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# ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF RALOXIFENE BY QBD APPROACH

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**ABSTRACT**

The number of new drugs introduced into the market is increasing day by day. These drugs may be either new entities or partial structural modification of the existing one. Very often there is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, reports of new toxicities (resulting in their withdrawal from the market), development of patient resistance and introduction of better drugs by competitors. Under these conditions, standards and analytical procedures for these drugs may not be available in the pharmacopoeias. It becomes necessary, therefore to develop newer analytical methods for such drugs. Using QbD, pharmaceutical quality is assured by understanding and controlling formulation and manufacturing variables, degradation products and related impurities in bulk drugs, pharmaceutical formulations and biological samples such as whole blood, plasma, serum and urine. Instances of these include estimation of Raloxifene through the HPLC method with UV detector , florescence detector , diode array detector , photodiode array detector , UPLC-ESIQ-MS , UPLC–MS-MS , LC–MS-MS method , etc.

**Keywords**: Pharmacopoeias, QbD, Raloxifene, Analytical Methods, HPLC, UV

## INTRODUCTION

QbD is a systematic approach to pharmaceutical development that begins with pre-defined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management.

Application of QbD will provide knowledge and scientific understanding to support pharmaceutical development.

Pharmaceutical QbD goals may include:

a) to achieve meaningful product quality specifications;

b) to increase process capability and reduce product variability;

c) to increase pharmaceutical development and manufacturing efficiencies; and

d) to enhance cause-effect analysis and regulatory flexibility.

The adoption of risk-based approaches and pharmaceutical QbD has been encouraged by most of regulatory agencies worldwide. Pharmaceutical QbD concepts have been used to enhance manufacturing pharmaceutical products in terms of "Six-sigma" approach.

"Six-sigma" is a system of practices to achieve process improvement, which leads to a significantly reduced chance of out-of-specification (OOS) products. The significant number of OOS results reported indicates that this is an issue to pharmaceutical industries. However, many times the problems in achieving the required "six-sigma" performance is not due to manufacturing issues, but due to poor analytical methods robustness and reliability.

Several authors described the application of the QbD concepts in the development of analytical methods. Analytical QbD is useful in the development and optimization of robust and cost-effective analytical methods. Implementation of analytical QbD provides a better solution to OOS results, as well as it also reduces the risk of method failure

Analytical methods are considered an integral part of pharmaceutical development. Thus, the application of QbD approach to analytical method development is justifiable and a recommended strategy to attain regulatory flexibility, to reduce out-of-specification results, to achieve a high degree of robustness and a cost-effective analytical method.

## MATERIALS AND METHODS

Raloxifene raw material obtained from CIPLA Ltd Mumbai was tested as per in house specification and the results are listed in table 01. The drug source is identified and found complying with the specifications.

**Table 01: Characterization of Raloxifene**

| S.No | Test | Specification | Results |
|---|---|---|---|
| 1 | Description | White to off-white crystalline powder | A white crystalline powder |
| 2 | Solubility | Slightly soluble in water, soluble in Methanol. | Complies |
| 3 | Melting point | 140-144 °C | 141-143 °C |

## PRODUCT IDENTIFICATION STUDIES

### Organoleptic properties

The colour, odour and taste of the drugs were studied

**Particle size and shape**

Particle size and shape of the drugs were studied by optical microscopic method.

**Melting point**

Melting points of the drugs were confirmed by capillary tube method.

**Solubility analysis**

Solubility is the important parameter for preformulation studies because,

1. It affects the dissolution of the drug.
2. Bioavailability of drug is directly affected by oral administration and also by dissolution.
3. Particle size, shape, surface area may affect the dissolution characteristics of drug hence it should be determined during preformulation.

## RESULTS AND DISCUSSION

### HPLC METHOD DEVELOPMENT

The column was equilibrated with mobile phase for saturation of the stationary phase prior to chromatographic analysis.

**Preparation of Standard Stock Solution**

A standard stock solution (i.e., 100 μg/mL) of Raloxifene was prepared in the mobile phase.

**Factor screening studies**

A seven-factor eight-run fractional factorial design was employed for factor screening studies to identify the CMPs/CPPs critically affecting the method CAAs

Table 1 show the Chromatographic Conditions Seven-Factor Eight-Run Taguchi Design Matrix for Screening of Method Variables and Process Parameters at Their Respect Low and High Levels

**Table No. 01: Chromatographic Conditions**

| **Runs** | **Mobile phase ratio** | **Flow rate** | **Injection volume** | **Column oven temperature** | **Buffer strength** | **Buffer pH** | **Column type** |
|---|---|---|---|---|---|---|---|
| 1 | +1 | +1 | −1 | +1 | −1 | +1 | +1 |
| 2 | +1 | +1 | +1 | −1 | +1 | +1 | +1 |
| 3 | +1 | −1 | −1 | −1 | −1 | −1 | +1 |
| 4 | −1 | −1 | +1 | −1 | −1 | +1 | −1 |
| 5 | −1 | +1 | −1 | −1 | +1 | −1 | −1 |
| 6 | −1 | +1 | +1 | +1 | −1 | −1 | |
| 7 | +1 | −1 | +1 | +1 | +1 | −1 | +1 |
| 8 | −1 | −1 | −1 | +1 | +1 | +1 | −1 |

**Method Development as per the Experimental Design**

Table 2 summarizes the design matrix as per the BBD with 17 experimental runs along with quintuplicate studies of center point (0, 0) runs.

A standard concentration 10 µg/mL was used for all experimental runs, which were analyzed for method CAAs, i.e., peak area, retention time, theoretical plates and peak tailing.

**Table 2: Design Matrix as per the BBD for Optimization of the HPLC Method of Raloxifene**

| Trial no. | Coded Factor Levels | | |
|---|---|---|---|
| | Factor 1 | Factor 3 | Factor 3 |
| 1 | 0 | 1 | 1 |
| 2 | −1 | 0 | 1 |
| 3 | −1 | 1 | 0 |
| 4 | −1 | 0 | −1 |
| 5 | 0 | 1 | −1 |
| 6 | 1 | 1 | 0 |
| 7 | 0 | 0 | 0 |
| 8 | 0 | −1 | 1 |
| 9 | −1 | −1 | 0 |
| 10 | 1 | −1 | 0 |
| 11 | 1 | 0 | −1 |
| 12 | 0 | −1 | −1 |
| 13 | 1 | 0 | 1 |

## ANALYTICAL METHOD VALIDATION

### Linearity

The linear calibration plot for Raloxifene in the concentration ranging between 1 and 200ng/mL showed good linearity with higher value of the coefficient of correlation ($r^2$) of 0.999. This revealed that all the responses were within the specified acceptance limit indicating high degree of closeness of the predicted data with the observed ones.

### Accuracy

SD, %RSD and SEM were calculated to check the accuracy of data within the specified limit. Accuracy Shown in Table No. 3.

**Table 3**

| **Levels (%)** | **Concentration (ng/mL)** | **Amount recovered (ng/mL) ± SD** | **Recovery (%)** | **RSD (%)** |
|---|---|---|---|---|
| LQC: 80 | 18 | 17.97 ± 0.21 | 99.8 | 1.16 |
| MQC: 100 | 20 | 19.26 ± 0.13 | 96.3 | 0.05 |
| HQC: 120 | 22 | 22.03 ± 0.31 | 100.13 | 1.45 |

**Precision**

Precision was assessed by assay of three different concentrations of Raloxifene (LQC: 10ng/mL, MQC: 30ng/mL and HQC: 50ng/mL) at different time intervals on the same day.

**Table 04: Intra- and Interday Precision Data for the Developed HPLC Method of Raloxifene**

| Standard concentration (ng/mL) | Amount recovered (ng/mL) ± S.D. | Recovery (%) | RSD (%) |
|---|---|---|---|
| Intraday precision | | | |
| LQC: 10 | 9.94 ± 0.21 | 99.4 | 2.21 |
| MQC: 30 | 30.31 ± 0.79 | 101.0 | 2.60 |
| HQC: 50 | 49.67 ± 1.06 | 99.3 | 2.13 |
| Interday precision | | | |
| LQC: 10 | 9.91 ± 0.31 | 99.1 | 3.12 |
| MQC: 30 | 29.32 ± 1.02 | 97.7 | 3.47 |
| HQC: 50 | 50.04 ± 0.95 | 100.8 | 1.91 |

**Limit of detection and limit of quantitation**

The LOD and LOQ were determined from the slope (S) of the linearity plot and standard deviation of the response to the blank sample (σ) as per the formulae enlisted in Equation.

$$\mathrm{LOD} = 3.3 \times \frac{\sigma}{S} \qquad \mathrm{LOQ} = 10 \times \frac{\sigma}{S}.$$

**Robustness**

A working standard 10 ng/mL was used during the experimentation, followed by calculation ofthe mean percent recovery and % RSD.

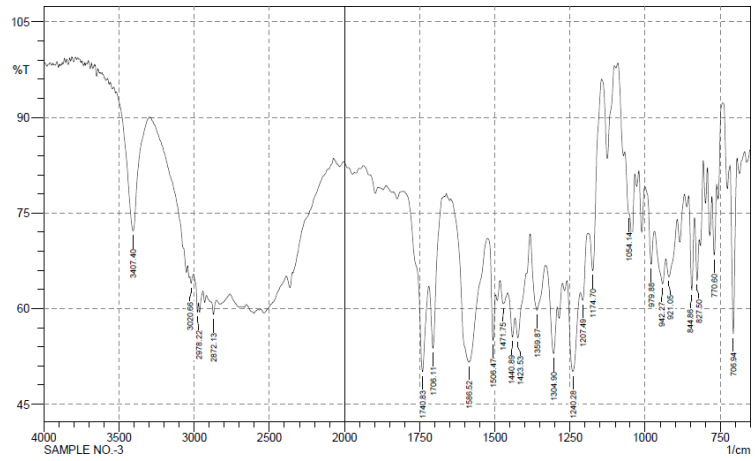
**System suitability**

System suitability was assessed by six replicate analyses of standard TM× 10ng/mL, followed by estimation of SD and %RSD for the peak area and retention time.

**Drug Identification by FTIR**

From FTIR of Raloxifene citrate pure drug in figure no. 1 we could interpret 3 major peaks of functional groups -C=C stretching C-H stretching and -NH2. Their Frequency is 1507cm$^{-1}$ and 1444cm$^{-1}$, 3027 cm$^{-1}$, 3200-3500 cm$^{-1}$ respectively.

**Figure No. 1: Drug Identification by FTIR**

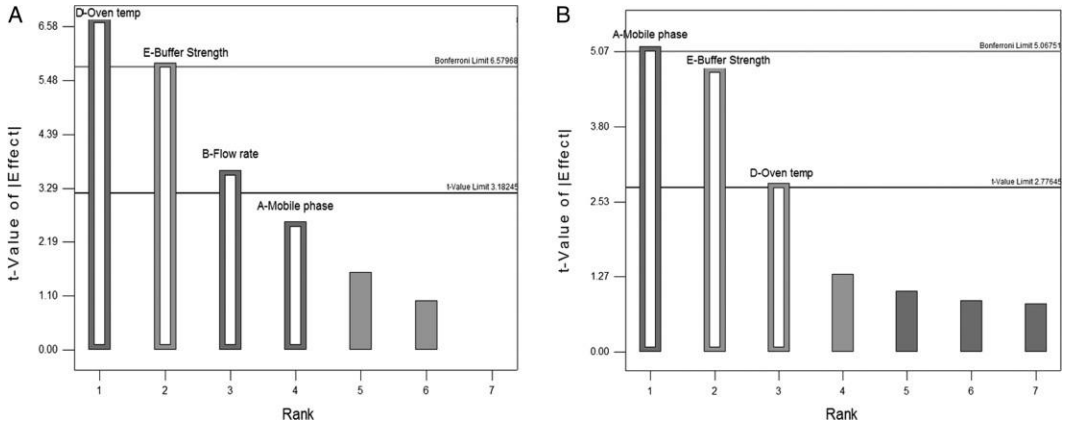


## PRELIMINARY METHOD DEVELOPMENT STUDIES

The preliminary studies were carried out as per the literature reports for developing the LC method for estimation of Raloxifene.

Figure 2 portrays the Pareto charts depicting the influence of MPs/PPs on method CAAs. Pareto ranking analysis revealed that the influence of factors, i.e., mobile phase ratio, buffer pH and oven temperature, on the CAAs was statistically significant ($P < 0.05$); thus they were selected asthe CMPs for further analytical optimization studies.

**Figure No. 2. Pareto charts depicting the influence of various factors on the CAAs, (A) theoretical plates and (B) peak tailing.**




### Optimization Data Analysis and Response Surface Mapping

The optimization data analysis was carried out by selecting the second-order quadratic polynomial model for detecting both main and interaction effects. Table 6 illustrates the coefficients of the second-order quadratic polynomial model along with ANOVA parameters such as model P-value, $r^2$ and PRESS.

**Table No. 7: Coefficient of Polynomial Equations as per the Second-Order QuadraticModel for each CAA**

| **Coefficient code** | **Polynomial coefficients for CAAs** | | | |
|---|---|---|---|---|
| | **Peak area** | **Retention time** | **Theoretical plates** | **Peak tailing** |
| $\beta_0$ | 84,437.40 | 3.80 | 6260.00 | 1.24 |
| $\beta_1$ | −9935.00 | 0.097 | 105.75 | −0.13 |
| $\beta_2$ | −2296.37 | −0.081 | −798.00 | 0.075 |
| $\beta_3$ | 8779.63 | −0.038 | 202.00 | −0.050 |
| $\beta_4$ | −9781.75 | 0.22 | −643.75 | −0.12 |
| $\beta_5$ | −316.75 | 0.094 | −756.75 | 0.23 |
| $\beta_6$ | 15,921.50 | − 0.098 | −306.50 | −0.025 |
| $\beta_7$ | −2691.95 | −0.15 | −1156.50 | −0.38 |
| $\beta_8$ | −9173.20 | −0.11 | 99.25 | −0.032 |
| $\beta_9$ | −11,100.70 | −0.35 | 230.25 | −0.033 |
| $\beta_{10}$ | 84,437.40 | −0.093 | 1208.75 | 1.24 |
| $\beta_{11}$ | −9935.00 | 0.15 | −317.75 | −0.13 |
| P-value | P <0.001 | P < 0.001 | P < 0.001 | P < 0.001 |
| $R^2$ | 0.9237 | 0.9381 | 0.9573 | 0.9964 |

Figures 3–6 illustrate the 2D-contour plots and 3D-response surface plots for each of the CAAs,i.e., peak area, retention time, theoretical plates and peak tailing.

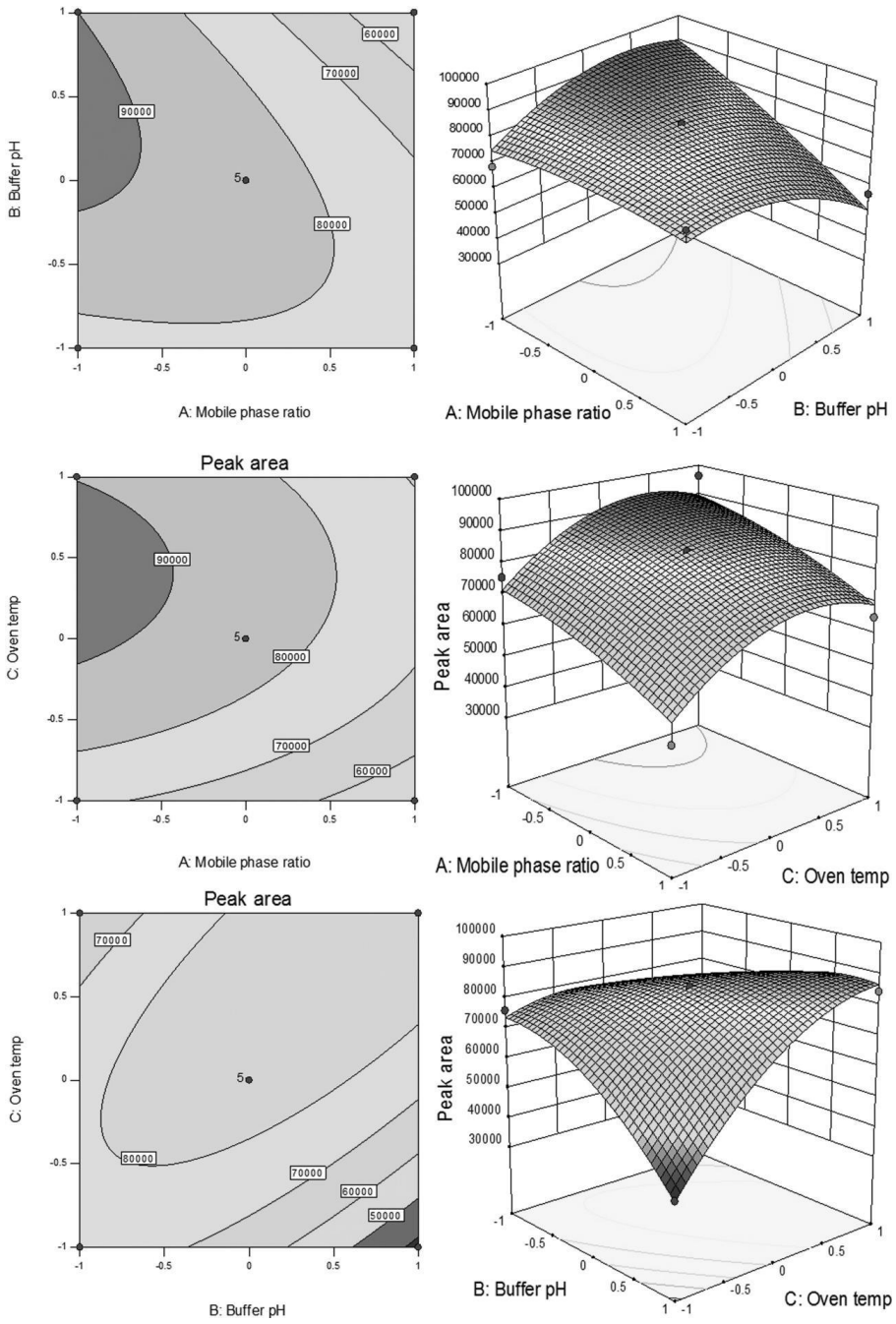



**Figure 3: 2D-contours and 3D-response surface plots showing the influence of CMPs, i.e., mobilephase ratio (A), buffer pH (B) and oven temperature (C) on the peak area as the CAA.**

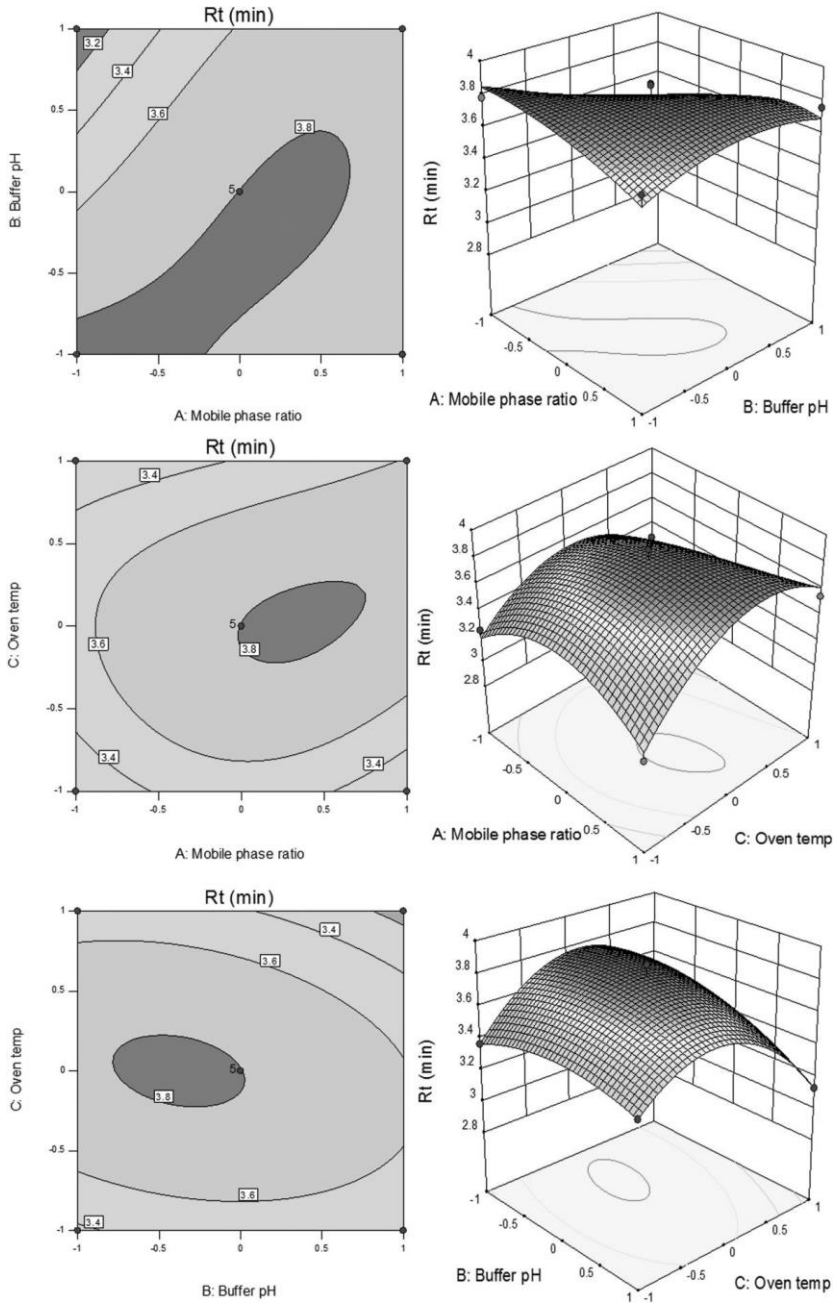



**Figure 4: 2D-contours and 3D-response surface plots showing the influence of CMPs, i.e., mobilephase ratio (A), buffer pH (B) and oven temperature (C) on Rt as the CAA.**

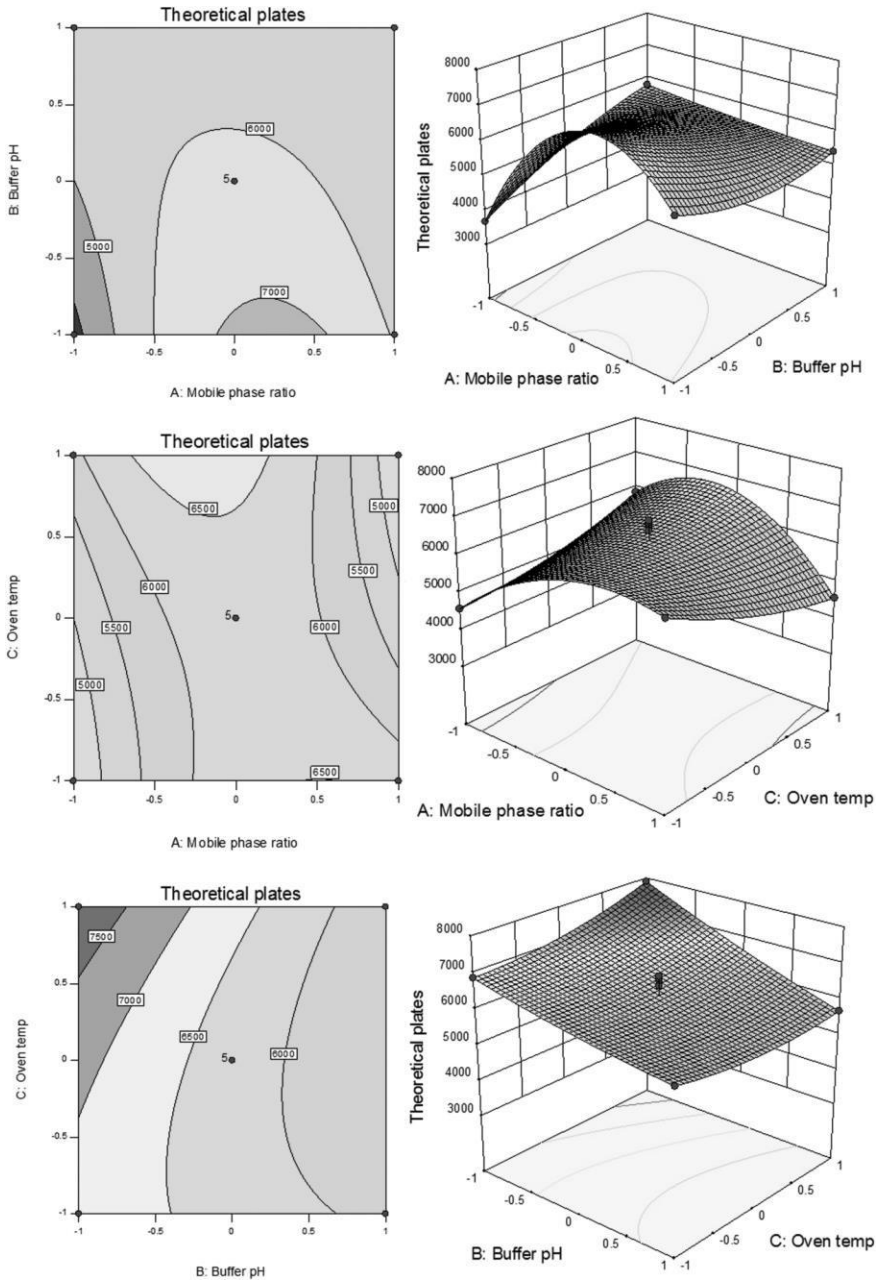



**Figure 5: 2D-contours and 3D-response surface plots showing the influence of CMPs, i.e., mobilephase ratio (A), buffer pH (B) and oven temperature (C) on theoretical plates as the CAA.**

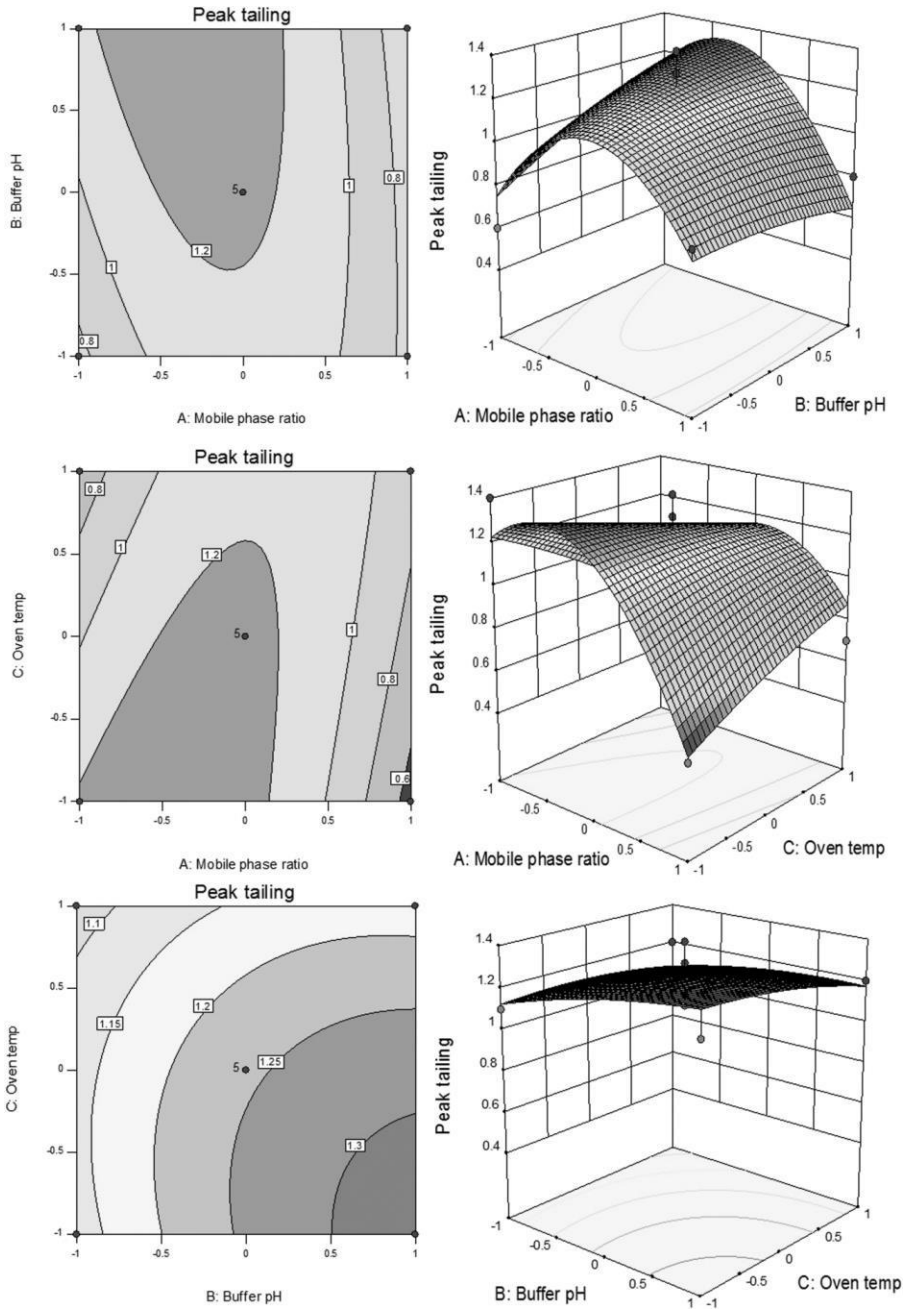



**Figure 6: 2D-contours and 3D-response surface plots showing the influence of CMPs, i.e., mobilephase ratio (A), buffer pH (B) and oven temperature (C) on peak tailing as the CAA.**

## SUMMARY & CONCLUSION

The present study, in a nutshell, demonstrated the successful application of QbD principles for establishment of a HPLC method for Raloxifene with improved robustness and performance. Extensive validation studies further ensured high degree of method robustness with extreme variation in the key variables influencing the method performance. In addition, the method showed improved sensitivity for the Raloxifene much beyond the values reported in the literature.

A simple, rapid, sensitive and economical stability-indicating analytical method has been successfully developed employing the systematic QbD-based approach for quantification of Raloxifene. Application of risk assessment studies helped in prioritizing the factors, critically influencing the method parameters, while factor screening and optimization studies employing experimental designs finally embarked upon the selection of CMPs/CPPs, thus facilitating the understanding of relationship among CMPs/CPPs with CAAs.

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