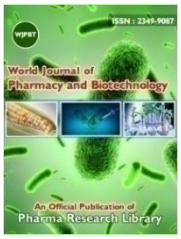

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Analytical Method for Simultaneous Estimation of Lumacftor and Ivacaftor in Pharamaceutical Dosage Form by Using RP-HPLC

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A B S T R A C T

A simple, precise, and robust reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Lumacaftor and Ivacaftor in bulk and pharmaceutical dosage forms in compliance with ICH Q2(R1) guidelines. Chromatographic separation was performed using an Agilent C18 column (4.5 × 150 mm, 5.0 µm) with a mobile phase of methanol and phosphate buffer (70:30 v/v) at a flow rate of 1.0 mL/min, and detection at 254 nm. The method exhibited excellent system suitability parameters, with a resolution of 5.23, tailing factors <2, and plate counts exceeding 2000 for both drugs. Linearity was established over the range of 20–100 µg/mL for Lumacaftor and 12.5–62.5 µg/mL for Ivacaftor, with correlation coefficients of 0.9996 and 0.9999, respectively. Precision studies showed %RSD values below 0.5%, and accuracy tests confirmed recoveries of 99.5% for Lumacaftor and 100.3% for Ivacaftor, all within the acceptance range of 98–102%. The limits of detection (LOD) were found to be 0.66µg/mL and 0.44 µg/mL, while the limits of quantification (LOQ) were 2.28µg/mL and 1.50 µg/mL for Lumacaftor and Ivacaftor, respectively. Robustness studies indicated no significant variation in performance with small deliberate changes in chromatographic conditions. The validated method is accurate, specific, sensitive, and reliable, making it suitable for routine quality control, regulatory compliance, and batch release testing of Lumacaftor and Ivacaftor in pharmaceutical formulations.

Keywords: Lumacaftor, Ivacaftor, cystic fibrosis therapy, simultaneous estimation, RP-HPLC validation, Agilent C18 column, methanol–phosphate buffer system, UV detection at 254 nm, sensitivity (LOD/LOQ), pharmaceutical quality control.

A R T I C L E I N F O

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1. Introduction

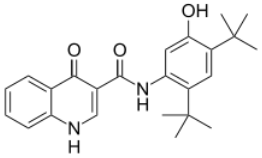

Fig.1: Ivacaftor

Table 1: Ivacaftor drug profile

IUPAC name	N-(2,4-di-tert-butyl-5-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide
Structural formula	$C_{24}H_2$, N_2O_3
Molecular weight	392.49072 g/mol

Solubility	Solubility: 0.002mg/ml in Water; practically insoluble in water and buffers at pH 7 Freely soluble in methyl ethyl ketone and water mixture Soluble in 2 methyl tetrahydro furan and PEG 400 Slighty soluble in ethanol methanol acetone.
pka	9.40-11.60
Category	cystic fibrosis transductase inhibitors
bioavalibility	1hour
Mechanism of action	Cystic fibrosis is caused by any one of several defects in a protein, cystic fibrosis transmembrane conductance regulator, which regulates fluid flow within cells and affects the components of sweat, digestive fluids, and mucus. The defect, which is caused by a mutation in the individual's DNA, can be in any of several locations along the protein, each of which interferes with a different function of the protein. One mutation, G551D, lets the CFTR protein reach the epithelial cell surface, but doesn't let it transport chloride through the ion channel. Ivacaftor is a potentiator of the CFTR protein. The CFTR protein is a chloride channel present at the surface of epithelial cells in multiple organs. Ivacaftor facilitates increased chloride transport by potentiating the channel-open probability (or gating) of the G551D-CFTR protein.

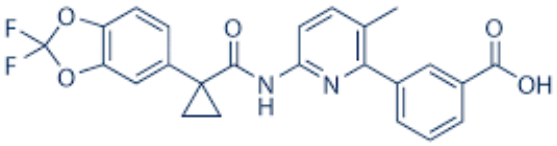

Fig.2: Lumacaftor

Table 2: Ivacaftor drug profile

IUPAC name	3-(6-(1-(2,2-di fluoro-1-3-benzodioxol-5-yl)cyclopropyl}carbonyl}amino}-3-methylpyridin-2-yl}benzoic acid.
Structural formula	C24H18F2N2O5
Molecular weight	452.41
Solubility	it is soluble in water and buffers at pH 1-8 Sparingly soluble in butanol
	freely soluble in formic acid and 2-methyl tetrahydrofuran
pka	Strong acidic (9.16)
Category	Cystic fibrosis transmembrane conductase regulator.
Mechanism of action	Orkambi is a combination of lumacaftor and ivacaftor, both of which are oral cystic fibrosis transmembrane conductance regulator (CFTR) modulators. The CFTR protein is a chloride channel present at the surface of epithelial cells in multiple.

2. Materials and Methods

Table 3: List of Materials Used

S.No	Instrument	Model
1	HPLC	WATERS, software: Empower, 2695 separation module.2487 UV detector.
2	UV/VIS spectrophotometer	LABINDIA UV 3000$^+$
3	pH meter	Adwa – AD 1020
4	Weighing machine	Afcoset ER-200A
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil

Table 4: Chemicals used

S.No	Chemical	Brand
1	Lumacaftor, Ivacaftor	Supplied by MSN LAB
2	KH_2PO_4	FINAR chemical LTD
3	Water and Methanol for HPLC	Standard solutions Ltd
4	Acetonitrile for HPLC	Standard solutions Ltd
5	HCl, H_2O_2, NaOH	MERCK

Wave length selection:

UV spectrum of 10µg/ml Lumacaftor and Ivacaftorin diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm and the isobestic λmax of both the drugs obtained at 225 nm.

Standard Solution Preparation of Lumacaftor:

Accurately weigh and transfer 10mg of Lumacaftor working standard into a 10ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)Further pipette 0.5ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Standard Solution Preparation of Ivacaftor:

Accurately weigh and transfer 10mg of Ivacaftor working standard into a 10ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make

volume up to the mark with the same solvent. (Stock solution). Further pipette 0.5ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Preparation of buffer and mobile phase:

Preparation of KH2PO4 Buffer pH 3.5:

To prepare KH2PO4 Buffer solution, by adding 6.8Grams of Potassium dihydrogen orthophosphate in 1000ml water. Adjust this solution to pH 3.5 by using sodium hydroxide.

Preparation of mobile phase:

Mix a mixture of above MEOH 700ml (70%), 300 ml KH2PO4 (30%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

MEOH: KH2PO4 PH 3.5 (700:300ml) ratio.

Optimization of Column:

Platisil C18 Column, (150×4.6mm, 3µm) was found to be ideal as it gave good peak shape and resolution at 1.0 ml/min flow.

Optimization of Chromatographic Conditions

Instrument: High performance liquid chromatography equipped with Auto Sampler and PDA or UV detector

Column : Agilent C18 (4.5×150 mm) 5.0 µm

Column temperature : Ambient

Wavelength : 254 nm

Mobile phase ratio:(70:30) methanol: phosphate buffer

Flow rate : 1 ml/min

Auto sampler temperature : Ambient

Injection volume :10µl

Run time :10.0 minutes

System Suitability:

- Tailing factor for the peaks due to Lumacaftor and Ivacaftor in Standard solution should not be more than 2.0.
- Theoretical plates for the Lumacaftor and Ivacaftor peaks in Standard solution should not be Less than 2000.

Calculation: (For Lumacaftor and Ivacaftor)

Calculation: (For Lumacaftor)

$$\%\ \text{Assay} = \frac{\text{AT}}{\text{AS}} * \frac{\text{WS}}{\text{DS}} * \frac{\text{DT}}{\text{WT}} * \frac{\text{Average weight}}{\text{Label Claim}} * \frac{\text{P}}{100} * 100$$

Where:

AT = average area counts of sample preparation.

AS = average area counts of standard preparation.

WS = Weight of working standard taken in mg.

P = Percentage purity of working standard

LC= Label Claim mg/ml.

Acceptance criteria of System Suitability:

Tailing factor should be less than 2

Theoretical Plates should be above 2000

Method validation parameters:

Assay:

Standard Solution Preparation:

Accurately weigh and transfer 20 mg of Lumacaftor and 10 mg Ivacaftor working standard into a 20 ml clean dry volumetric flasks add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.6ml of each of the above stock solutions into a 10ml volumetric flasks and dilute up to the mark with Diluents. (60ppm Lumacaftor and 37.5ppm Ivacaftor)

Sample Solution Preparation:

Accurately weigh and transfer 20 mg of Lumacaftor and 10 mg Ivacaftor working standard into a 20 ml clean dry volumetric flasks add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.6ml of each of the above stock solutions into a 10ml volumetric flasks and dilute up to the mark with Diluents. (60ppm Lumacaftor and 37.5ppm Ivacaftor)

Procedure:

Inject 20 µL of the standard, sample into the chromatographic system and measure the areas for the Lumacaftor and Ivacaftor peaks and calculate the % Assay by using the formulae.

Linearity:

Preparation of stock solution:

Accurately weigh and transfer 20 mg of Lumacaftor and 10 mg Ivacaftor working standard into a 20 ml clean dry volumetric flasks add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (20ppm of Lumacaftor and 12.5 ppm Ivacaftor):

0.2 ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Preparation of Level – II (40ppm of Lumacaftor and 25ppm Ivacaftor

0.4 ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Preparation of Level – III (60ppm of Lumacaftor and 37.5 ppm Ivacaftor

0.6ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Preparation of Level – IV (80ppm of Lumacaftor and 50 ppm Ivacaftor):

0.8ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Preparation of Level – V (100ppm of Lumacaftor and 62.5 ppm Ivacaftor):

1ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Procedure:

Inject each level into the chromatographic system and measure the peak area.

Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

Precision:

Preparation of stock Solution:

Accurately weigh and transfer 20 mg of Lumacaftor and 10 mg Ivacaftor working standard into a 20 ml clean dry volumetric flasks add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.6ml of each of the above stock solutions into a 10ml volumetric flasks and dilute up to the mark with Diluents.

Procedure:

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Intermediate precision/ruggedness:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day within the laboratory.

Preparation of stock solution:

Accurately weigh and transfer 20 mg of Lumacaftor and 10 mg Ivacaftor working standard into a 20 ml clean dry volumetric flasks add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.6ml of each of the above stock solutions into a 10ml volumetric flasks and dilute up to the mark with Diluents.

Procedure:

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Accuracy:

For accuracy determination, three different concentrations were prepared separately i.e. 50%, 100% and 150% for the analyte and chromatograms are recorded for the same.

Preparation of Standard stock solution:

Accurately weigh and transfer 20 mg of Lumacaftor and 10 mg Ivacaftor working standard into a 20 ml clean dry volumetric flasks add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.6ml of each of the above stock solutions into a 10ml volumetric flasks and dilute up to the mark with Diluents.

Preparation Sample solutions:

For preparation of 50% solution (With respect to target Assay concentration):

Accurately weigh and transfer 10 mg of Lumacaftor and 5 mg Ivacaftor working standard into a 20 ml clean dry volumetric flasks add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.6ml of each of the above stock solutions into a 10ml volumetric flasks and dilute up to the mark with Diluents.

For preparation of 100% solution (With respect to target Assay concentration):

Accurately weigh and transfer 20 mg of Lumacaftor and 10 mg Ivacaftor working standard into a 20 ml clean dry volumetric flasks add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)**.** Further pipette 0.6ml of each of the above stock solutions into a 10ml volumetric flasks and dilute up to the mark with Diluents. (60ppm Lumacaftor and 30ppm Ivacaftor)

For preparation of 150% solution (With respect to target Assay concentration):

Accurately weigh and transfer 30 mg of Lumacaftor and 15 mg Ivacaftor working standard into a 20 ml clean dry volumetric flasks add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)**.** Further pipette 0.6ml of each of the above stock solutions into a 10ml volumetric flasks and dilute up to the mark with Diluents..

Procedure: Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions. Calculate the Amount found and Amount added for Lumacaftor and Ivacaftor and calculate the individual recovery and mean recovery values.

Limit of detection:

Preparation of Lumacaftor and Ivacaftor solution:

Preparation of 0.66µg/ml solution:

Accurately weigh and transfer 20 mg of Lumacaftor working standard into a 20 ml clean dry volumetric flasks add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.6ml of each of the above stock solutions into a 10ml volumetric flasks and dilute up to the mark with Diluents. Further pipette 1 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent. Further pipette 1.1 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

Preparation of 0.44µg/ml solution:

Accurately weigh and transfer 12.5mg Ivacaftor working standard into a 20 ml clean dry volumetric flasks add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.6ml of each of the above stock solutions into a 10ml volumetric flasks and dilute up to the mark with Diluents. Further pipette 1 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent. Further pipette 1.16 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent

Limit of quantification:

Preparation of Lumacaftor and Ivacaftor solution:

Preparation of 2.28µg/ml solution: Accurately weigh and transfer 20 mg of Lumacaftor working standard into a 20 ml clean dry volumetric flasks add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.6ml of each of the above stock solutions into a 10ml volumetric flasks and dilute up to the mark with Diluents. Further pipette 4 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent. Further pipette 0.95 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

Preparation of 1.50µg/ml solution:

Accurately weigh and transfer 12.5 mg Ivacaftor working standard into a 20ml clean dry volumetric flasks add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.6ml of each of the above stock solutions into a 10ml volumetric flasks and dilute up to the mark with Diluents. Further pipette 2 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent. Further pipette 2 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

Robustness: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

The flow rate was varied at 0.8 ml/min to 1.2 ml/min: Standard solution 60µg/ml of Lumacaftor and 37.5µg/ml Ivacaftor prepared and analyzed using the varied flow rates along with method flow rate.

The Organic composition in the Mobile phase was varied from 63% to 77%: Standard solution 60µg/ml of Lumacaftor and 37.5µg/ml Ivacaftor was prepared and analyzed using the varied Mobile phase composition along with the actual mobile phase composition in the method.

3. Results and Discussion

Optimization of Column:

Agilent C18 (4.5×150 mm) 5.0 µm was found to be ideal as it gave good peak shape and resolution at 1.0 ml/min flow.

Optimization of Chromatographic Conditions

Column : Agilent C18 (4.5×150 mm) 5.0 µm
Column temperature : Ambient
Wavelength : 254 nm
Mobile phase ratio: (70:30) methanol: phosphate buffer
Flow rate : 1 ml/min
Auto sampler temperature : Ambient
Injection volume : 10µl
Run time : 10.0 minutes

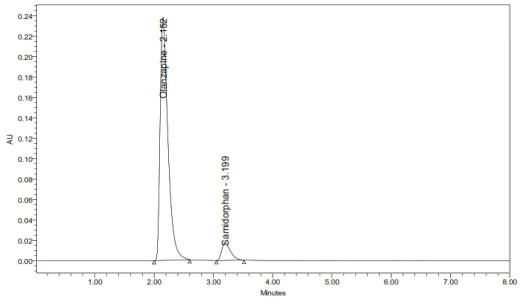

Fig.3: Optimized chromatogram

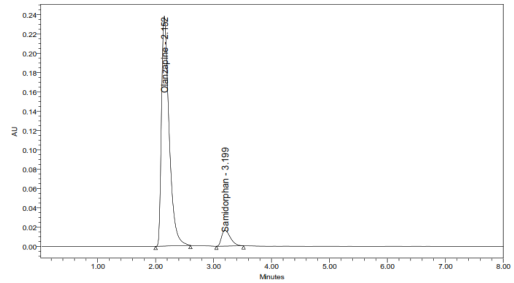

Fig.4: Chromatogram for system suitability

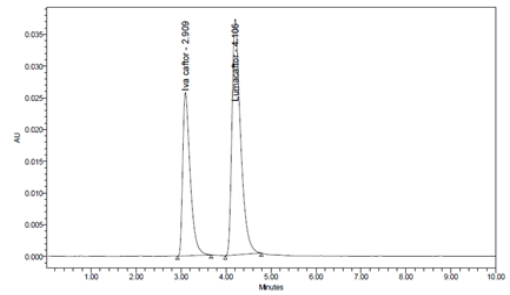

Fig.5: Chromatogram for Standard

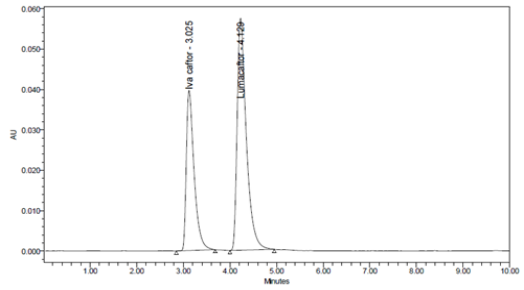

Fig.6: Chromatogram for Sample

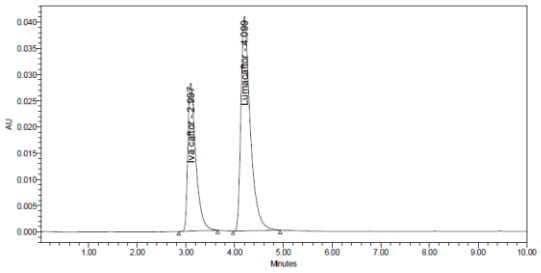

Fig.7: Chromatogram for linearity-5

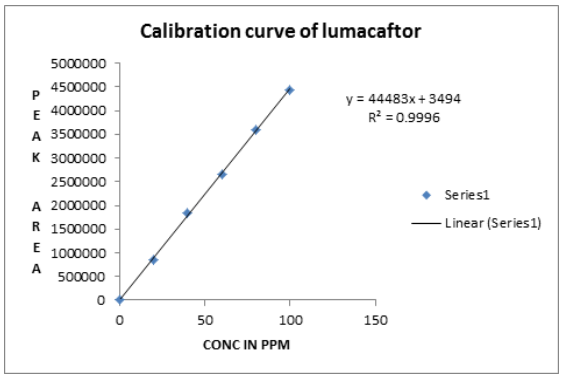

Fig.8: Calibration graph for Lumacaftor

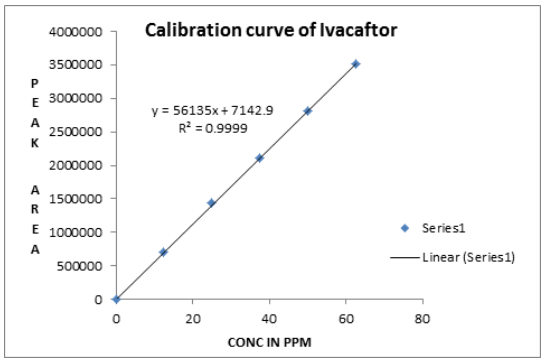

Fig.9: Calibration graph for Ivacaftor

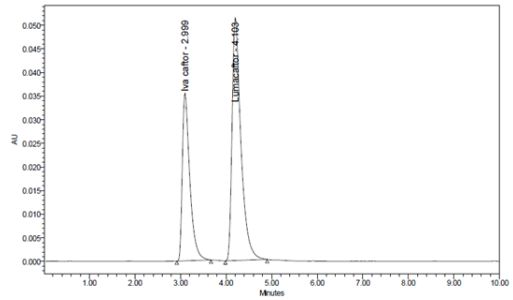

Fig.10: Chromatogram for Precision -6

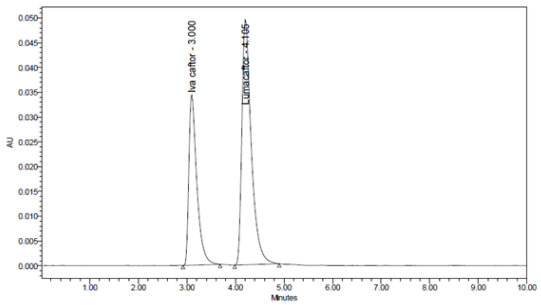

Fig.11: Chromatogram for ID Precision -6

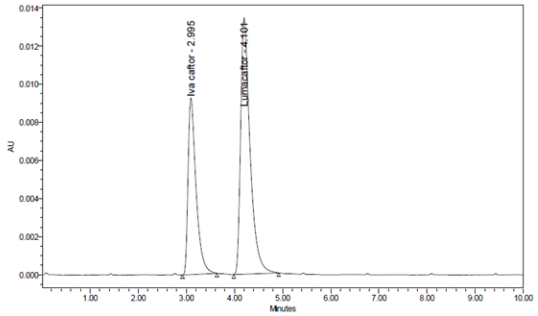

Fig.12: Chromatogram for Accuracy 50%-3

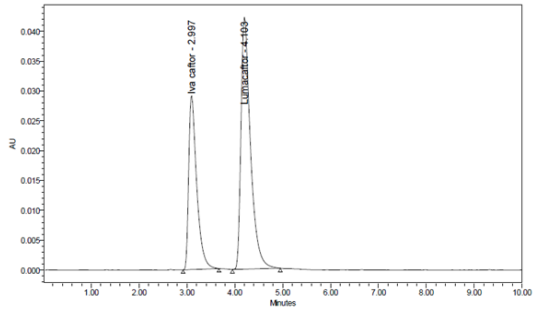

Fig.13: Chromatogram for Accuracy100%-3

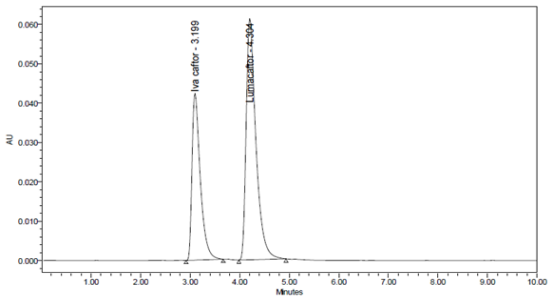

Fig.14: Chromatogram for Accuracy 150%-3

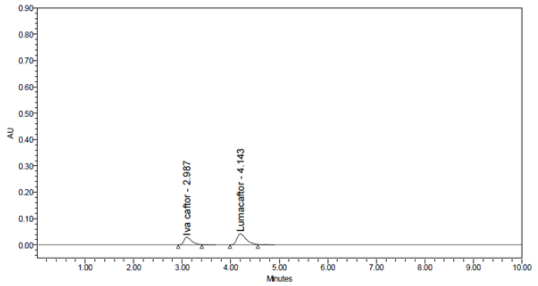

Fig.14: Chromatogram of Lumacaftor and Ivacaftor showing LOD

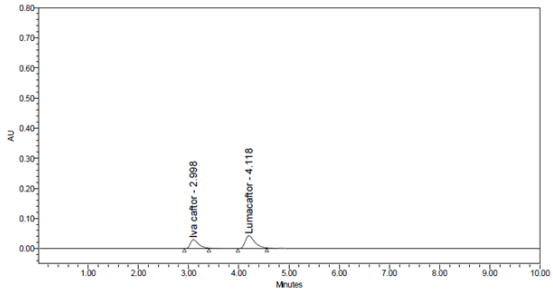

Fig.16: Chromatogram of Lumacaftor and Ivacaftor showing LOQ

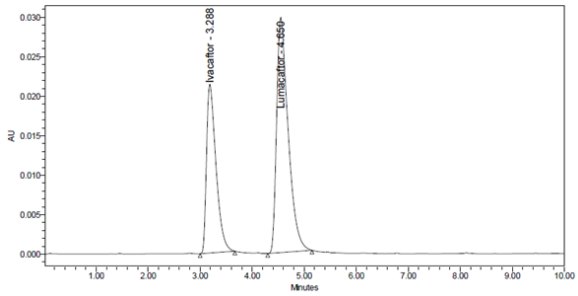

Fig.17: Chromatogram showing less flow

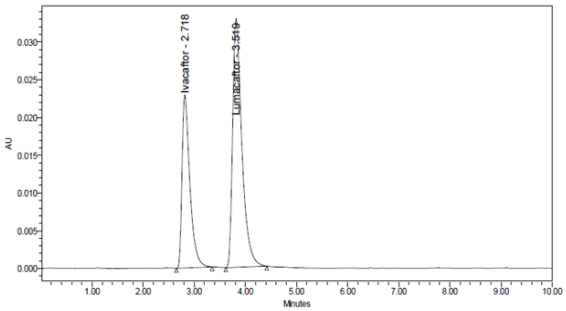

Fig.18: Chromatogram showing more flow

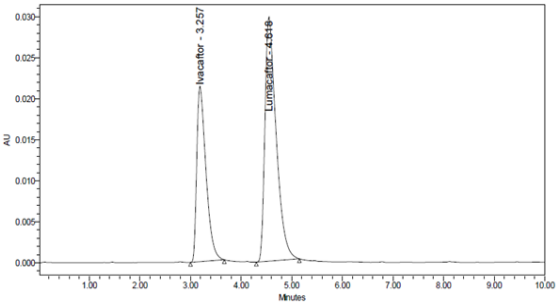

Fig.19: Chromatogram showing less organic composition

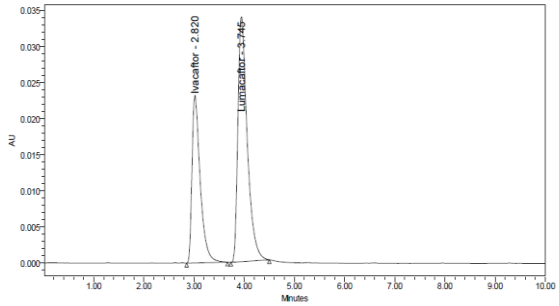

Fig.20: Chromatogram showing more organic composition

Table 5: Analytical performance parameters of Lumacaftor and Ivacaftor

Parameters	Lumacaftor	Ivacaftor
Slope (m)	44483	56135
Intercept (c)	3494	7142.9
Correlation coefficient (R^2)	0.9996	0.9999

Table 6: Results of Intermediate precision for Lumacaftor and Ivacaftor

Injection	Ivacaftor Area	Lumacaftor Area
Injection-1	2117452	2676523
Injection-2	2129563	2681458
Injection-3	2127852	2689851
Injection-4	2124589	2684263
Injection-5	2122358	2679854
Injection-6	2125872	2688542
Average	2124614.33	2683415.167
Standard Deviation	4311.47801	5146.602041
%RSD	0.20	0.19

Table 7: Accuracy (recovery) data for Lumacaftor and Ivacaftor

%Concentration of Ivacaftor	Area*	Amount Added (mg)	Amount Foun (mg)	% Recovery	Mean Recovery
50%	1067362	6.25	6.2	101.3	100.3
100%	2094251	12.5	12.4	99.4	
150%	3161512	18.75	18.75	100.1	

%Concentration of Lumacaftor	Area*	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	1352517	10	10.1	101.1	99.5
100%	2645236	20	19.8	98.9	
150%	3947542	30	29.5	98.4	

Table 19: Results for variation in flow for Lumacaftor and Ivacaftor

S. No	Flow Rate (ml/min)	System Suitability Results of Lumacaftor	
		USP Plate Count	USP Tailing
1	0.8	3652	1.04
2	1.0	3693	1.08
3	1.2	3610	1.1

S. No	Flow Rate (ml/min)	System Suitability Results of Ivacaftor	
		USP Plate Count	USP Tailing
1	**0.8**	6716	1.14
2	**1.0**	6780	1.01
3	**1.2**	6791	1.25

Table 20: Results for variation in mobile phase composition for Lumacaftor and Ivacaftor

S. No	Change in Organic Composition the Mobile Phase	System Suitability Results of Lumacaftor	
		USP Plate Count	USP Tailing
1	10% less(63ml)	3652	1.04
2	*Actual(70ml)	3693	1.08
3	10% more(77ml)	3610	1.1

S. No	Change in Organic Composition the Mobile Phase	System Suitability Results of Ivacaftor	
		USP Plate Count	USP Tailing
1	10% less(63ml)	6716	1.14
2	*Actual(70ml)	6780	1.01
3	10% more(77ml)	6791	1.25

4. Conclusion

A simple, precise, and robust RP-HPLC method was developed and validated for the simultaneous estimation of Lumacaftor and Ivacaftor in bulk and pharmaceutical dosage forms in accordance with ICH Q2(R1) guidelines. Chromatographic separation was achieved using an Agilent C18 column (4.5 × 150 mm, 5.0 µm) with a mobile phase consisting of methanol and phosphate buffer (70:30 v/v), at a flow rate of 1.0 mL/min, with detection at 254 nm. The method demonstrated excellent system suitability, with a resolution of 5.23, tailing factors within the acceptable limit (<2), and plate counts exceeding 2000 for both drugs. Linearity was observed in the range of 20–100 µg/mL for Lumacaftor and 12.5–62.5µg/mL for Ivacaftor, with correlation coefficients of 0.9996 and 0.9999, respectively. Precision studies yielded %RSD values below 0.5%, indicating high reproducibility. Accuracy studies confirmed mean recoveries of 99.5% for Lumacaftor and 100.3% for Ivacaftor, all within the acceptance range of 98–102%. Sensitivity evaluation revealed LOD values of 0.66 µg/mL and 0.44 µg/mL, and LOQ values of 2.28µg/mL and 1.50 µg/mL for Lumacaftor and Ivacaftor, respectively. Robustness testing showed no significant variation in results under minor changes in flow rate and mobile phase composition. The validated RP-HPLC method fulfills all ICH Q2(R1) validation requirements for specificity, linearity, accuracy, precision, LOD, LOQ, and robustness, confirming its suitability for routine quality control analysis of Lumacaftor and Ivacaftor in bulk and dosage forms. The method is highly sensitive, reliable, and reproducible, with good peak symmetry and short run times, making it both efficient and cost-effective for analytical laboratories. Its robustness under varied chromatographic conditions ensures consistent performance across different laboratory setups. This method can be confidently applied for routine pharmaceutical analysis, regulatory compliance, and batch release testing to ensure consistent product quality and therapeutic efficacy.

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