

# Analytical quality by design -assisted LC-MS method for quantification of Telmisartan and Amlodipine besylate fixed dose combination and its stability studies

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## Research Article

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**Analytical quality by design -assisted LC-MS method for quantification of Telmisartan and Amlodipine besylate fixed dose combination and its stability studies.**

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## ABSTRACT

An accurate, basic assessment and responsive technique ensue fixed towards the concurrent verification regarding Telmisartan and Amlodipine besylate formulation. Quality by design plays an important role for analytical technique development and validation towards the accurate results. The examination of fixed-dose combinations (FDCs) helps in saving time, resources, costs, and organic solvent based on this strategy LC-MS has been developed an accurate, basic assessment and sensitive method was fixed. The process that was created by employing an improved analytical quality by design (A QbD) almost entirely relies on a center composite design (CCD) that is based on design of experiment (DoE). A QbD based LC-MS approach (using Design Expert Software version 11) constituted, evolved also intentional apex clarity of Fixed Dose mix of Telmisartan and Amlodipine Besylate peak using Photo Diode Array detector. A stability-indicating LC-MS approach in existence and validated for the quantitative assessment of Telmisartan and Amlodipine besylate was accomplished directing Zorbax C18 column amid a mobile phase of Methyl alcohol: Ammonium ethanoate (90:10, v/v) at a flow rate of 0.6 mL/min with a DL temperature of 250°C. Observation achieved by electrospray ionization in positive-ion multiple reaction keep track of mode. The correlation coefficient for Telmisartan and Amlodipine besylate was found to be 0.9968 and 0.998 respectively. To date no LC-MS/MS method has been reported for the simultaneous determination of Telmisartan and Amlodipine besylate.

**Keywords:** Telmisartan, Amlodipine besylate, Method development, Validation, LC-MS and ICH guidelines.

### 1. Introduction:

Most hypertension patients, particularly those with damage to their organs, would likely need to take many medications to achieve their blood pressure aims and lower of their chance of negative vascular results. The justification for using a combination therapy involving medications with the result inhibit the renin-angiotensin-aldosterone system (RAAS) along with a calcium channel blocker (CCB) or diuretic is exceptionally established [1]. Telmisartan constitutes a angiotensin II blocking agent that has elevated affinity for the angiotensin II receptor type 1 (AT<sub>1</sub>) [2], influences in a discerning manner peroxisome proliferators-activated

receptor gamma (PPAR- $\gamma$ ), a core regulator of insulin and glucose[3] metabolism. It is chemically 4'-[(1,7'-Dimethyl-2'-propyl-1H,3'H-2,5'-bibenzimidazol-3'-yl) methyl]-2 biphenylcarboxylic acid and [4] shown in (Figure 1). Amlodipine hampers by inhibiting the arrival of extracellular calcium ions into myocardial and mural cells. This action guides to the relaxation of vascular and myocardial contraction. Consequently, cardiac contractility lowers, blood flow and oxygen delivery to myocardial tissue increase, the leading coronary and systemic arteries enlarge, and comprehensive peripheral resistance diminishes. It is 3-Ethyl 5-methyl 2-[(2-aminoethoxy) methyl] in chemical terms. 4-dihydro-3, 5-pyridine dicarboxylate, and 4-(2-chlorophenyl)-6-methyl-1 are displayed in [5-6] (Figure 2).

Like tablets, capsules, modified release formulations, controlled release formulations, and extended release variations, fixed dosage combinations of medications are used to treat different cardiovascular problems in different dosing kinds. Positive challenges are guided by the combined drugs' physiochemical effects. The necessity is ready to separate, identify, and control the corresponding sum of the ingredient in the mixture of samples during the analysis of the assay detected towards the discrete pharmaceuticals in official compendia [7]. Ensuring the identity, strength, quality, and chastity of pharmaceutical products is essential to guarantee their safety and effectiveness over their entire shelf life, including during storage, distribution, and usage. Fixed-dose combinations of medications play a significant role in treatment regimens by reducing the frequency of doses required, thereby enhancing patient compliance [8]. The composition of a marketed formulation is determined by analytical method by the process of determining an accurate assay procedure for the step by step of proving that an analytical procedure is accepted to utilize in the laboratory to measure the concentration of subsequent samples. In GMP and GLP environments, it is imperative to conduct analytical method validation using protocols and acceptance criteria outlined in the ICH guidelines Q2(R1). This ensures that the methods used for analyzing pharmaceutical products are robust, reliable, and accurate. Adhering to these guidelines helps maintain quality standards and ensures consistency in results, which are crucial for regulatory compliance and product safety. There exist many methods exists for the determination Telmisartan and Amlodipine besylate [9] by UV Spectroscopic and HPLC method. LC/MS is a strategy one integrate segregation space of liquid chromatography with mass analysis capability of Mass Spectrometry. It is an approach that alliance segregation authority of HPLC with detection power of Mass spectrometry [10]. In this method the detector is removing from the column of LC and fit the column to interface of MS. The sight of the investigation was to elevate a simple, accurate,

robust and precise method [11] for simultaneous estimation of antihypertensive drugs as active pharmaceutical ingredient (alone) and fixed dose formulation with forced degradation study by LC-MS methods. A systematic method development strategy was employed to refine the parameters/conditions for the analysis. The forced degradation study was done at various conditions and its parameters were studied and chromatogram recorded periodically to evaluate the percentage of degradation in stability studies, hardly LC-MS method for the proposed drug for [12] this combination available. This method proposed to be suitable to marketed dosage form with the same label contents. Therefore, LC-MS merge the outstanding separation resolution of liquid chromatography with exceptional superior abilities of mass spectrometry. The spectra from these examine measurements produce molecular mass and structural information for evade components, which complements the qualitative information based on retention times obtained using other LC detectors. An updated AQbD was executed based on failure mode critical effect analysis and DoE elements in order to evolve the LC-MS method for regulatory compliance. As stated by ICH Q9 guidelines, the analytical FMCEA has been carried out by identifying possible analytical negligence modes and investigating their crucial impacts using risk priority number (RPN) ranking. As directed by ICH Q8 (R2) [13], a DoE-based analytical FMCEA was implemented to connect high-risk analytical failure modes with critical method performance features using DoE-based center-composite design employing Design-Expert software (trial version). The new contribution lies in the application of AQbD, which optimizes method development and degradation studies, using a central composite design.

Examining fixed-dose combinations (FDCs) to reduce expenses, time, resources, and the use of organic solvents was the main goal of this study. Expanding upon this approach, an LC-MS method was created that is sensitive, clear, and accurate. An improved Analytical Quality by Design (AQbD) technique that mostly makes use of a central composite design (CCD) in the Design of Experiments (DoE) process. utilizing Design Expert Software (version 11), a QbD-based LC-MS technique was refined to investigate the peak clarity of a fixed-dose combination of Telmisartan and Amlodipine Besylate utilizing a Photo Diode Array (PDA) detector. ensures reliability and reproducibility in the analysis of telmisartan and amlodipine besylate by offering a strong LC-MS method backed by AQbD principles for the efficient quantitative evaluation of fixed-dose combinations.

As per the literature, several LC-MS/MS methods have been reported for the determination of Telmisartan and Amlodipine besylate individually or with other drugs. To date, no LC-MS/MS

method has been reported for the simultaneous determination of Telmisartan and Amlodipine besylate. The present work describes simple, sensitive and sensitive method which employs a simple method.

## **2. Experimental**

### *2.1. Materials and methods*

The active pharmaceutical ingredients (APIs) of Telmisartan (TEL) and Amlodipine (AMD) were obtained as samples provided by various reputable pharmaceutical industries in Gujarat, India. Methanol and acetonitrile of high-performance liquid chromatography (HPLC) grade were purchased from Merck Specialties Pvt. Ltd. (Mumbai, India), and Labsil Instruments (Bangalore, India) provided the Milli-Q grade water. Chemicals such as hydrochloric acid, sodium hydroxide, formic acid, glacial acetic acid, and 30% hydrogen peroxide were acquired from S.D. Fine-Chem. Ltd. (Mumbai, India). Fixed-dose combination (FDC) products of TEL with AMD were acquired against domestic market in Chennai, India. Membrane filters (0.45  $\mu\text{m}$ ) and syringe filters (0.22  $\mu\text{m}$ ) were belonging to Pall India Private Limited (Andheri, Mumbai, India).

### *2.2. Instrumentation*

The investigation utilized a Shimadzu LCMS 8030 Prominence Triple Quadrupole mass spectrometer from Shimadzu Scientific Instruments (India) Private Limited, located in Bangalore, India. Additionally, equipment included a Centrifuge R8c model from Remi Motors Ltd, India, and a Vortex Mixer also from Remi Motors Ltd, India. A UV-Visible spectrophotometer model 3092 from Lab India Instrument Private Limited, also based in Mumbai, Maharashtra, India, was used in conjunction with a sonicator water bath produced by PCI Analytics Private Limited in Mumbai. An LI 120 pH meter from Elico India and an electronic weighing balance from Shimadzu Scientific Instruments (India) Private Limited were used.

### *2.3 Test Assay Preparation Analytical Target Profile (ATP)*

Ten tablets weight were taken which is equivalent to 50milligram of Telmisartan and 50milligram of Amlodipine besylate withdrawn into a100 mL standard flask and diluted with 100 mL of methyl alcohol. Further dilutions efforts were directed towards obtaining the working concentration of 60ng/mL respectively.

## 2.4. Preparation of Solutions

- (a) *Preparations of Buffer (0.1% v/v)*: Weighed 0.750 g of Ammonium acetate dissolve and made up to 1 L escorted by water and adjust the pH to 5 accompanied by acetic acid. Separated by 0.45 micrometer.
- (b) *Preparation of Standard Solution*: Meticulous weighed and carried 0.01 g of Amlodipine besylate and Telmisartan standard to a separate 10 mL standard flask dissolved and reduced to 10 mL accompanied by acetonitrile.

## 2.5 Analytical Target Profile

This research implemented a risk-based strategy grounded in the fundamentals of Quality by Design (QbD) as delineated in ICH Q8 and Q9. The assessment included an examination of both ruggedness and robustness in accordance with these guidelines. Robustness, as per the definition in ICH Q8, pertains to a method's capacity to endure variations in materials, processes, and equipment without adverse consequences. The Analytical Target Profile (ATP) was established, covering method necessities such as target analytes (both drug and impurities), rational and product specifications. The primary focus exist on the determination of Telmisartan (TEL) and Amlodipine (AMD) Active Pharmaceutical Ingredients (APIs) using LC-MS. Method prerequisites encompassed aspects like diluents, mobile phase composition, and column selection.

## 2.6 Critical test parameters/Attributes and initial risk assessment

Experiments were conducted within the scope of Quality by Design (QbD) to determine the relationship between the technique or product's critical quality attributes (CQAs) and critical test parameters (CTPs). To obtain the intended process/test or product quality target test profile (QTTP), the collected data was then used. The concentration of trifluoroacetic acid (TFA) in the mobile phase at 0.2 mL/L, the use of an alternative kind of C18 column, and the replacement of TFA with orthophosphoric acid (OPA) in the mobile phase while keeping the same pH and pKa range were among the CTPs examined in this investigation. Statistical analysis was used to evaluate the design's efficacy, using techniques including analysis of variance (ANOVA) and goodness of fit evaluation. The effect of two independent variables, buffer concentration (BC%) and the organic ratio of the mobile phase (MP%), on three responses—retention time (RT), tailing factor (TF), and theoretical plate (TP)—was examined using a factorial design and Design Expert software version 11 (CCD). These settings were selected in order to optimize the suggested approach. Table 1 provides specifics on the chromatographic conditions

and ranges for the chosen variables. Table 2 displays the CCD matrix for method variable screening for the TEL & AML tablets assay technique using Design Expert software v11. The TEL & AML 100 mg tablet assay method's response surface plot for standard error of design (Figure 3) demonstrates an accurate response. The Supporting Information (Figures 4) displays the variable's responses.

## 2.7 Critical Analytical Attributes

AQbD testing exist preside over using Design Expert software v11 (CCD) to investigate the effects of two critical analytical attributes (CAAs): buffer concentration (BC %) and organic ratio of the mobile phase (MP %) on three responses: retention time (RT), tailing factor (TF), and theoretical plates (TP). The relevance of the strategy was demonstrated by the probabilities linked to these responses being less than 1 (Tables 3). Both variables A and B had a positive impact on RT, TF, and TP, according to an analysis of the quadratic model equations and three-dimensional (3D) response surfaces (Figure 5), indicating a linear link between factors and responses. Furthermore, simultaneous alterations of one or more factors resulted in similar response trends. ANOVA was employed for statistical analysis of the QbD data (Table 4). The predicted R-squared values for all responses (R1: -1.1842, R2: -2.2215, and R3: -3.5990) closely matched the adjusted R-squared values (-0.1961, -0.2856, and -0.1087, respectively) (Table 5), indicating good agreement. The significant model F-values (433.92, 10.80, and 3.60 for responses R1, R2, and R3, respectively) and low p-values for the model terms confirmed the significance of both variables A and B. Equations representing the relationships between retention time, tailing factor, and theoretical plates with respect to coded and actual factors are provided (eqs 1–6). Select the highest order polynomial where the additional terms are important and the example is not aliased. A negligible lack-of-fit should be present in the chosen model (Table 6).

Model terms are contemplated significant when the P-value is less than 0.0500.  $A^2$  is a crucial model term in the course of instance. The model terms are not significant if the values are higher than 0.1000. Model reduction may intensify the model if there are a lot of unwarranted terms (apart from those needed to maintain hierarchy) (Table 7). The Predicted  $R^2$  value of 0.1351 is notably distant from the Adjusted  $R^2$  value of 0.7915, escorted a difference exceeding 0.2. This deviation may suggest the presence of a substantial block effect or possible issues with the model and/or data integrity. It is advisable to explore options such as model simplification, response transformation, identification and handling of outliers, among others.

Additionally, it is essential to validate all empirical models through confirmation runs, as outlined in (Table 8). Adeq Precision, which assesses the signal-to-noise ratio, yields a favorable ratio of 8.984, indicating a satisfactory signal. Consequently, this model demonstrates suitability for navigating the design space.

Assuming all other factors stay constant, the coefficient estimate manifest the expected change in response for every unit change in factor value. The intercept in an orthogonal design represents the average response over all runs. Adjustments around this mean, as determined by the factor settings, are then represented by the coefficients. Variance Inflation components (VIFs) equal 1 when the components are orthogonal; values greater than 1 indicate multicollinearity, with larger VIFs signifying stronger correlations between the factors. According to Table 9, VIFs under 10 are generally regarded as acceptable.

$NTP = 22590.00 - 81.24A - 316.29B - 299.75AB + 1254.31A^2 + 31.31B^2$  with respect to actual factors

$NTP = 1.48577E+05 - 4.48702E+05FR + 2354.70617MP$   
 $5450.00000FR * MP + 4.14649E+05FR^2 + 31.31250MP^2$

The chromatogram and UV spectrum of TEL & AMD are shown in [Figure 6a and 6b](#). In QbD approaches, the CTPs satisfies all system criteria suitability parameters with a % RSD of 0.27–0.41% .

### ***Analytical Method Validation***

The refined LC-MS/MS procedure validated as per International Conference on Harmonization guidelines.

### ***Forced Degradation***

The stress examination was conveyed out on a drug product to illustrate the inherent stability. The Forced degradation was carried out on placebo and drug product with current ICH procedures.

## **3. Results and discussion**

### ***3.1 Chromatography Conditions***

The Analytical Quality by Design (AQbD) methodology was employed within develop the chromatography method for the compound. Utilizing the Waters HPLC system as previously described, the separation was executed. Additionally, Quality by Design (QbD) principles were explored through the utilization of Design Expert software version 11, employing Central Composite Design (CCD). CCD facilitated the identification of significant factors and the optimization of chromatography parameters with minimal experimental runs.

### **3.2. Assessment approach**

For the Design of Experiment (DOE) optimization, two variables—buffer concentration and mobile phase composition—were present, while retention time (RT), tailing factor (TF), and theoretical plates (TP) were designated as the key response variables. The mobile phase comprised a degasified mixture of 0.2% v/v TFA and ACN in a ratio of 80:20 (% v/v). Other parameters such as a flow rate of 1 mL/min, a run time of 15 min, a column temperature of 30 °C, and a detection wavelength of 290 nm with a 10 µL sample injection volume were maintained throughout the experimentation process (Figure 7 I, II & III).

#### **3.2.1. Acid degradation**

About 1mL of 100 µg /mL of Telmisartan formulation in methyl alcohol were taken into 10 mL standard flask and the dilution were adjusted with methyl alcohol 0.1 N hydrochloric acid placed strength of 10 µg/mL. The solution was refluxed at 60°C. The solution then examined at various period such as 0, 2, 4, 6, 8, 12 and 24 hours. About 1mL of 100 µg /mL of Amlodipine besylate formulation in methyl alcohol were taken into 10 mL standard flask and the dilution were adjusted with methyl alcohol 0.1 N hydrochloric acid placed strength of 10 µg /mL. The solution was refluxed at 60°C. The solution then examined at various period such as 0, 2, 4, 6, 8, 12 and 24 hours. The concentration of 60 ng/mL was produced by diluting with methyl alcohol. The degradation studies were performed at different condition after 24 hours of degradation at 60°C.

#### **3.2.2. Basic degradation**

About 1mL of 100 µg of Telmisartan and Amlodipine besylate tablet formulation in methyl alcohol was taken into 10 mL volumetric flask respectively and dilution were adjusted with 0.01 N Sodium hydroxide to placed strength of 10 µg/mL. The solutions were refluxed at 60°C. The solution was examined at various periods such as zero, two, four, six, eight, twelve

and twenty four hours. The solution was diluted with methyl alcohol to produce the working concentration 65 ng/mL of Telmisartan and 60 ng/mL of Amlodipine besylate respectively. The base degradation is represented by a Chromatogram.

### ***3.2.3. Neutral degradation***

About 1 mL of 100 µg/mL Telmisartan and Amlodipine besylate formulation with methyl alcohol were taken into 10 mL standard flask and the dilution were adjusted using water to place concentration of 10 µg/mL Telmisartan and Amlodipine besylate respectively. The solution was refluxed at 60°C. The solution was analyzed at various periods such as zero, two, four, six, eight, twelve and twenty four hours. The solution was reduced with methyl alcohol to obtain the strength of Telmisartan 65 ng/mL and 60 ng/mL of Amlodipine besylate respectively.

### ***3.2.4. Oxidative degradation***

To 1 mL of 100 µg/mL Telmisartan and Amlodipine besylate formulation in methyl alcohol was taken diluted to 10 mL standard flask and the capacity was made up with 30 % hydrogen peroxide to obtain strength of 10 µg/mL Telmisartan another Amlodipine besylate respectively. The solution was refluxed at 60°C. The solution was analyzed at various periods such as zero, two, four, six, eight, twelve and twenty four hours. The solution was reduced with methyl alcohol to obtain the strength of Telmisartan 65 ng/mL and 60 ng/mL of Amlodipine besylate respectively.

### ***3.2.5. Photo degradation.***

About 100 milligram of Telmisartan and Amlodipine besylate formulation was exposed to UV light and sunlight. The sample was dissolved in methyl alcohol and further diluted to produce a concentration of Telmisartan 65 ng/mL and 60 ng/mL of Amlodipine besylate respectively. The solution was analyzed at various periods such as zero, two, four, six, eight, twelve and twenty four hours. The reduction in the strength with photodecomposition of Telmisartan was indicated by a chromatogram in Fig. 9 (A& B) and the outcomes were shown in (Table 10).

## ***3.3. Method validation***

### ***3.3.1. Method Precision and Accuracy***

The three various dilutions of levels of 55 ng/mL, 65 ng/mL and 75 ng/mL of Telmisartan and 50 ng/mL, 60 ng/mL and 70 ng/mL of Amlodipine besylate adapted and resolved to examine the precision of the method. The average recovery, normal deviation (SD) and %RSD were tabulated. The interday and intraday precision was determined for both Telmisartan and Amlodipine besylate it is shown in (Table 11 & 12). The mean recovery for the refined method of accuracy and was established at three levels by diluting the known concentration of Telmisartan (55 ng/mL, 65 ng/mL and 75 ng/mL) and Amlodipine besylate (50 ng/mL, 60 ng/mL and 70 ng/mL) standard drug with the respective degradation solution.

### **3.3.2. Linearity**

The linearity was determined with standard solutions of concentration ranging from 20-120 ng/mL of Telmisartan and 15-105 ng/mL of Amlodipine besylate was analyzed to check the linearity of response. The linearity was determined with standard solutions of concentration ranging from 20-120 ng/mL of Telmisartan and 15-105 ng/mL of Amlodipine besylate is shown in (Table 13) and figure 8a & 8b/

**3.3.3. Sensitivity.** It is measured in terms of detection limit and quantification limit for Telmisartan in the strength of 5 ng/mL and 20 ng/mL respectively and Amlodipine besylate 5 ng/mL and 15 ng/mL respectively.

## **CONCLUSION**

This method proposed to be suitable to marketed dosage form with the same label contents. A forced degradation study on Telmisartan and Amlodipine besylate was performed to determine the percentage degradation under respective stress condition. The formulation was liable to degradation condition like acidic, alkaline, neutral, oxidative and photolytic condition. The percentage degradation was revealed thus it states an accurate, precise method to be used in the routine laboratory and industry. In addition, it is proposed to achieve the minimize sample size, the operational cost, time consumption and maximize specificity, accuracy and precision.

The average recoveries for the normal deviation (SD) and %RSD were calculated. The percentage recovery is from 98 to 100. To show the inherent stability, a medication product was the subject of a stress study. Using current ICH techniques, the forced deterioration was applied to both the medicinal product and the placebo. The degradation behavior of Telmisartan and Amlodipine besylate in acid medium, base, neutral, oxidation decomposition was signified

and the results were shown. Thus enabling maximum output per man-hour in industry and reduce expenditure directly and indirectly. It is used in routine laboratory analysis and with high degree of accuracy and precision to determine the quantitative estimation.

## CONFLICT OF INTEREST

The authors communicated that they have no conflict of interest.

## FUNDING

The research was presided over without any funding sources.

**Table 1: Coded values for variables**

| Factor | Variable     | Minimum | Maximum | Coded Low | Coded High | Mean   | Std. Dev. |
|--------|--------------|---------|---------|-----------|------------|--------|-----------|
| A      | Flow rate    | 0.5272  | 0.6828  | -1 ↔ 0.55 | +1 ↔ 0.66  | 0.6050 | 0.0449    |
| B      | Mobile phase | 8.59    | 11.41   | -1 ↔ 9.00 | +1 ↔ 11.00 | 10.00  | 0.8165    |

**Table 2: Optimization parameters for analysis of Olmesartan and Amlodipine using CCD**

| Std | Run | Factor | Response |
|-----|-----|--------|----------|
|-----|-----|--------|----------|

|    |    | <b>A:FR (ml/min)</b> | <b>B: MP (%)</b> | <b>A2:TF</b> | <b>B2: NTP</b> |
|----|----|----------------------|------------------|--------------|----------------|
| 8  | 1  | 0.605                | 102.728          | 1.1          | 15200          |
| 10 | 2  | 0.605                | 90               | 1.2          | 15616          |
| 4  | 3  | 0.66                 | 99               | 1.1          | 17600          |
| 6  | 4  | 0.682782             | 90               | 1.1          | 15498          |
| 13 | 5  | 0.605                | 90               | 1.2          | 15600          |
| 7  | 6  | 0.605                | 77.2721          | 1.2          | 13200          |
| 9  | 7  | 0.605                | 90               | 1.2          | 15600          |
| 11 | 8  | 0.605                | 90               | 1.2          | 15600          |
| 5  | 9  | 0.527218             | 90               | 1.1          | 15678          |
| 2  | 10 | 0.66                 | 81               | 1.2          | 18700          |
| 1  | 11 | 0.55                 | 81               | 1.1          | 15890          |
| 12 | 12 | 0.605                | 90               | 1.2          | 15600          |
| 3  | 13 | 0.55                 | 99               | 0.9          | 16789          |

**Table 3: Confirmation of Solution 1 of 100 Response**

| Solution<br>1 of 100<br>Response | Predicted<br>Mean | Predicted<br>Median | Std Dev   | n | SE Pred   | 95% PI<br>low | 95% PI<br>high |
|----------------------------------|-------------------|---------------------|-----------|---|-----------|---------------|----------------|
| TF                               | 1.08462           | 1.08462             | 0.0800641 | 1 | 0.0830864 | 0.903586      | 1.26565        |
| NTP                              | 23475.4           | 23475.4             | 492.894   | 1 | 560.47    | 22150.1       | 24800.7        |

**Table 4: QBD Data Statistical Analysis Using ANOVA for Responses: Assay of TEL & AMD mg Tablet (With TF)**

| Source             | Sum of Squares | df | Mean Square | F-value | p-value |
|--------------------|----------------|----|-------------|---------|---------|
| Mean vs Total      | 15.29          | 1  | 15.29       |         |         |
| Linear vs Mean     | 0.0003         | 2  | 0.0001      | 0.0164  | 0.9838  |
| 2FI vs Linear      | 0.0025         | 1  | 0.0025      | 0.3033  | 0.5952  |
| Quadratic vs 2FI   | 0.0244         | 2  | 0.0122      | 1.72    | 0.2471  |
| Cubic vs Quadratic | 0.0497         | 2  | 0.0249      |         |         |
| Residual           | 0.0000         | 5  | 0.0000      |         |         |
| Total              | 15.37          | 13 | 1.18        |         |         |

**Table 5: Summary Statistics**

| Source    | Std. Dev. | R <sup>2</sup> | Adjusted R <sup>2</sup> | Predicted R <sup>2</sup> | PRESS  |
|-----------|-----------|----------------|-------------------------|--------------------------|--------|
| Linear    | 0.0876    | 0.0033         | -0.1961                 | -1.1842                  | 0.1680 |
| 2FI       | 0.0908    | 0.0358         | -0.2856                 | -2.2215                  | 0.2478 |
| Quadratic | 0.0843    | 0.3533         | -0.1087                 | -3.5990                  | 0.3538 |
| Cubic     | 0.0000    | 1.0000         | 1.0000                  |                          |        |

Focus on the model maximizing the **Adjusted R<sup>2</sup>** and the **Predicted R<sup>2</sup>**.

**Table 6: Lack of Fit Tests**

| Source     | Sum of Squares | df | Mean Square |
|------------|----------------|----|-------------|
| Linear     | 0.0767         | 6  | 0.0128      |
| 2FI        | 0.0742         | 5  | 0.0148      |
| Quadratic  | 0.0497         | 3  | 0.0166      |
| Cubic      | 0.0000         | 1  | 0.0000      |
| Pure Error | 0.0000         | 4  | 0.0000      |

**Table 7: NTP**

| Source | Sum of Squares | Df | Mean Square | F-value | p-value |
|--------|----------------|----|-------------|---------|---------|
| Model  | 1.228E+07      | 5  | 2.456E+06   | 10.11   | 0.0042  |
| A-FR   | 52800.47       | 1  | 52800.47    | 0.2173  | 0.6552  |

|                  |           |    |           |        |        |
|------------------|-----------|----|-----------|--------|--------|
| B-MP             | 8.003E+05 | 1  | 8.003E+05 | 3.29   | 0.1124 |
| AB               | 3.594E+05 | 1  | 3.594E+05 | 1.48   | 0.2633 |
| A <sup>2</sup>   | 1.094E+07 | 1  | 1.094E+07 | 45.05  | 0.0003 |
| B <sup>2</sup>   | 6820.68   | 1  | 6820.68   | 0.0281 | 0.8717 |
| <b>Residual</b>  | 1.701E+06 | 7  | 2.429E+05 |        |        |
| Lack of Fit      | 1.701E+06 | 3  | 5.669E+05 |        |        |
| Pure Error       | 0.0000    | 4  | 0.0000    |        |        |
| <b>Cor Total</b> | 1.398E+07 | 12 |           |        |        |

**Table 8: Fit Statistics**

|                  |          |                                |        |
|------------------|----------|--------------------------------|--------|
| <b>Std. Dev.</b> | 492.89   | <b>R<sup>2</sup></b>           | 0.8784 |
| <b>Mean</b>      | 23381.15 | <b>Adjusted R<sup>2</sup></b>  | 0.7915 |
| <b>C.V. %</b>    | 2.11     | <b>Predicted R<sup>2</sup></b> | 0.1351 |
|                  |          | <b>Adeq Precision</b>          | 8.9836 |

**Table 9: Coefficients in Terms of Coded Factors**

| <b>Factor</b>  | <b>Coefficient Estimate</b> | <b>df</b> | <b>Standard Error</b> | <b>95% CI Low</b> | <b>95% CI High</b> | <b>VIF</b> |
|----------------|-----------------------------|-----------|-----------------------|-------------------|--------------------|------------|
| Intercept      | 22590.00                    | 1         | 220.43                | 22068.77          | 23111.23           |            |
| A-FR           | -81.24                      | 1         | 174.26                | -493.31           | 330.83             | 1.0000     |
| B-MP           | -316.29                     | 1         | 174.26                | -728.36           | 95.78              | 1.0000     |
| AB             | -299.75                     | 1         | 246.45                | -882.50           | 283.00             | 1.0000     |
| A <sup>2</sup> | 1254.31                     | 1         | 186.88                | 812.42            | 1696.21            | 1.02       |
| B <sup>2</sup> | 31.31                       | 1         | 186.88                | -410.58           | 473.21             | 1.02       |

**Table 10: Degradation parameters for Telmisartan and Ramipril**

| <b>Degradation</b> |  | <b>Concentration of drug Present</b> | <b>% Degradation</b> |
|--------------------|--|--------------------------------------|----------------------|
|--------------------|--|--------------------------------------|----------------------|

| Condition           | Withdrawal time (hr) | Telmisartan (65ng/ml) | Amlodipine besylate (60ng/ml) | Telmisartan | Amlodipine besylate |
|---------------------|----------------------|-----------------------|-------------------------------|-------------|---------------------|
| Acid degradation    | 0                    | 64.56                 | 59.03                         | 0.67        | 1.62                |
|                     | 2                    | 61.25                 | 50.47                         | 5.76        | 15.88               |
|                     | 4                    | 58.87                 | 47.72                         | 9.43        | 20.47               |
|                     | 6                    | 55.12                 | 41.02                         | 15.20       | 31.63               |
|                     | 8                    | 53.31                 | 34.69                         | 17.98       | 42.18               |
|                     | 12                   | 51.01                 | 24.32                         | 21.52       | 59.47               |
|                     | 24                   | 49.73                 | 12.36                         | 23.49       | 79.40               |
| Base degradation    | 0                    | 64.89                 | 59.43                         | 0.17        | 0.95                |
|                     | 2                    | 63.85                 | 53.44                         | 1.77        | 10.93               |
|                     | 4                    | 62.95                 | 51.37                         | 3.15        | 14.38               |
|                     | 6                    | 59.25                 | 46.12                         | 8.85        | 23.13               |
|                     | 8                    | 58.65                 | 33.02                         | 9.77        | 44.97               |
|                     | 12                   | 57.09                 | 29.59                         | 12.17       | 50.68               |
|                     | 24                   | 54.38                 | 23.01                         | 16.34       | 61.65               |
| Neutral degradation | 0                    | 63.61                 | 59.26                         | 2.14        | 1.23                |
|                     | 2                    | 61.07                 | 57.09                         | 6.05        | 4.85                |
|                     | 4                    | 59.11                 | 53.24                         | 9.06        | 11.27               |
|                     | 6                    | 55.30                 | 48.69                         | 14.92       | 18.85               |
|                     | 8                    | 50.41                 | 44.22                         | 22.45       | 26.30               |
|                     | 12                   | 45.52                 | 39.20                         | 29.97       | 34.67               |
|                     | 24                   | 42.38                 | 33.01                         | 34.80       | 44.98               |
| Sun light           | 0                    | 64.91                 | 59.81                         | 0.14        | 0.32                |
|                     | 2                    | 64.55                 | 59.01                         | 0.69        | 1.65                |
|                     | 4                    | 64.10                 | 58.47                         | 1.38        | 2.55                |
|                     | 6                    | 63.93                 | 57.17                         | 1.65        | 4.72                |
|                     | 8                    | 63.84                 | 56.28                         | 1.78        | 6.20                |
|                     | 12                   | 63.45                 | 54.79                         | 2.38        | 8.68                |
|                     | 24                   | 63.39                 | 53.36                         | 2.48        | 11.07               |
| UV light            | 0                    | 64.95                 | 59.92                         | 0.08        | 0.13                |
|                     | 2                    | 64.47                 | 59.11                         | 0.82        | 1.48                |
|                     | 4                    | 64.13                 | 58.97                         | 1.34        | 1.72                |
|                     | 6                    | 63.61                 | 58.22                         | 2.14        | 2.97                |
|                     | 8                    | 63.31                 | 57.27                         | 2.60        | 4.55                |
|                     | 12                   | 63.08                 | 56.39                         | 2.95        | 6.02                |
|                     | 24                   | 62.86                 | 54.85                         | 3.29        | 8.58                |

**Table 11. Precision studies for Telmisartan and Amlodipine besylate**

| Actual Concentration               |          | Mean Concentration (n=6) | SD ± %RSD    |
|------------------------------------|----------|--------------------------|--------------|
| Intraday precision studies (ng/ml) |          |                          |              |
| Telmisartan                        | 55 (LQC) | 53.96                    | 0.802 ± 1.48 |
|                                    | 65 (MQC) | 63.98                    | 0.675 ± 1.05 |
|                                    | 75 (HQC) | 74.35                    | 0.438 ± 0.58 |
| Amlodipine besylate                | 40 (LQC) | 49.09                    | 0.182 ± 0.36 |
|                                    | 50 (MQC) | 59.32                    | 0.320 ± 0.54 |
|                                    | 60 (HQC) | 68.89                    | 0.65± 0.95   |
| Interday precision studies (Day 2) |          |                          |              |
| Telmisartan                        | 55 (LQC) | 53.38                    | 0.99 ± 1.85  |
|                                    | 65 (MQC) | 63.08                    | 0.21 ± 0.32  |
|                                    | 75 (HQC) | 74.17                    | 0.65 ± 0.87  |
| Amlodipine besylate                | 50 (LQC) | 47.98                    | 0.14 ± 0.30  |
|                                    | 60 (MQC) | 58.01                    | 0.33 ± 0.56  |
|                                    | 70 (HQC) | 67.97                    | 0.53 ± 0.78  |
| Interday precision studies (Day 3) |          |                          |              |
| Telmisartan                        | 55 (LQC) | 52.28                    | 1.01 ± 1.94  |
|                                    | 65 (MQC) | 62.78                    | 0.20 ± 0.33  |
|                                    | 75 (HQC) | 73.52                    | 0.38 ± 0.51  |
| Amlodipine besylate                | 50 (LQC) | 47.20                    | 0.34 ± 0.72  |
|                                    | 60 (MQC) | 57.95                    | 0.24 ± 0.42  |
|                                    | 70 (HQC) | 67.80                    | 0.31 ± 0.45  |

**Table 12. Recovery studies for Telmisartan and Amlodipine besylate**

| Drug                | Actual Concentration (ng/ml) | Mean Concentration (n=6)(ng/ml) | SD ± %RSD   | Recovery % |
|---------------------|------------------------------|---------------------------------|-------------|------------|
| Telmisartan         | 55 (LQC)                     | 54.35                           | 0.65±1.20   | 98.81      |
|                     | 65 (MQC)                     | 64.40                           | 0.40 ± 0.62 | 99.10      |
|                     | 75 (HQC)                     | 74.92                           | 0.03 ± 0.04 | 99.89      |
| Amlodipine besylate | 50 (LQC)                     | 49.23                           | 0.64±1.31   | 98.46      |
|                     | 60 (MQC)                     | 59.81                           | 0.10±0.18   | 99.68      |
|                     | 70 (HQC)                     | 69.90                           | 0.02±0.03   | 99.85      |

**Table 13: System suitability parameters for Telmisartan and Ramipril**

| Parameters              | Telmisartan           | Ramipril               |
|-------------------------|-----------------------|------------------------|
| Linearity and range     | 20-120 ng/ml          | 15-105 ng/ml           |
| Regression equation     | $y = 17221x - 1113.6$ | $y = 2297.6x - 7666.7$ |
| Correlation coefficient | 0.9968                | 0.998                  |
| Theoretical plates      | 15147                 | 22519                  |
| Asymmetric factor       | 1                     | 1                      |
| Tailing factor          | 1.1                   | 1.1                    |
| Limit of detection      | 5 ng/ml               | 5ng/ml                 |
| Limit of quantification | 20 ng/ml              | 15 ng/ml               |

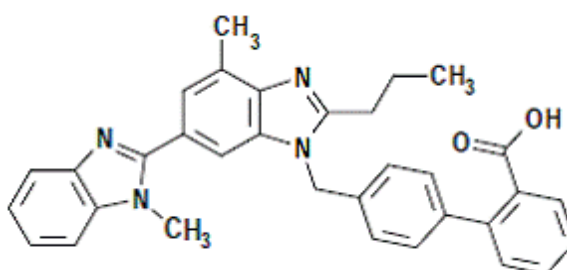


**Figure 1: Chemical structure of Telmisartan**

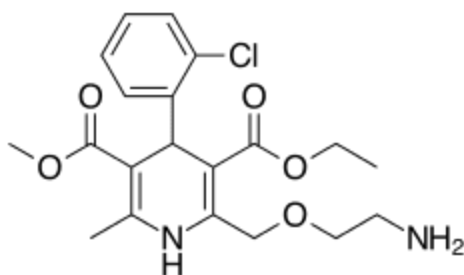


Figure 2: Structure of Amlodipine besylate

### Std Error of Design

Std Error Shading

0.500 1.500

X1 = A: FR

X2 = B: MP

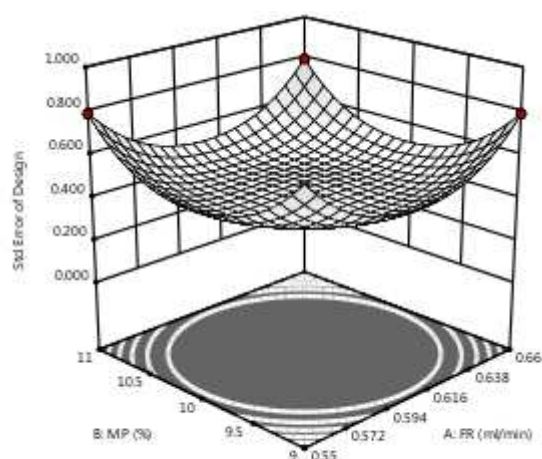


Figure 3: 3D Response surface plot for standard error design for drug

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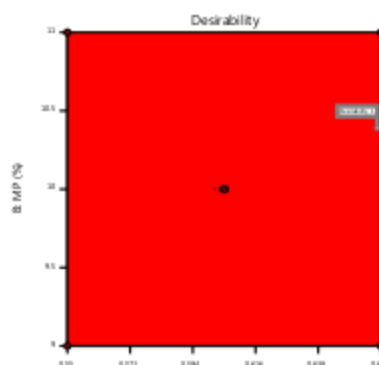
Factor Coding: Actual

### All Responses

● Design Points

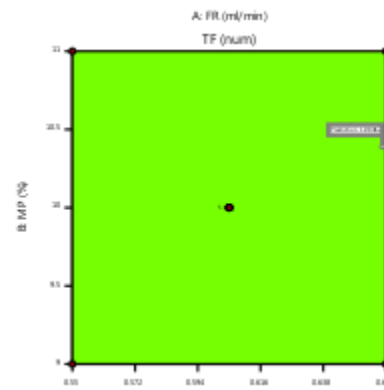
0 1

X1 = A: FR

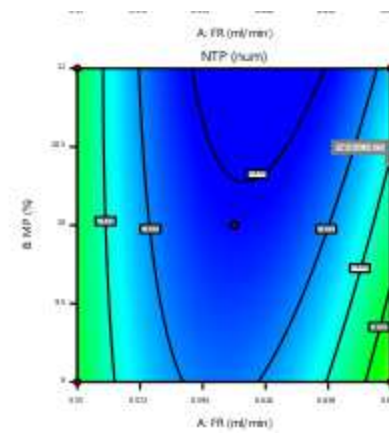


(A)

--- ---  
X2 = B: MP

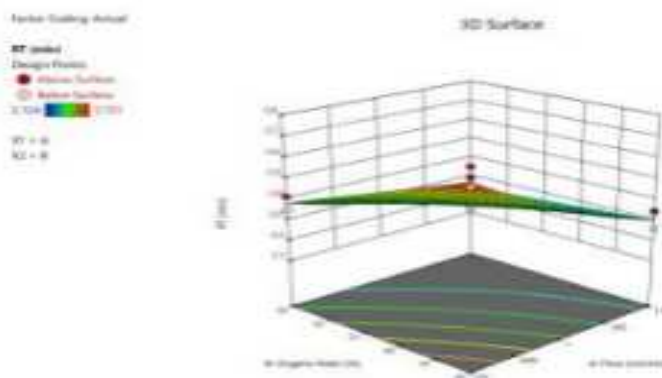


(B)



(C)

**Figure 4 : 2D Response Surface graph for CQA factors for response (A) retention time (RT), (B) tailing factor (TF), (C) theoretical plate (TP)**



(A)

TF (num)

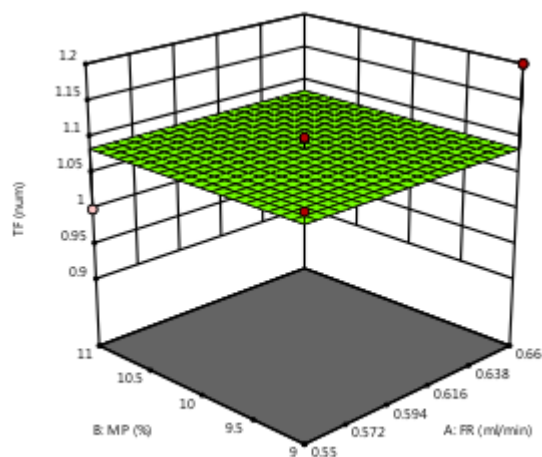
● Design points above predicted value

○ Design points below predicted value

0.9 1.2

X1 = A: FR

X2 = B: MP



(B)

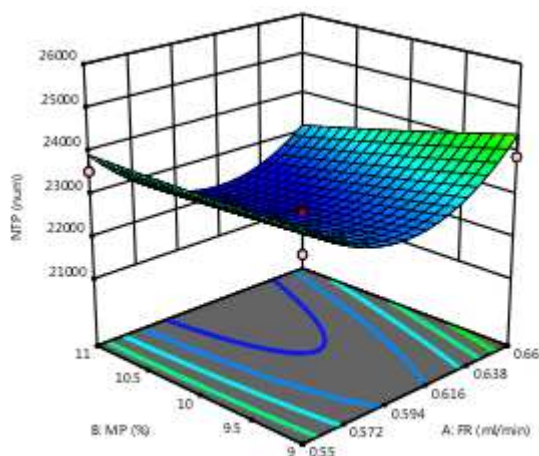
NTP (num)

○ Design points below predicted value

22569 25672

X1 = A: FR

X2 = B: MP



(C)

**Figure 5: 3D Response Surface graph for CQA factors for response (A) retention time (RT), (B) tailing factor (TF), (C) theoretical plate (TP)**

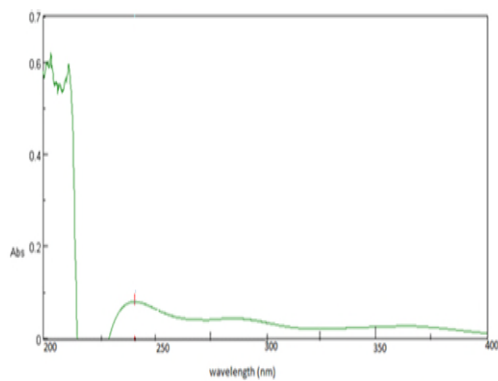


**Figure 6a: UV Spectrum of Telmisartan**

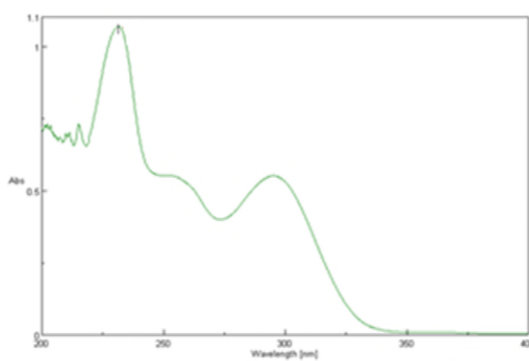


**Figure 6b: UV Spectrum of Amlodipine besylate**

**Design-Expert® Software**  
Factor Coding: Actual

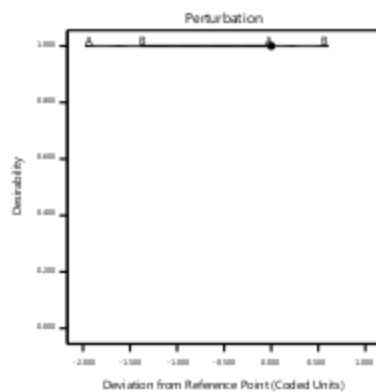
### All Responses

#### Actual Factors

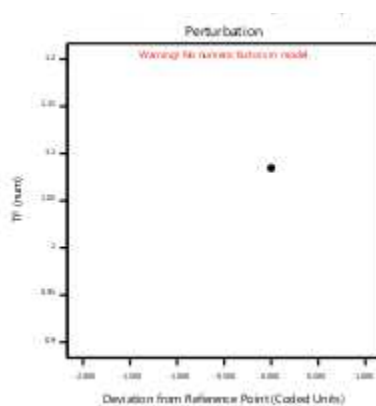
A: FR = 0.658737

B: MP = 10.3896

(A)



(B)



(C)

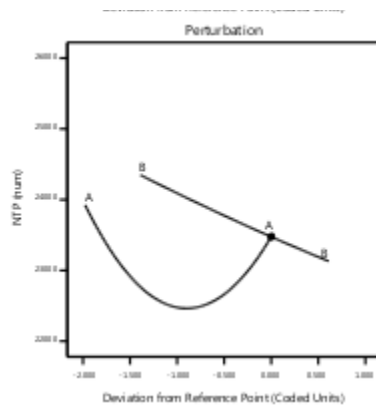


**Figure 7 (I) : Perturbation Plot vs Actual Plots of the response of CQAs (A) retention time (RT), (B) tailing factor (TF), (C) theoretical plates (TP)**

**Design-Expert® Software**  
Factor Coding: Actual

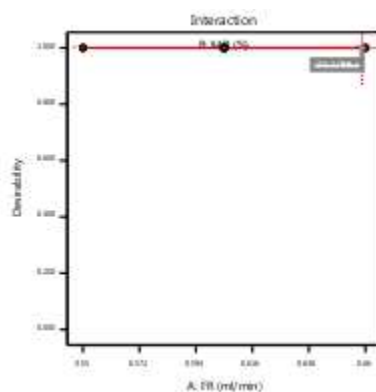
# **All Responses**

● Design Points

X1 = A: FR

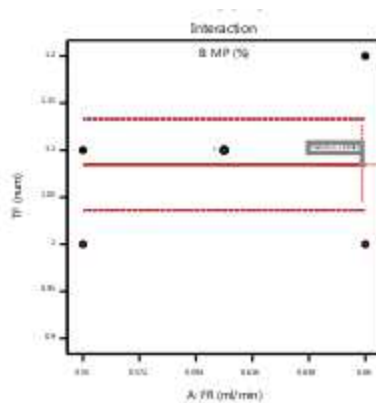
X2 = B: MP

B- 9

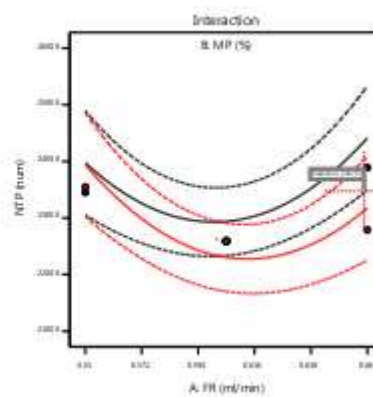


(A)

B+ 11

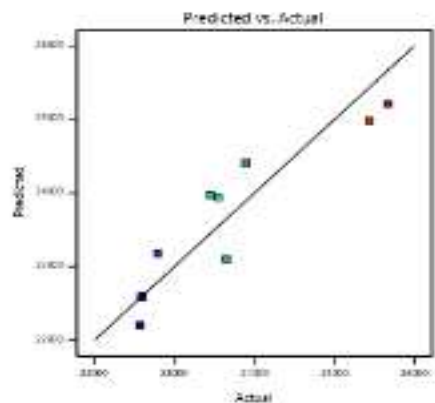


(B)



(C)

**Figure 7 (II): Interaction Plot vs Actual Plots of the response of CQAs (A) retention time (RT), (B) tailing factor (TF), theoretical plates (TP)**



(A)

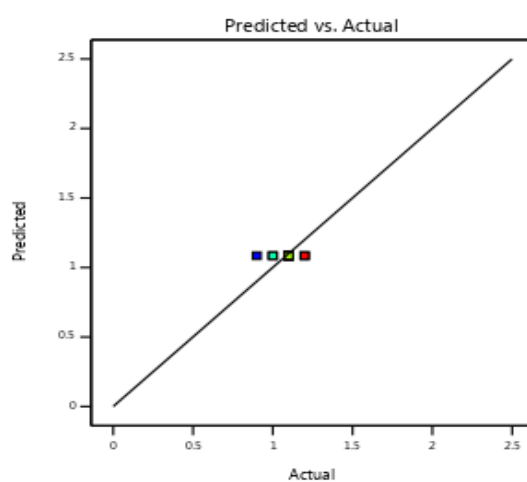
**Design-Expert® Software**

**TF**

Color points by value of

TF:

0.9 1.2



(B)

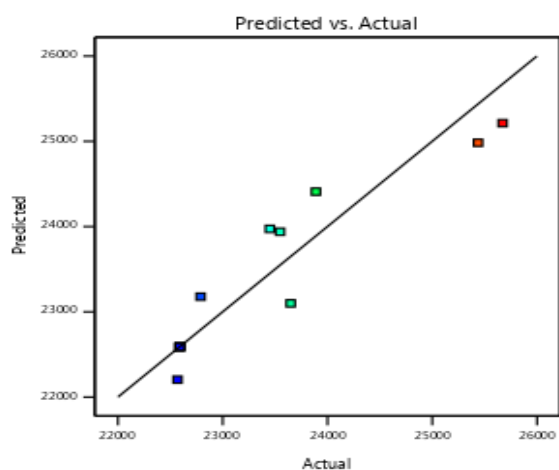
**Design-Expert® Software**

**NTP**

Color points by value of

NTP:

22569 25672



(C)

**Figure 7 (III): Predicated Actual Plots of the response of CQAs (A) retention time (RT), (B) tailing factor (TF), (C) theoretical plates (TP)**

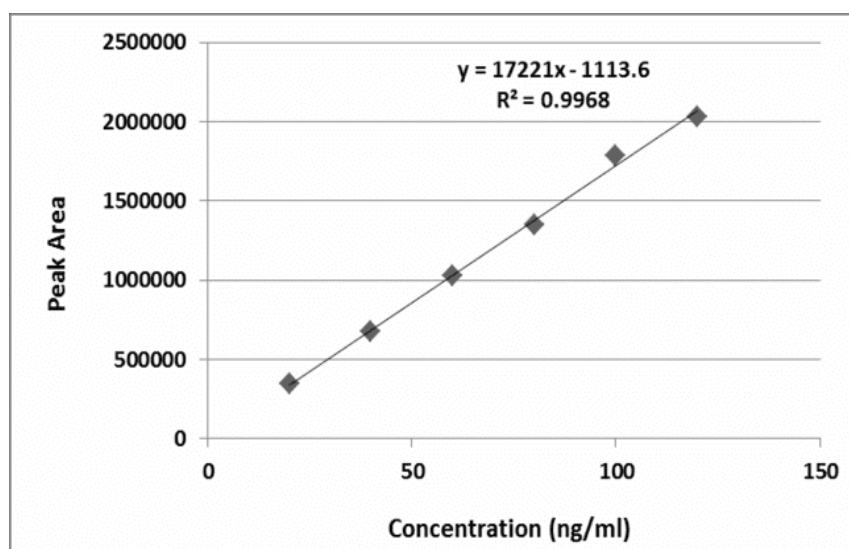


**Figure 8a. Calibration curve of Telmisartan**

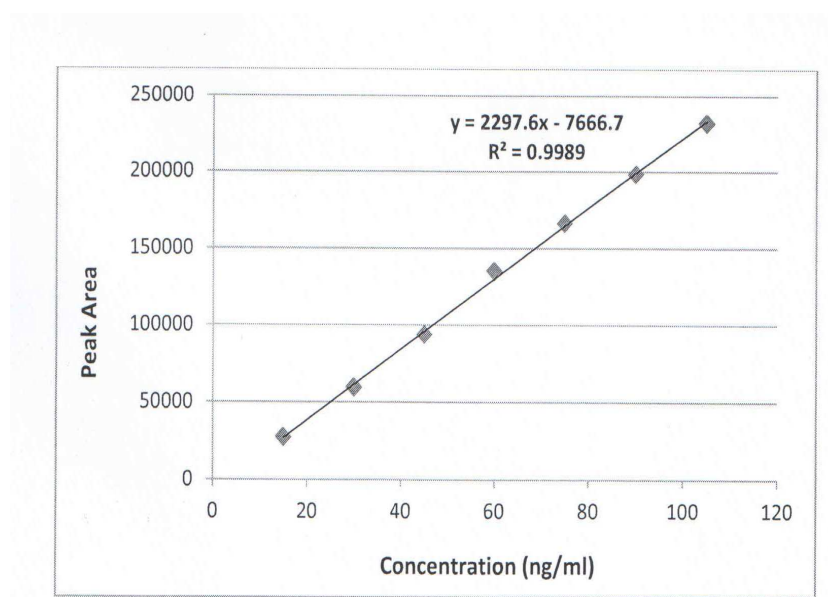


**Figure 8b. Calibration curve of Amlodipine besylate**

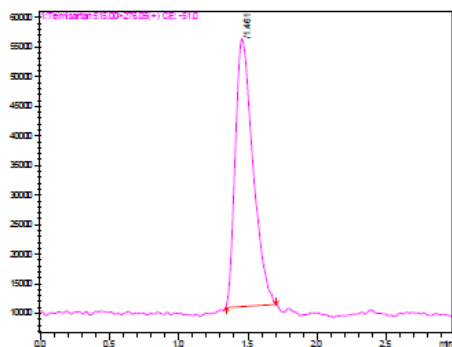


**Figure 9a: Chromatogram of  
Telmisartan**

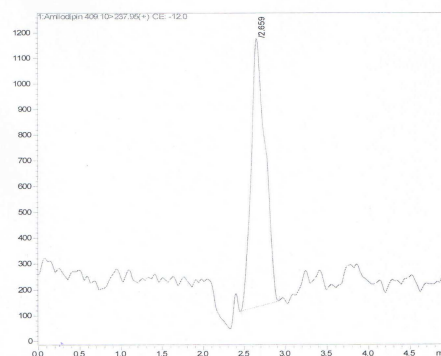


**Figure 9b: Chromatogram of  
Amlodipine besylate**

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**DATA AVAILABILITY DECLARATION:**

All authors declare that all the data are available in the manuscript.

**AUTHOR CONTRIBUTION DECLARATION:**

**Sonia K** –Article drafting and methodology writing

Nappinnai M – Article drafting till results and discussion.

Sujatha K – Conclusion writing.

Ajitha A – Final draft

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