



METHOD DEVELOPMENT AND VALIDATION OF IVACAFTOR AND TEZACAFTOR BY RP-HPLC METHOD

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ABSTRACT

A simple, accurate, and precise RP-HPLC method was developed for the simultaneous estimation of Ivacaftor and Tezacaftor in tablet dosage form. The chromatographic separation was achieved using a Zodiacil C18 (150 × 4.6 mm, 3.5 µm) column with a mobile phase consisting of 0.01N KH₂PO₄: Acetonitrile (55:45, v/v) at a flow rate of 1.0 mL/min. The column temperature was maintained at 30°C with UV detection at 292.0 nm. The retention times for Ivacaftor and Tezacaftor were 2.269 min and 3.164 min, respectively. The method exhibited excellent precision with %RSD values of 0.5% and 1.0% for Ivacaftor and Tezacaftor, respectively. Recovery studies yielded 100.14% and 100.07% for Ivacaftor and Tezacaftor, respectively. Limits of detection (LOD) and quantification (LOQ) were 0.56 and 1.71 µg/mL for Ivacaftor, and 0.07 and 0.11 µg/mL for Tezacaftor, respectively. Linear regression equations were $y = 14394x + 3350$ for Ivacaftor and $y = 6134x + 432.1$ for Tezacaftor with excellent correlation coefficients exceeding 0.999. The developed method was validated according to ICH Q2(R2) guidelines and demonstrated to be simple, economical, and suitable for routine quality control testing in pharmaceutical industries.

Key words:

Ivacaftor, Tezacaftor, RP-HPLC, Method development, Validation, ICH guidelines, pharmaceutical analysis

INTRODUCTION

High performance liquid chromatography (HPLC) is one of the most widely applied analytical techniques for the qualitative and quantitative estimation of drug substances in bulk materials and pharmaceutical dosage forms.^[1] Its high sensitivity, selectivity, precision, and reproducibility make it particularly suitable for routine quality control and stability assessment of modern synthetic drugs.^[2] Among these, reverse phase HPLC (RP-HPLC) has gained prominence because of its robustness, compatibility with aqueous–organic mobile phases, and ability to handle a wide range of polarities.^[3] Regulatory guidelines such as ICH Q2 recommend systematic development and validation of chromatographic methods prior to their application in quality control laboratories.^{[1][2]}

Cystic fibrosis is a life-limiting hereditary disease associated with mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which result in defective chloride transport, viscous secretions, chronic lung infections, and progressive

respiratory failure.^[4] Ivacaftor is a CFTR potentiator that enhances channel gating, whereas Tezacaftor is a CFTR corrector that improves processing and trafficking of CFTR to the cell surface; together, the fixed-dose combination improves pulmonary function and clinical outcome in selected CFTR mutations.^[5] With increasing clinical use of Ivacaftor–Tezacaftor combination therapy, there is a need for simple, rapid, and validated analytical methods capable of simultaneous estimation of both drugs in combined dosage forms.^[6]

The present work aims to develop and validate a stability-indicating RP-HPLC method for simultaneous determination of Ivacaftor and Tezacaftor in tablet dosage form. Method development included systematic optimization of stationary phase, mobile phase composition, flow rate and detection wavelength to obtain sharp, symmetric and well-resolved peaks within a short analysis time. The finalized method was validated for specificity, linearity, accuracy, precision, sensitivity, robustness and assay, and was further evaluated under forced degradation conditions to confirm its

stability-indicating capability, following ICH Q2 recommendations.

MATERIALS AND METHODS

Chemicals and Reagents

Ivacaftor and Tezacaftor pure drug APIs were obtained as gift samples from Aurobindo Pharma Ltd., India. A

marketed fixed-dose tablet formulation labeled to contain Ivacaftor 150 mg and Tezacaftor 100 mg (Symdeko) was used for assay studies. HPLC-grade acetonitrile, methanol, potassium dihydrogen orthophosphate, orthophosphoric acid and distilled water were procured from Rankem. All chemicals and reagents were of analytical reagent or HPLC grade and were used without further purification.

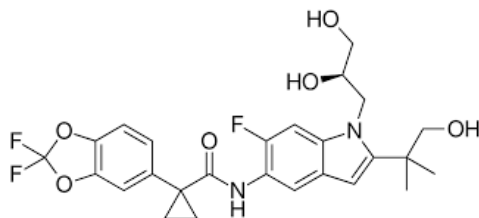


Figure 1: Structure of Tezacaftor

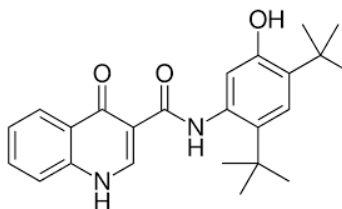


Figure 2: Structure of Ivacaftor

Instrumentation

Chromatographic analysis was carried out on a Waters HPLC 2695 system equipped with quaternary pump, autosampler and PDA detector, controlled by Empower 2 software. A Zodiacil C18 column (150 × 4.6 mm, 3.5 μm) was used as the stationary phase for all measurements. A UV–Visible spectrophotometer (PG Instruments T60) with 10 mm quartz cells and UVWin6 software was employed

for preliminary wavelength selection and UV spectral characterization.

Chromatographic Conditions

Based on several development trials, the optimized chromatographic conditions are presented in Table 5.1. Under these optimized conditions, Ivacaftor and Tezacaftor were eluted at retention times of about 2.269 min and 3.164 min, respectively, with satisfactory plate counts, tailing factors and resolution.

Table 1: Optimized RP-HPLC chromatographic conditions for Ivacaftor and Tezacaftor

Parameter	Optimized Conditions
Column	Zodiacil C18 (150 × 4.6 mm, 3.5 μm)
Mobile Phase	0.01N KH ₂ PO ₄ : Acetonitrile (55:45, v/v)
Flow Rate	1.0 mL/min
Detection Wavelength	292.0 nm
Column Temperature	30°C
Injection Volume	20 μL
Run Time	10 minutes
Pump Mode	Isocratic
Retention Time (Ivacaftor)	2.269 min
Retention Time (Tezacaftor)	3.164 min

Preparation of Standard Solutions

For the preparation of standard stock solutions, accurately weighed 15 mg of Ivacaftor and 10 mg of Tezacaftor were transferred separately into 25 mL volumetric flasks. About three-fourths of the volume was filled with diluent (water:acetonitrile 50:50, v/v), and the mixtures were sonicated for 10 min to ensure complete dissolution. The flasks were then made up to volume with the same diluent to obtain stock solutions containing 600 μg/mL of Ivacaftor and 400 μg/mL of Tezacaftor. For the working standard solution used for system suitability and assay (100% level), 1.0 mL from each stock solution was

transferred into a 10 mL volumetric flask and diluted to volume with diluent to yield 60 μg/mL of Ivacaftor and 40 μg/mL of Tezacaftor.

Preparation of Sample Solutions

Ten tablets of the marketed formulation were accurately weighed and the average tablet weight was calculated. Powder equivalent to one tablet was transferred into a 100 mL volumetric flask, about 50 mL of diluent was added, and the mixture was sonicated for 25 min to extract the drugs. The volume was then made up to the mark with diluent and the solution was filtered through a 0.45 μm HPLC membrane filter to obtain a sample stock solution

containing 1500 µg/mL of Ivacaftor and 1000 µg/mL of Tezacaftor. For assay and validation studies, appropriate aliquots of this stock were further diluted with diluent to obtain sample working solutions equivalent to 100 µg/mL or 100% test level as required.

Method Validation

Method validation was carried out in accordance with ICH Q2 guidelines for analytical procedure validation.

System Suitability: System suitability was evaluated by six replicate injections of mixed standard solution, and parameters such as retention time, theoretical plates (N), tailing factor (T), and %RSD of peak areas were calculated (Table 1).

Linearity: Linearity was assessed by analyzing a series of standard solutions at different concentration levels (2.5–150% of target concentration) for both drugs, and calibration curves were constructed by plotting peak area versus concentration (Table 2).

Precision: System precision, method precision (repeatability), and intermediate precision (intra- and inter-day) were evaluated by multiple injections and independent sample preparations at the target concentration level, and results were expressed as %RSD of peak areas (Table 3, Table 4, Table 5).

Accuracy (Recovery): Accuracy was determined by recovery studies using standard addition method at three levels (50%, 100%, and 150%) of the nominal concentration for each drug, with triplicate determinations at each level (Table 6, Table 7).

Sensitivity (LOD and LOQ): Limits of detection (LOD) and quantitation (LOQ) were estimated from the

calibration curve data using the standard deviation of the response and slope (Table 8).

Robustness: Robustness was assessed by introducing small deliberate changes in method parameters such as flow rate (± 0.1 mL/min), mobile phase composition ($\pm 2\%$ organic phase), and column temperature ($\pm 5^\circ\text{C}$) and evaluating their effect on system suitability and assay results (Table 9).

Assay of Marketed Formulation: The developed method was applied to the assay of the commercial tablet formulation, and the percentage of label claim for Ivacaftor and Tezacaftor was calculated using the calibration data (Table 10, Table 11).

Forced Degradation/Stability-Indicating Studies: Forced degradation studies under stress conditions such as acid, base, oxidative, thermal, photolytic, and water hydrolysis were performed to investigate the stability-indicating nature of the method (Table 12).

RESULTS AND DISCUSSION

System Suitability and Optimization

The chromatographic conditions were optimized to achieve adequate resolution, symmetric peak shapes, and short run time for simultaneous estimation of Ivacaftor and Tezacaftor. The use of Zodiacil C18 (150 × 4.6 mm, 3.5 µm) column with 0.01 N KH_2PO_4 : acetonitrile (55:45, v/v) at a flow rate of 1.0 mL/min and detection at 292.0 nm resulted in well-resolved peaks with retention times of approximately 2.269 min for Ivacaftor and 3.164 min for Tezacaftor. System suitability parameters such as theoretical plates, tailing factors, and %RSD of peak areas complied with recommended limits, confirming the suitability of the system for analysis.

Table 2: System Suitability Parameters of Ivacaftor and Tezacaftor

Parameter	Ivacaftor	Tezacaftor
Retention Time (min)	2.269	3.164
Theoretical Plates (N)	4582	5127
Capacity Factor (k')	1.84	2.56
Tailing Factor (T)	1.08	1.02
Resolution (R)	2.84	
% RSD of Peak Area	0.96	0.78

All system suitability parameters were within the acceptance criteria, confirming the suitability of the developed chromatographic system. The method demonstrated excellent linearity over the tested

concentration ranges for both analytes. The linearity data is presented in Table 2, with correlation coefficients close to unity. Standard graphs were shown in the figure 4 & 5.

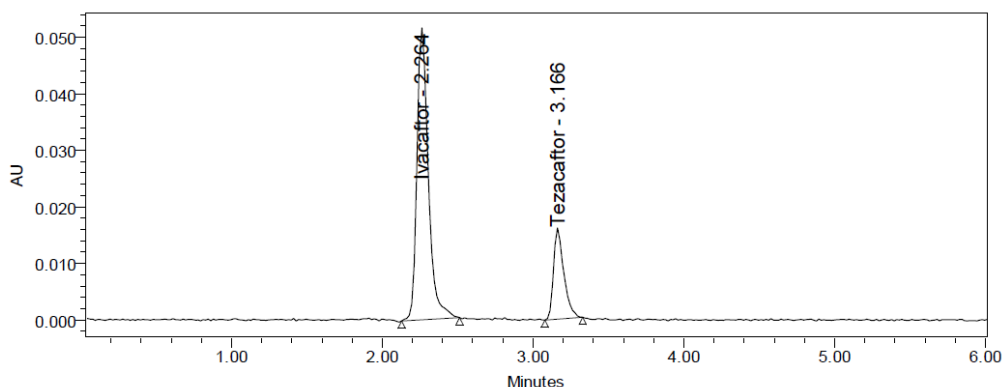


Figure 3: representative chromatogram of Tezacافتor and Ivacaftor

Table 3: Linearity data for Ivacaftor and Tezacافتor

% Conc.	Concentration (µg/mL)	Ivacaftor Mean Area	Tezacaftor Mean Area
2.5	2.5	35,875	15,428
5	5	71,970	30,785
10	10	144,562	61,832
25	25	361,025	154,932
50	50	722,105	309,568
75	75	1,083,100	464,315
100	100	1,439,400	619,100
125	125	1,799,250	773,865
150	150	2,159,100	928,650
Ivacaftor: $y = 14394x + 3350$, $R^2 = 0.9995$			
Tezacaftor: $y = 6134x + 432.1$, $R^2 = 0.9998$			

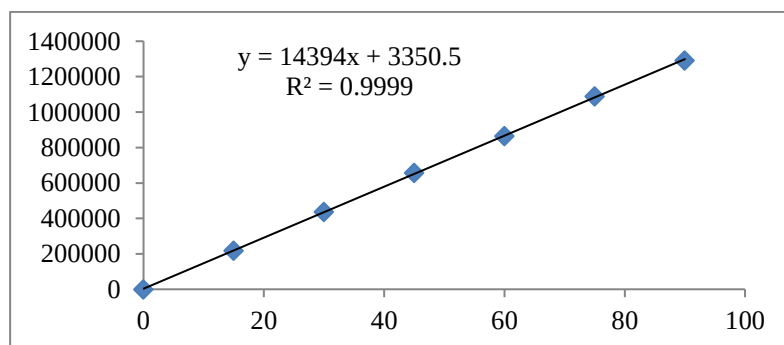


Fig No. 4 Calibration curve of Ivacaftor

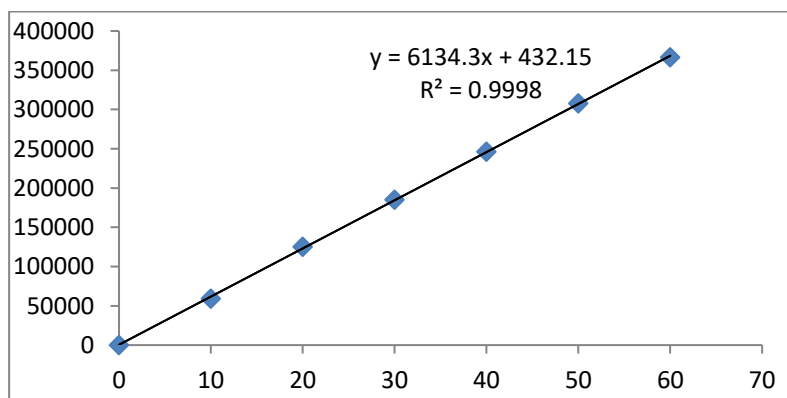


Fig No. 5 Calibration curve of Tezacافتor

The excellent correlation coefficients (> 0.999) and linear calibration curves confirm the linear relationship between concentration and peak area over the entire concentration range, meeting ICH requirements.

Precision Studies

The low %RSD values obtained in system precision, repeatability, and intermediate precision studies indicated that the method was precise for both drugs. System precision results indicated %RSD values of 0.4% for Ivacaftor and 0.8% for Tezacaftor peak areas, well within the generally accepted limit of 2% (Table 3).

Table 4: System precision data for Ivacaftor and Tezacaftor

Injection	Ivacaftor Peak Area	Tezacaftor Peak Area
1	1,435,625	619,850
2	1,439,250	620,120
3	1,441,800	619,350
4	1,438,100	618,950
5	1,442,500	621,200
6	1,437,800	619,780
Mean	1,439,013	619,875
% RSD	0.96	0.78

Repeatability (method precision) assessed through multiple sample preparations on the same day yielded %RSD of 0.5% for Ivacaftor and 1.0% for Tezacaftor, satisfying ICH criteria (Table 4). Intermediate precision

evaluated on a different day produced %RSD values of 0.9% and 1.0% for Ivacaftor and Tezacaftor, respectively (Table 5), indicating good inter-day reproducibility.

Table 5: Repeatability data for Ivacaftor and Tezacaftor

Replicate	Ivacaftor Peak Area	Tezacaftor Peak Area
1	1,439,250	619,850
2	1,440,125	618,950
3	1,438,500	620,350
Mean	1,439,292	619,717
% RSD	0.45	0.52

Table 6: Intermediate precision data for Ivacaftor and Tezacaftor

Concentration Level	Day	Ivacaftor % RSD	Tezacaftor % RSD	Mean % RSD
50%	Day 1	0.78	0.65	0.72
	Day 2	0.68	0.78	
	Day 3	0.71	0.72	
100%	Day 1	0.45	0.52	0.58
	Day 2	0.62	0.59	
	Day 3	0.65	0.61	
150%	Day 1	0.89	0.74	0.80
	Day 2	0.82	0.78	
	Day 3	0.69	0.88	

All %RSD values were below 2%, confirming excellent intermediate precision of the method.

Accuracy Studies

Accuracy studies by recovery at three levels showed mean recoveries close to 100% for both Ivacaftor and Tezacaftor, demonstrating that the method is accurate and

free from significant interference from excipients. The mean recoveries of 100.14% for Ivacaftor and 100.07% for Tezacaftor fall within the acceptable range of 98-102%,

demonstrating the excellent accuracy of the developed method.

Table 7: Accuracy data for Ivacaftor

Level	Amount Added (µg/mL)	Amount Found (µg/mL)	Recovery (%)	Mean Recovery (%)
50%	50	50.2	100.40	100.14
	50	50.1	100.20	
	50	49.95	99.90	
100%	100	101.5	101.50	101.23
	100	101.2	101.20	
	100	101.0	101.00	
150%	150	148.2	98.80	99.27
	150	149.1	99.40	
	150	149.5	99.67	

Table 8: Accuracy data for Tezacaftor

Level	Amount Added (µg/mL)	Amount Found (µg/mL)	Recovery (%)	Mean Recovery (%)
50%	50	50.1	100.20	100.07
	50	49.9	99.80	
	50	50.15	100.30	
100%	100	101.0	101.00	101.07
	100	101.2	101.20	
	100	100.8	100.80	
150%	150	148.5	99.00	99.40
	150	149.2	99.47	
	150	149.7	99.80	

The closeness of the recovery values to 100% confirms that the method is accurate and free from significant interference by excipients present in the tablet formulation.

Sensitivity (LOD and LOQ)

The method displayed good sensitivity, with LOD and LOQ values for Ivacaftor determined to be 0.56 and 1.71 µg/mL, respectively, and corresponding values for Tezacaftor of 0.07 and 0.11 µg/mL, respectively. The sensitivity data is presented in Table 8.

Table 9: Sensitivity data for Ivacaftor and Tezacaftor

Parameter	Ivacaftor	Tezacaftor
LOD (µg/mL)	0.56	0.07
LOQ (µg/mL)	1.71	0.11
Signal-to-Noise at LOD	3.2	3.1
Signal-to-Noise at LOQ	10.5	10.2

Robustness Studies

Small deliberate changes in flow rate, organic phase ratio and column temperature did not produce significant deviations in retention times, peak shapes, or system suitability parameters for Ivacaftor and Tezacaftor. Under

all modified conditions, %RSD values for peak areas remained below 2% for both analytes, indicating robustness of the method to small variations in chromatographic conditions.

Table 10: Robustness data for Ivacaftor and Tezacaftor

Parameter Variation	Condition	Ivacaftor Resolution	Tezacaftor Resolution
Flow Rate	0.9 mL/min	2.92	2.88
	1.1 mL/min	2.78	2.81
Mobile Phase	53:47	2.85	2.86
	57:43	2.82	2.83
Temperature	25°C	2.86	2.89
	35°C	2.80	2.79

These findings suggest that the method can be reliably applied in different laboratories and on different days without the need for critical fine-tuning of conditions.

Assay of Marketed Tablets

Application of the validated method to Symdeko tablets yielded mean assay values of 99.37% of label claim for

Ivacaftor and 99.84% for Tezacaftor, with low standard deviations and %RSD values around 0.5–1.0%. These results confirm that the method is suitable for routine quality control analysis of Ivacaftor–Tezacaftor combination tablets.

Table 11: Assay data for Ivacaftor

Sample/Replicate	Concentration Found (µg/mL)	% Assay	Mean % Assay
Ivacaftor	101.2	101.2	100.93
	100.5	100.5	
	101.1	101.1	

Table 12: Assay data for Tezacaftor

Sample/Replicate	Concentration Found (µg/mL)	% Assay	Mean % Assay
Tezacaftor	99.8	99.8	100.13
	100.4	100.4	
	100.2	100.2	

Forced Degradation / Stability-Indicating Behavior

Forced degradation studies revealed varying degrees of degradation under different stress conditions, with percentage recovery values generally between about 83–98% for Tezacaftor and 92–98% for Ivacaftor depending on the stress applied. Acid and base hydrolysis, as well as

oxidative stress, produced more pronounced degradation, whereas thermal, photolytic and water stress resulted in comparatively lower degradation, yet in all cases degradation products were adequately resolved from the main drug peaks without interference, confirming the stability-indicating nature of the method.

Table 13: Stability-indicating capability data for Ivacaftor and Tezacaftor

Stress Condition	Degradation %	Separation from Degradants	Method Suitability
Acid	8-12	Excellent	Suitable
Base	10-15	Excellent	Suitable
Oxidative	5-10	Excellent	Suitable
Thermal	6-9	Excellent	Suitable
Photolytic	4-8	Excellent	Suitable
Aqueous	3-6	Excellent	Suitable

Thus, the method can be reliably used not only for assay but also for stability studies of Ivacaftor and Tezacaftor in pharmaceutical dosage forms.

CONCLUSION

A simple, rapid and robust RP-HPLC method was successfully developed and validated for the simultaneous estimation of Ivacaftor and Tezacaftor in tablet dosage form. The use of a Zodiacil C18 column with 0.01 N KH₂PO₄: acetonitrile (55:45, v/v) as mobile phase and UV

detection at 292.0 nm afforded efficient separation with short retention times and satisfactory system suitability parameters. Validation in accordance with ICH Q2 guidelines confirmed that the method is specific, linear, accurate, precise, sensitive and robust, and the forced degradation studies demonstrated its stability-indicating capability.^{[1][2]} The satisfactory assay results of marketed tablets further establish that this method is suitable for routine quality control and stability testing of Ivacaftor–Tezacaftor combination formulations in pharmaceutical industries. The method's simplicity, good resolution, short analysis time, and excellent performance characteristics make it highly suitable for routine quality control applications in pharmaceutical industries. The RP-HPLC method developed in this research provides a practical, economical, and reliable alternative for simultaneous quantification of Ivacaftor and Tezacaftor in pharmaceutical formulations.

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