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Research Article

# ANALYTICAL QUALITY BY DESIGN APPROACH IN RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF DUVELISIB

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**ABSTRACT**

**Objective:** A simple, accurate, and robust RP-HPLC method was developed and validated for the estimation of Duvelisib using analytical quality by design approach.

**Methods:** The critical method parameters (CMP) were systematically optimized using box-Behnken design (BBD). The CMP's selected were % organic phase composition, column temperature, and flow rate. The critical quality attributes investigated were retention time and theoretical plates.

**Results:** Chromatographic separation was accomplished on Agilent Zorbax Eclipse C18 (150×4.6 mm, 5 µm) column. The optimized and predicted data from Design Expert software consist of mobile phase 0.1 % orthophosphoric acid (46.3%): Acetonitrile (53.7%), pumped at a flow rate of 0.91 ml/min at 32.6°C gave the highest desirability function of 1. The retention time of the drug was found to be 2.85 min. The developed method was validated as per the ICH Q2 (R1) guidelines.

**Conclusion:** Based on the analysis of variance values, the selected models were found to be significant with $p<0.05$. The results of the validation parameters were within the acceptable limit. The stability of the drug was examined under different stress conditions forcibly and significant degradation was found in acidic condition.

**Keywords:** Analytical quality by design, Box-Behnken design, Duvelisib, Desirability function, Analysis of variance.

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## INTRODUCTION

Duvelisib sold under the brand name Copiktra is a drug used for the treatment of chronic lymphocytic leukemia or small lymphocytic lymphoma when other treatments have failed. It is taken orally. It is soluble in ethanol and practically insoluble in water. Duvelisib was given orally 25 mg twice daily with or without food [1-4].

Design of experiments (DoE) has evolved as a powerful and cost-effective statistical technique which gives more information from fewest runs. Prerequisite tools needed for DoE include statistical analysis by analysis of variance (ANOVA), design validation, and optimization of the method by desirability function. Box-Behnken design (BBD) was used for the DoE. Design validation was done by predicted versus actual plots which tell how well the model fits the data.

An extensive literature survey has revealed that only one UPLC-MS/MS method [5] for the pharmacokinetic analysis was reported for the estimation of Duvelisib and there is no RP-HPLC method reported. Hence, the present work is aimed to develop and validate simple, rapid, precise, and robust RP-HPLC method for the estimation of Duvelisib assisted with DoE, followed by graphical interpretation of data by response surface methodology (RSM) [6].

## METHODS

### Chemicals

Acetonitrile, orthophosphoric acid (OPA), HPLC grade water, and methanol were purchased from Merck India Pvt. Ltd, Mumbai, India. API of Duvelisib was obtained as a gift sample from Sterling Biologicals, Ahmedabad, India.

### Equipment

FT-IR/ATR (BRUKER ALFA) spectrophotometer and UV-VIS spectrophotometer (Shimadzu -1800, Japan) were used for the authentication of drug sample. HPLC study was carried out on WATERS HPLC 2695 system with photo diode array detector. Software used is Empower 2 for HPLC method development and validation. Design Expert® (11.0.2.0) modeling software (Stat-Ease Inc., Minneapolis, MN, USA) was used for generation of 2D contour plots and 3D surface plots.

### Authentication and identification of sample

*By UV-visible spectra*

10 µg/mL concentration of Duvelisib was dissolved in methanol and UV spectrum was recorded. The absorption maxima was found to be 222 nm as shown in Fig. 1.

*By IR spectra*

Duvelisib was scanned in FT-IR spectrometer (Bruker-ALFA) from 4000 to 400 $cm^{-1}$ and characteristic peaks of functional groups were identified. The IR Spectra are shown in Fig. 2.

### Preparation of mobile phase

Mobile phase was prepared using HPLC grade acetonitrile and 0.1% OPA in 50:50 ratio.

### Preparation of diluent

Diluent was prepared using HPLC grade acetonitrile and water in 50:50 ratio.

### Preparation of Standard stock solution

Accurately weighed 6.25 mg of Duvelisib was transferred to 25 ml volumetric flask, 3/4$^{th}$ of final volume was filled with diluent and

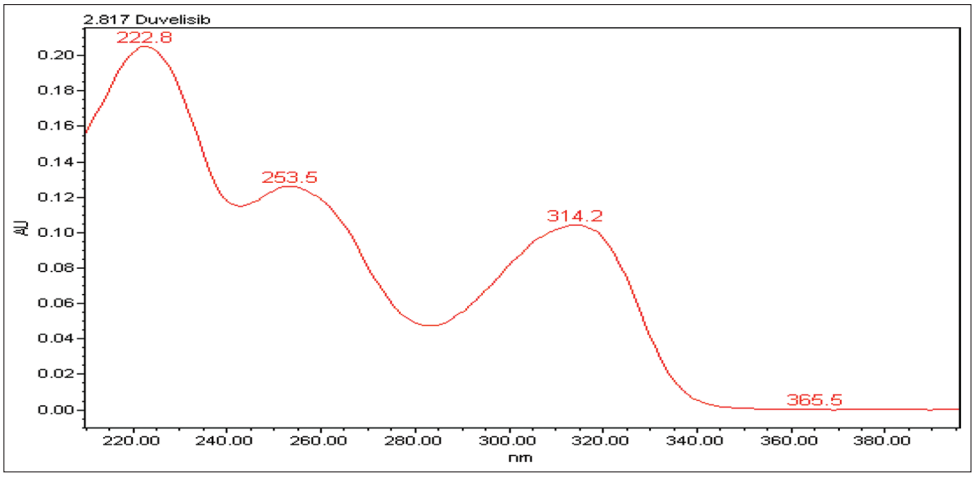


**Fig. 1: UV spectrum of Duvelisib**

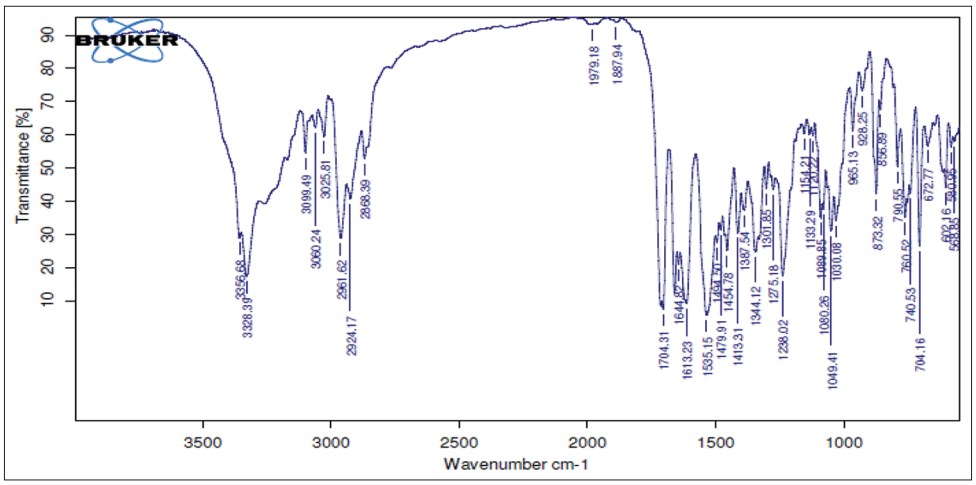


**Fig. 2: IR spectra of Duvelisib**

sonicated to dissolve completely. Final volume was made up to 25 ml with diluent and labeled as standard stock solution (250 µg/ml of Duvelisib). 1 ml of the above stock solution of Duvelisib was pipetted out and taken into 10 ml volumetric flask and made up to volume with diluent (25 µg/ml of Duvelisib).

**Preparation of laboratory synthetic mixture**

The laboratory synthetic mixture of Duvelisib was prepared using suitable excipients mentioned in the FDA label. In a motor and pestle accurately weighed 25 mg of Duvelisib, 5 mg of colloidal silicon dioxide, 5 mg of crossPovidone, 5 mg of magnesium stearate, and 113 mg of microcrystalline cellulose were taken and contents were thoroughly mixed.

**Preparation of sample solution**

The above prepared synthetic mixture was transferred into 100 ml clean dry volumetric flask; diluent was added to dissolve the drug and sonicated for 30 min. Then, the volume was made up to the mark with diluent. It is the stock solution having concentration of 250 µg/ml of Duvelisib. Then, it is filtered through 0.45 µm membrane filter. Further 1 ml of above solution was pipetted into 10 ml volumetric flask and diluted up to the mark with diluent (25 µg/ml of Duvelisib).

**Method development**

*Optimized chromatographic conditions*

The initial trials are needed to optimize the final method. Chromatographic separation was accomplished on Agilent Zorbax Eclipse C18 (150×4.6 mm, 5 µm) column at 30°C. A mixture of 0.1% OPA and acetonitrile (50:50 %v/v) was used a mobile phase pumped at a flow rate of 1.0 ml /min. The UV detector was set at 222 nm.

**Experimental design**

The method was optimized using BBD [7]. Total three factors, namely, % organic content in the mobile phase, flow rate, and temperature of the column were optimized. Hence, BBD was used to optimize these parameters which were varied over three level (high, mid, and low). Different ranges of three parameters 40–60% acetonitrile, flow rate of 0.9–1.1 ml/min, and column temperature 27–33°C were taken as shown in Table 1.

A 3-factor 3-level BBD design was established [8]. This study design of 17 experimental runs was generated and analyzed by Design-Expert software as shown in Table 2.

**Method validation**

The final optimized analytical method was validated as per the International Conference on Harmonization (ICH) Q2(R1) guidelines for system suitability, specificity, linearity, accuracy, precision, limit of detection, limit of quantification, and robustness [9].

**Linearity**

Standard calibration curve was generated with six different concentrations over the range of 6.25–37.5 µg/ml. Linear calibration

**Table 1: Design summary of BBD**

| **Design summary** | | | | | |
|---|---|---|---|---|---|
| File version: DX 11.0.0 | | | CQA: Retention time, Theoretical plates Runs:17 | | |
| Study type: Response surface | | | | | |
| Design type: Box-Behnken design | | | | | |
| **CMPs** | **Unit** | **Type** | **Subtype** | **Min.** | **Max.** |
| Flow rate | ml/min | Numeric | Continuous | 0.9 | 1.1 |
| % Organic composition | %v/v | Numeric | Continuous | 40 | 60 |
| Column temperature | $^0$C | Numeric | Continuous | 27 | 33 |

CMP: Critical method parameters, CQA: Critical quality attributes

**Table 2: Box-Behnken experimental design with responses**

| Run | S. No | Flow rate (FR) (ml) | % Organic composition (MP) | Temperature (Temp) (°C) | Retention time (RT) (min) | USP plate count (TP) |
|---|---|---|---|---|---|---|
| 8 | 1 | 1.1 | 50 | 33 | 2.459 | 3925 |
| 2 | 2 | 1.1 | 40 | 30 | 2.946 | 3911 |
| 10 | 3 | 1 | 60 | 27 | 2.197 | 2714 |
| 16 | 4 | 1 | 50 | 30 | 2.723 | 2924 |
| 12 | 5 | 1 | 60 | 33 | 2.107 | 3916 |
| 3 | 6 | 0.9 | 60 | 30 | 2.405 | 2894 |
| 14 | 7 | 1 | 50 | 30 | 2.713 | 2849 |
| 7 | 8 | 0.9 | 50 | 33 | 2.843 | 3610 |
| 5 | 9 | 0.9 | 50 | 27 | 2.803 | 2614 |
| 6 | 10 | 1.1 | 50 | 27 | 2.451 | 2356 |
| 13 | 11 | 1 | 50 | 30 | 2.739 | 2939 |
| 4 | 12 | 1.1 | 60 | 30 | 2.172 | 2524 |
| 15 | 13 | 1 | 50 | 30 | 2.724 | 2713 |
| 9 | 14 | 1 | 40 | 27 | 3.161 | 2482 |
| 1 | 15 | 0.9 | 40 | 30 | 3.386 | 2441 |
| 17 | 16 | 1 | 50 | 30 | 2.736 | 2904 |
| 11 | 17 | 1 | 40 | 33 | 3.045 | 3916 |

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 shown in the Fig. 9.

**Accuracy**

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

**Precision**

Precision of the optimized method was determined by studying the intermediate precision and repeatability. Six working sample solutions of 25 µg/ml are injected on the same day and next day of the preparation of samples and the % RSD of the peak area was calculated.

**Limits of detection (LOD) and limits of quantitation (LOQ)**

LOD and LOQ values were determined from the signal-to-noise ratio method. The detection limit refers to the lowest concentration level resulting in a peak area of 3 times the baseline noise. Quantification limit refers to the lowest concentration level that provided a peak area with a signal-to-noise ratio higher than ten.

**Robustness**

Small deliberate changes in the method were made such as flow rate (0.9–1.1 ml/min), proportion of organic composition in the mobile phase (40–60%), and temperature of the column (25–35°C). %RSD of the above conditions was calculated.

**System suitability**

The system suitability was determined by taking six replicates of the drug at same concentration of 25 µg/ml. The acceptance criteria were ± 2% for the percent coefficient of variation (% CV) for the peak area, retention time of drug, USP Plate Count, and asymmetry.

**Forced degradation studies [10]**

*Acid hydrolysis*

To 1 ml of stock solution, 1ml of 2N HCl solution was added. The degradation sample was placed for reflux in Radley apparatus (Veego) with continuous stirring at 70°C for 60 min. The sample was neutralized with 2N NaOH and diluted up to 10 ml with mobile phase.

*Base hydrolysis*

To 1 ml of stock solution, 1 ml of 2N NaOH solution was added. The degradation sample was placed for reflux in Radley apparatus (Veego) with continuous stirring at 70°C for 60 min. The sample was neutralized with 2N HCl and diluted up to 10 ml with mobile phase.

*Neutral hydrolysis*

1 ml of stock solution was diluted to 10 ml with HPLC grade water. The degradation sample was placed for reflux in Radley apparatus with continuous stirring at 70°C for 4 h.

**Oxidative study**

To 1 ml of stock solution, 1 ml of 20% $H_2O_2$ solution was added. The degradation sample was kept in dark area without disturbance at room temperature for 4 h. The sample was diluted up to 10 ml with mobile phase.

**Thermal degradation**

25 mg of Duvelisib was taken in a Petri dish and placed in hot air oven at 70°C for 60 min. The sample was diluted with mobile phase and analyzed using the HPLC system.

**Photo degradation**

25 mg of Duvelisib was uniformly spread in a Petri dish and was exposed to UV light for 24 h. The sample was diluted with mobile phase and analyzed by the HPLC system.

## RESULTS AND DISCUSSION

**Statistical analysis of experimental data by design-expert software**
ANOVA was applied to study the significance of the model [11].

The Model F-value of 130.99 implies the model is significant for the responses RT and TP given in the Tables 3-5. There is only a 0.01% chance that an F-value this large could occur due to noise. p<0.0500 indicates that model terms are significant. In this case, A, B, AB, and $C^2$ are significant model terms.

The predicted $R^2$ of 0.9088 is in reasonable agreement with the adjusted $R^2$ of 0.9865; that is, the difference is less than 0.2. Adequate precision measures the signal-to-noise ratio. A ratio greater than 4 is desirable. S/N ratio of 40.378 indicates an adequate signal shown in the Table 4. This model can be used to navigate the design space. 2D Contour and 3D Surface plots [12,13] were analyzed to visualize the effect of factors and their interactions on the responses using the Design Expert® software. The regions shaded in dark blue represents lower values and shaded in dark red represents higher values. The regions shaded in light blue, green, and yellow represent intermediate values.

From the above 2D Contour and 3D surface plots of retention time shown in Fig. 3, it was found that at a higher flow rate, higher temperature, and higher organic phase composition lower will be the value of retention time.

The Model F-value of 10.14 implies the model is significant. There is only a 0.09% chance that an F-value this large could occur due to noise. p<0.0500 indicates that model terms are significant. In this case, C and AB are significant model terms.

The predicted $R^2$ of 0.9015 is in reasonable agreement with the adjusted $R^2$ of 0.9242; that is, the difference is less than 0.2. Adequate precision measures the signal-to-noise ratio. A ratio greater than 4 is desirable. S/N ratio of 9.218 indicates an adequate signal. This model can be used to navigate the design space given in the Table 6.

From the above 2D Contour and 3D surface plots of theoretical plates shown in Fig. 4, it was found that at a higher flow rate, higher temperature, and lower the organic phase composition higher will be the value of theoretical plates.

**Design validation**
From the normal plot of studentized residuals for the two responses [14] shown in Fig. 5, it was observed that the selected models for the respective responses were suitable for the selected design as these plots indicated straight line. It was further evidenced from the ANOVA Tables 3 and 4 that the selected models were significant with p<0.05. Hence, the selected models were suitable for the design employed in this work.

**Table 3: ANOVA table for retention time using Box-Behnken design**

| **ANOVA for response surface quadratic model** | | | | | | |
|---|---|---|---|---|---|---|
| **Analysis of variance table (Partial sum of squares - Type III)** | | | | | | |
| **Source** | **Sum of squares** | **d f** | **Mean square** | **F value** | **p-value** | **Inference** |
| Model | 1.97 | 9 | 0.2191 | 130.99 | <0.0001 | Significant |
| A-FR | 0.2482 | 1 | 0.2482 | 148.37 | <0.0001 | Significant |
| B-MP | 1.67 | 1 | 1.67 | 999.50 | <0.0001 | Significant |
| C-Temp | 0.0031 | 1 | 0.0031 | 1.87 | 0.2142 | |
| AB | 0.0107 | 1 | 0.0107 | 6.40 | 0.0392 | Significant |
| AC | 0.0003 | 1 | 0.0003 | 0.1531 | 0.7073 | |
| BC | 0.0002 | 1 | 0.0002 | 0.1010 | 0.7599 | |
| $A^2$ | 0.0001 | 1 | 0.0001 | 0.0869 | 0.7767 | |
| $B^2$ | 0.0001 | 1 | 0.0001 | 0.0797 | 0.7859 | |
| $C^2$ | 0.0371 | 1 | 0.0371 | 22.19 | 0.0022 | Significant |
| Residual | 0.0117 | 7 | 0.0017 | | | |

df: Degrees of freedom, ANOVA: Analysis of variance

**Table 4: Fit statistics**

| **Std. Dev.** | **0.0409** | **$R^2$** | **0.9941** |
|---|---|---|---|
| Mean | 2.68 | Adjusted $R^2$ | 0.9865 |
| C.V. % | 1.52 | Predicted $R^2$ | 0.9088 |
| | | Adequate precision | 40.3777 |

**Table 5: ANOVA table for theoretical plates using BBD**

| **ANOVA for response surface 2F1 model** | | | | | | |
|---|---|---|---|---|---|---|
| **Analysis of variance table (Partial sum of squares - Type III)** | | | | | | |
| **Source** | **Sum of squares** | **d f** | **Mean square** | **F value** | **p-value Prob>F** | **Inference** |
| Model | 4.552E+06 | 6 | 7.587E+05 | 10.14 | 0.0009 | Significant |
| A-FR | 1.673E+05 | 1 | 1.673E+05 | 2.24 | 0.1656 | |
| B-MP | 61600.50 | 1 | 61600.50 | 0.8234 | 0.3855 | |
| C-Temp | 3.381E+06 | 1 | 3.381E+06 | 45.20 | <0.0001 | Significant |
| AB | 8.464E+05 | 1 | 8.464E+05 | 11.31 | 0.0072 | Significant |
| AC | 82082.25 | 1 | 82082.25 | 1.10 | 0.3195 | |
| BC | 13456.00 | 1 | 13456.00 | 0.1799 | 0.6805 | |
| Residual | 7.481E+05 | 10 | 74812.05 | | | |

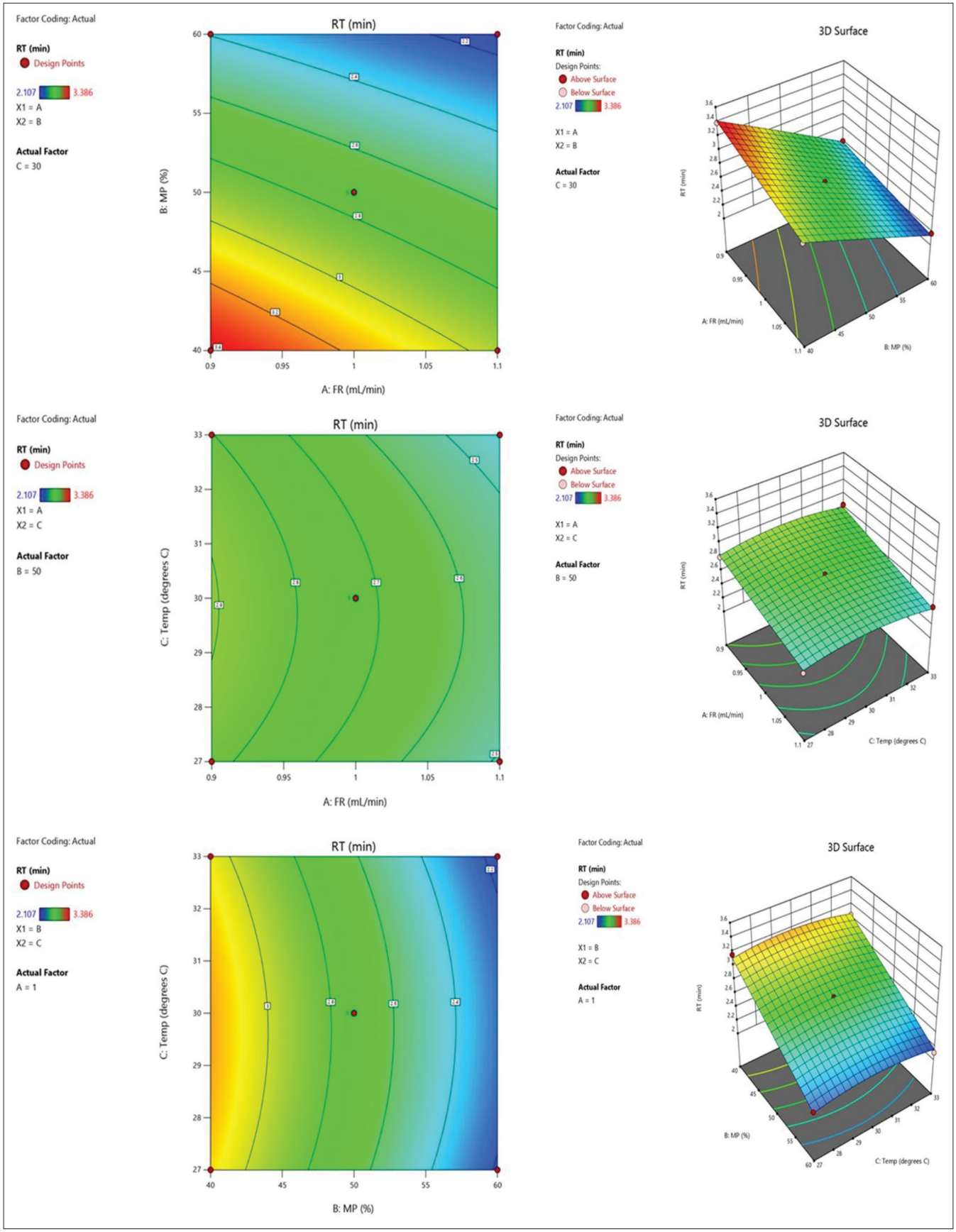


**Fig. 3: 2D contour and 3D surface plots of retention time as a function of flow rate, organic phase composition, and column temperature**

**Optimization by desirability function**

A composite desirability was applied to get an optimum set of conditions based on the specified goals and boundaries for the each response. This desirability function depends on a scale of desirability function ranges between d = 0 for a completely undesirable response, to d = 1 for a fully desirable response [15]. Based on the specified goals and boundaries

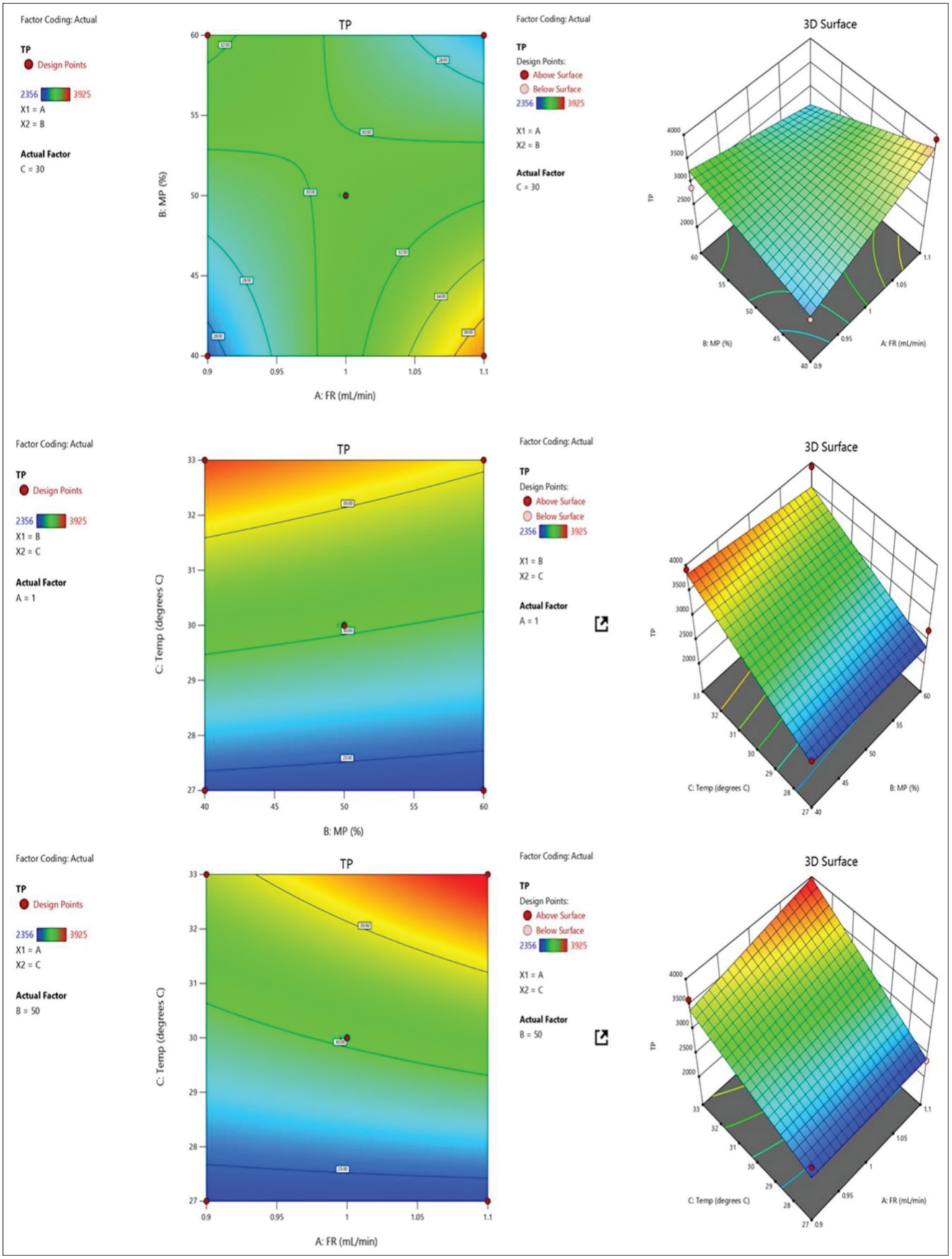


**Fig. 4: 2D contour and 3D surface plots of theoretical plates as a function of flow rate, organic phase composition, and column temperature**

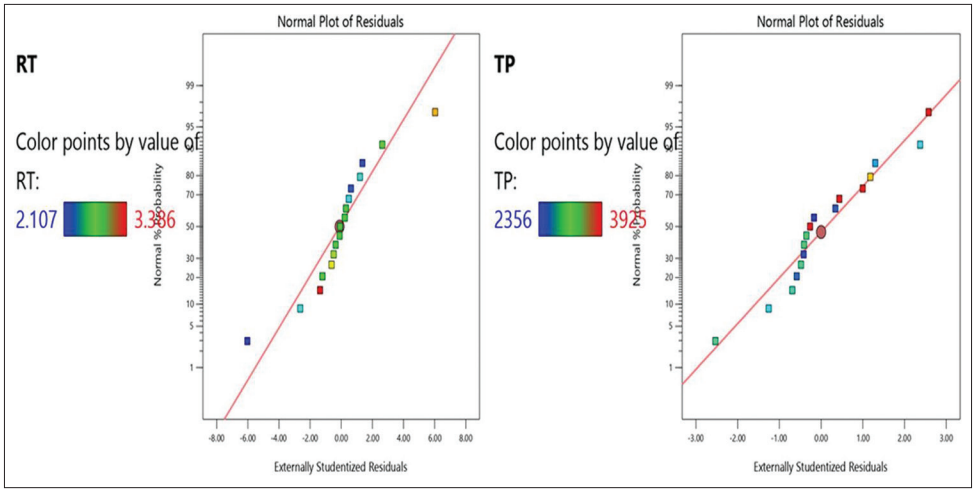


**Fig. 5: Normal plot of studentized residuals for retention time and theoretical plates**

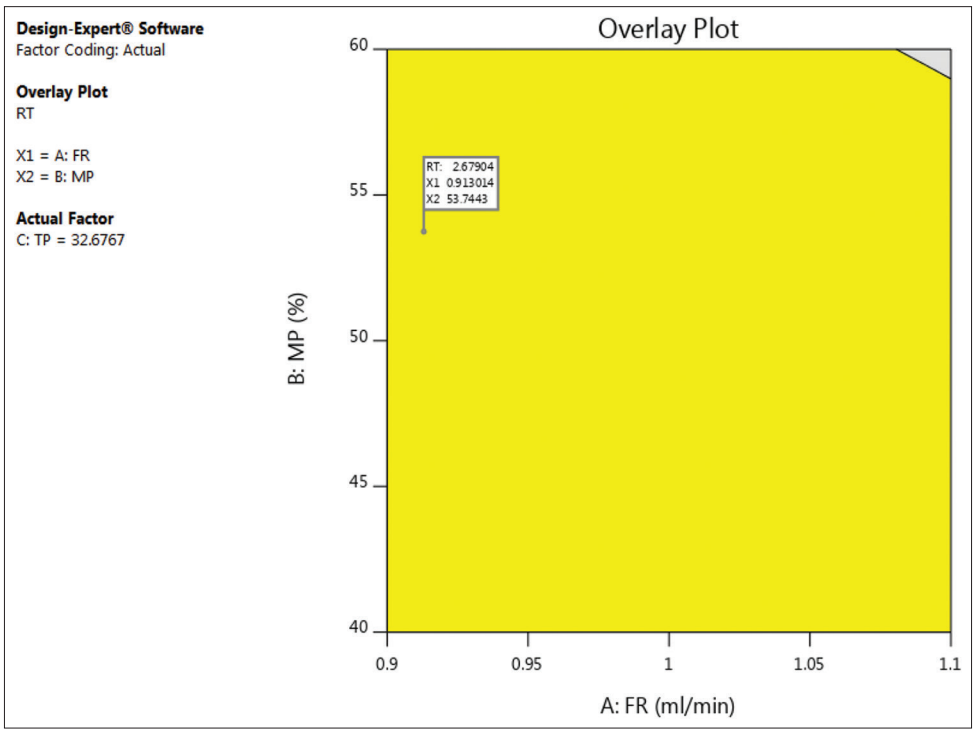


**Fig. 6: Overlay contour plot for design space**

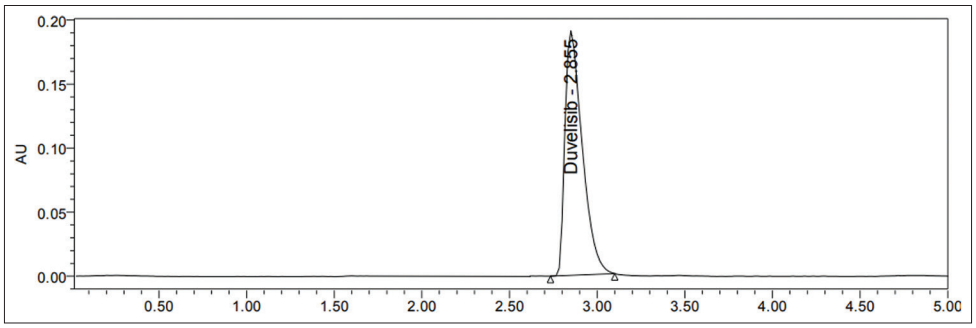


**Fig. 7: Chromatogram of the optimized method (standard chromatogram)**

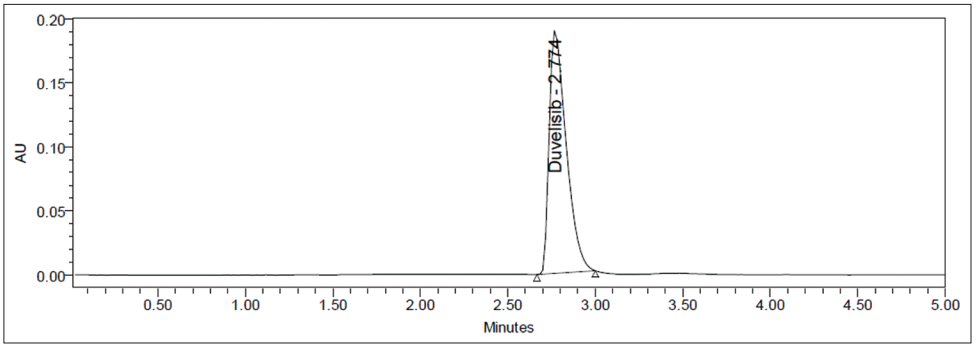


**Fig. 8: Chromatogram of sample**

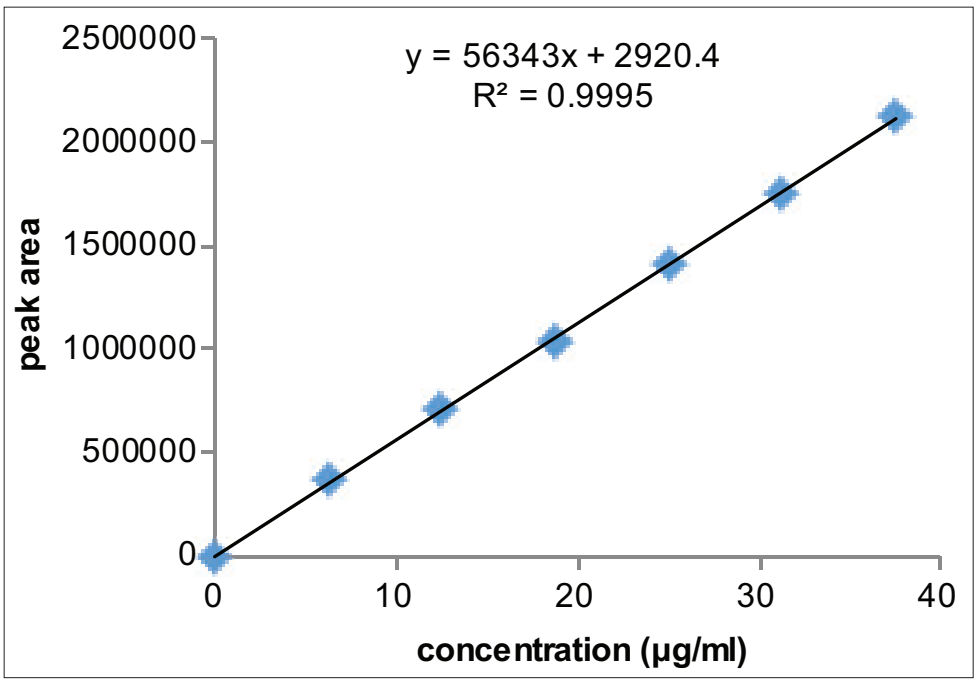


**Fig. 9: Linearity curve of Duvelisib**

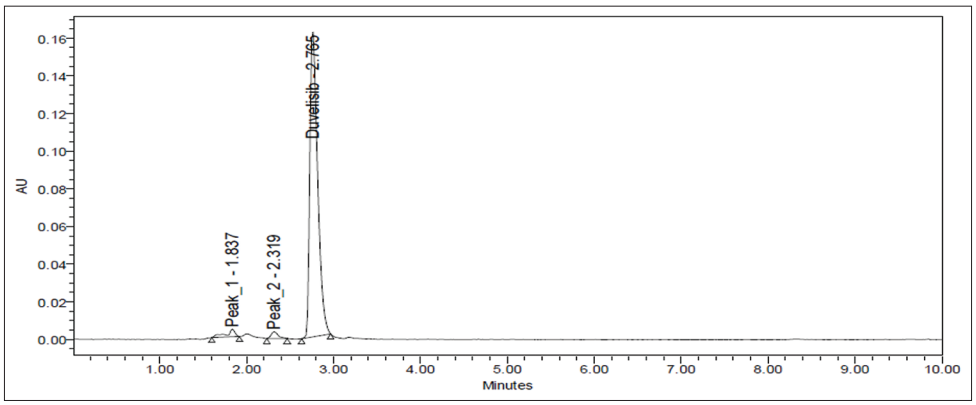


**Fig. 10: Chromatogram of acid degradation**

**Table 6: Fit statistics**

| | | | |
|---|---|---|---|
| Std. Dev. | 273.52 | $R^2$ | 0.9589 |
| Mean | 3037.18 | Adjusted $R^2$ | 0.9242 |
| C.V. % | 9.01 | Predicted $R^2$ | 0.9015 |
| | | Adequate precision | 9.2180 |

for the retention time (minimum) and theoretical plates (maximum) a composite desirability (D) of 1 was obtained. To confirm these optimum set of conditions gievn in the Table 7, three replicate injections of 25 µg/ml Duvelisib were analyzed to determine if their observed retention time and theoretical plates were within the predicted ranges shown

**Table 7: Final optimized HPLC chromatographic conditions**

| Chromatographic condition | Value |
|---|---|
| Mobile phase | 0.1% OPA (46.3%): Acetonitrile (53.7%) |
| Flow rate | 0.91 ml/min |
| Column temperature | 32.6°C |

**Table 8: Responses of the optimized method**

| S. No. | Response variables | Predicted value | Actual value | Desirable range |
|---|---|---|---|---|
| 1 | Retention time (min) | 2.679 | 2.855 | 2.49436–2.86364 |
| 2 | Theoretical plates | 3921.66 | 3508 | 3203.18–7423 |

**Table 9: Results of the validation parameters**

| S. No. | Parameter | Results |
|---|---|---|
| 1. | Linearity | |
| | Linearity range (µg/ml) | 6.25-37.5 |
| | Correlation coefficient | 0.999 |
| | Regression equation | y= 56343x+2920 |
| 2. | Accuracy (% recovery) | |
| | 50%, 100%, 150% levels | Between 99.08 and 99.94 |
| 3. | Precision (% RSD of peak area) | |
| | Intermediate precision | 0.3 |
| | Repeatability | 0.4 |
| 4. | Sensitivity | |
| | LOD (µg/ml) | 0.10 |
| | LOQ (µg/ml) | 0.31 |
| 5. | Robustness (% RSD of peak area) | |
| | Flow rate (±0.1 ml/min) | 0.5 |
| | Organic phase (±10%) | 0.4 |
| | Temperature (±5ºC) | 0.6 |
| 6. | System suitability | |
| | Retention time (min) | 2.72 |
| | Tailing factor | 1.5 |
| | Plate count | 3267 |

in the table 8 and the corresponding optimized chromatograms of the standard and sample(synthetic mixture)were shown in the Figs. 7 and 8 respectively.

**Over lay plot**

The overlay counter plot shows the QbD design space where the method meets the mean performance goals and robustness criteria [16] shown in Fig. 6. The flag represents optimized combination of the three selected independent factors, which gives the selected desirability of minimum retention time and maximum theoretical plates.

**Method validation**

The developed method was linear over the concentration range of 6.25–37.5 µg/ml with correlation coefficient of 0.999. For the accuracy studies at 50, 100, and 150% levels the % recovery of the drug was found to be within 98–102%. Intermediate precision and repeatability were carried out and the % RSD values were found to be less than 2%. LOD and LOQ values were found to be 0.10 µg/ml and 0.31 µg/ml. Robustness of the developed method was checked by making minor changes in the experimental conditions such as flow rate, % organic composition, and temperature and % RSD values for the peak area were found to be less than 2%. From the system suitability tests, the number of theoretical plates was found to be more than 3000 and tailing factor was found to be <2. The summary of the method validation parameters is shown in Table 9.

**Table 10: Results of forced degradation studies**

| S. No. | Stress condition | % Drug recovered | % Drug degraded |
|---|---|---|---|
| 1. | Acidic (2N HCl, 70°, 60 min) | 94.73 | 5.27 |
| 2. | Alkali (2N NaOH,70°, 60 min) | 95.82 | 4.18 |
| 3. | Neutral ($H_2O$, 70°, 4 h) | 98.13 | 1.87 |
| 4. | Oxidative (20% $H_2O_2$, 4 h) | 96.47 | 3.53 |
| 5. | UV light (24 h) | 97.85 | 2.15 |
| 6. | Thermal (70°, 60 min) | 97.43 | 2.57 |

**Forced degradation studies**

Forced degradation studies of Duvelisib in various conditions such as acidic, basic, peroxide, thermal, photolytic, and hydrolytic were observed. The drug showed significant degradation in acidic condition represented in Fig. 10. Results of forced degradation studies are presented in Table 10.

## CONCLUSION

A simple, accurate, and robust RP-HPLC method was developed for the estimation of Duvelisib using analytical quality by design approach. The critical method parameters (CMPs) selected were % of organic content in the mobile phase, flow rate, and column temperature. The critical quality attributes are retention time and theoretical plates. The CMPs were systematically optimized using Box-Behnken design (BBD). Optimized chromatographic conditions consist of mobile phase 0.1 % OPA (46.3%): Acetonitrile (53.7%), pumped at a flow rate of 0.91 ml / min. The retention time of the drug was found to be 2.85 min. Theoretical plates and asymmetry were found to be within the limits. The developed method was validated as per the ICH Q2 (R1) guidelines. Utilization of RSM provides a better insight for method development and robustness testing. Degradation studies were performed in various stress conditions and the drug was found to be degraded more in acidic condition.

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## AUTHORS' CONTRIBUTION

Dr. K. S. Nataraj, Dr. A. K. M. Pawar provided the mentorship and reviewed the data. Srujani has carried out the work as a part of her Ph.D and compiled the manuscript. Annapurna helped in analyzing the data.

## CONFLICTS OF INTEREST

Nil.

## AUTHORS' FUNDING

The authors declare that any funding or research grants are not received in this research work.

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