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.

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 Cmax...
and AUC for both Valsartan and Amlodipine achieved was less than 10% for all IVIVC models. **Conclusion:** The relatively low prediction errors for \( C_{\text{max}} \) 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); \( C_{\text{max}} \); 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.
2. MATERIALS AND METHODS

2.1 Drugs and Chemicals

Valsartan and Amlodipine Besylate USP Reference Standards were used to prepare a working standard microtablets. 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.

\[ \text{Percent prediction error:} \quad \% \text{ PE} = \frac{\text{Observed value} - \text{Predicted value}}{\text{Observed value}} \times 100 \]  

(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.

\[ \text{Absorption rate constant} = \text{slope} \times \text{dissolution rate constant} = \text{intercept} \]  

(2)

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

\[ y = F/Vd \times \text{(Dose)} \times \frac{Ka}{Ka - Ke} - Ke \left( e^{-Ket} - e^{-Kat} \right) \]  

(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 (\( \% \text{ PE} \)) is less than 10% the predictability is acceptable for both pharmacokinetic parameters of Cmax and AUC. Moreover, the \( \% \text{ PE} \) 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. \( \% \text{ PE} \) should be less than 10% for pharmacokinetic parameters like Cmax and AUC. If the \( \% \text{PE} \) 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.

The dissolution profile similarity 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:                          | Amlodipine (as amlodipine besylate):10 mg   |
| Valsartan:160 mg                                 | Valsartan:160 mg                            |
| The other ingredients are:                       | The other ingredients are:                  |
| Avicel PH 302                                    | Cellulose microcrystalline                  |
| Avicel PH 200                                    | Cross-povidone (type A)                     |
| Crospovidone XL                                  | Silica,                                     |
| Colloidal Silicon dioxide                        | Colloidal anhydrous magnesium stearate      |
| Magnesium stearate                               | Hypromellose [substitution type 2910 (3 m NS)] |
| Amlodipine micro tablet:                         | Macrogol 4000                               |
| Amlodipine (as amlodipine besylate):10 mg        | Talc,                                       |
| Avicel PH 102                                    | Titanium dioxide (E171)                     |
| Avicel PH 302                                    | Iron oxide, yellow (E172)                   |
| Dibasic Calcium Phosphate                        | Iron oxide, red (E172).                     |
| Sodium Starch Glycollate                         |                                             |
| Magnesium stearate                               |                                             |

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                 |
| 3       | 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     |     | Valsartan and Amlodipine Capsules 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 Tablets 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 |

Where,

\[
f_1 = \frac{|\sum_{i=1}^{n} (R_t - T_t)|}{\|\sum_{i=1}^{n} R_t\|} \times 100 \quad (2)
\]

and

\[
f_2 = 50 \times \log \{ [1 + 1/n \sum_{i=1}^{n} (R_t - T_t)^2]^{0.5} \times 100 \} \quad (3)
\]

Where,

\[n = \text{number of time points}
\]

\[R_t = \text{Reference products dissolution in percentage}
\]

\[T_t = \text{Test product’s dissolution in percentage}
\]

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) | 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 | 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    | 960.55 | 1345.15 | 1894.55 | 2568.1 | 2984.5 | 4251.1 | 3500.3 | 3168.25 | 2836.1 |
| SD      | 45.15 | 145.21 | 39.12 | 43.23 | 38.55 | 42.14 | 25.25 | 23.80 |
| Min     | 12   | 12   | 12   | 12   | 12   | 12   | 12   | 12   | 12   | 12   |
| Median  | 1056.1 | 1440.79 | 1990.19 | 2663.8 | 3080.2 | 4346.8 | 3455.1 | 3123.09 | 2795.9 |
| Max     | 1197.0 | 1581.60 | 2131.00 | 2804.6 | 3221.0 | 4487.6 | 3736.8 | 3404.70 | 3072.6 |

| Summary | 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    | 2504.04 | 2171.9 | 1839.0 | 1507.7 | 1175.6 | 843.54 | 511.4 | 179.3 | 94.16 | 6.86 |
| SD      | 45.15 | 145.21 | 39.12 | 43.23 | 38.55 | 42.14 | 25.25 | 23.80 |
| Min     | 12   | 12   | 12   | 12   | 12   | 12   | 12   | 12   | 12   | 12   | 12   |
| Median  | 1056.1 | 1440.79 | 1990.19 | 2663.8 | 3080.2 | 4346.8 | 3455.1 | 3123.09 | 2795.9 |
| Max     | 1197.0 | 1581.60 | 2131.00 | 2804.6 | 3221.0 | 4487.6 | 3736.8 | 3404.70 | 3072.6 |
| 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 | 2364.2 | 2936.3 | 2379.9 | 2147.8 | 1883.6 |
| 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 | 2259.0 | 2831.1 | 2274.8 | 2042.7 | 1778.5 |
| Median  | 0.00       | 412.69 | 797.29 | 1346.69 | 2020.31 | 2436.7 | 3008.8 | 2452.4 | 2220.3 | 1956.1 |
| Max     | 0.00       | 590.36 | 974.96 | 1524.36 | 2197.98 | 2614.3 | 3186.4 | 2630.1 | 2398.0 | 2133.8 |

| 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    | 1551.5     | 1219.4 | 1056.4 | 756.17 | 555.2 | 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     | 1446.4     | 1114.3 | 951.29 | 651.00 | 450.1  | 118.03 | 50.55  | 12.17  | 8.15   | 1.16  | 0.00 |
| Median  | 1624.0     | 1291.9 | 1128.9 | 828.66 | 627.7  | 295.69 | 200.35 | 148.99 | 82.33  | 8.16  | 0.00 |
| Max     | 1801.7     | 1469.6 | 1306.6 | 1006.3 | 805.4  | 473.35 | 350.16 | 285.81 | 156.50 | 15.18 | 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       | 967.71 | 1535.3 | 2184.7 | 2958.3 | 3974.73 | 5198.1 | 4210.1 | 3178.0 | 2545.9 |
| 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 | 1384.6 | 2034.0 | 2807.6 | 3824.08 | 5047.5 | 4059.5 | 3027.4 | 2395.3 |
| Median  | 0.00       | 957.86 | 1585.1 | 2234.5 | 3008.1 | 4042.58 | 5248.0 | 4260.0 | 3227.9 | 2595.8 |
| Max     | 0.00       | 1165.56 | 1785.6 | 2435.0 | 3208.6 | 4225.09 | 5448.5 | 4460.5 | 3428.4 | 2796.3 |

| 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 | 553.53 | 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 | 402.7 | 170.62 | 35.35  | 12.51  | 0.00  | 0.00 |
| Median  | 2263.73    | 1931.63 | 1599.53 | 1267.43 | 935.33 | 603.2 | 371.12 | 200.35 | 149.50 | 0.00  | 0.00 |
| Max     | 2464.23    | 2132.13 | 1800.12 | 1467.93 | 1135.38 | 803.7 | 571.63 | 365.35 | 286.48 | 0.00  | 0.00 |

Table 11. *In-vivo* data summary for Valsartan for B.No: FVA04
### 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 | 4357.8 | 3455.0 | 3022.9 | 2790.8 |
| 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 | 4207.3 | 3304.4 | 2872.3 | 2640.2 |
| Median  |            | 0.00 | 947.89 | 1199.84 | 1749.24 | 2422.86 | 2839.26 | 4357.8 | 3455.0 | 3022.9 | 2790.8 |
| Max     |            | 0.00 | 1194.0 | 1446.00 | 1995.40 | 2669.02 | 3085.42 | 4604.0 | 3701.2 | 3269.1 | 3037.0 |

| 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    |            | 2358.7 | 2026.6 | 1794.5 | 1462.4 | 1030.3 | 798.23 | 466.1 | 163.4 | 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     |            | 2208.1 | 1876.0 | 1643.9 | 1311.8 | 879.77 | 647.67 | 315.5 | 86.17 | 34.17 | 0.00 | 0.00 |
| Median  |            | 2358.7 | 2026.6 | 1794.5 | 1462.4 | 1030.3 | 798.23 | 466.1 | 163.4 | 86.14 | 0.00 | 0.00 |
| Max     |            | 2604.9 | 2272.8 | 2040.7 | 1708.6 | 1276.5 | 1044.4 | 712.3 | 409.6 | 332.3 | 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) | 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   |
| 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 | 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    | 4.83 | 4.22 | 3.53 | 2.88 | 2.51 | 2.06 | 1.27 | 0.63 | 0.35 | 0.00 |
| SD      | 19.08| 20.04| 25.65| 30.17| 25.17| 15.17| 10.17| 5.56 | 3.02 | 0.00 |
| Min     | 5.22 | 4.60 | 3.91 | 3.27 | 2.89 | 2.45 | 1.65 | 1.02 | 0.73 | 0.00 |
| Max     | 5.70 | 5.08 | 4.39 | 3.75 | 3.37 | 2.92 | 2.13 | 1.50 | 1.21 | 0.00 |

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

| Summary | 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    | 6.85 | 6.14 | 5.86 | 5.32 | 3.82 | 2.94 | 2.40 | 1.60 | 0.77 | 0.35 |
| SD      | 16.57| 11.14| 10.42| 23.80| 12.65| 12.25| 10.56| 16.86| 26.51| 0.00 |
| Min     | 7.23 | 6.53 | 5.25 | 4.20 | 3.32 | 2.78 | 1.98 | 1.15 | 0.73 | 0.00 |
| Max     | 7.71 | 7.00 | 6.73 | 5.68 | 3.80 | 3.26 | 2.46 | 1.63 | 1.21 | 0.00 |

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

| Summary | 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    | 6.21 | 5.72 | 5.46 | 5.36 | 3.65 | 3.03 | 2.58 | 1.87 | 1.01 | 0.60 |
| SD      | 12.65| 12.25| 10.56| 9.05 | 6.80 | 10.40| 34.17| 22.16| 8.17 | 45.16|
| Min     | 6.11 | 5.63 | 4.46 | 3.56 | 2.94 | 2.49 | 1.77 | 0.92 | 0.51 | 0.02 |
| Median  | 6.59 | 6.10 | 4.94 | 4.04 | 3.42 | 2.97 | 2.25 | 1.40 | 0.99 | 0.50 |
| Max     | 7.07 | 6.58 | 5.42 | 4.52 | 3.90 | 3.45 | 2.73 | 1.88 | 1.46 | 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.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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