Implementation of QbD Principles for Simultaneous Quantitative Expression of Olmesartan Medoxomil, Telmisartan and Hydrochlorothiazide by RP-HPLC

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Authors’ contributions
This work was carried out in collaboration among all authors. Author BM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors HJ and US managed the analyses of the study. Author PP managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim and Study Design: Aims: The current research paper describes the RP-HPLC Method for estimation of Olmesartan Medoxomil, Telmisartan, and Hydrochlorothiazide and implements the role of QbD for Data Analysis
Study design: Mentioned study is simple, rapid, economical, accurate, and robust RP-HPLC Method for Olmesartan Medoxomil, Telmisartan, and Hydrochlorothiazide and implementing QbD Approach for Data Analysis.
Place and Duration of Study: The present study was carried out at Smt. S. M. Shah Pharmacy College, Mahemdabad, Gujarat, India from October 2019 to February 2020.
Methodology: The separation was done on Hypersil ODS C18 column with dimensions (250mm x 4.6ID, Particle size: 5 microns) and Methanol: 0.02M potassium dihydrogen phosphate buffer (60:40%v/v) pH 3 used as mobile phase. The flow rate was 1.2ml/min; detection at 254nm. QbD
INTRODUCTION

Cardiovascular disease (CVD) is a class of disease that includes the heart or blood vessels. It involves coronary heart diseases like angina and heart attack. Other CVD includes heart failure, stroke, hypertensive heart disease, congenital heart disease, and venous thrombosis. Cardiovascular diseases are the leading cause of death in developed countries [1]. The utmost risk factors of cardiovascular diseases are low-density lipoprotein (LDL) cholesterol, hypertension, platelet aggregation, diabetes, smoking, sleep apnea, and obesity, which are primarily caused by an unhealthy diet and physical inactivity [2]. Among these, high blood pressure (hypertension), is a common condition that affects more than 1.13 billion people worldwide. The frequency of hypertension increases with advancing age and the risk of both fatal and nonfatal cardiovascular disease in adults is lowest with systolic blood pressures of less than 120 mm Hg and diastolic blood pressures less than 80 mm Hg. However, these risks increase progressively with higher systolic and diastolic blood pressures. Calcium channel blockers, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, and diuretics are commonly used for the management of hypertension [3,4]. Diuretics are often chosen as the first line of therapy for most people who have hypertension. ACE inhibitors are often a prime consideration for people with diabetes. Many dual and triple combinations are available for the management of hypertension and cardiovascular diseases. Olmesartan medoxomil (OLM) is chemically known as the (5- methyl- 2- o xo- 3- dioxol- 4- yl) methyl ester of 4- (1- hydroxy- 1-methyl ethyl)- 2- propyl- 1- {[2- (1H- tetrazol- 5-yl)] [1, 1′- biphenyl]- 4- yl} methyl]- 1H- imidazole-5-carboxylic acid (Fig. 1A), an angiotensin II type 1 (AT(1)) receptor antagonist (angiotensin receptor blocker [ARB]) that inhibits the actions of angiotensin II on the renin-angiotensin-aldosterone system that plays an important role in the pathogenesis of hypertension [5,6]. Telmisartan (TMS) is chemically described as 4′- [(1, 40-dimethyl-20-propyl [2, 60-bi-1Hbenzimidazol]-10-yl) methyl]- [1,1-biphenyl]-2-carboxylic acid (Fig. 1B). Telmisartan (TMS) is an angiotensin receptor blocker. It works by blocking the action of certain natural substances that tighten the blood vessels, allowing the blood to flow more smoothly and the heart to pump more efficiently. It is a long-lasting and potent drug [7,8]. Hydrochlorothiazide (HCTZ) is chemically described as 6-chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7 sulfonamide 1,1-dioxide (Fig. 1C), one of the oldest and widely used thiazide diuretics. Diuretics reduce blood pressure by reducing blood volume by increasing the excretion of sodium and water in the urine [9].

Several analytical methods were reported for analysis of this drug product in pharmaceutical formulations including RP-HPLC, a stability-indicating RP-HPLC, UV-spectrophotometry, [10-21] for the determination of OLM, TM, and HCTZ either single or in combination with other drugs. However, the development of the RP-HPLC method for simultaneous estimation of OLM, TM, and HCTZ in combined dosage form has not been reported mainly focused on QbD principles.

DoE focuses on the principle of use of experimental design, generation of mathematical equations, and graphical outcomes, employing various combinations of factors. Experimental research designs are concerned with the examination of the effect of the independent variable on the dependent variable where the independent variables undergo various treatments and the effect of these treatments is observed on the dependent variable [22-24].

Experimental design emphasis not only the selection of suitable independent, dependent,
and control variables but planning the delivery of the experiment under statistically optimal conditions. Experimental design methods have proved to be a useful tool for method validation, as it allows the investigation of simultaneously changing factors. There are multiple approaches for determining the set of design points (unique combinations of the settings of the independent variables) to be used in the experiment. Numerous experimental designs for robustness study includes Plackett Burman design, factorial, fractional factorial, and response surface designs [25,26].

The main objective of this research article is to determine the robustness of the RP-HPLC analytical method by factorial design. The full factorial experiment is an experiment whose design consists of two or more factors, each with discrete possible values or "levels", and whose experimental values take on all possible combinations of these levels across all such factors. The amount (% v/v) of the organic phase (X1), pH of mobile phase (X2), and flow rate (X3) were selected as independent variables for analysis. Among the several experimental designs, a Factorial design as a response surface design was selected due to its flexibility, in terms of experimental runs and information related to factor’s main and interaction effects. Preliminary trials of optimization study affirm that the methanol and potassium dihydrogen orthophosphate buffer content in the mobile phase produced a significant effect on the response.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

OLM, TEL, and HCTZ were provided from Cadila Pharmaceuticals Ltd, Ahmedabad respectively.

2.2 Equipment and Software

The chromatographic separation was done on a Shimadzu HPLC Prominence series, integrated with PDA (Photo Diode Array) detector, LC solution software which is a data acquiring software by Shimadzu was used for responses.

![Chemical Structures](image)

Fig. 1. Chemical structures of A. Olmesartan Medoxomil, B. Telmisartan and C. Hydrochlorothiazide
2.3 Mobile Phase

The trials were done using methanol: 0.02 M potassium dihydrogen orthophosphate buffer at various pH in various ratios with various flow rates. HPLC system was operated at room temperature (25 ± 2°C).

2.4 Preparation of Standard Solution

The standard working concentrations of mixed OLM (10 μg/ml), TEL (10 μg/ml) and HCTZ (10 μg/ml) were prepared in the mobile phase. This solution was subjected to liquid chromatographic analysis.

2.5 Chromatographic Conditions

The Shimadzu HPLC Prominence series, Binary gradient, integrated with PDA (Photo Diode Array) detector was used for chromatographic separation. The analytical Grace Smart C18 column (25cm x 4.6mm i.e., 5 μm) with an injection volume of 20 μl was used. The mobile phase consisted of methanol: 0.02 M potassium dihydrogen orthophosphate buffer at various pH in several ratios with various flow rates. HPLC system was operated at room temperature (25 ± 2°C).

2.6 Method Development

2.6.1 Optimized chromatographic conditions

The optimized and predicted data from Design Expert software consisted of mobile Methanol: 0.02M potassium dihydrogen phosphate buffer (60:40%v/v), pumped at pH 3 and a flow rate of (1.2ml/min) gave the desirability function around 1. The UV detector was set at 254 nm.

2.6.2 Experimental design for method development

A Full Factorial Design was used for method development. The amount (% v/v) of the organic phase (X1), pH of mobile phase (X2), and flow rate (X3) were selected as independent variables. The independent variables were varied at three levels (-1, 0, +1). Different ranges of three parameters (X1) 50-70% organic phase, (X2) pH 3-5, and (X3) flow rate of 0.8-1.2 mL/min were taken respectively as shown in Table 1. The factors and ranges selected were established in previous chromatographic separation studies. The dependent variables (or responses) were retention time, tailing factor, and Resolution. The study design of 27 experimental runs was generated and analyzed by Design-Expert software version 7, as shown in Table 2.

2.7 Method Validation

The final optimized chromatographic analytical method was validated as per the International Conference on Harmonization (ICH) Q2(R1) [27].

2.7.1 Linearity

For the following method the standard calibration curve was generated with six different concentrations over the range of 8-48 μg/ml for Telmisartan and Olmesartan Medoxomil and 5-30 μg/ml for Hydrochlorothiazide. A linear calibration curve was generated between peak area and drug concentration. The linearity was examined using linear regression, which was calculated by the least square regression method.

2.7.2 Accuracy

Accuracy was carried out by adding a known amount of standard to the sample solution at 50, 100, and 150% in triplicate, and samples were analyzed using the optimized method. Percentage recovery was calculated.

2.7.3 Precision

The precision is the ability of the method to get consistent results for the six individual preparations. Six working sample solutions of 24 μg/mL for Olmesartan Medoxomil and Telmisartan and 15 μg/mL for Hydrochlorothiazide are injected on the same day and the next day of the preparation of samples and the % RSD of the peak area was calculated.

Table 1. Variables in factorial design

| Factors                     | Factors level |
|-----------------------------|---------------|
| Amount of organic phase (X1)| -1 60 70      |
| pH(X2)                      | 3 4 5         |
| Flow rate (X3)              | 0.8 1 1.2     |
Table 2. Factorial design run for 3 variables (coded values)

| Run Set No. | Coded Value | Actual Value |
|-------------|-------------|--------------|
|             | Amount of org phase (X1) | pH (X2) | Flow rate (ml/min) (X3) | Amount of org phase | pH | Flow rate (ml/min) |
| 1           | -1          | 1           | 50             | 5               | 1.2 |
| 2           | 1           | 0           | -1             | 70              | 4   | 0.8 |
| 3           | 1           | -1          | -1             | 70              | 3   | 0.8 |
| 4           | 0           | 1           | 1              | 60              | 5   | 1.2 |
| 5           | 0           | -1          | 1              | 60              | 3   | 1.2 |
| 6           | -1          | 0           | 1              | 50              | 4   | 1.2 |
| 7           | -1          | -1          | -1             | 50              | 3   | 0.8 |
| 8           | -1          | 1           | 0              | 50              | 5   | 1   |
| 9           | 1           | -1          | 0              | 70              | 3   | 1   |
| 10          | 0           | 0           | 0              | 60              | 4   | 1   |
| 11          | -1          | -1          | 1              | 50              | 3   | 1.2 |
| 12          | 0           | -1          | 0              | 60              | 4   | 1.2 |
| 13          | 0           | 0           | 1              | 60              | 3   | 1   |
| 14          | -1          | 0           | 0              | 50              | 4   | 1   |
| 15          | 1           | 1           | 0              | 70              | 5   | 1   |
| 16          | 0           | 1           | -1             | 60              | 5   | 0.8 |
| 17          | 1           | 0           | 1              | 70              | 4   | 1.2 |
| 18          | 1           | 1           | 1              | 70              | 5   | 1.2 |
| 19          | -1          | 1           | -1             | 50              | 5   | 0.8 |
| 20          | -1          | 0           | -1             | 50              | 4   | 0.8 |
| 21          | 1           | 1           | -1             | 70              | 5   | 0.8 |
| 22          | 0           | 0           | -1             | 60              | 4   | 0.8 |
| 23          | 1           | 0           | 0              | 70              | 4   | 1   |
| 24          | 0           | 1           | 0              | 60              | 5   | 1   |
| 25          | -1          | -1          | 0              | 50              | 3   | 1   |
| 26          | 0           | -1          | -1             | 60              | 3   | 0.8 |
| 27          | 1           | -1          | 1              | 70              | 3   | 1.2 |

2.7.4 Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Small deliberate changes in the method were made like flow rate (0.9-1.1 mL/min), the proportion of organic components in the mobile phase (40-80%), and temperature of the column (25-35°C). %RSD of the above conditions was calculated.

3. RESULTS AND DISCUSSION

3.1 Statistical Analysis of Experimental Data by Design-expert Software

Twenty-seven experiments were conducted using miscellaneous factorial designs, to evaluate the effects of the amount of Organic phase, pH, and flow rate on responses. These independent variables were studied each at three levels (-1, 0, +1). The data generated were analyzed using Design-Expert software version 7. Factors and responses considered for the study are shown in Table 3.

A quadratic model was suggested by the Design-Expert software and the general equation for this model is as follows:

\[ Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_12X_1X_2 + \beta_{13}X_1X_3 + \beta_{23}X_2X_3 + \beta_1X_1^2 + \beta_2X_2^2 + \beta_3X_3^2 \ldots \ldots \ldots \ldots \ldots \ldots .(1) \]

Where \( \beta_0 \) represents the arithmetic averages of all the quantitative outcomes of all experimental runs; \( \beta_1, \beta_2, \) and \( \beta_3 \) are the coefficients computed from the observed experimental values of \( Y \); and \( X_1, X_2, \) and \( X_3 \) are the coded levels of factors. The equation represents the quantitative effect of factors \( X_1, X_2, \) and \( X_3 \) upon each of the responses; \( Y_1, Y_2, \) and \( Y_3, Y_4, Y_5, Y_6, Y_7, \) and \( Y_8 \). Mathematical models were generated by ANOVA for each dependent
variable and then 3D surface graphs were acquired for each of them along with their equations. The effect of independent variables (X1, X2, and X3) and their interaction terms (X1X2, X1X3, and X2X3) were assessed on each dependent variable. For identifying non-linearity exponential terms are investigated.

3.1.1 Effect of independent variables on retention time 1 (Y1)

After the application of experimental design, the suggested quadratic model was found to be significant with a model F value of 5.06, p-value less than 0.05, and R2 value of 0.9556. Adjusted R2 shows the good fit of the experimental model with the polynomial equation, which was found to be 0.9320.

\[
Y_1 = 3.26-1.25A+3.33B-0.39C+8.91AB-0.42AC+0.69BC+0.41A^2-1.66B^2+0.26C...
\] (2)

Where,

X1= A,
X2= B and
X3= C
A= Ratio of Organic Phase,
B= Ph,
C= Flow rate

In this case, A, C, AC, A^2 are significant model terms.

3.1.2 Effect of independent variables on retention time 2 (Y2)

After applying experimental design, the suggested quadratic model was found to be significant with a model F value of 5.40, p-value 0.0086, and an R2 value of 0.9554. Adjusted R2 shows the good fit of the experimental model with the polynomial equation, which was found to be 0.9318.

\[
Y_2 = 5.20-1.46A+0.011B-0.39C+4.167AB-0.49C-003BC+0.14B^2+0.31C^2....
\] (3)

In this case, A, C, AC, A^2, C^2 are significant model terms.

3.1.3 Effect of independent variables on retention time 3 (Y3)

After applying experimental design, the suggested quadratic model was found to be significant with a model F value of 28.56, a p-value of 0.0001, and an R2 value of 0.8955. Adjusted R2 shows the good fit of the experimental model with the polynomial equation, which was found to be 0.8641.

\[
Y_3 =10.31-1.41A+3.833B-0.50C-6.583E-003AB-0.50 AC-9.417E-003BC… (4)
\]

In this case A, C, AC are significant model terms.

3.1.4 Effect of independent variables on tailing factor 1 (Y4)

After applying experimental design, the suggested quadratic model was found to be significant with a model F value of 8.15, a p-value of 0.0002, and an R2 value of 0.7097. Adjusted R2 shows the good fit of the experimental model with the polynomial equation, which was found to be 0.6226.

\[
Y_4 = 1.36+0.12A+0.30B-0.020C-0.18AB-0.046AC+9.833BC…… (5)
\]

In this case A, B, AB are significant model terms.

3.1.5 Effect of independent variables on tailing factor 2 (Y5)

After applying experimental design, the suggested quadratic model was found to be significant with a model F value of 17.78, a p-value of 0.0002, and an R2 value of 0.8421. Adjusted R2 shows the good fit of the experimental model with the polynomial equation, which was found to be 0.7947.

\[
Y_5 =1.38+0.068A+0.38B-0.100C-0.077AB-0.16AC+0.081BC------- (6)
\]

In this case B, C, AC are significant model terms.
3.1.6 Effect of independent variables on tailing factor 3 (Y6)

After applying experimental design, the suggested quadratic model was found to be significant with a model F value of 30.65, a p-value of 0.0001, and an R2 value of 0.9019. Adjusted R2 shows the good fit of the experimental model with the polynomial equation, which was found to be 0.8725.

\[ Y6 = 1.25 - 0.054B + 0.31B - 0.15AB - 0.015AC + 0.086BC \] ........................................ (7)

In this case, A, B, AB, BC are significant model terms.

3.1.7 Effect of independent variables on resolution 1 (Y7)

After applying experimental design, the suggested quadratic model was found to be significant with a model F value of 561.13, a p-value of 0.0001, and an R2 value of 0.9966. Adjusted R2 shows the good fit of the experimental model with the polynomial equation, which was found to be 0.9949.

\[ Y7 = 4.27 - 1.57A + 0.039B - 1.000C + 1.917AB + 0.053AC - 7.917BC + 1.03A^2 + 7.778B^2 - 8.556C^2 \] ........................................ (8)

In this case, A, C, AC, A2 are significant model terms.

3.1.8 Effect of independent variables on resolution 2 (Y8)

After applying experimental design, the suggested quadratic model was found to be significant with a model F value of 1003.15, a p-value of 0.0001, and an R2 value of 0.9981. Adjusted R2 shows the good fit of the experimental model with the polynomial equation, which was found to be 0.9971.

\[ Y8 = 6.74 - 1.29A + 3.389E - 0.03B + 0.073C - 6.75OAB + 0.10AC + 6.917BC + 0.26A^2 + 0.013B^2 - 0.032C^2 \] ........................................ (9)

In this case, A, C, AC, A2 are significant model terms.

Table 3. Factorial experimental design matrixes with responses

| Amt | org | pH | Flow rate | Rs | Rs | Tf | Tf | Tf | Rt | Rt |
|-----|-----|----|-----------|----|----|----|----|----|----|----|
| 50  | 70  | 3  | 0.8       | 6.846 | 8.313 | 1.815 | 1.862 | 1.793 | 5.002 | 7.308 | 11.617 |
| 4   | 5   | 1.2|           | 3.66 | 5.44 | 1.66 | 1.8 | 1.19 | 3.8 | 5.8 | 10.6 |
| 5   | 1.2|    |           | 4.211 | 6.764 | 1.548 | 1.611 | 1.552 | 3.293 | 5.407 | 10.09 |
| 3   | 1.2|    |           | 4.102 | 6.739 | 0.823 | 0.916 | 0.887 | 3.291 | 5.334 | 10.09 |
| 2   | 1.2|    |           | 6.833 | 8.262 | 1.256 | 1.298 | 1.273 | 5.013 | 7.291 | 11.602 |
| 1   | 1.2|    |           | 6.848 | 8.241 | 0.843 | 0.914 | 0.866 | 5.246 | 7.413 | 12.021 |
| 1   | 1.2|    |           | 6.854 | 8.287 | 1.764 | 1.811 | 1.793 | 5.024 | 7.397 | 11.721 |
| 1   | 1.2|    |           | 3.549 | 5.682 | 0.911 | 0.835 | 0.941 | 2.035 | 3.827 | 8.241 |
| 1   | 1.2|    |           | 4.344 | 6.745 | 1.365 | 1.401 | 1.392 | 3.483 | 5.324 | 10.111 |
| 1   | 1.2|    |           | 6.913 | 8.244 | 0.836 | 0.919 | 0.859 | 5.009 | 7.312 | 11.654 |
| 1   | 1.2|    |           | 4.223 | 6.811 | 0.892 | 0.885 | 0.898 | 3.491 | 5.398 | 10.102 |
| 1   | 1.2|    |           | 4.154 | 6.666 | 1.241 | 1.253 | 1.38 | 3.284 | 5.362 | 10.089 |
| 1   | 1.2|    |           | 6.824 | 8.332 | 1.241 | 1.294 | 1.222 | 5.103 | 7.381 | 11.737 |
| 1   | 1.2|    |           | 3.714 | 5.691 | 1.725 | 1.819 | 1.219 | 2.097 | 3.831 | 8.207 |
| 1   | 1.2|    |           | 4.255 | 6.666 | 1.515 | 1.737 | 1.521 | 3.521 | 5.499 | 10.197 |
| 1   | 1.2|    |           | 3.767 | 5.888 | 1.301 | 1.298 | 1.236 | 1.911 | 3.759 | 8.144 |
| 1   | 1.2|    |           | 3.849 | 5.916 | 1.611 | 1.677 | 1.549 | 1.937 | 3.736 | 8.162 |
| 1   | 1.2|    |           | 6.922 | 8.259 | 1.701 | 1.759 | 1.697 | 5.208 | 7.419 | 12.087 |
| 1   | 1.2|    |           | 6.896 | 8.321 | 1.294 | 1.312 | 1.322 | 5.219 | 7.423 | 12.139 |
| 1   | 1.2|    |           | 3.66 | 5.54 | 1.66 | 1.8 | 1.19 | 3.8 | 5.8 | 10.6 |
| 1   | 1.2|    |           | 4.309 | 6.714 | 1.291 | 1.261 | 1.312 | 3.509 | 5.503 | 10.182 |
| 1   | 1.2|    |           | 3.772 | 5.711 | 1.299 | 1.365 | 1.219 | 2.046 | 3.819 | 8.258 |
| 1   | 1.2|    |           | 4.539 | 6.793 | 1.683 | 1.776 | 1.614 | 3.501 | 5.492 | 10.118 |
| 1   | 1.2|    |           | 6.796 | 8.298 | 0.912 | 0.959 | 0.888 | 5.011 | 7.393 | 11.709 |
| 1   | 1.2|    |           | 4.265 | 6.683 | 0.763 | 0.868 | 0.875 | 3.516 | 5.487 | 10.139 |
| 1   | 1.2|    |           | 3.854 | 5.928 | 1.767 | 0.784 | 0.824 | 1.903 | 3.714 | 8.136 |
Fig. 2. Three-dimensional plot for retention time (rt1) as a function of pH and amount of organic phase, constant factor (flow rate 1.0 ml/min), retention time (rt2) and amount of organic phase, constant factor (flow rate 1.0 ml/min), retention time (rt3) as a function of pH and amount of organic phase, constant factor (flow rate 1.0 ml/min), tailing factor 1 (tf1) as a function of pH and amount of organic phase, constant factor (flow rate 1.0 ml/min).

Fig. 3. Tailing factor 2 (tf2) as a function of pH and amount of organic phase, constant factor (flow rate 1.0 ml/min), tailing factor 3 (tf3) as a function of pH and amount of organic phase, constant factor (flow rate 1.0 ml/min), resolution 1 (rs1) as a function of pH and amount of organic phase, constant factor (flow rate 1.0 ml/min), resolution 1 (rs1) as a function of pH and amount of organic phase, constant factor (flow rate 1.0 ml/min).
Table 4. Design summary

| Study Type     | Initial Design | Design Model | Runs |
|----------------|----------------|--------------|------|
|                | Full Factorial | 2FI          | 27   |
|                | No Blocks      |              |      |

| Factor | Name                            | Units | Type  | Low Actual | High Actual | Low Coded | High Code | Mean | Std. Dev. |
|--------|---------------------------------|-------|-------|------------|-------------|-----------|-----------|------|-----------|
| A      | Amount of organic phase         | Numeric | 50    | 70         | -1          | 1         | 60        | 8.165|           |
| B      | pH of mobile phase              | Numeric | 3     | 5          | -1          | 1         | 4         | 0.816|           |
| C      | Flow rate                       | ml/min | 0.8   | 1.2        | -1          | 1         | 1         | 0.163|           |

| Response Name | Units | Obs | Analysis | Minimum | Maximum | Mean | Std. Dev. | Ratio | Trans | Model   |
|---------------|-------|-----|----------|---------|---------|------|-----------|-------|-------|---------|
| Y1            | Polynomia | 27  | 3.549    | 6.922   | 4.949   | 1.372| 1.95      | None  | Quadratic |
| Y2            | Polynomia | 27  | 5.44     | 8.332   | 6.907   | 1.065| 1.532     | None  | Quadratic |
| Y3            | Polynomia | 27  | 0.763    | 1.84    | 1.355   | 0.347| 2.412     | None  | 2FI      |
| Y4            | Polynomia | 27  | 0.784    | 1.96    | 1.377   | 0.381| 2.5       | None  | 2FI      |
| Y5            | Polynomia | 27  | 0.824    | 1.793   | 1.255   | 0.295| 2.176     | None  | 2FI      |
| Y6            | Polynomia | 27  | 1.903    | 5.246   | 3.706   | 1.154| 2.757     | None  | Quadratic |
| Y7            | Polynomia | 27  | 3.714    | 7.423   | 5.75    | 1.333| 1.999     | None  | Quadratic |
| Y8            | Polynomia | 27  | 8.136    | 12.139  | 10.311  | 1.335| 1.492     | None  | 2FI      |
The optimum Chromatagram for OLM, TEL, and HCTZ shown in Fig. 4. Optimized Chromatogram for OLM, TM and HCTZ 10µg/ml in Methanol: 0.02M potassium dihydrogen ortho phosphate buffer pH 3 (60:40%v/v), flow rate 1.2 ml/min.

| Parameter                  | Limit          | OLM Result | HCTZ Result |
|----------------------------|----------------|------------|-------------|
| Linearity and Range        | R2 > 0.995     | 0.9952     | 0.9991      |
| Inter-Day Precision        | C.V. < 2       | 0.71 – 1.10| 0.57 – 1.01 |
| % Recovery                 | 98 - 102%      | 98.11 – 102.29 | 98.77 – 101.28 | 98.72 – 103.15 |
| Robustness                 | C.V. < 2       | 0.18 – 1.87 | 0.21 – 0.83 | 0.06 – 1.33 |
| Intraday Precision         | C.V. < 2       | 0.84 – 1.56 | 0.80 – 1.26 | 0.91 – 1.53 |

The Design Summary is shown in Table 4 represents the Full Factorial type of the design, the factor names and levels, and the response names and levels. It summarizes the current model that has been chosen for each response, including whether a transformation has been applied. The factors chosen were the Amount of organic phase, Ph of mobile phase, and Flow rate. The response noted was Resolution, Tailing factor, and Retention time. The software used was Design-Expert, which provides powerful tools to layout an ideal experiment on your process, mixture, or combination of factors and components. Design-Expert offers a wide selection of graphs that helps to identify standout effects and visualize the results.

The optimum Chromatagram for OLM, TEL, and HCTZ shown in Fig. 4.

### 3.2 Method Validation

The developed method was linear over the concentration range with 5-30 µg/mL with a correlation coefficient ($r^2$) of 0.999. This showed that all the responses were within the specified acceptance limit indicating a high degree of closeness of the predicted data with the observed ones. For the accuracy studies at 50%, 100% and 150% levels, the % recovery of the drug was to be within 98-102. Inter-day precision and repeatability were carried out, and the % RSD values were found to be less than 2%. The robustness of the developed method was checked by making minor changes in the experimental conditions like flow rate, % organic composition, and pH, and %RSD values for the peak area were found to be less than 2%. The summary of the method validation parameters was shown in Table 5.

### 4. CONCLUSION

A robust RP-HPLC method for OLM, TEL, and HCTZ with mobile phase in Methanol: 0.02M potassium dihydrogen orthophosphate buffer pH 3 (60:40%v/v), flow rate 1.2 ml/min, which was optimized with the help of Design Expert 7 software. Before method optimization, screening studies were carried out on different mobile phases of varying composition. Based on the results obtained from these studies, a suitable mobile phase with appropriate composition was selected and utilized for method development using the QbD approach. The study was done by using $3^3$ Factorial Design response surface
designs. In this study interaction of 3 factors; Composition of Organic phase, flow rate, and pH vary at 3 levels. Totally 27 experimental runs were suggested by the software for analyzing the interaction of each response, i.e., retention time, resolution, and tailing factor, which were considered as dependent factors. The method was validated according to ICH guidelines. Validation of the analytical QbD method corroborated excellent linearity, accuracy, precision, and robustness for determination based on the knowledge of the method obtained through the method development and the result of risk assessment.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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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