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In-vitro Study and *In-vivo* Predictions of Valsartan and Amlodipine Capsules through Micro Tablets

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The present research work was carried out to develop Valsartan and Amlodipine capsules using micro tablets and to evaluate the *in-vitro* drug release characteristics. The study was targeted to determine the systemic concentrations using *in-vivo* prediction.
Study Design: The *in vivo* parameters along with the marketed Valsartan and Amlodipine product was predicted using WinNonlin® software external prediction method.
Place and Duration of the Study: The present work was carried out at Pacific Academy of Higher Education and Research University, Udaipur between the duration of February-2019 to November-2019.
Methodology: The dissolution studies were performed for test and reference products in 900ml Phosphate buffer (pH 6.8), and the USP Type II apparatus at 50 RPM with a sinker. The *in vivo* pharmacokinetic prediction was performed using WinNonlin® Software. A mechanistic oral absorption model was built in Phoenix® WinNonlin® 8.2 software (Certara, Princeton, NJ, 08540, USA).
Results: The *in-vitro* dissolution studies were comparable between the test product and the reference product. The Similarity factor achieved was 61.7 and 84.8 for Amlodipine and Valsartan test product in comparison with the reference product. An average percent prediction error for C_{max}

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and AUC for both Valsartan and Amlodipine achieved was less than 10% for all IVIVC models.
Conclusion: The relatively low prediction errors for C_{max} and AUC observed strongly suggest that the Valsartan and Amlodipine IVIVC models are valid. The average percent prediction error of less than 10% indicates that the correlation is predictive and allows the associated dissolution data to be used as a surrogate for bioavailability studies.

Keywords: In-vitro-in-vivo correlation (IVIVC); Cmax; AUC; bioavailability; valsartan; amlodipine.

ABBREVIATIONS

IVIVC : In-Vitro-in-Vivo Correlation
USFDA: The United States Food and Drug Administration
AUC : Area under Curve

1. INTRODUCTION

Valsartan is an angiotensin-receptor blocker used to manage hypertension alone or in combination with other antihypertensive agents and to manage heart failure in patients who are intolerant to ACE inhibitors. Valsartan was initially approved in 1996 in Europe for the treatment of hypertension in adults. The structure of valsartan is shown in [Fig.1]. Shortly after, in 1997, this drug was approved in the United States. Valsartan is generally well-tolerated with a side-effect profile superior to that of other antihypertensive drugs. Overall, valsartan's physiologic effects lead to reduced blood pressure, lower aldosterone levels, reduced cardiac activity, and increased excretion of sodium. Valsartan is commonly used for the management of hypertension, heart failure, and Type 2 Diabetes-associated nephropathy, particularly in patients who are unable to tolerate ACE inhibitors. ARBs such as valsartan have been shown in a number of large-scale clinical outcomes trials to improve cardiovascular outcomes including reducing risk of myocardial infarction, stroke, the progression of heart failure, and hospitalization [1, 2].

Amlodipine, initially approved by the FDA in 1987, is a popular antihypertensive drug belonging to the group of drugs called dihydropyridine calcium channel blockers, structure shown in [Fig. 2]. Due to their selectivity for the peripheral blood vessels, dihydropyridine calcium channel blockers are associated with a lower incidence of myocardial depression and cardiac conduction abnormalities than other calcium channel blockers. Amlodipine is commonly used in the treatment of high blood pressure and angina. Amlodipine has antioxidant properties and an ability to enhance the production of nitric oxide (NO), an important vasodilator that decreases blood pressure. The option for single daily dosing of amlodipine is an attractive feature of this drug [1, 2].

As defined by the USFDA, in-vitro in-vivo correlation (IVIVC) is an analytical model capable of predicting the correlation between the in vitro dissolving properties of an oral formulation and its plasma drug concentration in vivo. This tool can be useful in product development and can act as a substitute for bioequivalence studies [1]. The IVIVC tool is not only useful during drug development, but can also be used as an assessment tool during scaling and post-approval changes. The therapeutic efficacy of any product is governed by its solubility and bioavailability. Solubility is indicated by the *in vitro* dissolution characteristics of a formulation. Therefore, the correlation between *in vitro* and *in vivo* helps to make decisions about solubility improvement [3]. Furthermore, the IVIVC tool can be used to establish suitable dissolution media for formulation development [4]. IVIVC is gaining importance in the development of pharmaceutical formulations in recent times, especially in extended-release formulations where it is necessary to evaluate each batch before going to human consumption [4]. According to the USFDA website, there are at least 14 cases where IVIVC studies have been accepted as supporting data for various reasons, such as post-approval changes, pre-approval changes, or as a guidance tool for marketed formulation [4,5]. Furthermore, these days IVIVC also extends to non-oral products [6]. Transdermal drug delivery systems, while having few limitations in expanding IVIVC predictions, are gaining momentum [7]. The present research work was carried out to develop Valsartan and Amlodipine Capsules using micro tablets and to evaluate the *in-vitro* drug release characteristics and predict the *in vivo* parameters along with the marketed Valsartan and Amlodipine product using Phoenix® WinNonlin® 8.2 software (Certara, Princeton, NJ, 08540, USA) external prediction method. The study was targeted to determine the systemic concentrations using *in-vivo* prediction.

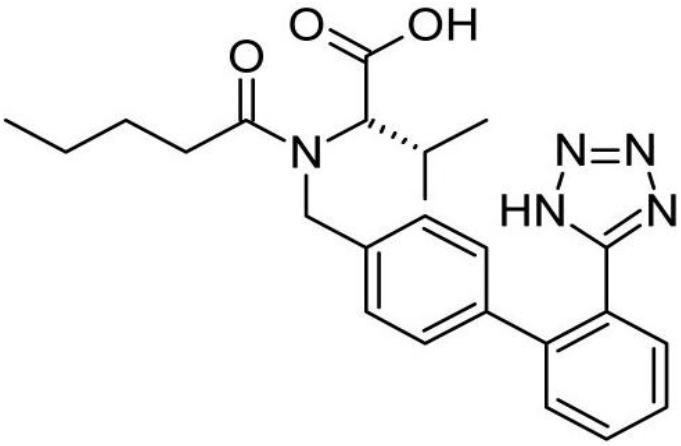

Fig. 1. Structure of Valsartan

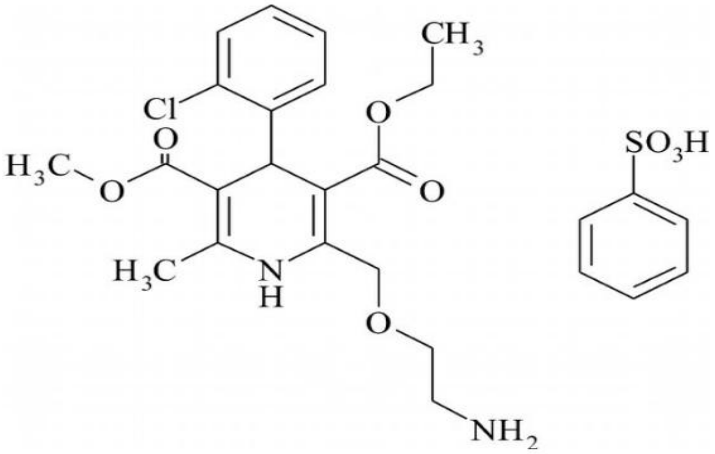

Fig. 2. Structure of Amlodipine

2. MATERIALS AND METHODS

2.1 Drugs and Chemicals

Valsartan and Amlodipine Besylate USP Reference Standards were used to prepare a working standard mircotablets. Exforge (AM 10 mg/VS 160 mg), manufactured by Novartis Pharmaceuticals Corp., Suffern, NY, was used in this study. All other excipient were procured from different vendors. All analytical grades chemicals were used.

2.2 Methods for Evaluation of Predictability

Based on the USFDA guideline there are four levels of correlation in IVIVC based on the ability of demonstration of correlations. The levels are known as A, B, C, and multiple levels C. Level A is a point-to-point correlation in which the fraction of drug absorbed is compared against a fraction of drug dissolved after the de-convolution procedure [1]. The drug absorbed fraction predicted is evaluated against the drug dissolved fraction detected. Model validity estimated is calculated for percentage error (%PE) through comparison of estimated vs. predicted [8]. The level A correlation is a linear correlation and the relationship is a point to point thus the *in-vitro* dissolution and *in-vivo* inputs are superimposable with the scaling factor. Whereas in level B correlation, the mean in *in-vitro* dissolution is compared against the mean residence time and thus it is called statistical moment analysis. Although level B correlation is not point-to-point evaluation it uses the whole dissolution in vivo data and it can't reflect the actual plasma curve [1]. Level C correlation is a single point dissolution parameter and a pharmacokinetic parameter thus it doesn’t reflect the complete curve [1]. Multiple level C correlation evaluates

several pharmacokinetic parameters against the amount of drug dissolved [1]. Phoenix WinNonlin® *IVIVC* toolkit version 8.2 was used for the assessment of both Amlodipine and Valsartan absorption studies. There are reported IVIVC studies using WinNonlin® toolkit for Nevirapine [7] and Nimesulide formulations [8].

The prediction is based on the dissolution characteristics of the test formulation. As it is a prediction based on the developed model the prediction error is identified. Normally internal or external predictability is recommended based on the different release profiles of the formulation [1]. So apart from the test formulation, two additional formulations with different release profiles are required for comparison. Internal prediction errors and external prediction errors were used for the evaluation of IVIVC. It was calculated for both Amlodipine and Valsartan drugs from the fixed-dose formulation capsule. The predicted vs observed values were compared for Cmax and AUC values.

Percent prediction error: (% PE) = $\frac{\text{Observed value} - \text{Predicted value}}{\text{Observed value}}$ x 100 ---------- (1)

2.3 Internal Predictability

Phoenix® WinNonlin® is a non-compartmental analysis (NCA) modeling tool. The statistical parameters like Area under the curve (AUC) and peak concentration (Cmax) are derived based in the two one sided tests procedure to determine the average values of pharmacokinetic parameters of test and reference formulations.

All formulations were studied for internal predictability using mean in vitro dissolution data. The mean dissolution rate constants were correlated to the mean absorption rate constants for the marketed product. The two data points along with zero intercept were used to calculate the expected absorption rate constant.

Absorption rate constant = slope x dissolution rate constant = intercept. ---------- (2)

The prediction of the plasma concentration was done by curve fitting modeling equation.

$y = F/Vd \times (Dose) \times Ka/Ka - Ke\ (e^{-Ket} - e^{-Kat})$ ---------- (3)

Where y = predicted plasma concentration
F= fraction absorbed
Vd= volume of distribution
Ka = absorption rate constant
Ke= elimination rate constant

Both reference and test formulations plasma concentration is predicted for Cmax and AUC from the dissolution data of respective formulation for establishing level C correlation. The predicted bioavailability is then compared to the observed bioavailability for each formulation and a prediction error is estimated.

If the average absolute percent prediction error (% PE) is less than 10% the predictability is acceptable for both pharmacokinetic parameters of Cmax and AUC. Moreover, the % PE for each formulation should not exceed 15%. If both these criteria are not met then external predictability should be performed to establish the IVIVC conclusively.

2.4 External Predictability

When IVIVC is used as the substitute to Bioequivalence study external predictability is very important to establish. This is established using the IVIVC to predict the in vivo performance for a formulation with known bioavailability. % PE should be less than 10% for pharmacokinetic parameters like Cmax and AUC. If the %PE is 10-20% then it indicates that the model is inconclusive and additional evaluations or studies to be conducted for its consistency. The only exception is for narrow therapeutic range formulations. Valsartan and Amlodipine Capsules were developed through Micro tablets technology. The qualitative composition of the test and reference product [10] is in Table 1.

The various formulation details studied for IVIVC are described in Table 5.

For the comparative study, the slow-release Valsartan and Amlodipine capsules were formulated with different release profiles shown in Table 6.

Dissolution profile similarity comparison using a model-independent approach.

The dissolution profile comparison was performed at identical conditions by virtual comparison of dissolution at every time point and overall dissolution comparison through model-independent models through similarity (f2) and dissimilarity (f1) analysis. The (f1) and (f2)

calculates the percentage difference between two formulations at each time point and the relative error measured between two dissolution profiles using the formula [11,12].

Table 1. The qualitative composition of test and reference product

Test product: Valsartan and Amlodipine Capsules	Reference product: Exforge Tablets 160/10 mg
Valsartan Micro tablet: Valsartan:160 mg The other ingredients are: Avicel PH 302 Avicel PH 200 Crospovidone XL Colloidal Silicon dioxide Magnesium stearate Amlodipine micro tablet: Amlodipine (as amlodipine besylate):10 mg Avicel PH 102 Avicel PH 302 Dibasic Calcium Phosphate Sodium Starch Glycollate Magnesium stearate	Amlodipine (as amlodipine besylate):10 mg Valsartan:160 mg The other ingredients are: Cellulose microcrystalline Cross-povidone (type A) Silica, Colloidal anhydrousmagnesium stearate Hypromellose [substitution type 2910 (3 m Pas)] Macrogol 4000 Talc Titanium dioxide (E171) Iron oxide, yellow (E172) Iron oxide, red (E172).

Table 2. Solubility Profile for Valsartan

Sr. No.	Media	Solubility(mg/ml)	Solubility (mg/250 ml)
1	pH 1.0	0.092	23.00
2	pH 4.5	0.888	222.00
2	pH 6.8	1.264	316.00
3	Water	0.175	43.75

Table 3. Solubility Profile for Amlodipine

Sr. No.	Media	Solubility(mg/ml)	Solubility (mg/250 ml)
1	pH 1.0	0.31	77.5
2	pH 4.5	0.28	70
3	pH 6.8	0.1	25
4	Water	0.08	20

Table 4. The API PSD

Sample	D(0.1) µm	D(0.5) µm	D(0.9) µm
Amlodipine Besylate	2.973	15.595	55.75
Valsartan	2.654	11.723	57.540

Table 5. The Test and Reference product details used for IVIVC

Sr.No	RLD	Test product
1	Exforge Tablet 160/10 mg Each film-coated tablet contains Valsartan: 160 mg Amlodipine Besylate equivalent to Amlodipine: 10 mg B.No: BCY17 Mfg. Date: 04-2018 Exp. Date: 03-2021	Valsartan and Amlodipine Capsules 160/10 mg Each capsule contains Valsartan: 160 mg Amlodipine Besylate equivalent to Amlodipine: 10 mg B.No : FVA02

Table 6. Release Data

Sr.No.	USP Apparatus	Speed (RPMs)	Medium	Volume (mL)	Time points (minutes)
1	Type II (Paddle)	50	pH 6.8 phosphate Buffer	900	5,10,15,20,30, 45,60,90,120

$f1 = \{[\sum_{t=1}^{n}[|Rt - Tt|] / [\sum_{t=1}^{n} Rt]\}$ x 100---------- (2)
and
$f2 = 50 \times \log \{[1 + 1/n \sum_{t=1}^{n}(Rt - Tt)^2]^{-0.5}$ x 100}
---------- (3)
Where,

n = number of time points
Rt = Reference products dissolution in percentage
t = time
Tt = Test product's dissolution in percentage

Similarity factor (f2) is the logarithmic reciprocal square root transformation of the sum of squared error and similarity between two dissolution profiles.

The acceptable similarity factor (f2) should be more than 50 and 100 being the maximum, close to 100 indicates maximum similarity. Similarly, the (f1) vale is an acceptable maximum of 20, and lower the value is more preferable [13].

3. RESULTS AND DISCUSSION

3.1 *In-vitro In-vivo* Correlation Studies (IVIVC)

3.1.1 IVIVC for valsartan

Various formulations identified for the IVIVC study are FVA02 (test formulation), FVA04 (Fast release formulation), and FVA03 (slow-release formulation). The dissolution was performed for reference products and various Valsartan drug release formulations. The dissolution results are in Table 7.

The similarity factor f2 was below the acceptable limit of 50 which indicates that there is a clear difference in the drug release between the target formulation and both alternate formulations. Thus this formulation can be selected for IVIVC study in Win onlin software.

Similarity factor (f2) was calculated considering test formulation and other formulations in Table 8.

3.2 Calculated *In-Vivo* Data of Various Valsartan Formulations

The summary statistics were calculated for various formulations with different release profiles of Valsartan for *In-vivo* data are in Tables 9, 10, and 11. The *in-vivo* data for Valsartan from the reference product Exforge 160/10 mg is in Table 12. The resulted graph is shown in Fig. 3, 4, and 5.

Here we have seen that the R-square value 92% which indicates that the model is well fitted with a slope coefficient is 0.2447.

3.3 Summary of Calculated in vivo Parameters for Valsartan Test Product

The average absolute percent prediction error (% PE) of less than ±10% for Cmax and AUC establishes the predictability of the IVIVC shown in Table 13.

3.4 Amlodipine IVIVC Study

Various formulations identified for the IVIVC study are FVA02 (test formulation), Fast release formulation (FVA04), and slow-release formulation (FVA03). The dissolution was performed for reference products and various Amlodipine drug release formulations. The dissolution was media 900ml pH 6.8 Phosphate buffer, USP Type II at 50 RPM with a sinker. The dissolution results are in Table 14.

The similarity factor f2 shows that there is a clear difference in the drug release between the target formulation and both alternate formulations. Thus this formulation can be selected for IVIVC study in WinNonlin® software shown in Table 15.

3.5 Calculated *In-Vivo* data of Various Amlodipine Formulations

The summary statistics were calculated for various formulations with different release profiles of Amlodipine for In-vivo data are in Table 16, 17, and 18. The *in-vivo* data for

Amlodipine from the reference product Exforge 160/10 mg is in Table 18. The resulted graph is shown in Fig. 6, 7, and 8.

Here we have seen that the R-square value 89% which indicates that the model is well fitted with a slope coefficient is 0.276.

Table 7. Comparative Valsartan drug release of various test formulation and reference product

Time (Min)	pH 6.8 buffer			
	FVA02 (Test product)	FVA04 (Fast release)	FVA03 (Slow release)	RLD-BCY17 (Reference product)
5	68.8	80.9	58.9	72.3
10	86.4	89.7	65.8	84
15	89.6	95.7	72.6	88.1
20	97.2	99.9	78.2	98.1
30	99.4	100.2	82.4	99.8
45	99.8	100.8	85.3	99.9
60	100.4	101.3	86.4	100.2
90	100.8	101.4	91.8	100.6

Table 8. Similarity factor (f2) comparison for Valsartan drug release

Reference formulation	Test formulation	Similarity factor (f2)
FVA02	FVA04	49.45
FVA02	FVA03	40
FVA04	FVA03	36
RLD-BCY17	FVA02	84.8

Table 9. *In-vivo* data summary for Valsartan for B.No: FVA02

Summary	Time (hr.)									
	0.00	0.50	1.00	2.00	2.50	3.00	3.50	4.00	4.50	5.00
N	12	12	12	12	12	12	12	12	12	12
Mean	0.00	960.55	1345.15	1894.55	2568.17	2984.57	4251.17	3500.35	3168.25	2836.15
SD	0.00	45.15	145.21	157.12	39.12	43.23	38.55	42.14	25.25	23.80
Min	0.00	915.39	1299.99	1849.39	2523.01	2939.41	4206.01	3455.19	3123.09	2790.98
Median	0.00	1056.19	1440.79	1990.19	2663.82	3080.22	4346.82	3595.99	3263.89	2931.79
Max	0.00	1197.00	1581.60	2131.00	2804.63	3221.03	4487.63	3736.80	3404.70	3072.60

Summary	Time (hr.)										
	6.00	7.00	8.00	10.00	12.00	14.00	16.00	20.00	24.00	36.00	48.00
N	12	12	12	12	12	12	12	12	12	12	1
Mean	2504.04	2171.94	1839.84	1507.74	1175.64	843.54	511.44	179.34	94.16	6.86	0.00
SD	12.65	12.25	10.56	9.05	6.80	10.40	34.17	22.16	8.17	45.16	0.00
Min	2458.88	2126.78	1794.68	1462.58	1130.48	798.38	466.28	134.18	81.16	2.16	0.00
Median	2599.69	2267.59	1935.49	1603.39	1271.29	939.19	607.09	274.99	205.89	122.74	0.00
Max	2740.50	2408.40	2076.30	1744.20	1412.10	1080.00	747.90	415.80	330.62	243.32	0.00

Table 10. *In-vivo* data summary for Valsartan for B.No FVA03

Summary	Time (hr.)									
	0.00	**0.50**	**1.00**	**2.00**	**2.50**	**3.00**	**3.50**	**4.00**	**4.50**	**5.00**
N	12	12	12	12	12	12	12	12	12	12
Mean	0.00	340.20	724.80	1274.20	1947.82	2364.22	2936.32	2379.99	2147.89	1883.69
SD	0.00	63.08	98.71	89.24	28.53	22.52	16.86	26.51	17.02	21.46
Min	0.00	235.03	619.63	1169.03	1842.65	2259.05	2831.15	2274.83	2042.73	1778.53
Median	0.00	412.69	797.29	1346.69	2020.31	2436.71	3008.81	2452.49	2220.39	1956.19
Max	0.00	590.36	974.96	1524.36	2197.98	2614.38	3186.48	2630.15	2398.05	2133.85

Summary	Time (hr.)										
	6.00	**7.00**	**8.00**	**10.00**	**12.00**	**14.00**	**16.00**	**20.00**	**24.00**	**36.00**	**48.00**
N	12	12	12	12	12	12	12	12	12	12	1
Mean	1551.59	1219.49	1056.46	756.17	555.29	223.19	100.00	35.65	22.66	6.13	0.00
SD	16.57	11.14	10.42	23.80	12.65	12.25	10.56	16.86	26.51	6.31	0.00
Min	1446.43	1114.33	951.29	651.00	450.13	118.03	50.55	12.17	8.15	1.16	0.00
Median	1624.09	1291.99	1128.95	828.66	627.79	295.69	200.35	148.99	82.33	8.16	0.00
Max	1801.75	1469.65	1306.62	1006.33	805.45	473.35	350.16	285.81	156.50	15.18	0.00

Table 11. *In-vivo* data summary for Valsartan for B.No: FVA04

Summary	Time (hr.)									
	0.00	**0.50**	**1.00**	**2.00**	**2.50**	**3.00**	**3.50**	**4.00**	**4.50**	**5.00**
N	12	12	12	12	12	12	12	12	12	12
Mean	0.00	967.71	1535.31	2184.71	2958.33	3974.73	5198.16	4210.18	3178.08	2545.98
SD	0.00	170.82	256.78	206.71	93.70	61.41	49.58	41.96	22.97	22.05
Min	0.00	750.16	1384.66	2034.06	2807.68	3824.08	5047.50	4059.53	3027.43	2395.33
Median	0.00	957.86	1585.16	2234.56	3008.18	4024.58	5248.00	4260.03	3227.93	2595.83
Max	0.00	1165.56	1785.66	2435.06	3208.69	4225.09	5448.51	4460.53	3428.43	2796.33

Summary	Time (hr.)										
	6.00	**7.00**	**8.00**	**10.00**	**12.00**	**14.00**	**16.00**	**20.00**	**24.00**	**36.00**	**48.00**
N	12	12	12	12	12	12	12	12	12	12	12
Mean	2213.88	1881.78	1549.68	1217.58	885.48	553.38	321.28	115.00	36.13	0.00	0.00
SD	19.08	20.04	25.65	30.17	25.17	12.17	15.17	10.17	9.56	0.00	0.17
Min	2063.23	1731.12	1399.02	1066.92	734.82	402.72	170.62	35.35	12.51	0.00	0.00
Median	2263.73	1931.63	1599.53	1267.43	935.33	603.23	371.12	200.35	149.50	0.00	0.00
Max	2464.23	2132.13	1800.03	1467.93	1135.83	803.73	571.63	365.35	286.48	0.00	0.00

Table 12. *In-vivo* data summary for Valsartan of Reference product B.No: BCY17

Summary	Time (hr.)									
	0.00	**0.50**	**1.00**	**2.00**	**2.50**	**3.00**	**3.50**	**4.00**	**4.50**	**5.00**
N	12	12	12	12	12	12	12	12	12	12
Mean	0.00	947.89	1199.84	1749.24	2422.86	2839.26	4357.86	3455.04	3022.94	2790.84
SD	0.00	68.54	57.01	65.08	76.12	54.50	37.96	34.70	30.79	79.06
Min	0.00	797.33	1049.28	1598.68	2272.30	2688.70	4207.30	3304.47	2872.37	2640.27
Median	0.00	947.89	1199.84	1749.24	2422.86	2839.26	4357.86	3455.04	3022.94	2790.84
Max	0.00	1194.06	1446.00	1995.40	2669.02	3085.42	4604.02	3701.20	3269.10	3037.00

Summary	Time (hr.)										
	6.00	**7.00**	**8.00**	**10.00**	**12.00**	**14.00**	**16.00**	**20.00**	**24.00**	**36.00**	**48.00**
N	12	12	12	12	12	12	12	12	12	12	1
Mean	2358.74	2026.63	1794.53	1462.43	1030.33	798.23	466.13	163.44	86.14	0.00	0.00
SD	46.48	28.60	28.17	8.57	13.28	71.11	55.17	36.65	11.15	0.00	0.00
Min	2208.17	1876.07	1643.97	1311.87	879.77	647.67	315.57	86.17	34.17	0.00	0.00
Median	2358.73	2026.63	1794.53	1462.43	1030.33	798.23	466.13	163.44	86.14	0.00	0.00
Max	2604.90	2272.80	2040.70	1708.60	1276.50	1044.40	712.30	409.60	332.30	0.00	0.00

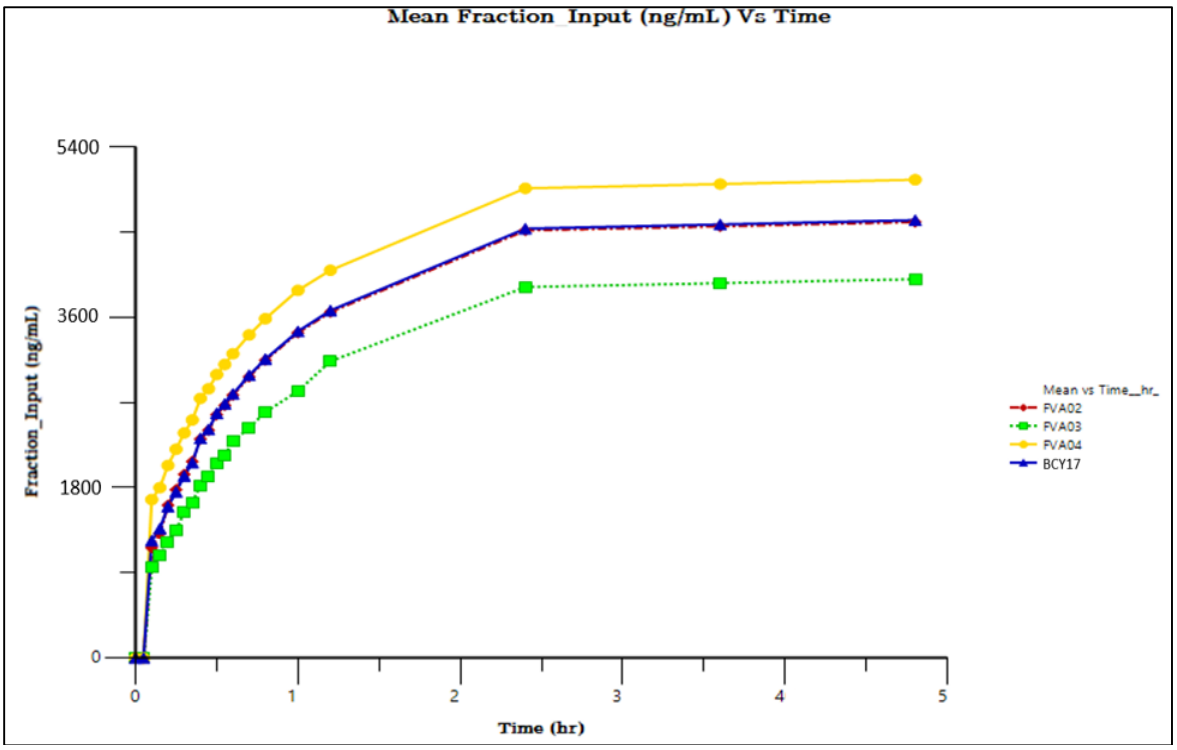

Fig. 3. *In-vivo* De-convolution Fraction input for Valsartan: Output generated using Win Nonlin IVIVC Toolkit V8.2 software

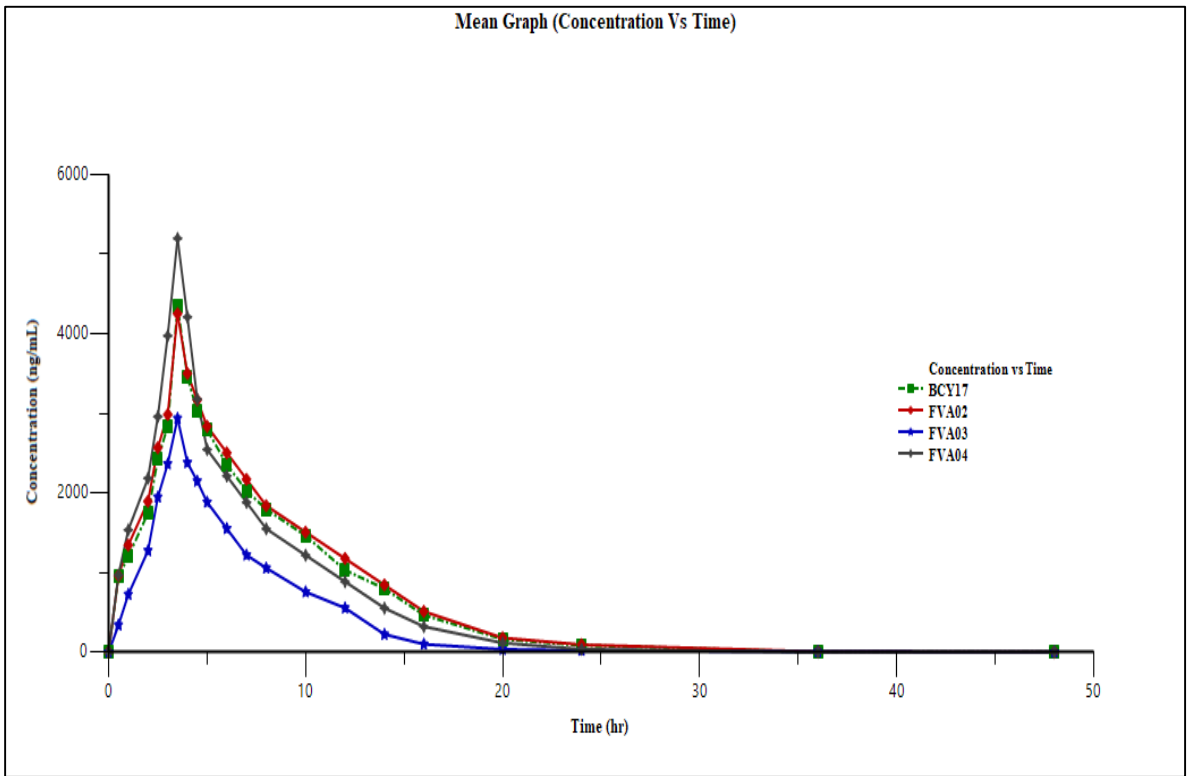

Fig. 4. *In-vivo* Time vs. Concentration Graph of Valsartan test and reference product

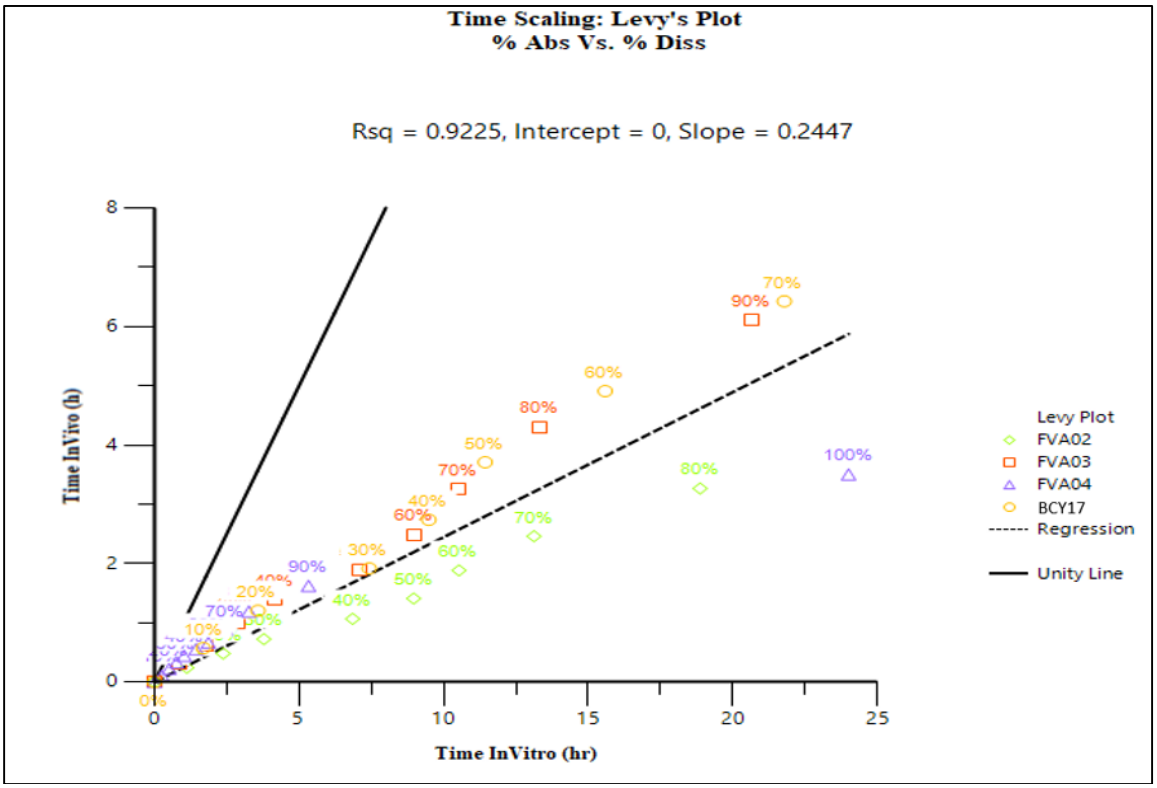

Fig. 5. Time Scaling: Levy's Plot: %absorption vs. % Dissolution of Valsartan test and reference product

Table 13. Valsartan *in-vivo* parameters for combination product

Sr. No	Formulation	Parameter	Predicted	Observed	%PE	Ratio
1	FVA02	AUC last	344407.034	32459.466	6.39	1.06
2.	FVA02	Cmax	3867.4909	4249.99	-9.34	0.91

Table 14. Comparative Amlodipine drug release formulation of test formulation and reference product

Time (Min)	pH 6.8 buffer			
	FVA02 (test formulation)	FVA04 (Fast release formulation)	FVA03 (Slow release formulation)	Reference BCY17
5	54.5	77.4	48.5	69.3
10	82.8	92.7	72.6	80.6
15	88.9	98.9	78.3	87.7
20	93.6	100.0	82.7	92.4
30	98.6	100.0	86.4	96.8
45	99.2	100.0	88.1	98.9
60	100.0	100.0	89.6	100.0
90	100.0	100.0	92.3	100.0

Table 15. Similarity factor (f2) comparison for Amlodipine drug release

Formulation		F2
FVA02	FVA04	49.98
FVA02	FVA03	49.44
FVA04	FVA03	37.08
BCY17	FVA02	61.7

Table 16. *In-vivo* data summary for Amlodipine for B.No: FVA02

Summary	Time (hr.)									
	0.00	1.00	2.00	4.00	6.00	7.00	8.00	8.50	9.00	9.50
N	12	12	12	12	12	12	12	12	12	12
Mean	0.00	0.17	0.53	1.13	1.82	2.43	2.95	3.78	4.17	4.86
SD	0.00	68.54	57.01	65.08	76.12	54.50	37.96	34.70	30.79	79.06
Min	0.00	0.07	0.44	1.04	1.73	2.34	2.85	3.68	4.07	4.76
Median	0.00	0.55	0.92	1.52	2.21	2.82	3.33	4.16	4.55	5.24
Max	0.00	1.03	1.40	2.00	2.69	3.30	3.81	4.64	5.03	5.72
Summary	**Time (hr.)**									
	10.00	10.50	11.00	12.00	13.00	14.00	16.00	20.00	24.00	36.00	48.00
N	12	12	12	12	12	12	12	12	12	12	12
Mean	5.67	4.72	4.23	3.55	3.11	2.56	1.77	0.93	0.52	0.10	0.00
SD	46.48	28.60	28.17	8.57	13.28	71.11	55.17	36.65	11.15	15.17	0.00
Min	5.58	4.62	4.13	3.45	3.01	2.47	1.67	0.84	0.42	0.01	0.00
Median	6.06	5.10	4.61	3.93	3.49	2.95	2.15	1.32	0.90	0.49	0.00
Max	6.54	5.58	5.09	4.41	3.97	3.42	2.63	1.80	1.38	0.97	0.00

Table 17. *In-vivo* data summary for Amlodipine for B.No:FVA03

Summary	Time (hr.)									
	0.00	**1.00**	**2.00**	**4.00**	**6.00**	**7.00**	**8.00**	**8.50**	**9.00**	**9.50**
N	12	12	12	12	12	12	12	12	12	12
Mean	0.00	0.02	0.13	0.53	1.12	1.63	2.15	2.59	3.17	3.51
SD	0.00	170.82	256.78	206.71	93.70	61.41	49.58	41.96	22.97	22.05
Min	0.00	0.07	0.04	0.43	1.03	1.54	2.05	2.49	3.07	3.42
Median	0.00	0.47	0.52	0.91	1.51	2.02	2.53	2.97	3.55	3.90
Max		0.88	1.00	1.39	1.99	2.50	3.01	3.45	4.03	4.38
Summary	Time (hr.)									
	10.00	**10.50**	**11.00**	**12.00**	**13.00**	**14.00**	**16.00**	**20.00**	**24.00**	**36.00**	**48.00**
N	12	12	12	12	12	12	12	12	12	12	12
Mean	4.83	4.22	3.53	2.88	2.51	2.06	1.27	0.63	0.35	0.00	0.00
SD	19.08	20.04	25.65	30.17	25.17	12.17	15.17	10.17	9.56	0.00	0.00
Min	4.74	4.12	3.43	2.79	2.41	1.97	1.17	0.54	0.25	0.00	0.00
Median	5.22	4.60	3.91	3.27	2.89	2.45	1.65	1.02	0.73	0.00	0.00
Max	5.70	5.08	4.39	3.75	3.37	2.92	2.13	1.50	1.21	0.00	0.00

Table 18. *In-vivo* data summary for Amlodipine for B.No: FVA04

Summary	Time (hr.)									
	0.00	**1.00**	**2.00**	**4.00**	**6.00**	**7.00**	**8.00**	**8.50**	**9.00**	**9.50**
N	12	12	12	12	12	12	12	12	12	12
Mean	0.00	0.25	0.73	1.45	2.57	3.85	4.65	5.03	5.72	7.17
SD	0.00	63.08	98.71	89.24	28.53	22.52	16.86	26.51	17.02	21.46
Min	0.00	0.15	0.63	1.36	2.47	3.75	4.56	4.93	5.62	7.07
Median	0.00	0.63	1.11	1.84	2.95	4.23	5.04	5.41	6.10	7.55
Max	0.00	1.11	1.59	2.32	3.43	4.71	5.52	5.89	6.58	8.03
Summary	Time (hr.)									
	10.00	**10.50**	**11.00**	**12.00**	**13.00**	**14.00**	**16.00**	**20.00**	**24.00**	**36.00**	**48.00**
N	12	12	12	12	12	12	12	12	12	12	12
Mean	6.85	6.14	4.86	3.82	2.94	2.40	1.60	0.77	0.35	0.00	0.00
SD	16.57	11.14	10.42	23.80	12.65	12.25	10.56	16.86	26.51	0.00	0.00
Min	6.75	6.05	4.77	3.72	2.85	2.30	1.50	0.67	0.26	0.00	0.00
Median	7.23	6.53	5.25	4.20	3.32	2.78	1.98	1.15	0.73	0.00	0.00
Max	7.71	7.00	5.73	4.68	3.80	3.26	2.46	1.63	1.21	0.00	0.00

Table 19*. In-vivo* data summary for Amlodipine reference product B.No: BCY17

Summary	Time (hr.)									
	0.00	**1.00**	**2.00**	**4.00**	**6.00**	**7.00**	**8.00**	**8.50**	**9.00**	**9.50**
N	12	12	12	12	12	12	12	12	12	12
Mean	0.00	0.18	0.56	1.26	2.04	2.62	3.12	3.96	4.25	4.93
SD	0.00	45.15	145.21	157.12	39.12	43.23	38.55	42.14	25.25	23.80
Min	0.00	0.08	0.46	1.17	1.94	2.52	3.02	3.86	4.16	4.84
Median	0.00	0.56	0.94	1.65	2.42	3.00	3.50	4.34	4.64	5.32
Max	0.00	1.04	1.42	2.13	2.90	3.48	3.98	4.82	5.12	5.80
Summary	Time (hr.)									
	10.00	**10.50**	**11.00**	**12.00**	**13.00**	**14.00**	**16.00**	**20.00**	**24.00**	**36.00**	**48.00**
N	12	12	12	12	12	12	12	12	12	12	12
Mean	6.21	5.72	4.56	3.65	3.03	2.58	1.87	1.01	0.60	0.11	0.11
SD	12.65	12.25	10.56	9.05	6.80	10.40	34.17	22.16	8.17	45.16	45.16
Min	6.11	5.63	4.46	3.56	2.94	2.49	1.77	0.92	0.51	0.02	0.02
Median	6.59	6.10	4.94	4.04	3.42	2.97	2.25	1.40	0.99	0.50	0.50
Max	7.07	6.58	5.42	4.52	3.90	3.45	2.73	1.88	1.46	0.97	0.97

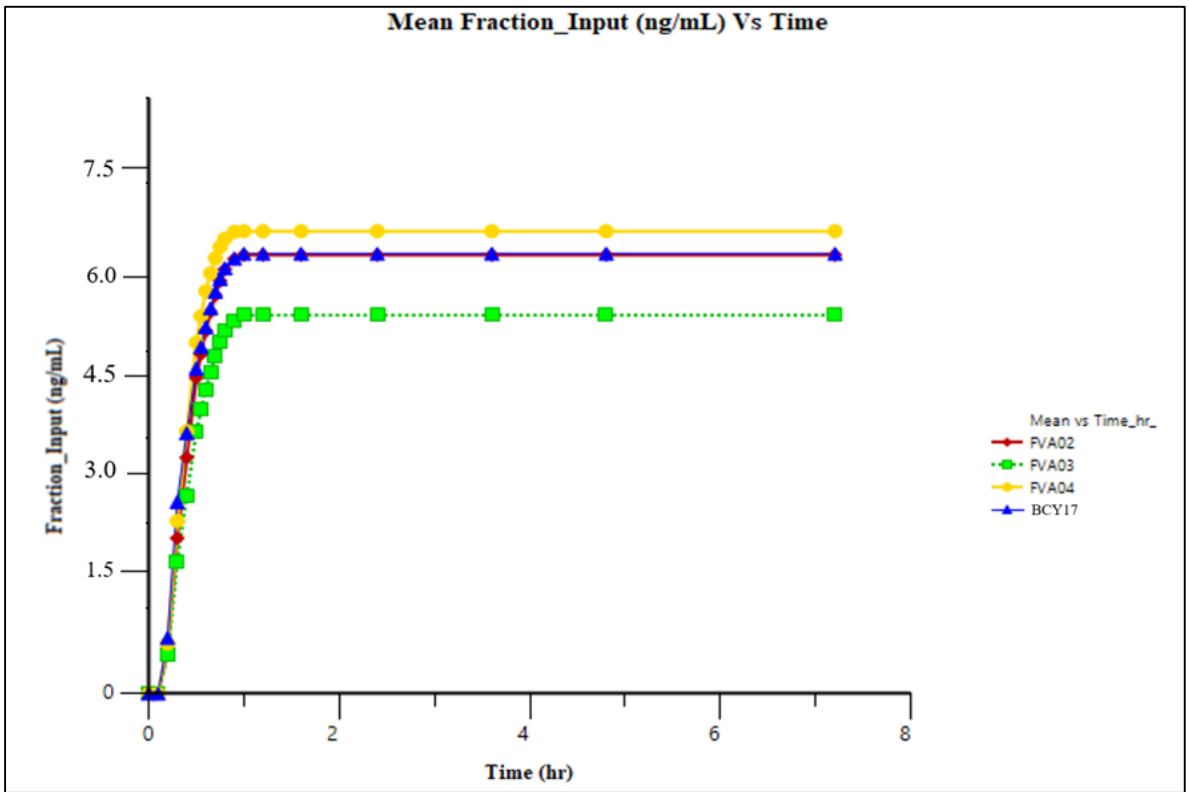

Fig. 6. *In- vivo* De-convolution Fraction input: Output generated using WinNonlin IVIVC Toolkit V8.2 software

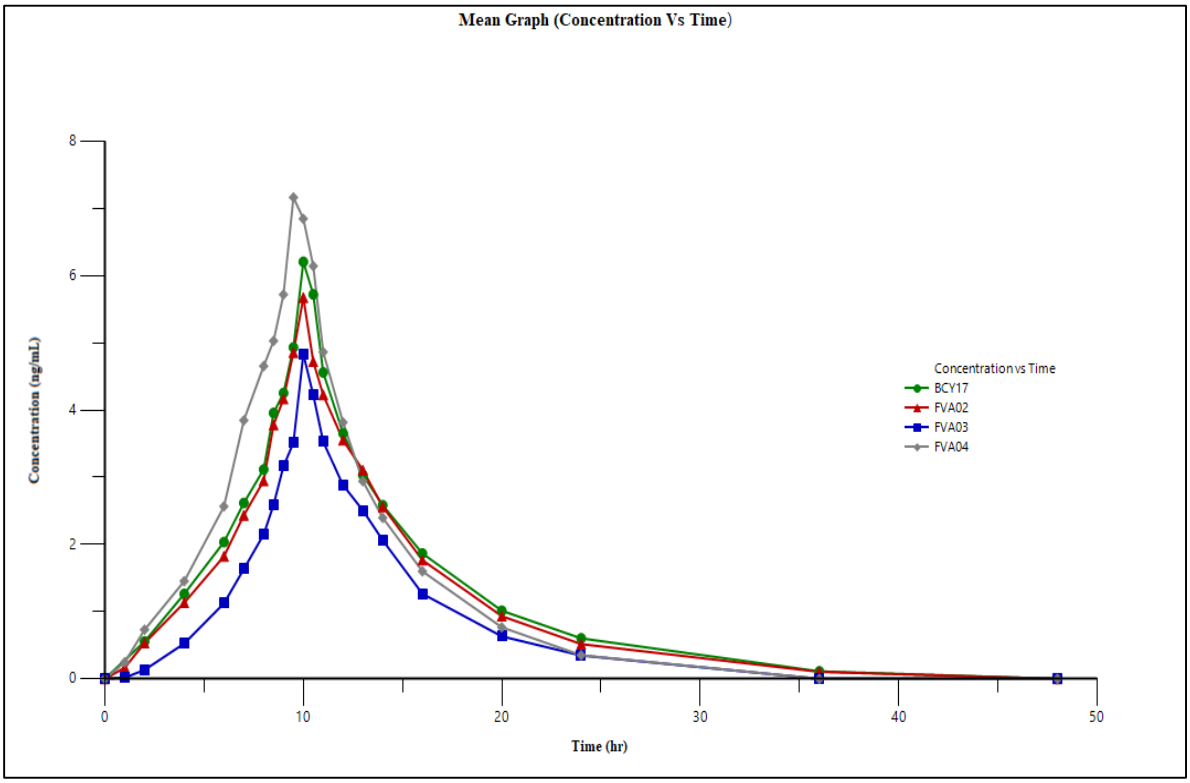

Fig. 7. *In-vivo* Time vs. Concentration Graph of Amlodipine test and reference product

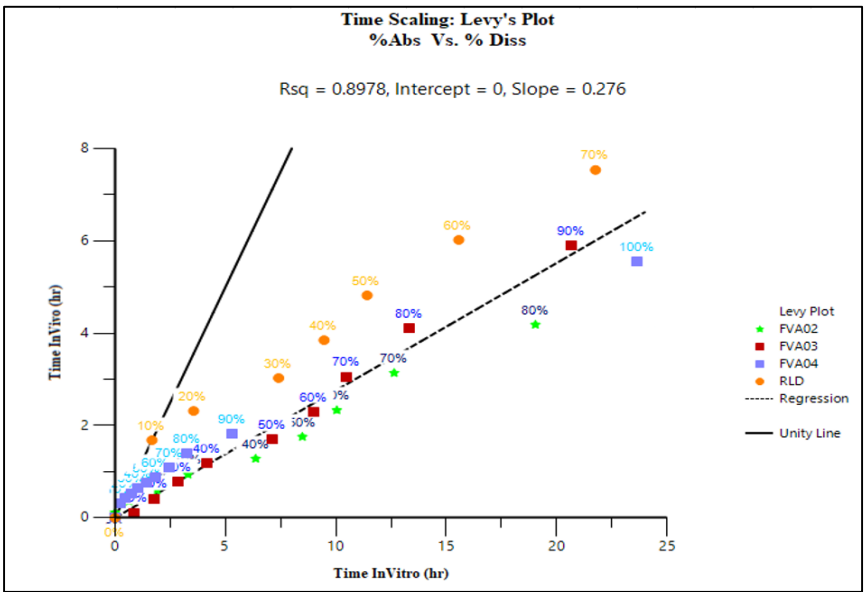

Fig. 8. Time Scaling: Levy's Plot- %absorption vs. % Dissolution of Amlodipine test and reference product

Table 20. Amlodipine in vivo parameters for combination product

Sr. No.	Formulation	Parameter	Predicted	Observed	%PE	Ratio
1.	FVA02	AUC last	249.76367	242.489	6.077	1.03
2.	FCA02	Cmax	5.5488	5.78	-9.016	0.96

3.6 Summary of Calculated in Vivo Parameters for Amlodipine Combination Product

The average absolute percent prediction error (% PE) of less than ±10% for Cmax and AUC establishes the predictability of the IVIVC shown in Table 20.

4. CONCLUSION

The objective of this study was to develop and evaluate an IVIVC simulation approach capable of predicting the likelihood of success in a human pharmacokinetic study. The developed model can guide pharmaceutical development by predicting the acceptability of the bioequivalence of the developed capsule formulation. Based on the software-assisted predicted results, it is deduced that the best bioavailability obtained from the capsules developed through the micro-tablet technology may correspond to the marketed reference product. Therefore, the developed formulation when the physicochemical properties and stability conditions meet the requirements along with the manufacturability of the final product, the developed formulation is expected to exhibit similar pharmacokinetic profiles.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENT

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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