*Section A-Research paper*
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# An HPLC Method for Tenofovir Alafenamide Hemifumarate Estimation Driven by QbD: Development and Validation

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**ABSTRACT:**
Tenofovir alafenamide has a new HPLC technology that is easy to use, precise, and accurate. An effective experimental design was carried out by thoroughly surveying all essential elements of the HPLC procedure. In accordance with ICH standards, the procedure has been validated. Optimization was performed using an analytical QbD strategy. QbD relies heavily on the Quality target product profile (QTPP). QbD ensures the quality of the pharmaceutical product for patients' protection. Based on QTPP expertise, the drug product's critical quality attribute (CQA) was determined. Three levels and three factors were utilized in the optimization process, which was carried out using a central composite design. All three variables, such as flow rate, wavelength, and mobile phase, were optimized for HPLC analysis of tenofovir alafenamide using a 23-factorial design. The compounds were separated using a C-18 column (250 mm x 4.6 mm) packed with 5.0 µm particles and equilibrated with a mobile phase (0.05%) consisting of 55 parts (v/v) methanol to 45 parts (v/v) orthophosphoric acid. The flow rate was kept constant at 0.8 ml/min, while the column temperature was kept at room temperature. The eluents were tracked using a PDA detector with a 258.0 nm wavelength setting. Good separation and peak symmetry for the drug were achieved under the following chromatographic settings. For concentrations between 10 and 50 µg/ml, the technique provided a linear response (r2 = 0.999). Repeatability, intraday accuracy, and interday precision all proved the reliability of the modified process, with percent RSDs of less than 2%. The LOQ was 0.41 µg/ml and the LOD was 0.13 µg/ml. The % recovery of spiked samples ranged from 999.69 ± 0.56 to 100.50 ± 0.47, which is within the range deemed accepTable No.. by the ICH criteria. The present approach was effectively verified for the detection of Tenofovir alafenamide in bulk drug substances and pharmaceutical dosage forms, demonstrating high selectivity, linearity, sensitivity, precision, and accuracy.

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**KEYWORDS: Tenofovir alafenamide, HPLC, QbD, Central composite design, dosage forms.**

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## INTRODUCTION :

About 257 million peoples are globally affected by hepatitis B virus (HBV) and around 350 million peoples affected with the chronic hepatitis B (CHB) [1]. Antiviral therapy comprises of Nucleotide analogue (NA) which has been proven to restrict viral replication, arrest HBV progression and increase the rate of survival [2]. At present the recommended first-line treatment for CHB includes Tenofovir alafenamide hemifumarate(TAF), a prodrug of tenofovir (TFV), is a highly effective nucleotide analogue inhibitor of HBV polymerase/reverse transcriptase [3]. The structure of TAF is shown in Fig.no 1. Intracellular enzymes, with carboxylesterase 1 convert Tenofovir alafenamide hemifumarate(TAF) to tenofovir (TFV). In hepatocytes TFV is phosphorylate to tenofovir diphosphate which is last active metabolite of TAF [4]. TAF has been proven in clinical phase 3 trials to be as effective as antiretroviral agent at a 30-fold lower dose 10 mg as compare to tenofovir disoproxil fumarate (TDF) which is administered orally as 300mg and also proved to be safer than TDF in accordance with the renal disturbances and bone mineral density abnormalities [5]. "A systemic approach to method development that begins with established objectives and promotes product and process understanding and process control, based on strong science and quality risk management," as per the concept of QbD [6]. QbD ensures higher product quality, greater regulatory leeway, and ongoing enhancement by integrating product and process knowledge with quality risk control. The QbD methodology was created with the help of the ICH Q8, ICH Q9, and ICH Q10 guidelines [7–10]. To attain optimal method performance, high resilience, ruggedness, and flexibility for continuous improvement, analytical QbD aims to grasp the established objectives and regulate the important method factors affecting the critical method attributes [11-14]. Analytical QbD's main goal has been to uncover modes of failure as well as provide a robust method operable design region or design space that meets significant system suitability criteria while also allowing for continuous life cycle management. According to a literature review, QbD approaches for HPLC methods have been documented [15]

## MATERIALS AND METHODS:

### Materials

### Drugs and Chemicals

Tenofovir alafenamide hemifumarate was procured as a gift sample from Hetero Lab Ltd. (Unit-II) Formulation division, Baddi, Dist. Solan, Himachal Pradesh, India. Ortho-phosphoric acid, methanol used was purchased from Merck Life sciences Pvt. Ltd. Mumbai. The solvent used was of HPLC quality, while all other reagents and compounds were of analytical grade. The marketed formulation Tenvir-AF 25 mg by Cipla Ltd. was used for assay. The double distilled water was obtained from a local pharmaceutical company

### Equipment

The system used to develop this method is AGILENT (1100) including column oven (G1316A) a pump (G1310A) an automatic injector (G1313A), C18 (4.6 x 250 mm) 5.0 column and PDA detector (G1314B) which is set at 258 nm. The chromatography software suite is used to process the data (version 10.0)

## Conditions for Chromatography

To separate the compounds, a C-18 column (250 mm x 4.6 mm) with a particle size of 5.0 µm was utilized and equilibrated with a mobile phase (0.05 %) of 55 parts (v/v) methanol to 45 parts (v/v) orthophosphoric acid. The column temperature was held at room temperature, and the flow rate was maintained at 0.8 ml/min. A PDA detector set to 258.0 nm was used to monitor the eluents. The following chromatographic conditions resulted in the decent separation and peak symmetry for the medication. Using a 23-factorial design, the three variables were optimized for Tenofovir alafenamide hemifumarate via HPLC.

## Development of a working and a standard stock solution

Tenofovir alafenamide, 10 mg, was dissolved in methanol, 10 millilitres at a time, to make the 1000 micrograms per millilitres standard stock solution. The stock solution was diluted to 0.1 ml with methanol to make the 10 µg/ml solution. As before, a stock solution of 0.2, 0.3, 0.4, and 0.5 ml was diluted to 10 ml with methanol to create a solution with concentrations of 20, 30, 40, and 50 µg/ml.

### Screening of wavelength

The optimal wavelength for detecting 10 µg/ml of Tenofovir alafenamide hemifumarate was found to be 258 nm after scanning from 200 to 400 nm.

## Method development for HPLC using the Quality-by-Design technique

The following describes the Analytical QbD approach to the HPLC technique. The list of all the parameters is illustrated below:

## Selection of quality target product profile (QTPP)

The QTPP plays a crucial role in identifying the factors that affect the QTPP settings. It was determined that QTPP describes the retention duration, theoretical plates, and peak asymmetry for the planned HPLC operation.

## Determine critical quality attributes (CQA)

Technical parameters that directly affect QTPP are known as critical quality attributes (CQAs). To maintain an acceptable response of QTPP, three significant technique parameters must be controlled: mobile phase, wavelength, and flow rate.

## Factorial design

The QTPP and CQAs were outlined, and then the HPLC method's mobile phase, wavelength, and flow rate was optimized and selected using a three-level factorial design. Using a three-level factorial statistical screening methodology, the effects of mobile phase composition, wavelength, and flow rate on the tailing factor,

theoretical plates, and peak area were investigated. Design Expert® (Version 11.0, Stat-Ease Inc.) was used to create a three-factor design including varying amounts of mobile phase, wavelength, and flow rate, with the optimal response being a second-order polynomial exploring quadratic response surfaces.

$$Y=\beta0 + \beta1A+ \beta2B + \beta3C + \beta12\ AB + \beta13\ AC + \beta27\ B2B+ \beta27\ B2\ A+ \beta11\ A2 \quad \text{(equation 1)}$$

Where Y is the observed experimental response associated with each combination of factor levels, β0 is an intercept, and β1 to β27 are regression coefficients generated from tests performed on the obtained measurement results of Y. Each of the words AB, A2, and B2 is an example of a quadratic term or an interaction term.

The factors were chosen using primary analysis that took prior research on the multivariate interaction of variables and process parameters into account. Table No.. 1 displays relationships between mobile phase, wavelength, and flow rate as functions of these independent variables. Peak area, tailing factor, and theoretical plates were chosen as the dependent and independent variables, respectively.

**Table No. 1 Independent Variable Coded Value**

| Independent variables | Coded value | Levels | | |
|---|---|---|---|---|
| | | -1 | 0 | +1 |
| Flow rate | A | 0.7 | 0.8 | 0.9 |
| Wavelength | B | 255 | 258 | 261 |
| Mobile phase | C | 50 | 55 | 60 |

## Analytical method validation

Method validation is performed in accordance with ICH principles to verify the method's fitness for its intended function. The system's robustness, intra-day precision, inter-day precision, limit of detection (LOD), limit of quantitation (LOQ), and linearity were all evaluated according to ICH standards [16].

**Linearity**:

Five different concentration levels (10-50 µg/ml) of Tenofovir alafenamide hemifumarate were analysed to determine the linearity of the compound. The concentration was compared to the peak area, which served as the y-axis in the construction of the calibration curve. Calculations of the regression line equation and the correlation coefficients were completed.

**Table No. 2 Linearity of Tenofovir alafenamide hemifumarate**

| S.No | Concentration (µg/ml) | Peak area (mean ± SD) (n = 3) |
|---|---|---|
| 1 | 10 | 291.98 ± 0.85 |
| 2 | 20 | 594.28 ± 0.09 |

| | | |
|---|---|---|
| 3 | 30 | 878.95 ± 0.36 |
| 4 | 40 | 1187.99 ± 3.61 |
| 5 | 50 | 1487.99 ± 1.27 |

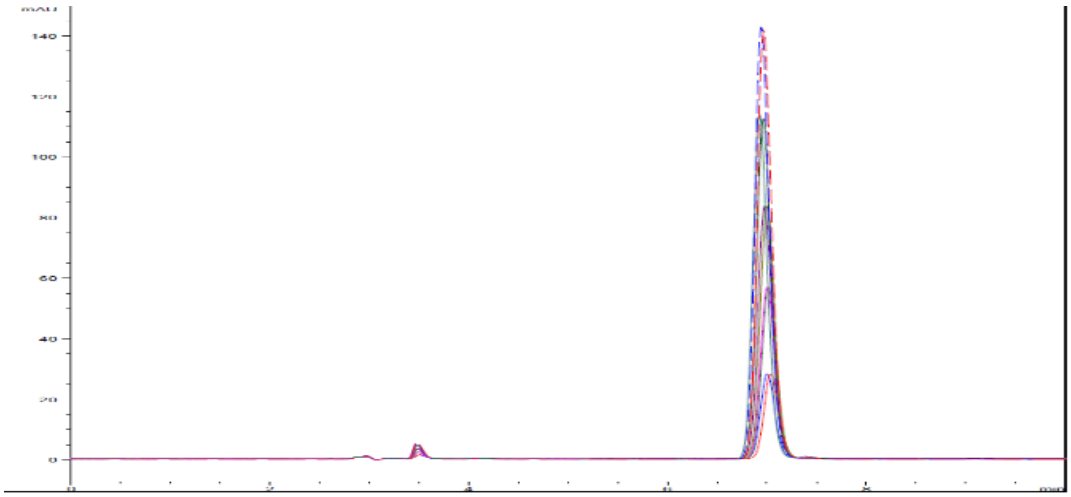

**Fig.1 Linearity of 10–50 µg/ml Tenofovir alafenamide**

**Precision:**

Using a sample size of six, we determined that the percent RSD for repeatability of Tenofovir alafenamide hemifumarate at 50 µg/ml was less than 0.06. Table No. 2 displays the accuracy rates between days and within days. The created approach was confirmed to be precise, with an RSD value of less than 2.

**Table No. 3 Data for intraday and interday of Tenofovir alafenamide**

| **Precision period** | **Concentration(µg/ml)** | **(Mean ± SD) (n = 3)** | **%RSD** |
|---|---|---|---|
| **Intraday precision** | 10 | 291.93 ± 0.80 | 0.27 |
| | 40 | 1192.04 ± 2.73 | 0.23 |
| | 50 | 1484.90 ± 0.56 | 0.04 |
| **Interday precision** | 10 | 291.36 ± 0.98 | 0.33 |
| | 40 | 1193.97 ± 0.534 | 0.04 |
| | 50 | 1484.5 ± 0.521 | 0.03 |

**Accuracy:**

To ensure precision, a recovery study was conducted. Three different spike concentrations (80%, 100%, and 120%) were used to create the sample solutions. Table No. 3 displays the results of using the proposed HPLC technique to measure recovery in terms of percentage. The developed method's accuracy according to the ICH

Q2 (R1) recommendations is supported by the fact that the percentage of recovery falls within the range of 98-102%.

**Table No. 4 Recovery of Tenofovir alafenamide hemifumarate**

| **Assay level** | **Amount equivalent to tablet powder (µg/ml)** | **Standard added** | **Total found** | **Recovered amount (mg) ± SD (n = 3)** | **% Recovered spiked amount ± SD (n = 3)** |
|---|---|---|---|---|---|
| **80%** | 10 | 8 | 18.04 ± 0.037 | 8.04 ± 0.037 | 100.50 ± 0.47 |
| **100 %** | 10 | 10 | 20.09± 0.043 | 10.0961809 ± 0.043 | 100.96 ± 0.43 |
| **120 %** | 10 | 12 | 21.96± 0.067 | 11.96318258 ± 0.067 | 99.69 ± 0.56 |

**Robustness and ruggedness studies:**

Tenofovir alafenamide hemifumarate was diluted to a concentration of 50 µg/ml for the toughness tests. Using small but systematic adjustments to technical parameters such as mobile phase composition, wavelength, and flow rate, resilience was investigated. Analyst turnover was used to investigate toughness as a potential outside influence. Modifying the mobile phase ratio, the wavelength, the flow rate, and the analyst all resulted in a % RSD for the peak area that was less than 2.

**LOD and LOQ**

Tenofovir alafenamide's LOD and LOQ were calculated to be 0.13 µg/ml and 0.41 µg/ml, respectively, using the standard deviation of the slope and intercept.

**System suitability**

System appropriateness was viewed as a crucial element for guaranteeing the method's adequate performance. Six replicate injections of 10 µg/ml of the medication were analyzed for their retention time (Rt), the number of theoretical plates (N), and tailing factor (T).

**Assay**

When assayed from Table No..ts, Tenofovir alafenamide's optimized chromatogram displayed a resolved peak at a retention time of 6.96 min. Tenofovir alafenamide hemifumarate was assayed to have a drug content percentage of 99.53 ± 0.125 (n = 3) . The assay result demonstrated the method's precision and accuracy for measuring tablet powder in the presence of excipients.

## RESULTS:

### Method optimization using factorial design

These conditions of the methodology were evaluated using the CCD method. Initially, we checked how things were going with regard to retention time, theoretical plates, and peak asymmetry. This led to different chromatographic circumstances for tenofovir alafenamide. AccepTable No.. ranges have been shown to originate from sTable No.. intervals where careful tweaks to the procedure parameters have no noticeable effect on the final product's quality. This safeguards against invalidation issues in subsequent phases of assessing the method's reliability. If the outcomes of the modelling trials are not as expected, the corresponding variable must be optimized at increasing values until the outcomes are within the desirable range. By utilizing the provided Design Expert tools, the optimum chromatographic conditions can be achieved.

**Table No. 5 the following Table displays the results of optimizing Tenofovir alafenamide hemifumarate analytical parameters using a three-level factorial design.**

| | | Factor 1 | Factor 2 | Factor 3 | Response 1 | Response 2 | Response 3 |
|---|---|---|---|---|---|---|---|
| Std | Run | A: Flow Rate | B: Wavelength | C: Methanol | PA | TP | TF |
| | | mL/min | nm | % | AUC | | |
| 21 | 1 | 0.9 | 255 | 60 | 1258.8 | 9707 | 0.8 |
| 24 | 2 | 0.9 | 258 | 60 | 1364.4 | 9359 | 0.8 |
| 5 | 3 | 0.8 | 258 | 50 | 1386.7 | 8796 | 0.8 |
| 16 | 4 | 0.7 | 261 | 55 | 1805.7 | 10536 | 0.74 |
| 2 | 5 | 0.8 | 255 | 50 | 1395 | 10115 | 0.7 |
| 15 | 6 | 0.9 | 258 | 55 | 1357.9 | 8957 | 0.7 |
| 10 | 7 | 0.7 | 255 | 55 | 1609.1 | 10437 | 0.7 |
| 20 | 8 | 0.8 | 255 | 60 | 1418 | 10048 | 0.8 |
| 8 | 9 | 0.8 | 261 | 50 | 1564.3 | 10196 | 0.7 |
| 1 | 10 | 0.7 | 255 | 50 | 1586.7 | 10823 | 0.69 |
| 17 | 11 | 0.8 | 261 | 55 | 1578.1 | 9606 | 0.7 |
| 23 | 12 | 0.8 | 258 | 60 | 1526.5 | 9616 | 0.8 |
| 14 | 13 | 0.8 | 258 | 55 | 1464.9 | 9654 | 0.7 |
| 27 | 14 | 0.9 | 261 | 60 | 1357.5 | 9178 | 0.8 |
| 22 | 15 | 0.7 | 258 | 60 | 1687.5 | 10631 | 0.7 |
| 7 | 16 | 0.7 | 261 | 50 | 1726.5 | 10874 | 0.6 |
| 13 | 17 | 0.7 | 258 | 55 | 1672.2 | 10850 | 0.7 |
| 4 | 18 | 0.7 | 258 | 50 | 1656.9 | 11066 | 0.6 |
| 26 | 19 | 0.8 | 261 | 60 | 1522.2 | 10204 | 0.8 |
| 18 | 20 | 0.9 | 261 | 55 | 1343.9 | 9483 | 0.7 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 12 | 21 | 0.9 | 255 | 55 | 1468.1 | 9493 | 0.7 |
| 9 | 22 | 0.9 | 261 | 50 | 1184 | 9948 | 0.7 |
| 19 | 23 | 0.7 | 255 | 60 | 1712.5 | 10802 | 0.7 |
| 6 | 24 | 0.9 | 258 | 50 | 1175.01 | 9942 | 0.7 |
| 3 | 25 | 0.9 | 255 | 50 | 1468.1 | 9494 | 0.7 |
| 25 | 26 | 0.7 | 261 | 60 | 1474.8 | 10847 | 0.7 |
| 11 | 27 | 0.8 | 255 | 55 | 1343.8 | 9922 | 0.7 |

There were 27 trials of a model based on a three-level factorial design. Using the proposed three-level experimental methodology, we compared the effects of varying the flow rate, wavelength, and mobile phase composition to the three responses: theoretical plates, peak asymmetry, and tailing factor.

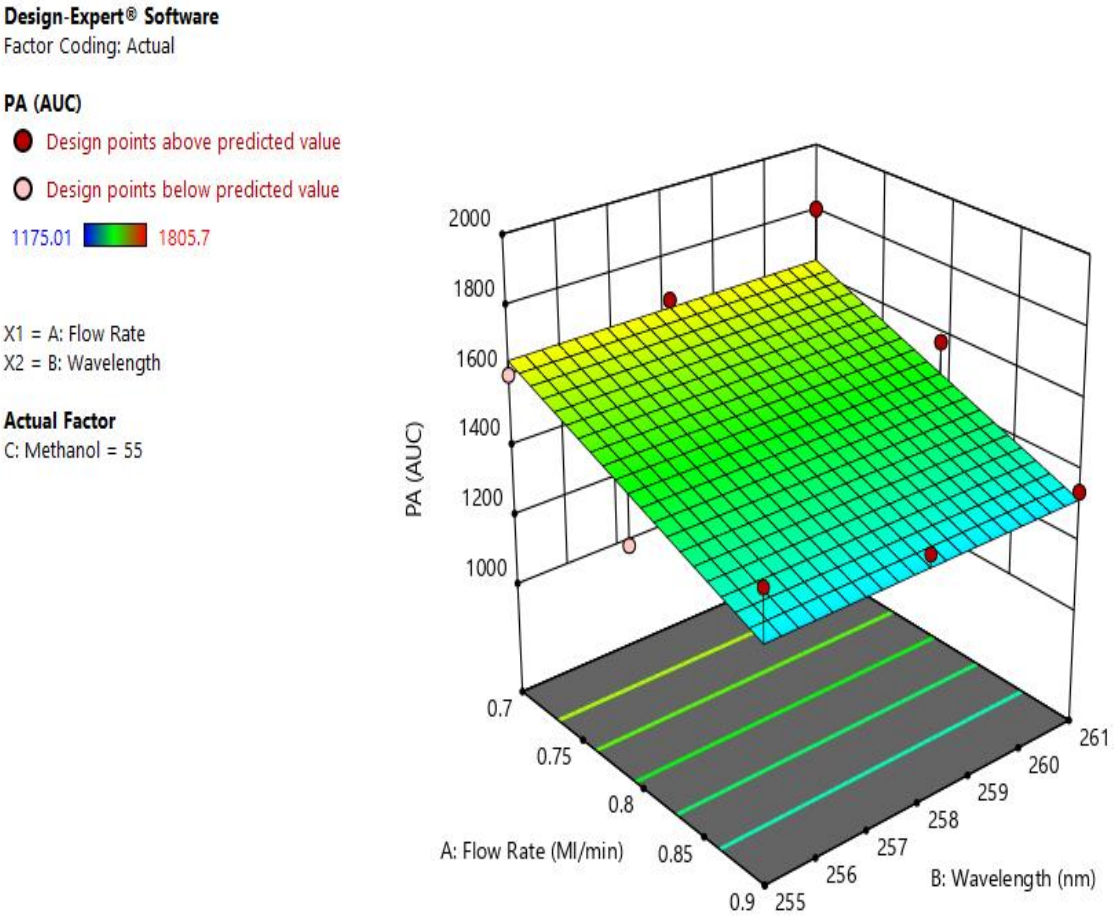


**Fig.2 (a) Tenofovir alafenamide hemifumarate peak area as a function of multiple parameters displayed as a three-dimensional surface plot**

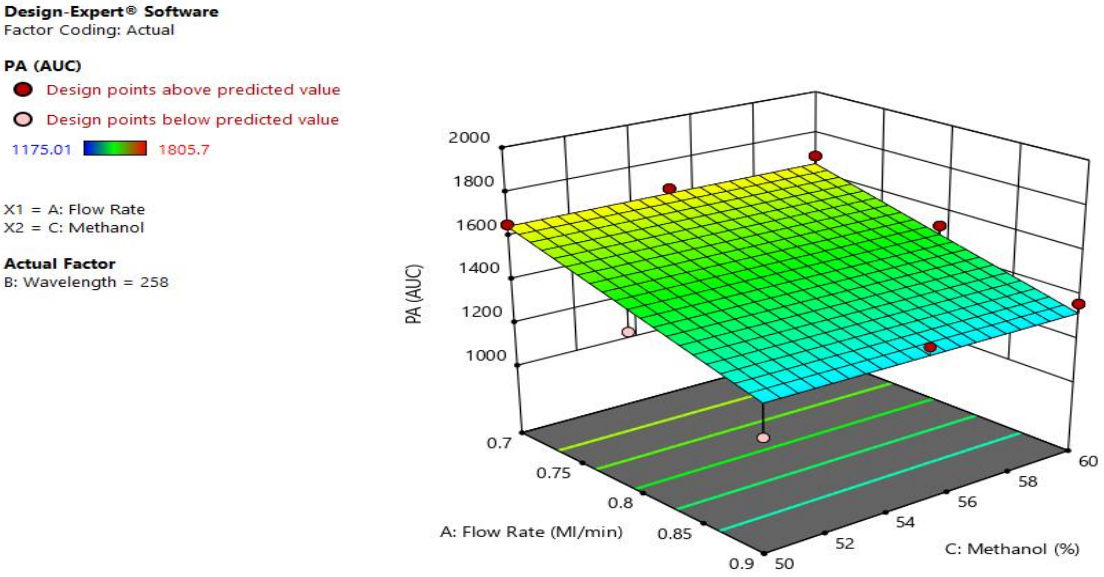


**Fig.2 (b) Three-dimensional Fig.showing the influence of multiple variables on the Tenofovir alafenamide hemifumarate peak area**

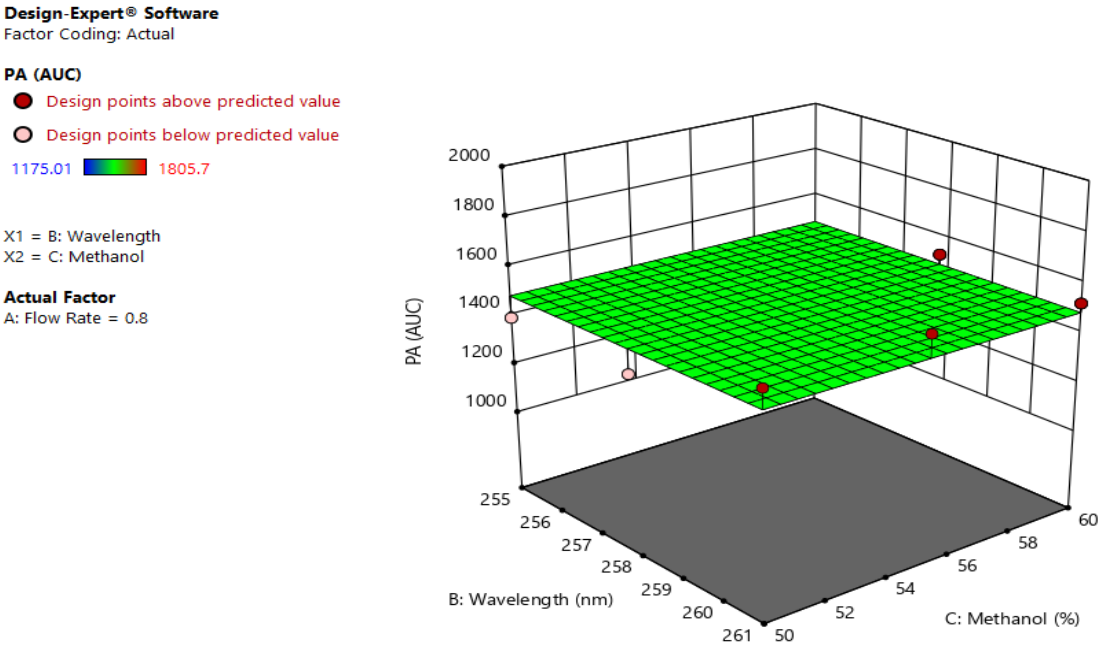


**Fig.2 (c) Tenofovir alafenamide hemifumarate peak area as a function of many parameters, displayed as a three-dimensional surface plot**

**Implications of distinguishing factors on peak asymmetry**

Incorporating experimental design, the "model F-value" of the proposed simplified linear model was 53.76, indicating its significance. The likelihood of observing an F-value this high as a result of random chance is less than 0.01%. If the p-value for a model term is less than 0.0500, then it is significant. Close agreement between the anticipated $R^2$ value of 0.6261 and the modified $R^2$ value of 0.6699 demonstrates the good fit of the model system with the polynomial equation, which has been determined to be 0.6826. (i.e., the variation is less than 0.2).

**The final equation in terms of the coded factor for peak asymmetry as follows**

PA (Y1) = +1485.52-164.12 A (**equation 2)**

Where A = Flow rate

Thus, an increase in flow rate will decrease the peak asymmetry. With the help of a response surface 3 D graph, the effect can be easily interpreted. The equation 2 is applicable for all three-dimensional plot of peak asymmetry. As shown in fig no. 01 the response model is plotted against two of the factors, while the third is held constant at a predetermined level, usually the proposed optimum, to produce three-dimensional graphs. The flow rate (A) and the wavelength (B) are represented graphically in Fig. 1 (a), while the Methanol (C) is kept constant at its optimum of value 55. An increase in flow rate will decrease the peak asymmetry. Fig 2 (b) shows graphical presentation of flow rate (A) and methanol (B) while the wavelength(C) is maintained constant at optimum value 258 nm. An increase in flow rate resulted in decrease in peak asymmetry and if the wavelength is varied within predetermine limit it will not have significant effect on peak asymmetry. If the flow rate (A) is kept constant at the optimum value 0.8 ml/min and other two parameters i.e. methanol (B) and wavelength (C) were varied within the predetermined limit it will not have significant effect on peak asymmetry as represented graphically in fig 1(c).

**Implications of distinguishing factors on theoretical plates**

The "Model F Value" for the suggested simplified quadratic model is 33.97, demonstrating its statistical significance from the perspective of experimental design. The likelihood of observing an F-value this high as a result of random chance is less than 0.01%. If the p-value for a model term is less than 0.0500, then it is significant. For this particular model, terms A and $A^2$ are crucial. With a difference of less than 0.2 between them, the predicted $R^2$ of 0.6696 and the adjusted $R^2$ of 0.7172 are rather close. This demonstrates the excellent agreement between the experimental model and the polynomial equation, which was found to be 0.7390.

**The final equation in terms of the coded factors for theoretical plates is as follows**

TP (Y2) = +9795.22-628.06A+339.61A² **(equation 3)**

Thus, it was concluded that increase in flow rate will decrease the number of theoretical plates. With the help of a response surface 3 D graph, the effect can be easily interpreted. The equation 3 is applicable for all three-dimensional plot of peak asymmetry. To create three-dimensional graphs, the response model is plotted against

two of the factors, while the third is kept constant at a fixed level, usually the proposed optimum, as seen in fig. 02. In Fig. 2 (a), the flow rate (A) and Methanol (C) are graphically depicted, while the wavelength (B) is kept constant at its optimum of 258 nm. An increase in flow rate will decrease the theoretical plates. The flow rate (A) and wavelength (C) are graphed while the methanol (B) is kept constant at the optimum value of 55 in Fig 2 (b). Based on the 3 D surface plots in figures 2 (a) and 2 (b) and equation 3, it was determined that only one parameter, flow rate (A), is statistically significant, whereas the other two parameters, wavelength (C) and methanol (B), had no effect on the number of theoretical plates.

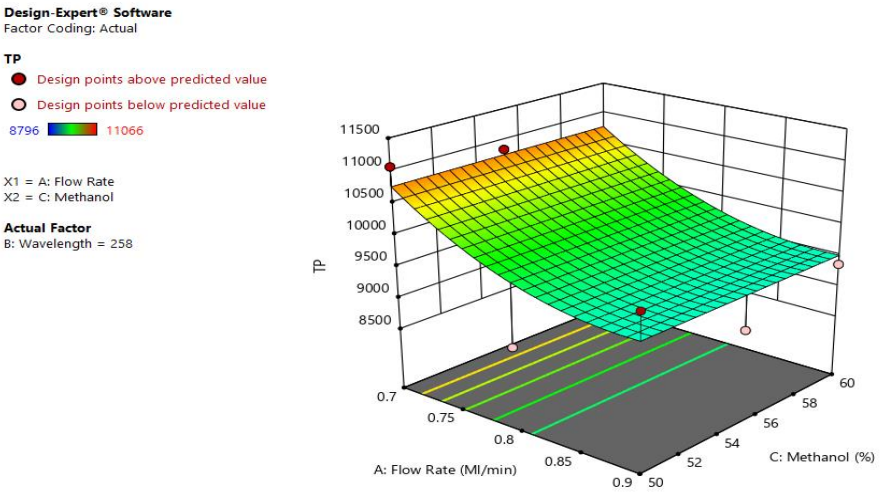


**Fig.3 (a) 3D surface plot for effect of combination of factors on Theoretical plates of tenofovir alafenamide**

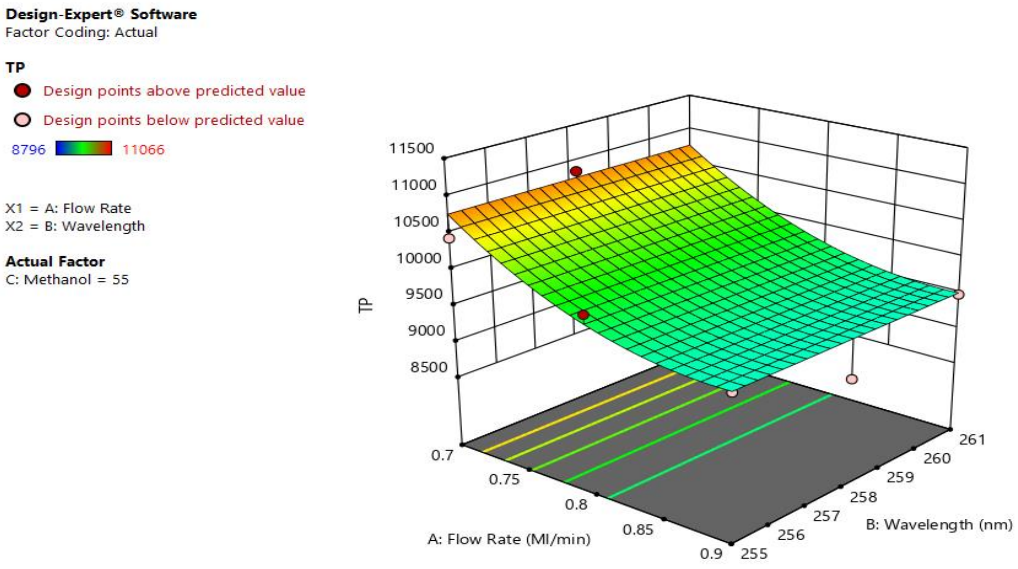


**Fig.3 (b) 3D surface plot for effect of combination of factors on Theoretical plates of tenofovir alafenamide**

**Implications of distinguishing factors on tailing factor**

By the application of experimental design, the proposed reduced linear model was found to be significant with a "**Model F Value**" of 11.78. A noise-induced F-value this high has a 0.03% chance of occurring. Model terms are statistically significant when the **p-values** is less than 0.0003. Model terms A and C are very important here.

The Adjusted $R^2$ of 0.6699 is reasonably close to the Predicted $R^2$ of 0.6261; that is, the difference is less than 0.2. This shows that the experiment model fits the polynomial equation well, with a value of 0.4954.

**Final equation in terms of coded factor for tailing factor as follows**

$$TF\ (Y3) = +\ 0.7196 + 0.0261\ A + 0.0394\ C \qquad \textbf{(equation 4)}$$

Form the equation, it was determined that increasing the flow rate increases the tailing factor, similarly methanol ratio (C) has the positive effect on tailing factor. The effect can be easily interpreted with the help of a response surface 3 D graph. The equation 4 applies to all three-dimensional tailing factor plots. To generate three-dimensional graphs, the response model is plotted against two of the factors, while the third is held constant at a fixed level, typically the proposed optimum, as shown in Fig.03. The flow rate (A) and wavelength (B) are graphically represented in Figure. 3 (a), while the Methanol (C) is kept constant at its optimum value of 55, exhibiting a positive relationship between the flow rate and tailing factor while the wavelength (B) has a less significant effect on tailing factor. The flow rate (A) and methanol (C) are graphically depicted in fig 3 (b), while the wavelength is held constant at the optimum range of 258 nm. The flow rate (A) and methanol ratio (C) have a synergistic effect on the tailing factor if they vary within a certain range. In fig 3 (c) the flow rate (A) was kept constant at the optimum level of 0.8 m/min, the wavelength (B) and methanol (C) ratio was varied within specified range. It was determined that methanol(C) ratio has positive correlation with the tailing factor on the other hand wavelength has less significant effect on tailing factor.

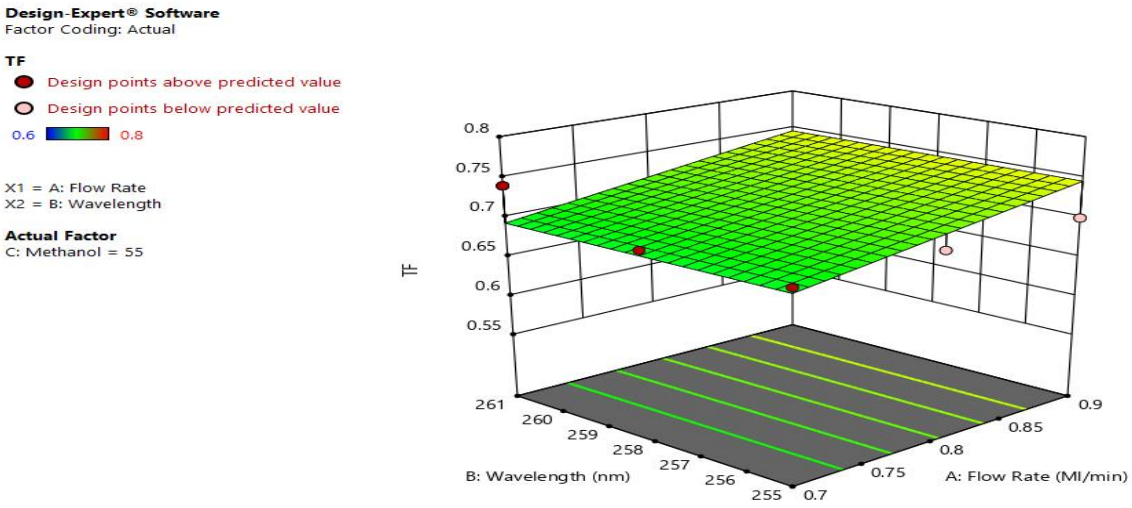


**Fig.4 (a) Tenofovir alafenamide's tailing factor's 3D surface plot shows the influence of several elements**

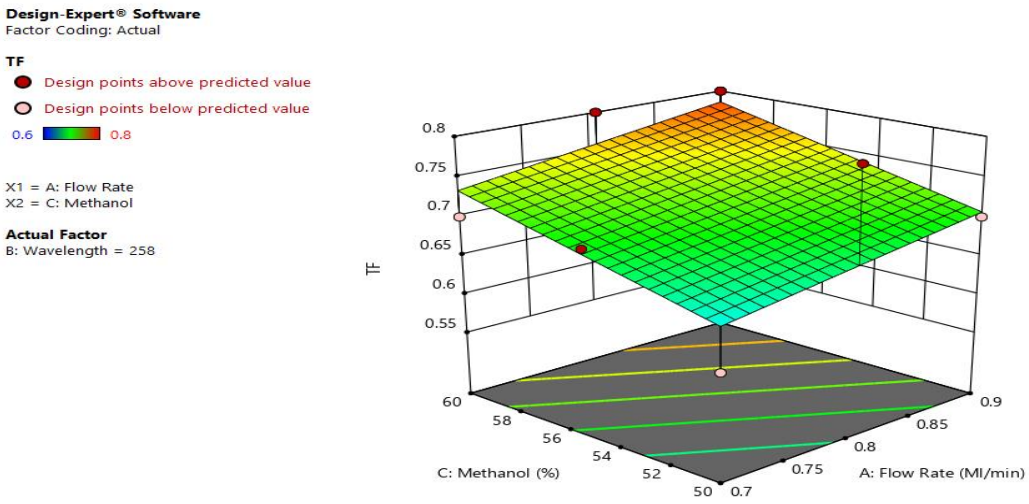


**Fig.4 (b) Tenofovir alafenamide hemifumarate theoretical plate 3D surface plot for interaction effects**

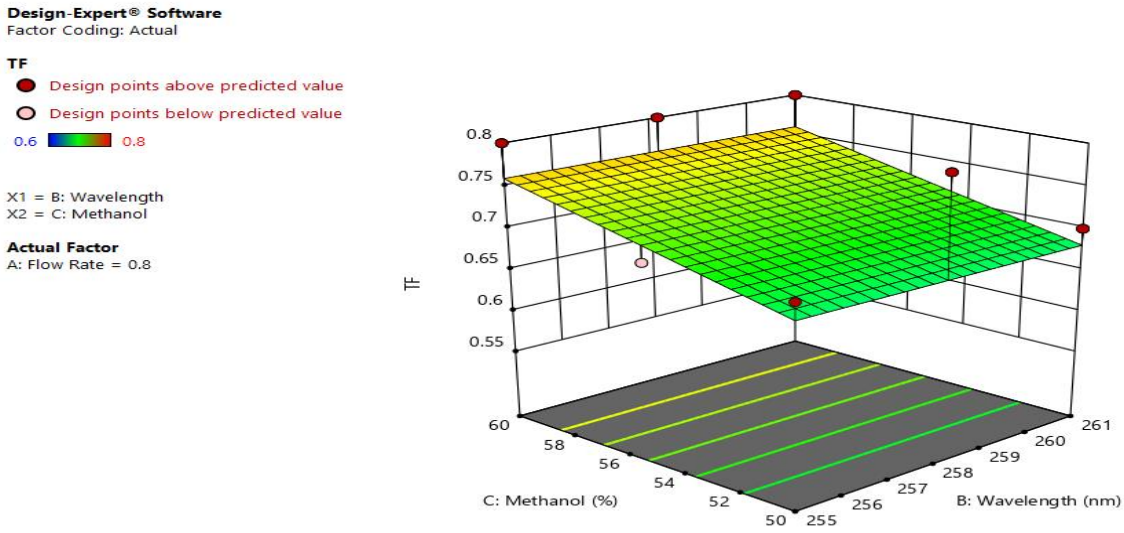


**Fig.4 (c) Tenofovir alafenamide's tailing factor's 3D surface plot showing the influence of several elements**

**Table No. 6 Regression analysis results for Y1, Y2, and Y3 responses for fitting to model by 3-level factorial as suggested by software**

| Response | Model | $R^2$ | Adjusted $R^2$ | Predicted $R^2$ | Adequate precision | SD | % CV |
|---|---|---|---|---|---|---|---|
| Peak asymmetry | Linear model | 0.6826 | 0.6699 | 0.6261 | 12.6995 | 94.97 | 6.39 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| (Y1) | | | | | | | |
| Theoretical plates (Y2) | Quadratic model | 0.7390 | 0.7172 | 0.6696 | 11.1270 | 338.67 | 3.38 |
| Tailing factor (Y3) | Linear model | 0.4954 | 0.4534 | 0.3667 | 9.5143 | 0.0413 | 5.74 |

**Table No. 7 Obtained solution for optimized formulation**

| Flow Rate | Wavelength | Methanol | Peak Asymmetry | Theoretical plate | Tailing Factor |
|---|---|---|---|---|---|
| **0.8** | **258** | **55** | **1386.75** | **8796** | **0.80** |

## DISCUSSION:

For the purpose of evaluating Tenofovir alafenamide hemifumarate in pharmaceutical formulation, an HPLC method was designed using a QbD strategy. Peak asymmetry, theoretical plates, and the tailing factor were all part of the analytic target product profile for Tenofovir alafenamide hemifumarateHPLC analysis. Important characteristics include the flow rate (A), wavelength (B), and mobile phase composition (C). The central composite design was implemented across three components and three levels using Design Expert Software Version 11.0. Chromatographic separation variables such as column type, instrument settings, and injection volume were held constant, while others including mobile phase composition, flow rate, and wavelength were tested for stability. With the use of a QbD strategy, an HPLC technique for Tenofovir alafenamide hemifumaratewas developed. The C-18 column (250mm x 4.6mm) with a 5.0 µm particle size was equilibrated with a mobile phase of methanol to ortho-phosphoric acid (0.05%) (55:45, v/v) in order to determine Tenofovir alafenamide hemifumaratein an RP-HPLC method that was optimized for this purpose. The method had a linear response between 10 and 50 µg/ml (correlation coefficient = 0.999). Reliability of the optimized process was demonstrated by a percent RSD of less than 2% for repeatability, intraday accuracy, and interday precision. The LOD was 0.13 µg, while the LOQ was 0.41 µg. The % recovery of spiked samples fell within the range of 999.69 ± 0.56 to 100.50 ± 0.47, which is within the acceptability standards of the ICH recommendations. The strategy was developed in accordance with ICH recommendations.

## CONCLUSION:

The use of the QbD strategy in the evolution of HPLC techniques has been discussed. The experimental plan specifies the flow rate, mobile phase composition, and wavelength that will be investigated as integral parts of the HPLC process. The HPLC method for Tenofovir alafenamide hemifumaratewas developed using analytical QbD principles, and a multidimensional assessment of various essential process parameters was undertaken to find the optimal system and the final design space. Their connections were studied in depth with a central composite design, and improvements were made at many stages. The purpose of this research is to improve our understanding of the factors that affect chromatographic separation and the efficacy of the procedures used to achieve those goals. With the information gained from this procedure, a future-proof chromatographic

optimization can be constructed. All validated parameters were found to be within accep Table No.. ranges. The validated method was found to be linear, exact, accurate, specific, robust, and rugged when used for the determination of tenofovir alafenamide. With the help of the QbD method creation process, we now have a more thorough understanding of the variables that can affect the success or failure of a method's validation and transfer. The automated QbD method development process using the Design Expert software resulted in a higher-performing, more robust method in a shorter amount of time compared to the manual production of such a method. Statistical testing shows that the method is reliable, consistent, precise, and repeaTable No... QbD is an approach for developing methods that has helped researchers gain a deeper comprehension of the factors that can affect the success or failure of a method's validation and transfer efforts. The design expert software and the automated QbD method development technique led to a more effective and robust method in a shorter amount of time compared to the manual construction of methods. Statistical analysis demonstrates that the method is repeaTable No.., selective, accurate, and resilient.

**CONFLICT OF INTEREST:**

The authors have no conflicts of interest regarding this investigation**.**

**REFERENCES:**

1. Patel KY, Dedania ZR, Dedania RR, Patel U. QbD approach to HPLC method development and validation of ceftriaxone sodium. Futur J Pharm Sci. 2021;7(1). Available from: http://dx.doi.org/10.1186/s43094-021-00286-4
2. Patil SS, Kumbhar DD, Manwar JV, Jadhao RG, Bakal RL, Wakode S. Ultrasound-assisted facile synthesis of nanostructured hybrid vesicle for the nasal delivery of indomethacin: Response surface optimization, microstructure, and stability. AAPS PharmSciTech. 2019;20(3):97. Available from: http://dx.doi.org/10.1208/s12249-018-1247-1
3. Zhao L, Li Z, Zhou Z, Kang X, Fang B, Ma H, et al. Simultaneous determination of Tenofovir alafenamide hemifumarateand tenofovir in human plasma by LC-MS/MS and its application to pharmacokinetics study in clinic. J Chromatogr B Analyt Technol Biomed Life Sci. 2019;1117:148–57. Available from: http://dx.doi.org/10.1016/j.jchromb.2019.04.011
4. Ramaswamy A, Arul Gnana Dhas AS. Development and validation of analytical method for quantitation of Emtricitabine, Tenofovir, Efavirenz based on HPLC. Arab J Chem. 2018;11(2):275–81. Available from: http://dx.doi.org/10.1016/j.arabjc.2014.08.007
5. Arabzadeh V, Sohrabi MR, Goudarzi N, Davallo M. Using artificial neural network and multivariate calibration methods for simultaneous spectrophotometric analysis of Emtricitabine and Tenofovir alafenamide hemifumaratefumarate in pharmaceutical formulation of HIV drug. Spectrochim Acta A Mol Biomol Spectrosc. 2019;215:266–75. Available from: http://dx.doi.org/10.1016/j.saa.2019.02.077
6. Bhavsar DS, Patel BN, Patel CN. RP-HPLC method for simultaneous estimation of tenofovir disoproxil fumarate, lamivudine, and efavirenz in combined Table No..t dosage form. Pharm Methods. 2012;3(2):73–8. Available from: http://dx.doi.org/10.4103/2229-4708.103876

7. Hummert P, Parsons TL, Ensign LM, Hoang T, Marzinke MA. Validation and implementation of liquid chromatographic-mass spectrometric (LC–MS) methods for the quantification of tenofovir prodrugs. J Pharm Biomed Anal. 2018;152:248–56. Available from: http://dx.doi.org/10.1016/j.jpba.2018.02.011
8. Kurmi M, Jayaraman K, Natarajan S, Kumar GS, Bhutani H, Bajpai L. Rapid and efficient chiral method development for lamivudine and tenofovir disoproxil fumarate fixed dose combination using ultra-high performance supercritical fluid chromatography: A design of experiment approach. J Chromatogr A. 2020;1625(461257):461257. Available from: http://dx.doi.org/10.1016/j.chroma.2020.461257
9. Xiao D, Ling KHJ, Tarnowski T, Majeed SR, German P, Kearney BP, et al. An LC-MS/MS method for determination of tenofovir (TFV) in human plasma following Tenofovir alafenamide hemifumarate(TAF) administration: Development, validation, cross-validation, and use of formic acid as plasma TFV stabilizer. Anal Biochem. 2020;593(113611):113611. Available from: http://dx.doi.org/10.1016/j.ab.2020.113611
10. Nn A, Ki B, Jt J. Stability indicating assay method development and validation for Tenofovir alafenamide hemifumaratefumarate by RP-HPLC. Pharm Anal Acta. 2018;9(12). Available from: http://dx.doi.org/10.4172/2153-2435.1000601
11. Attaluri T, Seru G, Varanasi SNM. Development and validation of a stability-indicating RP-HPLC method for the simultaneous estimation of bictegravir, emtricitabine, and Tenofovir alafenamide hemifumaratefumarate. Turk J Pharm Sci. 2021;18(4):410–9. Available from: http://dx.doi.org/10.4274/tjps.galenos.2020.70962
12. Dutta Tejaswi JK, Rajan R G. Reverse-phase high-performance liquid chromatography method development and validation for simultaneous estimation and forced degradation studies of emtricitabine, rilpivirine, and Tenofovir alafenamide hemifumaratein solid dosage form. Asian J Pharm Clin Res. 2019;12(1):112. Available from: http://dx.doi.org/10.22159/ajpcr.2019.v12i1.28765
13. Devrukhakar PS, Borkar R, Shastri N, Surendranath KV. A validated stability-indicating RP-HPLC method for the simultaneous determination of tenofovir, emtricitabine, and a efavirenz and statistical approach to determine the effect of variables. ISRN Chromatogram. 2013;2013:1–8. Available from: http://dx.doi.org/10.1155/2013/878295.
14. Krishna MV, Dash RN, Jalachandra Reddy B, Venugopal P, Sandeep P, Madhavi G. Quality by Design (QbD) approach to develop HPLC method for eberconazole nitrate: Application oxidative and photolytic degradation kinetics. J Saudi Chem Soc. 2016;20:S313–22. Available from: http://dx.doi.org/10.1016/j.jscs.2012.12.001
15. Tome T, zigart N, Casar Z, Obreza A. Development and optimization of liquid chromatography analytical methods by using AQbD principles: Overview and recent advances. Org Process Res Dev. 2019;23(9):1784–802. Available from: http://dx.doi.org/10.1021/acs.oprd.9b00238
16. Karmarkar S, Garber R, Genchanok Y, George S, Yang X, Hammond R. Quality by design (QbD) based development of a stability indicating HPLC method for drug and impurities. J Chromatogr Sci. 2011; 49(6):439–46. Available from: http://dx.doi.org/10.1093/chrsci/49.6.439
17. Babar SA, L. Padwal S. Qbd approach to analytical method development and its validation for estimation of Lenvatinib in bulk and pharmaceutical formulation. Int j appl pharm. 2021; 183–8. Available from: http://dx.doi.org/10.22159/ijap.2021v13i5.41786

18. Chaphekar MM, Hamrapurkar P. Development and validation of RP-HPLC assay method for Vildagliptin using QbD approach and its application to Forced degradation studies. Int j pharm sci drug res. 2016;8(03). Available from: http://dx.doi.org/10.25004/ijpsdr.2016.080306
19. Sandhu PS, Beg S, Katare OP, Singh B. QbD-driven development and validation of a HPLC method for estimation of tamoxifen citrate with improved performance. J Chromatogr Sci. 2016;54(8):1373–84. Available from: http://dx.doi.org/10.1093/chromsci/bmw090
20. The International Conference on Harmonisation ICH Technical Requirements for Registration of Pharmaceuticals for Human Use on Pharmaceutical Quality System Q10 (2008) https://database.ich.org/sites/default/files/Q10%20Guideline.pdf
21. The International Conference on Harmonisation ICH Technical Requirements for Registration of Pharmaceuticals for Human Use on Quality Risk Management Q9 (2005) https://database.ich.org/sites/default/files/Q9%20Guideline.pdf
22. The International Conference on Harmonisation ICH Technical Requirements for Registration of Pharmaceuticals for Human Use on Pharmaceutical Development Q8(R2)(2009) https://database.ich.org/sites/default/files/Q8%28R2%29%20Guideline.pdf