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ORIGINAL ARTICLE / ÖZGÜN MAKALE

STRATEGIC QBD IMPLEMENTATION IN RPHPLC-PDA METHOD FOR SIMULTANEOUS QUANTIFICATION OF CYSTIC FIBROSIS DRUGS TEZACAFTOR AND IVACAFTOR

KİSTİK FİBROZİS İLAÇLARI TEZACAFTOR VE IVACAFTOR'UN EŞ ZAMANLI KANTİFİKASYONU İÇİN RPHPLC-PDA YÖNTEMİNDE STRATEJİK QBD UYGULAMASI

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ABSTRACT

Objective: *A novel RP-HPLC PDA method was developed using a Quality by Design (QbD) approach for the simultaneous quantification of Tezacaftor and Ivacaftor medications employed in the management of cystic fibrosis.*

Material and Method: *Optimization was performed by Central Composite Design by selecting mobile phase ratio of methanol, pH of buffer and flow rate as factors and evaluating responses namely retention time and tailing factor. This technique makes use of an Inertial ODS C18 column (250 x 4.6 mm, 5 µm particle size) in conjunction with a Waters module fitted with a photo diode array detector. The chromatographic conditions including a flow rate of 1.0 ml/min, a mobile phase composed of methanol and buffer in a 45:55 ratio, and a detection wavelength of 210 nm, were thoughtfully designed to effectively separate Tezacaftor and Ivacaftor.*

Result and Discussion: *The method demonstrated remarkable accuracy, with average recoveries of 99.69% for ivacaftor and 100.06% for tezacaftor. The % assay results for system suitability, method precision, and intermediate precision consistently fell within the range of 99.91% to 100.37%. Linearity data exhibited correlation coefficient values of one for both Tezacaftor and Ivacaftor. The LOD and LOQ values for Tezacaftor and Ivacaftor were determined to be 0.56, 0.57, 1.69, and 1.74, respectively. The results obtained from the validation parameters demonstrate that this RP-HPLC method, developed using the QbD approach, is robust and dependable. It serves as a valuable tool for routine analysis and plays a pivotal role in bioanalytical and bioequivalence research within the realm of cystic fibrosis treatment. This method ensures precise and accurate quantification of Tezacaftor and Ivacaftor in combination tablet formulations.*

Keywords: *Central composite design, ivacaftor, method development, method validation, RP-HPLC, tezacaftor*

ÖZ

Amaç: *Kistik fibrozis tedavisinde kullanılan Tezacaftor ve Ivacaftor ilaçlarının eş zamanlı ölçümü için Tasarıma Göre Kalite (QbD) yaklaşımı kullanılarak yeni bir RP-HPLC PDA yöntemi geliştirildi.*

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Gereç ve Yöntem: *Optimizasyon, metanolün mobil faz oranı, tamponun pH'ı ve akış hızının faktör olarak seçilmesi ve tepkilerin, yani alıkonma süresi ve kuyruk faktörünün değerlendirilmesi yoluyla Merkezi Bileşik Tasarım ile gerçekleştirildi. Bu teknik, bir foto diyot dizisi dedektörü ile donatılmış bir Waters modülü ile birlikte bir Inertial ODS C18 kolonundan (250 x 4,6 mm, 5 µm parçacık boyutunda) yararlanır. 1.0 ml/dak'lık bir akış hızı, 45:55 oranında metanol ve tampondan oluşan bir mobil faz ve 210 nm'lik bir tespit dalga boyunu içeren kromatografik koşullar, Tezacaftor ve Ivacaftor'u etkili bir şekilde ayırmak için iyi bir şekilde tasarlanmıştır.*
Sonuç ve Tartışma: *Yöntem, ivacaftor için %99.69 ve tezacaftor için %100.06'lık ortalama geri kazanımlarla dikkate değer bir doğruluk gösterdi. Sistem uygunluğu, yöntem kesinliği ve ara kesinlik için % miktar tayini sonuçları sürekli olarak %99.91 ile %100.37 aralığında tespit edildi. Doğrusallık verileri, hem Tezacaftor hem de Ivacaftor için bir korelasyon katsayısı değeri gösterdi. Tezacaftor ve Ivacaftor için LOD ve LOQ değerleri sırasıyla 0.56, 0.57, 1.69 ve 1.74 olarak belirlendi. Doğrulama parametrelerinden elde edilen sonuçlar, QbD yaklaşımı kullanılarak geliştirilen bu RP-HPLC yönteminin sağlam ve güvenilir olduğunu göstermektedir. Rutin analizler için bu teknik değerli bir araç olarak hizmet eder ve kistik fibroz tedavisi alanında biyoanalitik ve biyoeşdeğerlik araştırmalarında önemli bir rol oynar. Bu yöntem, kombinasyon tablet formülasyonlarında Tezacaftor ve Ivacaftor'un kesin ve doğru miktarının belirlenmesini sağlar.*
Anahtar Kelimeler: *Merkezi kompozit tasarım, ivacaftor, RP-HPLC, tezacaftor, yöntem geliştirme, yöntem validasyonu*

INTRODUCTION

Ivacaftor is identified by the molecular formula $C_{24}H_{28}N_2O_3$ and is chemically described as N-(2,4-di-tert-butyl-5-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide [1]. Enhances the movement of chloride by boosting the likelihood of the G551D-CFTR protein channel to open, thereby facilitating chloride transport (see figure 1B) [2]. Genetic mutations affecting the CFTR gene, responsible for controlling the transport of ions like chloride and water in the body, are the root cause of cystic fibrosis [3]. The molecular formula of Tezacaftor is $C_{26}H_{27}F_3N_2O_6$, and its chemical structure is represented by 1-(2,2-Difluoro-1,3-benzodioxol-5-yl)-N-[1-[(2R)-2,3-dihydroxypropyl]-6-fluoro-2-(2-hydroxy-1,1-dimethylethyl)-1H-indol-5-yl]cyclopropane carboxamide. It functions as a corrector by aiding in the proper folding and presentation of the protein on the cell surface, ultimately enhancing its functionality in individuals with the F508 delmutation (Figure1A) [4].

Analytical Quality by Design (AQbD) is the practice of applying the Quality by Design (QbD) principle specifically to the development of analytical methods and procedures. It claims that rather than determining quality merely by testing of end results, quality should be integrated into the design of the analytical process. The foundation of this approach is the Quality Target Method Profile (QTMP), which begins the process. The term "ATP" stands for Analytical Target Profile, describing the method itself. This method profile outlines the desired outcome and directs decision-making throughout the research and development phases of a project [5]. Adhering to the definition of QTMP can aid in the identification of CAAs. CAAs are similar to critical quality attributes (CQA) in the context of product development. To ensure the required product quality, limits, ranges, or distributions for CQAs, which the ICH Q8 (R2) defines as chemical, physical, biological, or microbiological qualities, must be established.

According to a literature review, there are few developed RP-HPLC PDA methods for determining tezacaftor and ivacaftor simultaneously in a fixed dose combination [6-13]. Despite the extensive analytical methods available for individual quantification of Tezacaftor and Ivacaftor, there is a scarcity of robust, validated RP-HPLC methods developed using the QbD approach for their simultaneous estimation. Existing methods often lack systematic optimization strategies, leading to variability in retention time, peak symmetry, and sensitivity. Conventional HPLC methods often do not account for critical factors such as mobile phase composition, buffer pH, and flow rate in a systematic manner, leading to variability in retention time, resolution, and peak symmetry. Furthermore, few studies have provided comprehensive validation data, including system suitability, method precision, intermediate precision, and sensitivity parameters such as LOD and LOQ. Additionally, limited research has focused on the application of QbD-based RP-HPLC methods for

routine pharmaceutical analysis and bioequivalence studies in cystic fibrosis treatment. This study addresses these gaps by employing a QbD approach to develop a novel, highly accurate, and validated RP-HPLC method for the simultaneous estimation of Tezacaftor and Ivacaftor.We couldn't find any earlier studies that used design of experiments approach to build an AQbD-based HPLC method for quantitatively analysing Tezacaftor and Ivacaftor in commercially available formulations and pure medication. As a result, our current study represents a successful attempt to develop an AQbD-driven HPLC technique for measuring Tezacaftor and Ivacaftor in pure drug and formulations utilising Design of Experiments principles (DoE). The validated method can be applied in bioanalytical research to assess drug concentration profiles in biological matrices, essential for regulatory approval of generic formulations. With a run time optimized for efficiency, this method is suitable for routine quality control analysis in pharmaceutical industries.

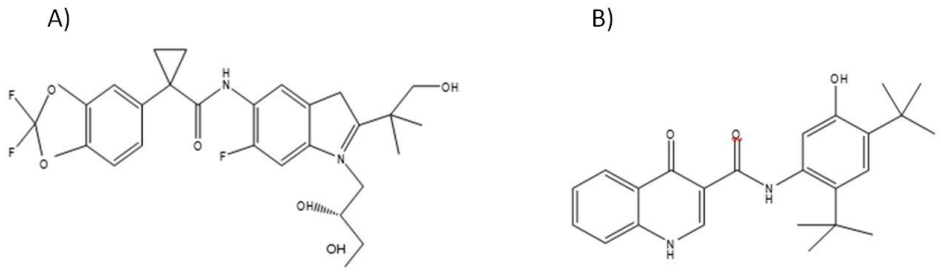

Figure 1. A) Structure of Tezacaftor, B) Structure of Ivacaftor

MATERIAL AND METHOD

Instrumentation

1.HPLC Waters Model series No .2690/95 with PDA using Empower software.
2.Electronic balance (SARTORIOUS)
3.Sonicator (FASTCLEAN).

Material and Reagents

Tezacaftor and Ivacaftor sample with 99.8% w/w purity was obtained from a Hetero labs Pvt.Ltd. Analytical grade potassium dihydrogen phosphate, orthophosphoric acid, HPLC grade methanol and Mill-Q water was procured from Merck. Drug combination tablets of dose 100mg Tezacaftor and 150mg Ivacaftor with a brand name Symkevi were procured from local pharmacy.

Table 1. Experimental factors and levels used in design

Factor	Lower Limit(-1)	Upper Limit(+1)
A:Mobile phase of methanol	40	50
B: pH of Buffer	2.40	4.40
C: Flow rate	0.80	1.20

Optimisation

The Design-Expert software employed a numerical optimisation method to evaluate the model's accuracy. Based on desirability 1.0, the software chose one of 100 solutions. Design Expert specified the optimal conditions for the experiment. The chromatographic settings recommended by Design-Expert were mobile phase, pH, and flow rate [14-16]. As shown in Figure 2, the model projected a method response of retention time and tailing factor. The HPLC equipment was used in the same experiment under the same conditions and optimized conditions are tabulated in Table 3. Figure 3, 4, 5, 6 and Table 2 shows that the predicted and observed values have a 0.999 correlation. As illustrated

in the Figure 2, all of these variables have been demonstrated to have a considerable impact on retention time and tailing factor. The chromatogram of optimized condition by HPLC is represented in Figure 7.

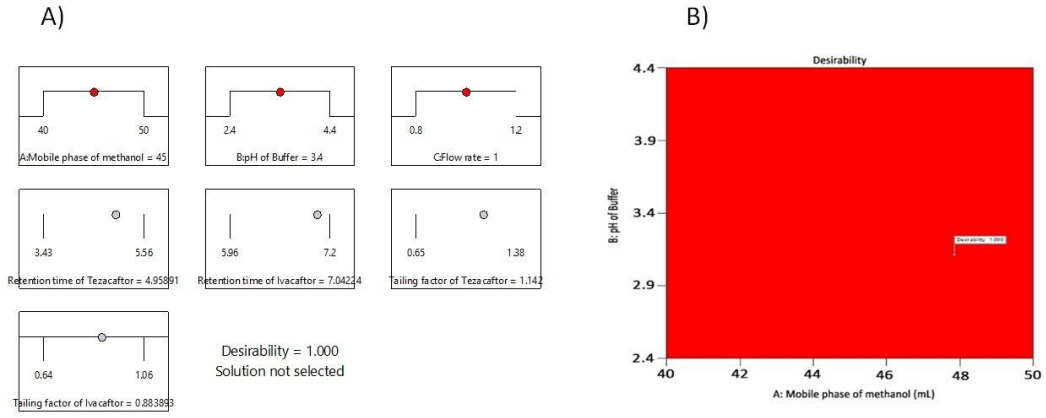

Figure 2. A), B) Optimisation and prediction of method responses by model

Table 2. Summary of central composite design 3 factors; 20 runs

			F 1	F 2	F 3	R 1	R 2	R 3	R 4
S.No.	**Std**	**Run**	**A:Mobile phase of methanol**	**B:pH of Buffer**	**C:flow rate**	**RT of Tezacaftor**	**RT of Ivacaftor**	**Tailing factor of Tezacaftor**	**Tailing factor of Ivacaftor**
			ml	ml	mL/min	mins	mins		
1	5	1	40	2.5	1.2	3.9	6.39	0.77	0.75
2	8	2	50	4.4	1.2	5.19	7.2	1	0.99
3	1	3	40	2.5	0.8	3.88	6.63	0.66	0.72
4*	16	4	45	3.4	1	4.97	7.07	1.15	0.89
5	3	5	40	4.4	0.8	4.06	6.54	0.85	0.7
6	10	6	53.4	3.4	1	5.56	6.8	1.26	1.06
7	11	7	45	1.8	1	4.35	6.9	1	0.88
8	7	8	40	4.4	1.2	3.87	6.3	0.95	0.72
9	12	9	45	5	1	4.62	7.02	0.99	0.83
10	6	10	50	2.5	1.2	5.27	7.2	1.15	1
11*	17	11	45	3.4	1	4.97	7.07	1.15	0.89
12*	15	12	45	3.4	1	4.89	6.91	1.1	0.85
13	4	13	50	4.4	0.8	5.42	7.1	1.2	0.95
14	13	14	45	3.4	0.7	4.89	6.98	1.12	0.84
15*	20	15	45	3.4	1	4.97	7.07	1.15	0.89
16*	19	16	45	3.4	1	4.97	7.07	1.15	0.89
17	9	17	36.6	3.4	1	3.43	5.96	0.65	0.64
18	14	18	45	3.4	1.3	4.99	7.1	1.07	0.92
19*	18	19	45	3.4	1	4.97	7.07	1.15	0.89
20	2	20	50	2.5	0.8	5.19	7.1	1.38	0.97

*F1,F2,F3- Factors of 1,2,3
*R1,R2,R3,R4- Response of 1,2,3,4

Table 3. Optimized chromatographic conditions

Parameters	Method
Stationary phase	Inertsil-ODSC_{18}(250x4.6mm,5µ)
Mobile Phase	Methanol:Buffer(45:55)
Flowrate	1.0ml/min
Runtime	12min
Column temperature	Ambient
Volume of injection loop	20µl
Detection wavelength	210nm
Drug RT(min)	4.977min for Tezacaftor and 7.077 for Ivacaftor.

Method Development

In our present study, we fine-tuned the chromatographic parameters of the HPLC method by employing the Central Composite Design. This design was selected because it provides the flexibility to modify experimental parameters whenever necessary. Our main objective was to create and confirm a Quality by Design approach based on HPLC. Table 1 outlines the recommended values for the low, medium, and high levels of the variables under consideration as suggested by the software. We conducted experimental runs to evaluate the impact of these factors on the Critical Analytical Attributes (CAAs).

Preparation of Diluent

Creating the standard and sample solutions required diluting them with the mobile phase.

pH 3.4 Phosphate Buffer Preparation

To produce a pH 3.4 phosphate buffer, begin by measuring 2.7218 grams of KH_2PO_4. Add this measured amount of KH_2PO_4 to a 1000ml beaker with 1000ml of HPLC water. Use orthophosphoric acid to adjust the solution's pH to 3.4.

Mobile Phase

Methanol degassed and buffered in a 45:55v/v ratio.

Preparation of Standard Stock Solution

Place 10 milligrams of each drug into a 10-ml volumetric flask, and then add 7 ml of ethanol and sonicate for half an hour. After half an hour add remaining 3ml upto the mark and sonicate it to 5 mins (i.e.,1000 ppm).

Preparation of Working Standard Preparation

Add 1 ml of Tezacaftor standard solution and 1ml of Ivacaftor in a 10 ml volumetric flask. Add methanol upto the mark, then sonicate it for five minutes (100 ppm).

Method Validation

The method parameters are validated as per ICH guidelines.

RESULT AND DISCUSSION

Response Surface Modeling by Central Composite design (CCD)

The Central Composite design was used to screen and optimise the chromatographic conditions. The mobile phase of methanol, pH of Buffer, and flow rate were all varied in the 40-50 ratio, 2.4-4.4, and 0.8-1.2 ml/min respectively. The levels of the selected method responses are shown in Table 1. Table 2 displays the outcomes of 20 runs conducted with the response surface method's . The results of utilising Design of Experiments software to build a quadratic model of ANOVA regression parameters

for retention time are shown in Table 1. The model's F-value 29.82, 117.52, 259.34, 201.24 indicates its importance. Model terms are considered significant if their p-value is less than 0.0500.

Retention Time of Ivacaftor

A quadratic model represented the built-in ANOVA. The model is crucial. Table 4 provides an overview of the model i.e., quadratic and lack of the fit test. The drug's retention time's built-in value suggested that it is substantial. The coded equation =+7.04+0.3035A+0.0037B-0.0080C+0.0258AB+0.0850AC-0.0015BC-0.2291A2-0.0273B2+0.0078C2 with the factors coded. The contour plot is depicted by the built-in model graph in Figure 3A, while the specifics of the 3D surface design points are shown in Figure 3B.

Table 4. Model summary statistics and lack of fit test

	Response	Source	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	F-value	p-value
Ivacaftor	RT	Quadratic	0.0883	0.9641	0.9317	0.7860	29.82	<0.0001
Ivacaftor	Tailing Factor	Quadratic	0.0147	0.9906	0.9822	0.9654	117.59	<0.0001
Tezacaftor	RT	Quadratic	0.0536	0.9957	0.9919	0.9738	259.34	<0.0001
Tezacaftor	Tailing Factor	Quadratic	0.0195	0.9945	0.9896	0.9759	201.24	<0.0001

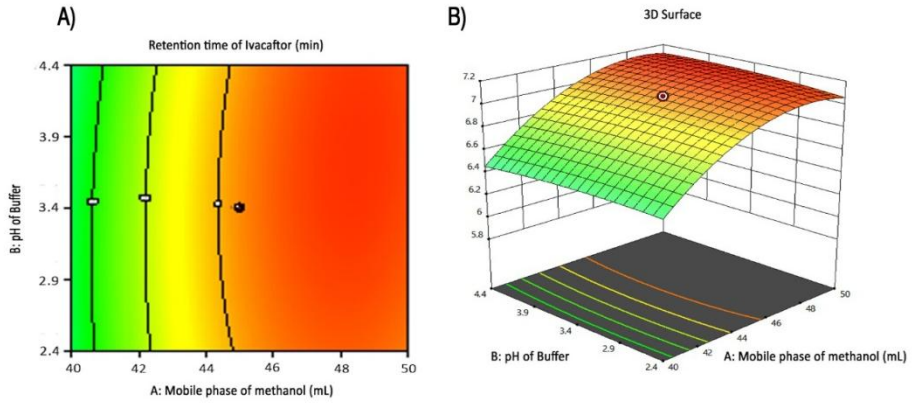

Figure 3. A) Contour plot for retention time of Ivacaftor, B) 3D Response surface retention time of Ivacaftor

Tailing Factor of Ivacaftor

The tailing factor was optimized using the built-in quadratic ANOVA model in the CCD programme to verify the peak summary. The lack-of-fit test and the quadratic model are summarized in Table 4. +0.08839+0.1264A-0.0120B+0.0192C+0.0027AB+0.0025AC-0.0002BC-0.0137A^2-0.0130B^2-0.0044C^2 is the final coded equation. Figure 4A built-in model graph shows a contour plot and Figure 4B specifies the 3D surface design points.

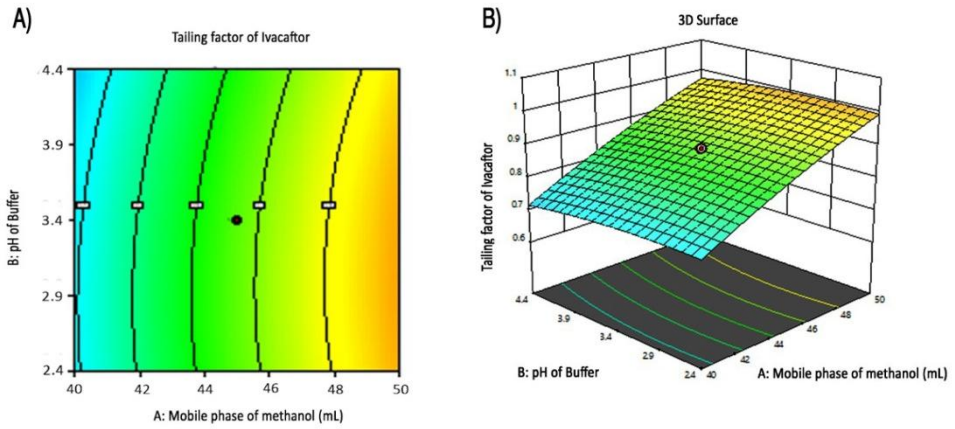

Figure 4. A) Contour plot for tailing factor of Ivacaftor, B) 3D Response surface tailing factor of Ivacaftor

Retention Time of Tezacaftor

Quadratic modelling was used for the built-in ANOVA. It is important to use the model. The quadratic model is summarised in Table 4 along with the "lack of the fit test." The retention duration for Tezacaftor was predetermined to be important based on its built-in value. The solution contained the coded factors $+4.96+0.6551A+0.0690B-0.0114C+0.0008AB+0.0025AC-0.0698BC-0.1687A^2-0.1904B^2-0.0152C^2$. The built-in model graph in Figure 5A shows the contour plot, whereas Figure 5B shows the specifics of the 3D surface design points.

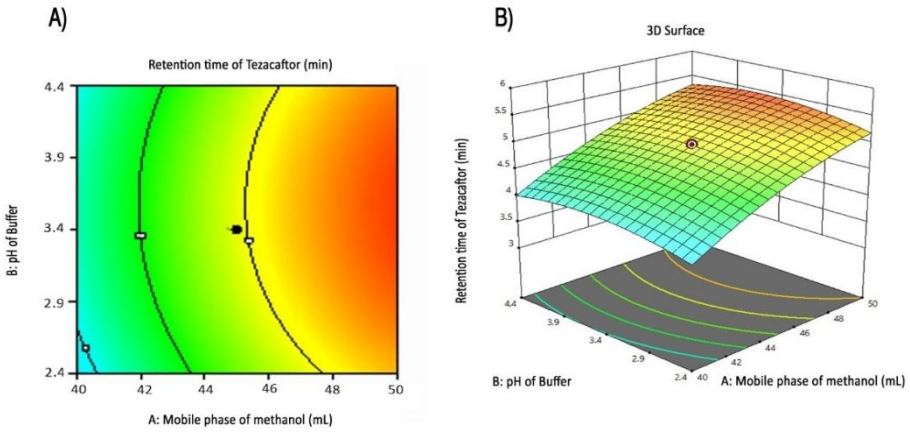

Figure 5. A) Contour plot for retention time of Tezacaftor, B) 3D Response surface retention time of Tezacaftor

Tailing Factor of Tezacaftor

To optimise the tailing factor and verify the peak summary, the CCD application's built-in quadratic ANOVA model was employed. The lack-of-fit test and the quadratic model are summarised in Table 4. The final coded equation is $+1.14+0.1877A+0.0051B-0.0237C-0.0919AB-0.0800AC+0.0024BC-0.0678A^2-0.0588B^2-0.0233C^2$.The contour plot is depicted by the built-in model graph in Figure 6A, while the 3D surface design points are described in more depth in Figure 6B.

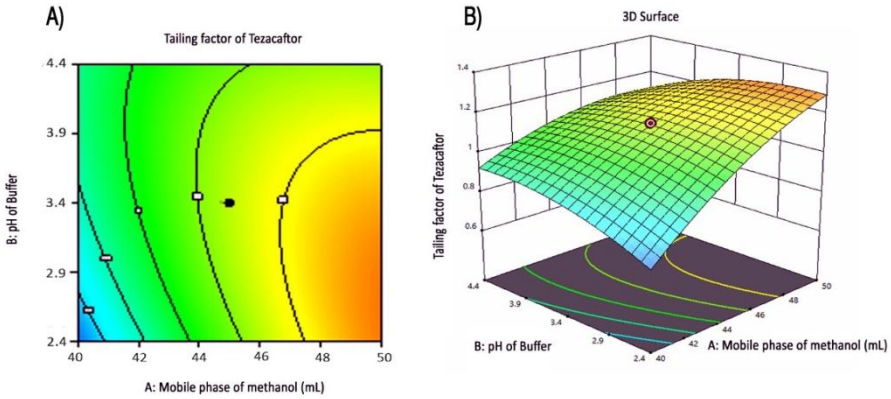

Figure 6. A) Contour plot for tailing factor of Tezacaftor, B) 3D Response surface tailing factor of Tezacaftor

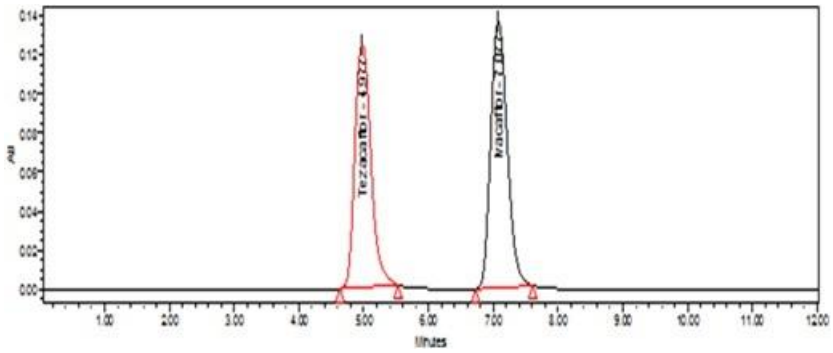

Figure 7. Optimized chromatogram

Method Validation

System Suitability

The system suitability results are presented in Table 5 and Table 6, and chromatogram of system suitability is represented in Figure 8.

Table 5. Data on Tezacaftor system suitability

Injection	RT	Peak Area	USP Plate count	USP Tailing
1	4.975	674753	10953.6097	1.15
2	4.976	674261	10951.0146	1.15
3	4.974	675298	10003.2730	1.15
4	4.975	679221	10986.9427	1.15
5	4.979	688636	10946.8723	1.15
6	4.972	674326	10964.9081	1.15
Mean	**4.975167**	**677749.2**	**10768.3467**	**1.15**
SD	**0.002115**	**5156.873**	-------	-------
%RSD	**0.0425**	**0.76**	-------	-------

Table 6. Data on Ivacaftor system suitability

Injection	RT	Peak Area	USP Plate count	USP Tailing
1	7.075	1218805	9478.3171	0.899633
2	7.076	1214014	9452.1967	0.893423
3	7.074	1215474	9569.9285	0.894443
4	7.070	1227655	9619.6337	0.882222
5	7.075	1267019	9749.9072	0.892316
6	7.072	1225625	9620.7336	0.889233
Mean	**7.0736**	**1228099**	**9573.9971**	**0.892407**
SD	**0.002055**	**18098.07**	-------	-------
%RSD	**0.029**	**1.47**	-------	-------

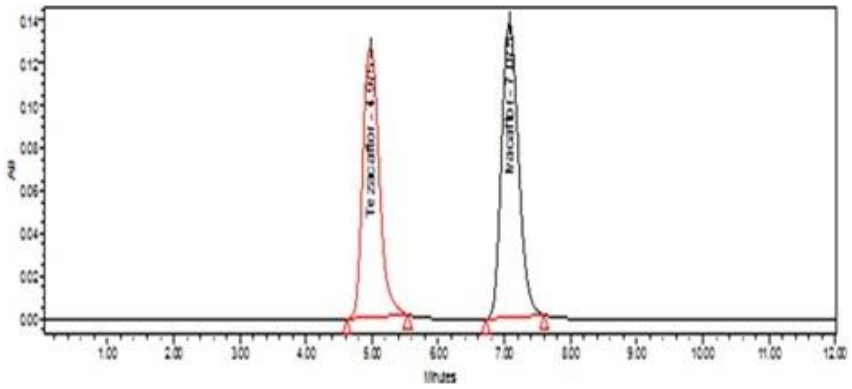

Figure 8. Chromatogram of system suitability

Specificity

By comparing the drug with the blank solution and analyzing for drug and blank solution interference, the specificity of this created approach is assessed [17-23]. The chromatogram of Blank and standard is represented in Figure 9A and 9B.

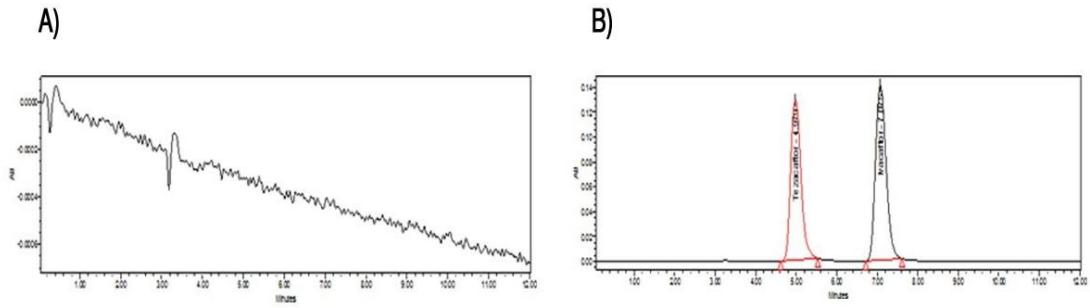

Figure 9. A) Blank chromatogram, B) Standard chromatogram

Precision

There are three types of precisions which are used in the development of HPLC those three precisions were system precision, intermediate precision and method precision [24-27]. It is measured at the concentration at 100 ppm, the peak area and the %assay were calculated based on the data. The results of precision is tabulated in Table 7-9.

Table 7. Results of system precision

Injection no.	Peak Area	
	Tezacaftor	Ivacaftor
1	678433	1228593
2	675498	1215374
3	679321	1226655
4	676341	1216454
5	679642	1224568
6	677541	1226548
Mean	677796	1223032
S.D	1505.65	5175.06
%RSD	0.22	0.42

Table 8. Results for intermediate precision

Inj.No.	Analyst-I		Analyst-II	
	Tezacaftor	Ivacaftor	Tezacaftor	Ivacaftor
1	644614	1206333	644607	1206333
2	645622	1216481	645245	1203264
3	642361	1205632	643216	1206513
4	647413	1216548	646648	1215484
5	647614	1205632	647012	1206513
6	645622	1213245	645146	1204516
Mean	645541	1210645	645312.3	1207104
SD	1769.49	4907.783	1264.72	3936.173
%RSD	0.27	0.40	0.19	0.32

Table 9. Results for method precision

Injection no.	Peak Area	
	Tezacaftor	Ivacaftor
1	637312	1202687
2	635732	1204628
3	634623	1205416
4	633214	1213268
5	637216	1202846
6	636632	1205416
Mean	635788.2	1205710
S.D	1475.349	3554.527
%RSD	0.23	0.29

Accuracy

Different concentrations (50%, 100%, 150%) of spiked solutions containing Tezacaftor and Ivacaftor were prepared and injected into the HPLC system [28-30]. The percentage recovery was calculated for each concentration. The accuracy results derived from these experiments are documented and presented in Table 10.

Table 10. Tezacaftor and Ivacaftor data with accuracy

	Concentration % of spiked level								
	50%			100%			150%		
	Amount added (ppm)	Amount found (ppm)	% recovery	Amount added (ppm)	Amount found (ppm)	% recovery	Amount added (ppm)	Amount found (ppm)	% recovery
Tezacaftor	20	19.93	99.69	40	39.93	99.83	60	59.98	99.97
Ivacaftor	20	20.19	100.06	40	40.01	100.04	60	60.01	100.02

Limit of Detection and Limit of Quantification

The values for Limit of Detection (LOD) and Limit of Quantification (LOQ) have been tabulated and are presented in Table 11. These values represent the sensitivity and lowest detectable and quantifiable levels of the substances under study.

Table 11. LOD and LOQ

Drug	LOD (µg/ml)	LOQ (µg/ml)
Tezacaftor	0.56	1.69
Ivacaftor	0.57	1.74

Linearity

The method's linearity was assessed by preparing concentration levels ranging from 20 to 70 ppm of Tezacaftor and Ivacaftor, which were subsequently injected into the HPLC system. Figures 10A and 10B depict the linearity plots of Tezacaftor and Ivacaftor, respectively. Tables 12 and 13 present the linearity data for Tezacaftor and Ivacaftor, showcasing the relationship between concentration levels and the analytical response, demonstrating the method's ability to provide accurate and consistent results across a range of concentrations.

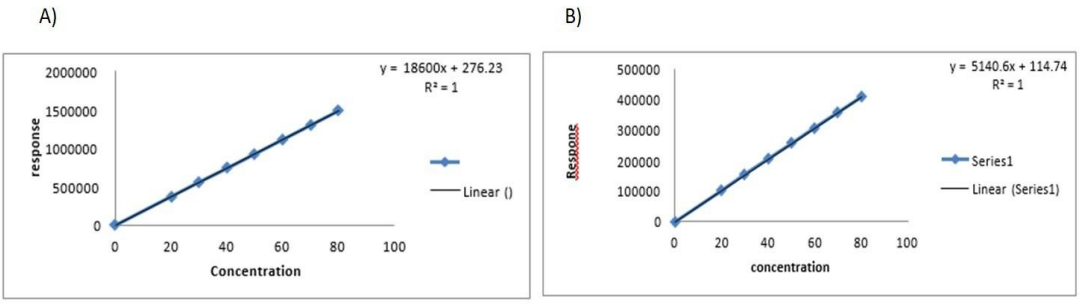

Figure 10. A) Tezacaftor's linearity plot, B) Ivacaftor's linearity plot

Table 12. Linearity data on Tezacaftor

Conc.(ppm)	Average Area
0	0
20	632546
30	658296
40	694400
50	730308
60	916282
70	9402046

Table 13. Linearity data on Ivacaftor

Conc.(ppm)	Average Area
0	0
20	1202965
30	1254371
40	1295856
50	1297167
60	1308577
70	1359903

During the development of the QbD analytical method various parameters were explored. Initially, Tezacaftor showed the highest absorbance at 241 nm, while Ivacaftor exhibited its peak absorbance at 254 nm. Consequently, 254 nm was chosen as the standard wavelength due to its excellent purity in peak. An injection volume of 20 µl was selected, providing a satisfactory peak area. The Inertsil C18 column (ODS) was chosen for its ability to generate well-defined peaks. The pharmaceutical solution proved suitable for analysis at room temperature. A flow rate of 1.0 ml/min was established offering acceptable peak area, retention time, and good resolution. Multiple mobile phase ratios were examined with the methanol:buffer ratio of 45:55v/v ultimately selected for its superior peak symmetry and strong resolution. This mobile phase was then applied in the suggested analysis. Both the system and method precision demonstrated adherence to acceptable limits. Successful curve fitting, correlation coefficient, and linearity studies indicated the linear behavior of the analytical methods for both Tezacaftor and Ivacaftor across the desired concentration range of 20-70 ppm. Accuracy tests also showed successful results, with excellent percentage recovery at all concentration levels, affirming the dependability of the analytical method for both Tezacaftor and Ivacaftor.

The RP-HPLC method developed through the QbD approach is a robust and reliable analytical tool. Its successful optimization chromatographic conditions and comprehensive validation parameters make it a valuable asset for the pharmaceutical industry and research community. This method not only ensures the accurate quantification of Tezacaftor and Ivacaftor but also contributes to the advancement of knowledge in the field of cystic fibrosis treatment and pharmaceutical quality assurance.

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AUTHOR CONTRIBUTIONS

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CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

REFERENCES

1. National Center for Biotechnology Information Web site. (2024). PubChem Compound Summary for CID 72722243, Symkevi. Retrieved March 25, 2024, from https://pubchem.ncbi.nlm.nih.gov/. Accessed date: 25.03.2024.
2. Wikipedia Web site. (2024). Retrieved March 25, 2024, from https://en.wikipedia.org/. Accessed date: 25.03.2025.
3. Tripathi, K.D. (2018). Essentials of medical pharmacology. Jaypee Brothers Medical Publishers, 539-550.
4. PubMed Web site (2025). Retrieved March 25, 2025, from https://pubmed.ncbi.nlm.nih.gov/30489718/. Accessed date: 25.03.2025.
5. ICH Topic Q8 (R2), "ICH harmonized tripartite guideline," in Proceedings of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH '09), Pharmaceutical Development. 2009.
6. Bhattacharyya I, Bhattacharyya SP, and Sen S (2010). Reverse phase high-performance chromatographic liquid system for Tezacaftor hydrochloride analysis in pharmaceutical dose type, International Pharmacy and Technology Review, vol.2, pp.224-232.
7. Shivaradha, B., Rani, S.S. (2022). RP-HPLC method development and validation for the simultaneous determination of elexacaftor, ıvacator and tezacaftor in pharmaceutical dosage forms. World Journal of Pharmaceutical Sciences, 12-21. [CrossRef]
8. Priyadarshini, G.I., Mounika, V., Anjani, G., Sowmya, B. (2020). Stability indicating RP-HPLC method development and validation for the simultaneous estimation of tezacaftor and ıvacaftor in bulk and pharmaceutical dosage form. Asian Journal of Pharmaceutical Analysis, 10(1), 19-26. [CrossRef]
9. Ramanjaneyulu, K.V., Ramana, K.V., Rao, M.P. (2020). Stability indicating LC method development and validation for the simultaneous analysis of cystic fibrosis drugs-ivacaftor and tezacaftor in pharmaceutical formulations. Research Journal of Pharmacy and Technology, 13(5), 2076-2080.
10. Kiranjyothi, R., Balakrishnan, M., Chandrasekhar, K.B. (2018). Method development and validation for the stability indicating simultaneous estimation of tezacaftor and ivacaftor in bulk and its dosage forms. International Journal of Pharmaceutical Research, 10(4), 173.
11. Donakonda, M., Indrakanti, S., Pasala, P.K., Desari, M., Kammari, S. (2021). A rapid RP-HPLC method for the simultaneous estimation of Ivacaftor and Tezacaftor and *in silico* study of their metabolitic products. Future Journal of Pharmaceutical Sciences, 7, 1-14. [CrossRef]
12. Singh, N., Bansal, P., Maithani, M., Chauhan, Y. (2020). Development and validation of a novel stability-indicating RP-HPLC method for simultaneous determination of tezacaftor and ivacaftor in fixed dose combination. Journal of Chromatographic Science, 58(4), 346-354. [CrossRef]
13. Kandula R.K., Sundararajan R. (2020). Stability indicating RP-HPLC method development and validation for the simultaneous estimation of tezacaftor, ıvacaftor and elexacaftor in bulk and its combined dosage form. Journal of Global Trends in Pharmaceutical Sciences, 11 (4), 8552-8557.
14. Sane, R.T., Francis, M., Khedkar, S., Menezrs, A., Moghe, A., Patil, P. (2003). Gas chromatographic determination of bupropion hydrochloride from its pharmaceutical formulations. Indian drugs, 40(4), 231-233.
15. Ravali, T., Marakatham, S., Kumar, M.S., Valli, R.V. (2019). Analytical method development and validation of tezacaftor and ıvacaftor by RPHPLC methodin bulk and marketed formulation. International Journal of Pharmacy and Biological Sciences, 9(4), 67-73.
16. Kumari, P.V.K., Yarraguntla, S.R., Sharmila, M., Gulibindala, E. (2021). Application of box-behnken design for formulation parameters of eslicarbazepine tablets. Indian Journal of Pharmaceutical Sciences, 83(3). [CrossRef]
17. Swathi, S., Kumar, H.T., Rao, P.K. (2015). Validated RP-HPLC method for simultaneous determination of rosuvastatin calcium and ezetimibe in pharmaceutical dosage form. Int. J. Pharm. And Pharm. sci, 7(4), 209-213.
18. Rao, K.V., Tanuja, M., Rao, Y.S., Kumar, T.H. (2015). Development and validation of spectrophotometric methods for estimation of zileuton in bulk and its dosage form. International Journal Chemical Sciences, 13(2), 714-24.
19. Lakshmi, G.T., Rao, Y.S., Rao, K., Prasada, V., Kumar, T.H. (2015). RP-HPLC method for estimation of atomoxetine hydrochloride in bulk and pharmaceutical dosage form. Research Journal Of Pharmaceutical Biological and Chemical Sciences, 6(2), 1208-1214.
20. Lakshmi, T.G., Rao, S.Y., Rao, V.P.K., Kumar, H.T. (2015). Visible spectrophotometric methods for the estimation of orlistat in bulk and pharmaceutical dosage form. Journal of Pharmaceutical Sciences and Research, 7(3), 155.

21. Siridevi, M.P., Hemant, K.T., Srinivasa, R.Y., Varaprasad, R.K. (2019). Simultaneous spectrophotometric estimation of amlodipine besylate and Perindopril Erbumine in tablet Formulation. Research Journal of Pharmacy and Technology, 12(12), 6101-6106. [CrossRef]
22. Srinivasarao, Y., Kumar, T.H., Chiranjivi, P., Rao, K.V. (2021). Simultaneous estimation of solifenacin succinate and tamsulosin hydrochloride in combined dosage form by using first order derivative spectrophotometric method. Indian Journal of Pharmaceutical Sciences, 83(2), 331-335. [CrossRef]
23. Anusha, G., Nargiz, S., Sireesha, A., Poojitha, K., Rao, K.V., Rao, Y.S. (2024). Green solvent-based UV spectrophotometric technique for quantifying molnupiravir in bulk and pharmaceutical formulation. Research Journal of Pharmacy and Technology, 17(11), 5210-5214. [CrossRef]
24. Gandi, A., Dudi, P.K., Pantala, E.S., Yarguntla, S.R. (2024). A comprehensive new approach to method development and validation of encorafenib using uv spectroscopy in bulk and pharmaceutical formulation. Hacettepe University Journal of the Faculty of Pharmacy, 44(4), 318-327. [CrossRef]
25. Gandi, A., Dudi, P.K., Pantala, E.S., Challa, G.N. (2024). Analyzing paxlovid: Examining its properties, characteristics, and analytical and bio-analytical methods-A comprehensive review. Separation Science Plus, 7(12), e202400117. [CrossRef]
26. Gandi, A., Rao, N.K., Anusha, G., Preethi, G., Mounika, D. (2020). Method development and validation of rupatadine fumarate in bulk by UV spectroscopy. International Journal of Ayurveda and Pharma Research, 51-54.
27. International Conference on Technical Requirement Harmonization for Pharmaceuticals for Human Use. (2005). Registration.Validation of analytical techniques:text and approach1-13 in ICHQ2(R1).
28. Analytical Techniques and Method Validation: Chemistry, Performance, and Controls. (2000) FDA. 65: 776-777 in the Federal Register.
29. Theoretical Methodological Validation (1996). Methodology, ICH Harmonized Tripartite Recommendations, Federal Register, Q2B(R1), 1-8.
30. Vogt, F.G., Kord, A.S. (2011). Development of quality-by-design analytical methods. Journal Of Pharmaceutical Sciences, 100(3), 797-812. [CrossRef]