUC prediction error are within the specified limit and hence, the *IVIVC* is considered as validated, both in terms of internal and external validation.

CONCLUSION

Assumed *IVIVC* model for Venlafaxine.HCl modified release capsule was generated using literature information and in house bio study. A target *in vitro* profile was generated from the *IVIVC* model. Assumed *IVIVC* was developed and a target profile in biorelevant media was selected for the formulation development. The prediction errors obtained were within the limits. Based on the target profile, a pharmacokinetic profile was predicted for formulation TPD and TPC. It was identified that formulation TPD is the best formulation to develop once daily modified release formulation of Venlafaxine.HCl. Assumed *IVIVC* was successfully utilized in product development.

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