Research Article

Analytical Method Development for Ivacaftor in Bulk and Pharmaceutical Dosage Forms

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Received: 10-04-2026; **Revised:** 26-06-2026; **Accepted:** 02-07-2026; **Published online:** 20-07-2026.

ABSTRACT

A simple, precise, and reliable reverse-phase high-performance liquid chromatographic (RP-HPLC) method was developed and validated for the quantitative estimation of Ivacaftor in bulk and pharmaceutical dosage forms. Chromatographic separation was achieved using a Waters X-Bridge Phenyl C18 column (150 × 4.6 mm, 5 µm) with an isocratic mobile phase consisting of 0.2% trifluoroacetic acid buffer and acetonitrile in the ratio of 40:60 (v/v). The detection was carried out at 225 nm with a flow rate of 1.0 mL/min and a runtime of 10 minutes. Ivacaftor exhibited a well-resolved peak with a retention time of approximately 2.3 minutes. The method demonstrated excellent linearity over the concentration range of 10–50 µg/mL with a correlation coefficient (R^2) of 0.9998. The method was validated in accordance with ICH guidelines for parameters such as accuracy, precision, specificity, robustness, limit of detection (LOD), and limit of quantification (LOQ). The % recovery ranged between 100.05% and 100.95%, indicating high accuracy, while %RSD values were found to be less than 2%, confirming precision. The LOD and LOQ were found to be 0.06 µg/mL and 0.21 µg/mL, respectively. Forced degradation studies under various stress conditions demonstrated the stability-indicating capability of the method. The proposed method was successfully applied for the assay of Ivacaftor in marketed tablet formulations, with results in good agreement with labeled claims. Overall, the developed RP-HPLC method is simple, sensitive, accurate, and suitable for routine quality control analysis of Ivacaftor in pharmaceutical formulations as well as for stability studies.

Keywords: Cystic fibrosis, Validation, RP-HPLC & Chromatogram.

INTRODUCTION

Ivacaftor (IVF) is a quinolinone-3-carboxamide derivative that acts as a Cystic Fibrosis Transmembrane Regulator (CFTR) gene potentiator, enhancing the function of the CFTR gene [1]. The CFTR gene mutation in cystic fibrosis patients causes excess mucus in the lungs. IVF helps by improving airway hydration, decreasing mucus build up, enhancing mucus the clearance from lungs [2]. The United States Food and Drug Administration (USFDA) approved IVF in 2012 for patients with the G551D mutation. It's approved for use in cystic fibrosis sufferers aged 6 or older, with a 150 mg dose administered with a fat-containing meal [3]. The FDA approved the IVF drug, known by brand name Kalydeco or VX-7703, which is marketed by Vortex Pharma [4].

Cystic fibrosis (CF) is a progressive, autosomal recessive disorder caused by mutations in the CFTR (Cystic Fibrosis Transmembrane Conductance Regulator) gene, which leads to impaired chloride ion transport across epithelial cells [5]. This results in the accumulation of thick mucus in the lungs, pancreas, and other organs, causing recurrent infections and severe respiratory and digestive complications [6]. Recent advances in CF pharmacotherapy have introduced CFTR modulators such as Ivacaftor and Tezacaftor, which significantly improve clinical outcomes by enhancing the function of the defective CFTR protein [7]. The increasing clinical use of these modulators necessitates the development of highly specific, sensitive, and validated analytical methods for their accurate quantification in pharmaceutical formulations and biological matrices [8]. Techniques such as reversed-phase highperformance liquid chromatography (RP-HPLC), Ultraperformance liquid chromatography (UPLC), and Liquid chromatography-tandem mass spectrometry (LC-MS/MS) have emerged as indispensable tools for this purpose due to their precision, robustness, and high analytical throughput. Method validation must adhere to the International Council for Harmonisation (ICH) Q2(R1) guidelines, which define the essential validation parameters, including specificity, linearity, accuracy, precision, detection limit, quantitation limit, robustness, and system suitability [9, 10].

Ivacaftor, (Figure 1) which has the chemical name N-(2, 4-di-tertbutyl-5-hydroxyphenyl)-1, 4-dihydro-4-oxoquinoline-3-carboxamide. Ivacaftor is a hydrophobic molecule and most of it (approximately 99%) is bound to plasma proteins, mainly alpha 1-acid glycoprotein and albumin.

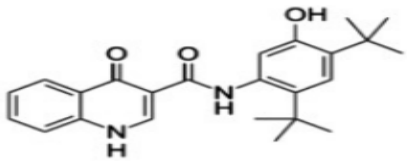


Figure 1: Structure of Ivacaftor

MATERIALS AND METHODS

Chemicals:

Pharmaceutical grade reference standards of Ivacaftor were obtained as gift samples from Pharma train, Hyderabad, India. Kaldeco 150mg Tablet was obtained from market Formulation. All chemicals were HPLC grade purchased from S. D. Fine Chem & Merck, Mumbai, India. Milli 'Q'water was used throughout the study.

Instrumentation:

Chromatographic analysis was carried out on Waters 2695 separation module (Waters Corporation, USA) equipped with auto sampler and waters 2998 PDA detector, and X-bridge Phenyl (150 4.6 mm, 5.6 m particle size) column.

PREPARATION OF BUFFER AND MOBILE PHASE:

Pipette 2 mL of Trifluoroacetic acid, which has a pH of 3.0, into 1000-mL volumetric flask and dissolve it using Orthophosphoric acid. Mixing the 400 mL (40%) of the buffer with 600 mL (60%) of Acetonitrile is necessary for preparing the mobile phase.

Diluent Preparation:

The Mobile Phase is used and was pre-prepared.

Standard solution Preparation:

25 mg of Ivacaftor working standard was taken into 25 mL flask, which is cleaned and dried, diluted and this stands as the Primary Stock. A 0.3 mL of the above was drawn up and diluted to get 30 µg/mL level solution.

Sample Solution Preparation:

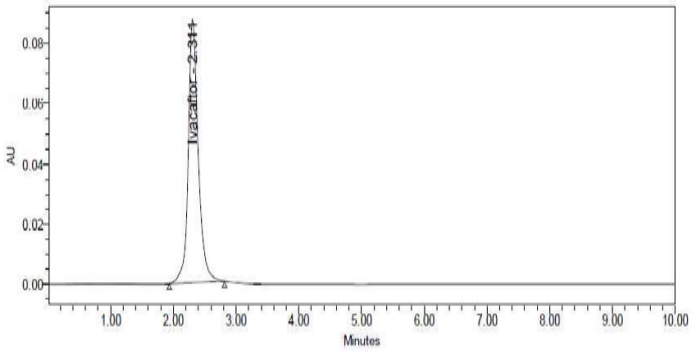
5 tablets of Ivacaftor formulation = 25 mg of the marketed tablet = 43.3 mg powder were weighed with accuracy and dissolved in a volumetric flask. The same solvent was added and make up the volume. Add 0.3 mL of above solution and diluted to 10 mL to get 30 µg/mL Ivacaftor.

Chromatographic trails:

Standard solutions (100µg/mL) of Ivacaftor were loaded in the injector and different trials were performed using different columns and experimental conditions. By using different mobile phase like 0.1% OPA: Methanol (40:60), 0.1% OPA: Acetonitrile (20: 80), 0.1% Trifluroacetic acid: Methanol (35:65), 0.1% Trifluroacetic acid: Methanol (45:55) & 0.2% Trifluroacetic acid buffer: Acetonitrile (40:60). By observing the peak resolution for the different mobile phase using UV detector. Optimized chromatographic condition was selected based on resolution of peak. Below are the following optimized conditions and chromatogram shown in Table 1 and Figure 2.

Table 1: Optimized Chromatographic Conditions

Parameter	Value
Column	Waters X-bridge phenylC18 column (150x4.6 mm,5 µm)
Mobile phase	0.2% Trifluroacetic acid buffer : Acetonitrile (40:60,v/v)
Elution mode	Isocratic
Detection wavelength	225 nm
Column temperature	Ambient
Volume of injection	20 µL
Run time	10 min.



	Peak Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Ivacaftor	2.311	962392	87554	1.13	3142.13

Figure 2: Optimized Chromatogram of Ivacaftor

METHOD VALIDATION:

The developed RP-HPLC method was validated for the linearity, precision, system suitability, robustness, and LOD and LOQ characteristics as per ICH guidelines [15].

Preparation of Stock Solution:

The 25 mg of Ivacaftor working standard was weighed into a 25mL volumetric flask, dissolve it completely and make volume up to the mark using diluents to get 1mg/mL solution. From stock solution, 0.3 mL was taken and made up to 10 mL with diluent to get 30 µg/mL solution.

Linearity:

From 30 µg/mL solution, the series of concentrations were prepared by taking 0.1; 0.2; 0.3; 0.4 & 0.5 mL of solution and diluting each to 10 mL with diluent to get the concentrations of 10; 20; 30; 40 & 50 µg/mL respectively.

These solutions were analyzed and the peak area was obtained from the respective chromatograms. The calibration curve was plotted with concentration on x-axis and the relevant peak area on y-axis.

Precision:

System Precision: The standard 30 µg/mL was injected 6 times and evaluated the %RSD for peak area of 6 injections using designed method.

Method Precision: Six different solutions of concentration 30 µg/mL were prepared and injected. The %RSD for peak area of 6 injections was evaluated using the current method.

Intermediate Precision: The standard solution of 30 µg/mL prepared was analyzed six times on next day and measured the %RSD for peak area of 6 injections.

The % RSD of obtained values were calculated to verify whether the method is precise or not.

Accuracy:

Accuracy is evaluated by 'Standard addition method', conducted at three levels of 50%, 100% and 150% of accuracy. These were analyzed in triplicate at each level as

per the proposed method. The % Recovery and % RSD were calculated at each accuracy level.

Preparation of Stand solution:

From 1mg/mL stock solution, 0.3 mL is taken in 10 mL, diluted to get 30 µg/mL concentration and used as standard.

Preparation Sample solutions:

The samples of 12.5 mg, 25 mg and 37.5 mg were taken each in 25 mL flasks and diluted with diluents. From these, each of 0.3 mL was taken and diluted to 10 mL and analyzed. The final concentrations of the solutions stand as 15 µg/mL, 30 µg/mL & 45 µg/mL.

Limit of Detection (LOD) and Limit of Quantification (LOQ):

The limit of Detection and limit of Quantification were determined by injecting progressively low concentrations of standard solutions using the proposed method. The smallest concentration of analyte at which it is detected is called Limit of Detection (LOD) (with S/N ratio of 3) and the least concentration of analyte where it is quantifiable is called Limit of Quantification (QL) (with S/N ratio 10).

Robustness:

This study was performed by making deliberate changes in the optimized chromatographic conditions such as organic Mobile phase percentage (± 10%, i.e., 50:50 (lesser organic) and 30:70 (more organic)) of 0.2% Trifluoroacetic acid buffer, pH-3.0: ACN, and flow rate (± 10%, i.e., 0.9 and 1.0 mL/min) using 30µg/mL of standard solution. Each experiment was repeated three times and the mean peak area was calculated from the resulting chromatograms obtained.

RESULTS AND DISCUSSION

In order to develop and validate a chromatographic method for simultaneous quantification of Ivacaftor by RP-HPLC, several trials were undertaken. Initially the drug solution was analyzed using a mixture of 0.2% Trifluroacetic acid buffer: Acetonitrile (40:60, v/v) at a flow rate of 1 mL/min, in which the peak resolution and symmetry were satisfactory. The proposed method obeyed linearity (Fig. 3) for Ivacaftor in the range of 10–50 µg/mL (r2=0.9998) and calibration data shown in table 2.

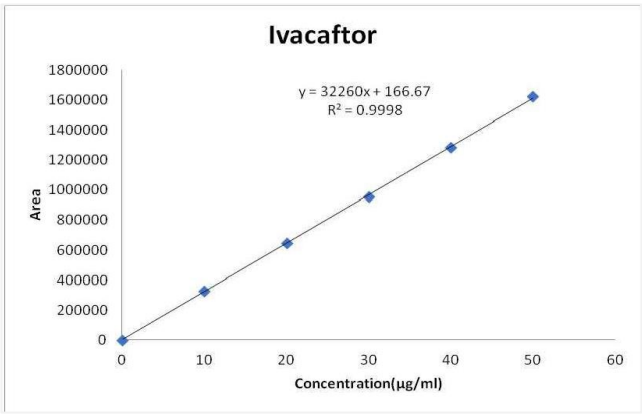


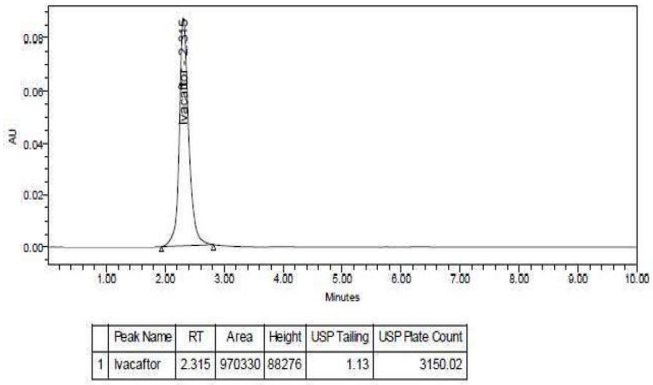
Figure 3: Calibration curve of Ivacaftor.

Table 2: Calibration data of Ivacaftor

S.No	Conc. (mcg/ml)	Average peak area	R1 (minutes)
1.	10	329394	2.311
2.	20	646880	2.321
3.	30	956645	2.328
4.	40	1282894	2.321
5.	50	1624112	2.318

Table 3: Assay of Tablet Components in Marketed Formulation

Brand Name	Label claim	Amount recovered (mg)	% Recovery
Kaldeco	150 mg	150.39	100.26



	Peak Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Ivacaftor	2.315	970330	88276	1.13	3150.02

Figure 4: Chromatogram of Assay marketed formulation

Specificity:

After testing blank and standard chromatograms, the technique proved to be specific since the elution patterns showed no interference from the excipients/ solvents (Refer Blank and Standard chromatograms from Figures 5 & 6).

Table 4: Data of specificity

S. No	Peak Name	Observation	
1	Blank	Nil	
2	Standard	R t = 2.307 min	ƛ max =225 nm

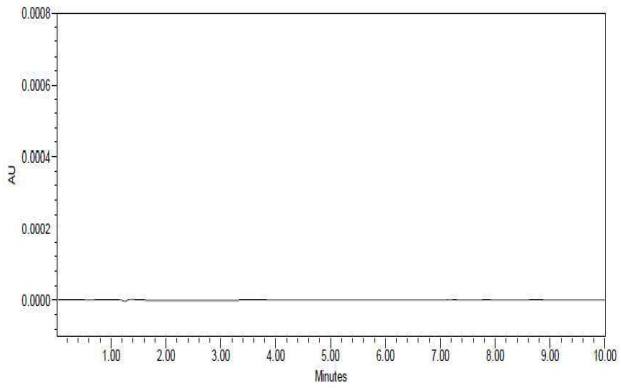


Figure 5: Chromatogram of blank solution

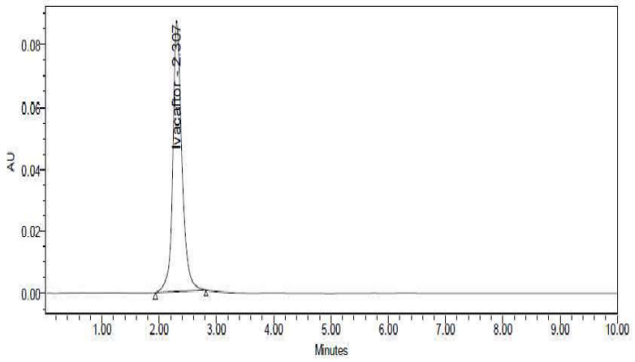


Figure 6: Chromatogram of standard solution

Linearity:

The system was linear for the Ivacaftor at the concentration range of 10-50 µg/mL, and the correlation coefficient was obtained as 0.999. The results of Linearity are given in Table 5.

Table 5: Results of linearity

S. No	Parameters	Ivacaftor
1	Slope	32260
2	Y intercept	166.67
3	Correlation coefficient R^2	0.9998
4	Regression equation	Y = 32260x + 166.67
5	Linearity range	10-50 µg/mL

System Suitability:

Based on the values obtained for all the parameters, the developed method was found to be reliable. The results are tabulated in Table 6.

Table 6: System suitability results

S.No	Parameters	Ivacaftor	Acceptance Criteria
1	Retention time (min)	2.3	As obtained
2	Theoretical plates (N)	3126.01	≥ 2000
3	Tailing factor (T)	1.13	≤ 2

Precision:

Ivacaftor satisfied the approval requirements with reproducibility of % RSD using current methods, which is meeting the acceptance criteria, i.e., ≤ 2.0% and results are tabulated in Table 7.

Accuracy:

The recovery rate was determined for Ivacaftor at 50%, 100%, and 150% recovery, and it was found to be 100.95%, 100.65%, and 100.05%, with a mean recovery of 100.55% and results are given below in Table 8 and the respective accuracy chromatograms are shown in Figures 7, 8 & 9.

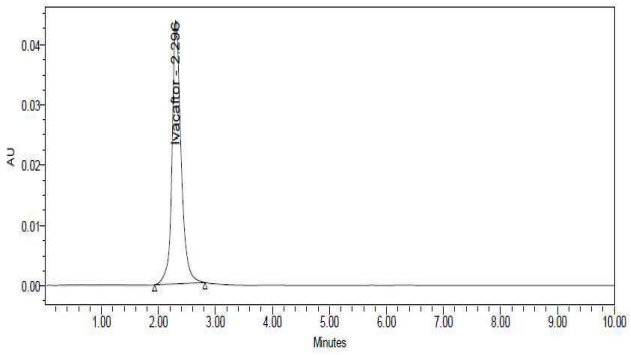


Figure 7: Chromatogram showing 50% Accuracy

Table 7: Results of Precision

Sample No.	Conc. (µg/mL)	System Precision (Repeatability)	Method Precision (Reproducibility)	Intermediate precision
Injection-1	30	965386	965977	962374
Injection-2	30	968864	970355	961365
Injection-3	30	963847	965846	963142
Injection-4	30	964837	968533	962846
Injection-5	30	963543	969586	959887
Injection-6	30	967454	969725	960875
Mean± SD		965655± 2097.9	968337±1968.3	961748.2±1258
% RSD		0.2	0.2	0.1

Table 8: Results of Accuracy

Conc. Taken (µg/mL)	Recovery level	Amount added (µg/mL)	Amount found (µg/mL)	Peak area (n=3)	% Recovery	% RSD
30	50%	12.5	12.50	483637.3	100.03	0.15
	100%	25	25.02	967634.3	100.07	
	150%	37.5	37.62	14550557	100.31	

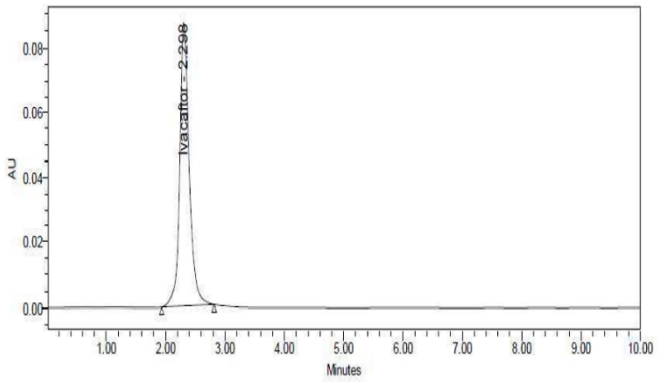


Figure 8: Chromatogram showing 100% Accuracy

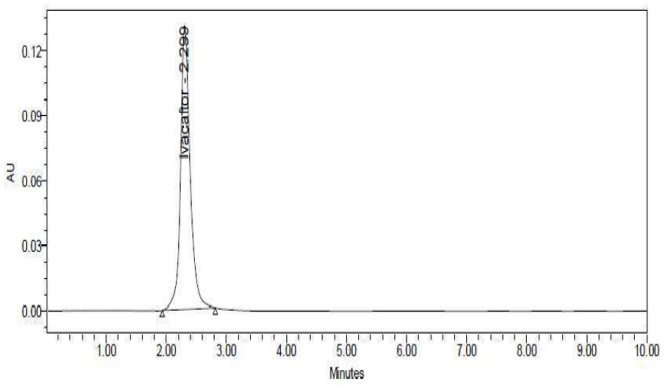


Figure 9: Chromatogram showing 150% Accuracy

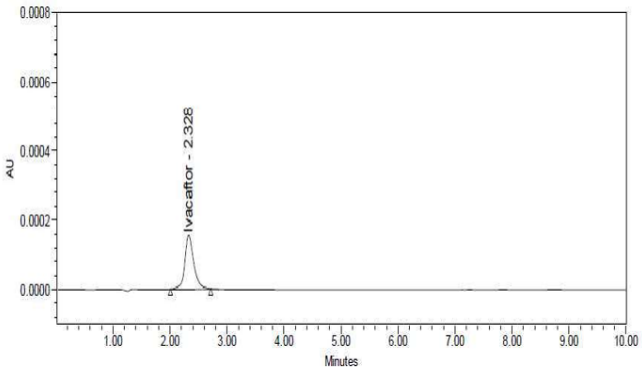


Figure 10: Chromatogram of LOD

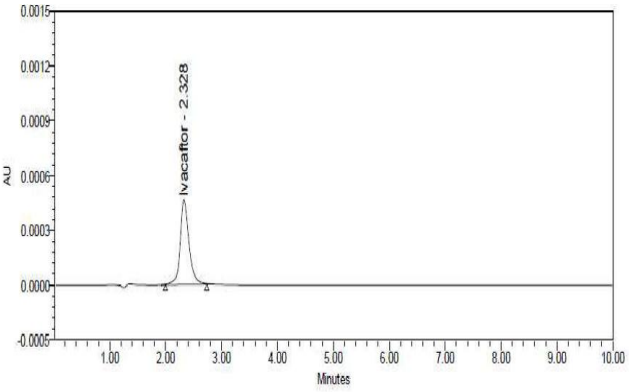


Figure 11: Chromatogram of LOQ

Table 9: Results of Robustness

Parameter	Condition	R_t (in min)	Plate Count	Tailing	Peak Area (*n=3)
Optimised	1.0mL/min, 0.2% Trifluro acetic acid Buffer (pH-3.0) Acetonitrile (40:60 v/v)	2.307	3129.42	1.14	963697
Flowrate	0.9mL/min	2.607	3512.17	1.31	967356
	1.1mL/min	2.153	3722.26	1.34	965293
Mobile Phase Composition	10% less organic 0.2% Trifluro acetic acid Buffer of pH 0 Acetonitrile (50:50v/v)	2.785	3529.24	1.56	961735
	10% more organic 0.2% Trifluroacetic acid Buffer of pH 0 Acetonitrile (30:70v/v)	2.199	3486.28	1.45	969538

Robustness:

Based on the findings above, it is clear that the technique was modified concerning flow rate variation (± 10%) and mobile phase composition (± 10%). The system appropriateness results satisfy the acceptance requirements and results are tabulated below in Table 9.

Limit of Detection and Limit of Quantification:

The results of LOD and LOQ obtained for Ivacaftor are as given below in Table 10 & Figures 10 & 11.

Table 10: Results of LOD & LOQ

Parameter	(µg/mL)	S/N Ratio	Area
LOD (µg/mL)	0.06	3	1912
LOQ (µg/mL)	0.21	10	6375

Table 11: Results of stability studies

Stress Conditions	Peak Area	% Degradation	% Recovery
Acidic / 0.1N HCl for 24hrs at room temperature	936498	2.96	97.04
Basic / 1N NaOH for 24hrs at room temperature	930638	3.57	96.43
Oxidative 3% v/v Hydrogen peroxide for 24 hrs at room temperature	927465	3.90	96.10
Thermal at 110 ° C for 24 hrs	922417	4.42	95.58
Photo: Sunlight for 24 hrs	935268	3.09	96.91

Stability Studies:

Ivacaftor resistance to various degradation routes is evaluated for the current project to understand its stability requirements better. Results are shown Table 11.

CONCLUSION

A simple precise and selective RP-HPLC method was developed for the determination of Ivacaftor. Chromatographic separation was achieved by using mobile phase consisting of a mixture of 0.2% Trifluroacetic acid buffer: Acetonitrile (40:60, v/v) on Waters X-bridge phenylC18 column (150x4.6 mm, 5 µm) column, with detection limit of 225 nm. Linearity was observed in the range of 10 - 50 µg / ml for Ivacaftor the amount of drugs estimated by the proposed methods was in good agreement with the label claim. The proposed method was validated. The accuracy of the methods was assessed by recovery studies at three different levels. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form. The proposed RP-HPLC (Reverse phase High Performance Liquid Chromatography) method has been evaluated for the accuracy, precision and linearity. The method was found to be precise, accurate and linear over the concentration range. The analytical method validation of Ivacaftor by RP-HPLC was found to be satisfactory and could be used for the routine pharmaceutical analysis of Ivacaftor. Method was validated as per ICH guidelines like system suitability, accuracy, precision, linearity, specificity, forced degradation studies, ruggedness, robustness, therefore, this HPLC method can be used as a routine analysis of these drugs in bulk, pharmaceutical formulations and also for stability studies.

Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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