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Research Article

IN-VITRO AND *IN-VIVO* RELATIONSHIP AND INFLUENCE OF COVARIATES ON PHARMACOKINETICS OF URAPIDIL SUSTAINED RELEASE CAPSULES

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ABSTRACT

Objective: The aim of this study was to evaluate the variability associated with formulation and population level. Correlation of absorption phase in-vitro against in-vivo was considered to measure variability related to formulation level, while covariates linked variability was studied to assess population related variability.

Methods: A comparative evaluation between percent drug release in-vitro and percent in-vivo absorption was assessed to calculate the variability that occurs during the absorption phase of Urapidil using Wagner-Nelson method. In addition to in-vitro and in-vivo relationship method, influence of covariates on Urapidil pharmacokinetics was evaluated using linear mixed effect model.

Results: A linear relationship was observed between the percentage release in-vitro and the percentage absorbed in-vivo for a portion of the curve. However, percentage prediction error was within the regulatory limit for most of the time points. Impact of covariates analysis showed that study conducted following parallel design did not show any significant covariates influence in terms of inter and intra-subject coefficient of variation. Similarly, there were no significant intra subject coefficients of variation observed for study conducted under 2-way crossover design. However, obtained inter-subject coefficients of variation explained that significant influence on Urapidil pharmacokinetics may expected for some of the covariates, even same subjects exposed with different innovator products lot.

Conclusion: Prediction of pharmacokinetic behavior at the early stage of drug development could be more beneficial for mitigating unsuccessful outcome. The major obstacle in making such predictions is the inability to correlate the *in-vitro* data to the in-vivo data.

Keywords: Urapidil, Variability, Covariates, Eupressyl, In-vitro and in-vivo relationship

INTRODUCTION

Variability in the design and analysis of bioequivalence studies has been the topic of discussion for many years. Drug absorption can be influenced by physicochemical properties of the drug substance, the choice of the excipients and the dosage form technology required to dissolve the drug substance, particularly for Biopharmaceutical Classification System Classes 2 and 4 drugs. For instance, swollen dosage forms may be retained in the stomach so that some unusual absorption time profiles are observed and which could hinder the achievement of a successful bioequivalence study [1]. A number of factors can contribute to high variability in bioequivalence parameters [2]. The formulation factors that may impact on bioavailability and bioequivalence can be classified into two categories: (a) In the first group belong factors that can affect drug dissolution or release which is considered as a prerequisite to the drug absorption process. (b) The second category comprises factors related to excipients or inactive ingredients which can influence drug stability, absorption and metabolism [3].

The terms variability and uncertainty are used almost interchangeably in the pharmacokinetic and metabolism literature. It should be noted, however, that variability is an inherent property of the system of interest; it can be observed and recorded but not changed. Accordingly, uncertainty in the information available can be decreased and theoretically eliminated by implementing "ideal" experiments and data-processing techniques[4]. Formulation-related aspects of drug release can be studied in vitro. The identification and quantification of covariates, particularly using population pharmacokinetics is now seen as an integral part of drug development. Nonetheless, integration of such information for a priori identification of the potential covariates has been less than optimal and many pharmaceutical companies go through unnecessary cycles of clinical studies involving formulation optimization without attention to the feasibility of reducing inter-individual variability and the source of such variation[5].

Thus, present study was aimed to assess the variability factors influencing on pharmacokinetics of Urapidil from typical pharmacokinetics studies. The main goal of this study was to evaluate the variability associated with formulation and population level. Correlation of absorption phase in-vitro against in-vivo was considered to measure variability related to formulation level, while covariates linked variability was studied to assess population related variability.

MATERIALS AND METHODS

All materials used in this study were complied with current United States Pharmacopeia (USP) /European Pharmacopoeia compendial specifications.

Formulations

Formulation 1: Eupressyl 60mg (Urapidil Retard Capsules 60mg), Manufactured by Altana Pharma, France, Lot no.126662.

Formulation 2: Eupressyl 60mg (Urapidil Retard Capsules 60mg), Manufactured by Altana Pharma, France, Lot no.113494.

In-vitro studies

Dissolution test was performed on Urapidil using USP Type I apparatus at 75 rpm in 900 ml of media change method (0.1N HCl followed by pH 6.8) maintained at 37ºC and analyzed using validated HPLC method at Lupin Research Park, Pune, India. Dissolution samples were collected at 01, 02, 03, 04, 05, 06, 07 and 08hrs and analyzed spectrophotometrically at wavelengths of 269 nm for Urapidil.

Pharmacokinetic studies

An open, randomized, fasting, single-dose, parallel/two way crossover studies were performed with 30 healthy, non-smoking, male subjects. The study protocols for Urapidil were approved by the Institutional Review Board at the clinical site and the Drug General Controller of India [DCGI approval no. 12-09/2009/BE EXP/LUPIN-28/DC]. Written informed consent was obtained from all subjects prior to enrolment in the study. Study details are presented in [Table 1].

Table 1: Details of study design

Study No.	Study Design (IRB approval number)	Subjects enrolled and completed	Formulations (Form) and Lot number
1	Single dose parallel study under fasting conditions (07/01/11)	10	1 (126662)
2	Single dose parallel study under fasting conditions (49/11/09)	10	2 (113494)
3	Single dose two way cross over study under fasting conditions (04/08/10)	Period I - 10 Period II- 10	Form 1: (126662) Form 2: (113494)

In each study period, after an overnight fast of at least 10 hrs, single oral dose of Urapidil Retard Capsules 60mg (Eupressyl) was orally administered with 240mL of drinking water in sitting posture at ambient temperature in the morning, as per the randomization schedule. In each period, 21 blood samples were collected. The pre-dose blood sample (1x5-mL) was collected within 1 hour prior to dosing. The post-dose blood samples (1×5-mL each) were collected at 1.00, 2.00, 2.50, 3.00, 3.50, 4.00, 4.50, 5.00, 5.50, 6.00, 7.00, 8.00, 10.00, 12.00, 14.00, 16.00, 20.00, 24.00, 30.00 and 36.00 hours after dosing. Subjects were seated upright for the first two hours following drug administration and prohibited from any strenuous or athletic activity during housing period of the study. Plasma samples were stored at -80^{o}C before analysis. Plasma samples were separated and analyzed for Urapidil plasma concentrations.

Analytical methods

The in-house validated UPLC-MS/MS method was applied to determine the concentration of Urapidil in human plasma using Urapidil D4 as internal standard. The method was validated for selectivity, linearity, reproducibility, recovery, precision accuracy and stability.

The study sample analysis was performed on UPLC-MS/MS system using Betasil C18 (50 x 4.6mm, 5µ). Electrospray ionization was carried out in positive ion mode $[M+H]^+$ using triple quadruple mass spectrometer and multiple reaction monitoring (MRM) transition used for detection of Urapidil and Urapidil D4 were m/z 388.3/190.1 and 392.3/190.1, respectively. The data was acquired and calculated by using Masslynx version 4.1 Software.

The slope, intercept and correlation of coefficient were determined by least squares linear regression with 1/x2 (1/conc2) weighting for the calibration curve standards. The measured concentrations for each subject for all the time points are calculated against the calibration curve prepared with known standards.

Pharmacokinetic data analysis

The Urapidil plasma concentration versus time data were evaluated using the WinNonlin software version 6.3. Pharmacokinetic parameters Cmax, the maximum observed concentration, AUC, the area under the concentration time curve, Cl, total body clearance, Vz, volume of distribution and $t_{1/2}$, half-life of the drug were determined for each subject and formulation.

To calculate the elimination rate constant (Kel), regression analyses were performed on the natural log (Ln) of plasma concentration values (Y-axis) versus time (X-axis). Calculations were made between a time point where log-linear elimination phase begins and the time at which the last concentration above the limit of quantitation occurred.

The Kel was taken as the slope multiplied by (-1) and the apparent half-life ($t_{1/2}$) as (ln2) /Kel. Areas under the concentration - time curves (AUC) was calculated using the linear trapezoidal rule. Volume of distribution is estimated based on the terminal phase. Clearance was calculated using the formula CL= Dose/AUC.

Correlation of in-vitro and *in-vivo* dissolution absorption kinetics

Wagner-Nelson (WN)method was employed to assess the Urapidil absorption kinetics from Eupressyl Capsules 60mg. WN equation was used for calculating fraction of dose absorbed from plasma drug concentration-time profile. The relationship between mean fraction of dose dissolved versus mean fraction of dose absorbed was evaluated for two different lots of Eupressyl. Deconvolution, IVIVC model-prediction and Convolution process were carried out as per the steps described below.

Step 1: Deconvolution

Deconvolution in pharmacokinetics has been widely used for almost 40 years. It is an algorithm-based process employing the reverse of the effects of convolution [6]. Fraction absorbed at various time points 01, 02, 03, 04, 05, 06, 07 and 08 hrs from in-vivo data were obtained by the WN method (equation 1). For formulations 1 and 2, regression analysis was performed for percentage of drug absorbed against percentage of drug dissolved at selected time points. Slope, intercept and R was estimated. The predicted fraction of the drug absorbed values from the observed fraction of the drug dissolved data were calculated using the equation 2. Predicted fraction of the drugs absorbed was convolved to the predicted values of plasma drug concentration values at selected time points using the equation 3.

Equation 1

$$\frac{\mathbf{At}}{\boldsymbol{A\infty}} = \frac{\mathbf{Ct} + \mathbf{Ke} * \int_{\mathbf{t=0}}^{\mathbf{t=t}} \mathbf{Cdt}}{\mathbf{Ke} * \int_{\mathbf{t=0}}^{\mathbf{t=\infty}} \mathbf{Cdt}}$$

Where,
A_t =Amount of drug absorbed at time 't'
A_∞ =Amount of drug absorbed at time 'infinite'
K_e=Elimination rate constant of the drug.

$\int_{t=0}^{t=t} Cdt$=Area under the curve of the plasma concentration versus time profile of drug, for time period between t=0 to t=t.

Step 2: IVIVC model (prediction)

The predicted fraction of the drug absorbed values were obtained from observed fraction of the drug dissolved data using the below mentioned formula[7]. Fraction of the drug dissolved values from in-vitro study were plotted against fraction of drug absorbed values from in-vivo study. Regression analysis was carried out to obtain intercept and slope values from in-vitro and in-vivo relationship curve.

Equation 2

$$\%\boldsymbol{in\ vivo}\ \mathbf{input}\ (\mathbf{t}) = \propto + \boldsymbol{\beta} \times \%\boldsymbol{in\ vitro}\ \mathbf{input}\ (\mathbf{t})$$

Where,α and β are intercept and slope of the regression line, respectively

Steps 3: Convolution (back calculation of WN equation)

For the purpose of model validation the rearranged form of the WN equation was used. The observed and the calculated values of plasma drug concentration at selected time points were compared to obtain prediction error.

Equation 3

$$Ct+1=\frac{\left[\frac{2*\Delta F*D}{Vd}\right]+Ct\,(2-Ke*\Delta t)}{(2+Ke*\Delta t)}$$

Where,
K_e is elimination rate constant of the drug
V_d is volume of distribution
ΔF is the difference between fractions of drug dissolved at two successive sampling time points
Δt is the difference between two time points used for calculating ΔF

The Deconvolution, Prediction and Convolution process was accomplished using excel spreadsheet. Predictability was evaluated by comparing the observed and model predicted concentration data for given time points. Prediction error (PE) was determined as follows [7].

$$\%PE=\frac{[Predicted-Observed]}{Observed}\times 100$$

Where, Predicted is the mean pharmacokinetic parameters C max and AUC values from predicted profiles and Observed is corresponding mean values obtained from the raw data.

Analysis of covariates

The influence of covariates (age, height, body weight, body mass index, hematocrit, albumin, total protein, total bilirubin, serum creatinine, serum glutamic oxaloacetic transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) on the pharmacokinetics of Urapidil were evaluated by analyzing the data from three different pharmacokinetic studies. The final data for covariate analysis includes 30 south Asians, adult male healthy subjects. Covariate analysis was carried out following a two-stage approach. At first stage, individual pharmacokinetic parameters (Cmax, AUC inf, CL/F, Vd/F and $t_{1/2}$) for Urapidil were calculated. The relationships between log transformed pharmacokinetic parameters versus effect of covariates were analyzed using linear mixed effects model. The linear mixed effects models were carried out using Win Nonlin Software. In the model definition, pharmacokinetic parameters used as categorical independent variables and covariates were used as continuous independent variables.

RESULTS AND DISCUSSION

Over the years, different types of correlation approaches between in-vitro and in-vivo study have been examined and proposed for the evaluation of influence of variability in pharmacokinetics outcome of drug product during pharmacokinetics studies.

***In-vitro* studies**

In vitro dissolution has been recognized for the past four decades as an important element both in drug development and quality assessment especially in controlled release formulations. Release and further dissolution of the drug from the solid dosage forms often constitute a determining step in the in-vivo absorption process and is thus used in conjunction with in-vivo and in-vitro correlations to establish quality control parameters[8]. Media change method (0.1N HCl followed by pH 6.8) was adopted to mimic the general physiology changes during drug transit from stomach to intestine. Results indicate that there was no significant difference between two lots of Eupressyl formulation in percentage drug dissolved.

***In*-vivo studies**

A total of 30 healthy adult human male subjects were enrolled in 3 different studies. Subjects' baseline characteristics are presented in [Table 2].Samples from all 30 subjects (20 subjects from parallel studies and 10 subjects from cross over study) were analyzed to determine the plasma concentrations of Urapidil. Pharmacokinetic and statistical analyses were performed on data obtained from 30 subjects, who completed the studies as per the protocol.

Table 2: Subjects' baseline characteristics

Parameters	Mean±SD (Range)		
	Study 1 (N=10)	Study 2 (N=10)	Study 3 (N=10)
Age (years)	30.50±5.169 (25-38)	27.80±4.131 (22-34)	31.70±5.539 (20-39)
BMI (kg/m²)	20.81±2.247 (18-24)	21.58±2.111 (18-24)	21.68±1.725 (19-24)
Height (cm)	166.90±7.901 (154-176)	166.25±3.795 (161-172)	167.80±4.962 (160-176)
Weight (kg)	58.53±7.415 (50-71)	59.56±6.435 (50-72)	61.06±4.845 (55-70)
Hematocrit (%)	43.60±3.239 (38-49)	41.26±1.953 (38-44)	41.64±3.077 (37-46.70)
Serum Creatinine (mg/dL)	0.86±0.099 (0.75-1.00)	0.99±0.117 (0.80-1.17)	1.08±0.232 (0.73-1.37)
Total Bilirubin (mg/dL)	0.53 ±0.195 (0.30-0.79)	0.48±0.261 (0.18-0.99)	0.53±0.156 (0.32-0.84)
Total Protein (gm/dL)	7.59±0.569 (6.9-8.30)	6.95±0.357 (6.4-7.40)	7.21±0.441 (6.6-8.10)
Albumin (gm/dL)	4.32 ±0.202 (4.03-4.73)	4.36±0.135 (4.15-4.61)	4.58±0.257 (4.26-4.94)
SGOT (U/L)	23.30 ±5.034 (17-31)	19.40±5.835 (13-30)	17.00±8.380 (5-35)
SGPT (U/L)	17.10±4.557 (11.0-24)	13.20±3.458 (5-17)	17.00±8.957 (5-30)

The pharmacokinetic parameters Cmax, AUC inf, CL/F, Vd/F and $t_{1/2}$ for Urapidil were calculated by non-compartmental method using Win Nonlin Professional Software (Version 5.3).Mean pharmacokinetic parameters are presented in [Table 3].

Table 3: Mean obtained pharmacokinetic parameters for Urapidil

PK Parameters	Mean±SD (Range)			
	Study 1 Formulation 1 (N=10)	Study 2 Formulation 2 (N=10)	Study 3 - Formulation 1(N=10)	Study 3 - Formulation 2(N=10)
Cmax (ng/mL)	583.11±229.729 (330.83-921.390)	841.93±276.939 (302.84-1177.890)	842.03±442.261 (334.19-1976.200)	876.66±272.097 (394.32-1310.260)
AUC inf (ng*hr/mL)	5560.67±1944.049 (3403.69-9360.500)	7311.73±2736.647 (2377.34-11465.180)	7015.38±2988.105 (3343.02-13642.480)	6829.76±1870.372 (4336.33-9839.970)
Cl/F (gm/L)	0.01±0.004 (0.01-0.02)	0.01±0.006 (0.01-0.03)	0.01±0.004 (0.00-0.02)	0.01±0.003 (0.01-0.01)
Vz/F(l/kg)	0.11±0.043 (0.06-0.20)	0.08± 0.030 (0.04-0.14)	0.09±0.051 (0.03-0.21)	0.08±0.025 (0.05-0.12)
$t_{1/2}$(hr)	6.30±1.045 (4.55-7.95)	5.78±1.168 (3.95-7.94)	6.01±0.885 (5.20-7.94)	5.71±0.575 (4.75-6.71)

***In-vitro* and *in-vivo* relationship**

Understanding of in-vitro and in-vivo relationship generates useful information while developing a generic drug solid dosage form, especially for extended-release formulations.

WN method is one of the important tools for such assessments for prediction of drug absorption and further adjustment of the drug release profile to match that of innovator formulation.

A comparative evaluation between percent drug-release in-vitro and percent in-vivo absorption was assessed with selected time points to calculate the variability that occurs during the absorption phase of Urapidil from Urapidil SR Capsules formulations. The data generated using WN model is presented in [Table 4-6].

Table 4: *In-vitro* and in-*vivo* relationship for study 1 (Formulation 1)

Time (hrs)	Drug dissolved (%)	%Drug absorbed (Observed)	%Drug absorbed (Predicted)	Prediction Error (%)
1	29.30	33.47	28.36	-15.27
2	63.90	72.25	66.53	-7.91
3	78.20	100.67	82.31	-18.24
4	85.00	109.30	89.81	-17.83
5	88.90	101.83	94.11	-7.58
6	90.80	101.40	96.21	-5.12
7	92.60	100.36	98.19	-2.16
8	93.70	99.05	99.41	0.36

From the in-vitro study results, it was observed that there were no significant differences observed between formulations 1 and 2. In-vivo (study-1) absorption profile for formulation-1 was slightly faster than that of (33% vs 29% for 1hr and 72 vs 64% for 2hr for in-vivo and in-vitro, respectively), in-vitro absorption profile. Similarly, in-vivo (study-2) absorption profile for formulation-2 was reasonably lower than (27% vs 17% for 1 hr and 53 vs 69% for 2hr for in-vivo and in-vitro, respectively) in-vitro absorption profile.

Likewise, in-vivo (study-3) absorption profile from in-vivo and in-vitro results for formulation-1 found to be 46% vs 29% for 1hour and 93% vs 64% for 2hour, respectively. Whereas, for formulation-2 absorption profile from in-vivo and in-vitro results was observed with 34% vs 27% for 1hour and 83 vs 69% for 2hour, against respective in-vitro absorption profile. Result from study-3 was suggested that observed in-vivo absorption much faster than in-vitro absorption for both formulation-1 and formulation-2 showed much faster in-vivo absorption than in-vitro absorption profile.

Moreover, obtained data suggested that the observed in-vivo variability between the different lots of formulation when administering to the same subjects in different occasions could affect the pharmacokinetics of Urapidil from Eupressyl formulation.

Table 5: *In-vitro* and *in-vivo* relationship for study 2 (Formulation 2)

Time (hrs)	Drug dissolved (%)	%Drug absorbed (Observed)	%Drug absorbed (Predicted)	Prediction Error (%)
1	26.90	17.17	13.21	-50.90
2	69.30	53.47	60.99	-12.00
3	82.40	67.87	75.75	-8.07
4	88.90	86.06	83.07	-6.55
5	92.90	89.77	87.58	-5.72
6	94.10	89.13	88.93	-5.49
7	94.90	91.72	89.84	-5.34
8	95.10	94.24	90.06	-5.30

Table 6: *In-vitro* and *in-vivo* relationship for study 3 (Formulations 1 and 2)

	Formulation 1				Formulation 2			
Time (hrs)	Drug dissolved (%)	%Drug absorbed (Observed)	%Drug absorbed (Predicted)	Prediction Error (%)	Drug dissolved (%)	%Drug absorbed (Observed)	%Drug absorbed (Predicted)	Prediction Error (%)
1	29.30	46.29	49.40	6.72	26.90	34.03	31.88	-6.29
2	63.90	92.94	86.15	-7.30	69.30	83.04	86.72	4.44
3	78.20	100.91	101.33	0.42	82.40	97.25	103.67	6.60
4	85.00	113.75	108.56	-4.56	88.90	118.61	112.07	-5.51
5	88.90	104.26	112.70	8.10	92.90	115.27	117.25	1.72
6	90.80	100.25	114.72	14.43	94.10	107.20	118.80	10.82
7	92.60	98.20	116.63	18.77	94.90	150.60	119.84	-20.43
8	93.70	99.48	117.80	18.42	95.10	104.36	120.09	15.08

Correlation obtained in the present study was observed with certain deviations from the ideal 1:1 relationship [Figure 1-3]. Yet, obtained percentage prediction error was within the regulatory limit for most of the time points suggesting the pertinence of point to point correlation used in this study.

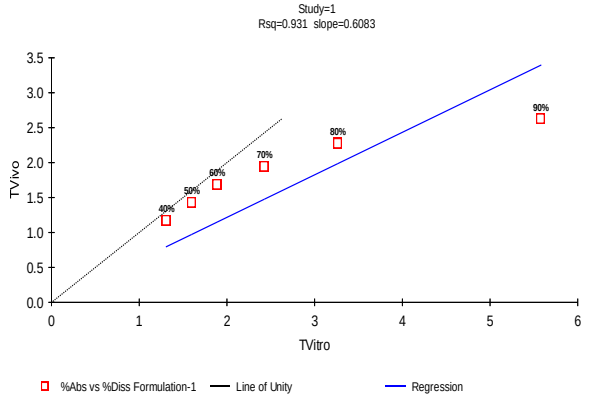

Fig. 1: Percentage drug absorbed versus dissolved for study-1

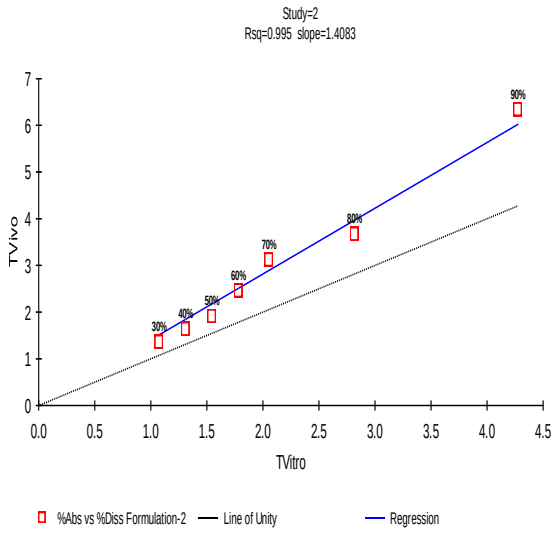

Fig. 2: Percentage drug absorbed versus dissolved for study-2

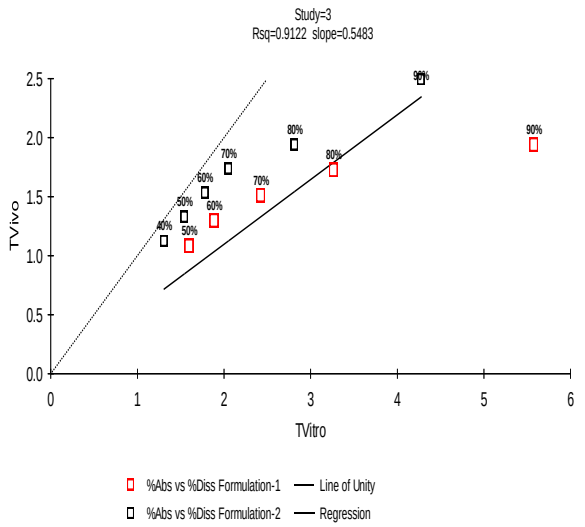

Fig. 3: Percentage drug absorbed versus dissolved for study-3

Covariates influence on outcome of Urapidil pharmacokinetics

The Linear Mixed Effects function is a statistical analysis system for analysis of variance for crossover and parallel studies, including unbalanced designs [9] and performs analyses using linear mixed effects models.

A two-stage pharmacokinetic analysis approach was used in this study to evaluate the covariates influence on pharmacokinetics of Urapidil. Results from the first stage analyses showed that there is no significant effect (P>0.05) on Urapidil pharmacokinetic parameters against evaluated covariates. However, obtained inter and intra-subject coefficient of variation for pharmacokinetic parameters Cmax and AUC from parallel and two way cross-over study indicates that significant influence of covariates effect on Urapidil pharmacokineticsis expected, though obtained P value is not statistically significant in the regression model[Table 7].

At second stage, using linear mixed effect models, pre-study laboratory parameters were correlated with obtained pharmacokinetic parameters among the subjects. Study conducted following a parallel design did not show any significant covariates influence in terms of inter and intra-subject coefficient of variation. Similarly, there were no significant intrasubject coefficients of variation observed for study conducted under 2-way crossover design.

Table 7: Obtained intersubject coefficient of variation for pharmacokinetic parameters

PK Parameters	Inter-subject CV (%)				Intra-subject CV (%)	
	Study 1 Form-1 (N=10)	Study 2 Form-2 (N=10)	Study 3 - Form-1 (N=10)	Study 3 - Form-2 (N=10)	Study 1 & 2 Form 1 vs 2	Study 3 Form 1 vs 2
Cmax	39	32	53	33	36	17
AUCinf	33	44	41	30	36	15
Cl/F	21	19	26	19	35	15
Vz/F	24	28	23	17	31	23
$t_{1/2}$	15	41	15	10	19	12

However, obtained intersubject coefficients of variation for laboratory parameters SGPT (69%) and SGOT (57%) explained that significant influence on Urapidil pharmacokinetics may expect, even same subjects exposed with different innovator products lot [Table 8].

Table 8: Obtained inter- and intra-subject coefficient of variation for covariates

Covariates	Inter-subject CV (%)		Intra-subject CV (%)	
	Study 1 vs 2 Form 1 vs 2 (N=20)	Study 3 Form 1 vs 2 (N=20)	Study 1 vs 2 Form 1 vs 2 (N=20)	Study 3 Form 1 vs 2 (N=20)
Age	8.0	9.0	13.0	14.0
BMI	NE	8.0	7.0	0.5.0
Height	2.0	3.0	3.0	2.0
Weight	6.0	8.0	7.0	2.0
Hematocrit	NE	5.0	6.0	6.0
Serum Creatinine	NE	22.0	15.0	0.5.0
Total Bilirubin	NE	29.0	6.0	11.0
Total Protein	3.0	6.0	5.0	3.0
Albumin	3.0	5.0	2.0	3.0
SGPT	23.0	69.0	2.0	18.0
SGOT	NE	57.0	3.0	7.0

NE-Not estimated

CONCLUSION

Successful generic drug development needs a comprehensive approach of adequate pharmacokinetic knowledge of the molecule. Prediction of pharmacokinetic behavior at the early stage of drug development could be more beneficial for mitigating unsuccessful outcome due to variability. The major obstacle in making such predictions is the inability to correlate the in-vitro data to the in-vivo data.

A comparative evaluation between percent drug-release in vitro and percent in vivo absorption was assessed with selected time points to calculate the variability that occurs during the absorption phase of Urapidil from Urapidil SR Capsules formulations. The data generated using WN model. In-vitro and in-vivo relationships were studied to predict the rate of drug absorption. Further, results confirm that the variability observed between different studies and study subjects are large, hence the same batch of formulation did not correlate well while observing the similar in-vitro results. However, it is important to note that a linear relationship was observed for a portion of the curve from in-vitro and in-vivo relationship. In addition to in-vitro and in-vivo relationship method, influence of covariates on Urapidil pharmacokinetics was evaluated using linear mixed effect model. The results evidencing that the reasonable influence of covariates on Urapidil pharmacokinetics parameters were observed even for different lot of innovator products, when administering to the same subjects. Thus, characterizing effect of few of the covariates on pharmacokinetics outcome will definitely reduce the number of pharmacokinetic studies using healthy human subjects and also development time and cost in generic R&D establishments. Although, the present study results can be while considered indicative, more realistic, if limitations like less number of sampling time points used for in-vitro and in-vivo release profiles evaluation and the adopted methodology to estimate influence of covariates were further improved.

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