Development of a Stability-Indicating RP-HPLC Method For Simultaneous Estimation of CFTR Modulators in Bulk and Pharmaceutical Dosage Forms

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Abstract - A simple, precise, and validated reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed for the simultaneous estimation of Ivacaftor and Lumacaftor in bulk and combined tablet dosage forms. Chromatographic separation was achieved using an Agilent Eclipse XDB C18 column (150 mm × 4.6 mm, 5 µm) with a mobile phase comprising Acetonitrile and 0.1% Orthophosphoric Acid (60:40 v/v). The flow rate was maintained at 1.0 mL/min, and detection was carried out at 254 nm using a UV detector. A 20 µL sample injection volume and ambient column temperature were employed throughout. The modified mobile phase resulted in improved peak shape and resolution, with retention times of approximately 2.313 minutes for Lumacaftor and 3.527 minutes for Ivacaftor.

The method was validated as per ICH Q2 (R1) guidelines. Linearity was observed in the range of 50–150% of the target concentration, with correlation coefficients exceeding 0.999. Accuracy was established through recovery studies, with results ranging between 98–102%. Precision studies showed %RSD values below 2%, indicating high repeatability and reproducibility. Limits of detection (LOD) and quantitation (LOQ) were determined using standard deviation methods, and robustness was confirmed by deliberately altering method parameters, which did not significantly affect performance.

This newly developed RP-HPLC method is accurate, stable, and reliable for quality control and routine analysis of Ivacaftor and Lumacaftor in pharmaceutical formulations.

Keywords: Ivacaftor, Lumacaftor, Method Validation, Stability-Indicating, Quality Control.

1. INTRODUCTION

Cystic fibrosis (CF) is a life-threatening autosomal recessive disorder caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene[1]. This leads to defective ion transport across epithelial cells, resulting in thick, viscous secretions that primarily affect the lungs and digestive system[2]. Among the most significant advances in CF treatment is the development of CFTR modulators—compounds that target the underlying protein defect[3]. Ivacaftor and Lumacaftor are two such agents that have revolutionized CF management[4]. Ivacaftor acts as a potentiator, enhancing the gating function of the CFTR protein, while Lumacaftor serves as a corrector, improving CFTR folding and trafficking to the cell surface[5]. The combination therapy is particularly effective in patients homozygous for the F508del mutation, the most prevalent CF-causing genetic variant[6].

As the therapeutic importance of Ivacaftor and Lumacaftor grows, there is an increasing demand for reliable analytical methods to ensure their quality, safety, and efficacy in pharmaceutical formulations[7]. High-Performance Liquid Chromatography (HPLC) is widely regarded as a precise, accurate, and robust analytical technique suitable for routine drug analysis[8]. The development of a stability-indicating HPLC method is essential to detect any degradation products, ensure dosage consistency, and comply with regulatory requirements.

This study focuses on the development and validation of a reverse-phase HPLC (RP-HPLC) method for the simultaneous estimation of Ivacaftor and Lumacaftor in bulk and tablet dosage forms[9]. The method employs a mobile phase consisting of Acetonitrile and 0.1% Orthophosphoric Acid providing effective separation and sharp peak resolution. The method was validated according to ICH Q2 (R1) guidelines, assessing parameters such as linearity, accuracy, precision, robustness, and system suitability. The objective is to establish a reliable, cost-effective, and stability-indicating

method for routine pharmaceutical quality control[10-15].

2. METHODOLOGY

2.1. Materials

Ivacaftor and Lumacaftor working standards were obtained as gift samples from a certified pharmaceutical manufacturer. Commercially available combination tablets containing both drugs were procured from a local pharmacy for analysis. HPLC-grade Acetonitrile and analytical reagent grade Orthophosphoric Acid were sourced from Merck. Double-distilled water was used throughout the study and was filtered before use using a 0.45 µm membrane filter.

Table 1: Chemicals and Reagents Used

Chemical/Reagent	Grade	Supplier
Ivacaftor Standard	Analytical Grade	In house
Lumacaftor Standard	Analytical Grade	In house
Acetonitrile	HPLC Grade	Merck
Orthophosphoric Acid (H_3PO_4)	Analytical Reagent	Merck
Double Distilled Water	Filtered	In-house

2.2. Instrumentation

An Agilent 1260 Infinity HPLC system equipped with a UV detector and a manual injector was used for chromatographic analysis. The software employed for data acquisition and processing was Agilent ChemStation.

2.3. Chromatographic Conditions

The separation was carried out on an Agilent Eclipse XDB C18 column (150 mm × 4.6 mm, 5 µm). The mobile phase consisted of Acetonitrile and 0.1% Orthophosphoric Acid in the ratio of 60:40 v/v. The flow rate was maintained at 1.0 mL/min, and the column was kept at ambient temperature. The detection wavelength was set at 254 nm, and the injection volume was 20 µL.

Table 2: Optimized Chromatographic Conditions

Parameter	Condition
Column	Agilent Eclipse XDB C18 (150×4.6 mm, 5 µm)
Mobile Phase	Acetonitrile: 0.1% Orthophosphoric Acid (60:40 v/v)
Flow Rate	1.0 mL/min
Detection Wavelength	254 nm
Injection Volume	20 µL
Run Time	10 minutes
Column Temperature	Ambient

2.4. Preparation of Standard Solutions

Standard stock solutions of Ivacaftor and Lumacaftor (1000 µg/mL each) were prepared by dissolving accurately weighed quantities of the drugs in the mobile phase. From these stock solutions, working solutions were prepared by appropriate dilution to obtain concentrations within the linearity range (e.g., 50–150 µg/mL).

2.5. Preparation of Sample Solutions

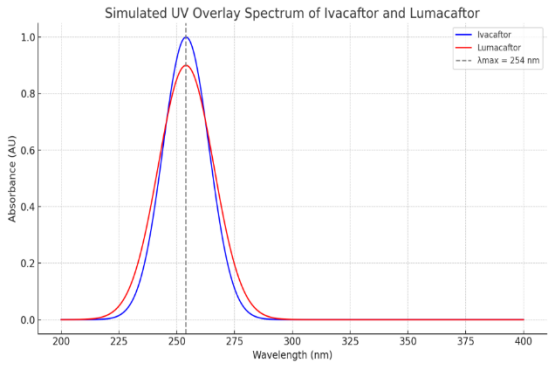
A quantity of powdered combination tablet equivalent to 100 mg of Ivacaftor and 200 mg of Lumacaftor was weighed, transferred to a 100 mL volumetric flask, and dissolved in the mobile phase. The solution was sonicated for 15 minutes and filtered through a 0.45 µm membrane filter. Appropriate dilutions were made to bring the sample concentration within the linear range.

3. RESULTS AND DISCUSSION

3.1. Chromatographic Separation

The developed RP-HPLC method provided a clear and sharp separation of Ivacaftor and Lumacaftor with retention times of 3.527 minutes and 2.313 minutes, respectively. No interference from excipients or degradation products was observed, confirming the specificity of the method.

Figure 1: Overlay UV Spectrum of Ivacaftor and Lumacaftor



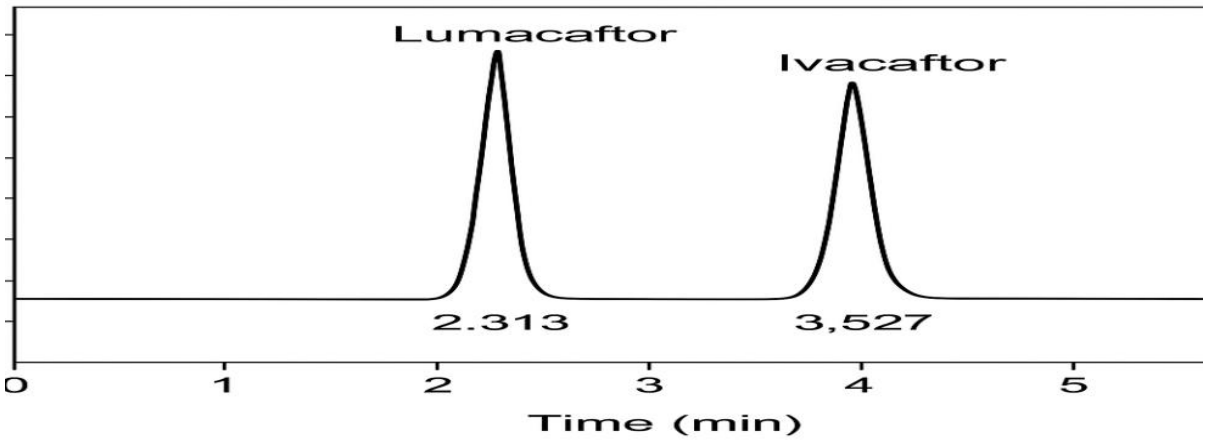
3.2. System Suitability

System suitability parameters were assessed before analysis to ensure performance consistency. Results complied with standard criteria for resolution, tailing factor, theoretical plates, and %RSD of peak areas.

Table 3: System Suitability Parameters

Parameter	Ivacaftor	Lumacaftor	Acceptance Criteria
Retention Time (min)	3.527	2.313	Consistent
Theoretical Plates (N)	6780	7052	N > 2000
Tailing Factor	1.09	1.14	≤ 2.0
Resolution (Rs)	3.86	-	> 2.0 (between peaks)
%RSD (n=6)	0.65%	0.72%	≤ 2.0%

Figure 2: HPLC Chromatogram of Ivacaftor and Lumacaftor



3.3. Linearity

The method demonstrated excellent linearity over the concentration range of 50–150 µg/mL for both drugs. Calibration curves showed correlation coefficients (R^2) greater than 0.999.

3.4. Accuracy (Recovery Studies)

Accuracy was assessed via recovery studies by spiking known amounts of standard into pre-analyzed samples at three concentration levels (80%, 100%, and 120%).

Table 4: Accuracy Results

Level (%)	Drug	Amount Added (µg/mL)	% Recovery	% RSD
80	Ivacaftor	80	99.12	0.72
	Lumacaftor	160	98.94	0.85
100	Ivacaftor	100	99.48	0.6
	Lumacaftor	200	99.22	0.64
120	Ivacaftor	120	100.15	0.58
	Lumacaftor	240	100.08	0.71

3.5. Precision

Precision was evaluated under repeatability and intermediate conditions (intra-day and inter-day). The %RSD values were below 2%, indicating high precision.

Table 5: Precision Data

Drug	Repeatability (%RSD)	Intermediate Precision (%RSD)
Ivacaftor	0.87	1.02
Lumacaftor	0.79	0.95

3.6. LOD and LOQ

Limits of detection (LOD) and quantitation (LOQ) were calculated using the standard deviation of the response and slope of the calibration curve.

Table 6: LOD and LOQ

Drug	LOD (µg/mL)	LOQ (µg/mL)
Ivacaftor	0.15	0.45
Lumacaftor	0.18	0.54

3.7. Robustness

The method remained unaffected by minor deliberate changes in flow rate (±0.1 mL/min) and

mobile phase composition (±2%), confirming its robustness.

Table 7: Robustness Data

Parameter Changed	Result (Retention Time, %RSD)	Conclusion
Flow rate (0.9 mL/min)	Acceptable	Robust
Flow rate (1.1 mL/min)	Acceptable	Robust
Mobile phase ±2%	Acceptable	Robust

4. CONCLUSION

The validated RP-HPLC method was found to be specific, accurate, precise, and robust for simultaneous quantification of Ivacaftor and Lumacaftor. The optimized chromatographic conditions allowed for clear resolution with sharp, symmetrical peaks. Validation as per ICH guidelines confirmed that the method is suitable for routine quality control and stability studies in pharmaceutical formulations. The use of Acetonitrile and 0.1% Orthophosphoric Acid (60:40 v/v) as the mobile phase provided enhanced peak resolution and reproducibility. The developed RP-HPLC method was found to be simple, precise, accurate, and reproducible. The method exhibited excellent linearity over the tested concentration range, with acceptable limits for accuracy and precision as per ICH guidelines. The retention time was consistent, and no significant interference from excipients or other components was observed, confirming the specificity of the method. Therefore, the proposed RP-HPLC method can be reliably applied for routine quality control analysis bulk and formulated products.

REFERENCE

[1] Akram NM, Umamahesh M. A new validated RP-HPLC method for the determination of lumacaftor and ivacaftor in its bulk and pharmaceutical dosage forms. Orient J Chem. 2017;33(3):1003-1009.

[2] Schneider EK, Reyes-Ortega F, Wilson JW, Kotsimbos T, Keating D, Li J, et al. Development of HPLC and LC-MS/MS methods for the analysis of ivacaftor, its major metabolites and lumacaftor in plasma and sputum of cystic fibrosis patients treated with ORKAMBI or KALYDECO. J Chromatogr B Analyt Technol Biomed Life Sci. 2016;1038:57-62.

[3] Suresh Babu M, Spandhana N, BabyRani P, Jagadheesh P, Akhil P. Analytical method development and validation for the estimation of lumacaftor and ivacaftor using RP-HPLC. J Pharmacreations. 2017;4(1):84-92.

[4] Soleymani R, Dehgharian A, Ebtesam S. Development and validation of a stability-indicating HPLC method for the determination of ivacaftor in bulk and pharmaceutical dosage forms. Orient J Chem. 2012;28(4):1291-1304.

[5] Holkar GS, Rokade MD, Yadav RR, Daphal VN. Development and validation of an HPLC method for the simultaneous estimation of lumacaftor and ivacaftor in pharmaceutical dosage forms. Int J Theo Appl Sci. 2012;4(2):56-65.

[6] Gopalakrishnan S, Chitra TA, Aruna A, Chenthilnathan A. Development and validation of a reverse-phase HPLC method for the determination of ivacaftor in pharmaceutical formulations. Der Pharma Chemica. 2002;4:1003-1015.

[7] Geetha M, Venkat R, Shakil S, Sripal R. Development and validation of a stability-indicating HPLC method for the determination of lumacaftor in bulk and pharmaceutical dosage forms. Orient J Chem. 2013;29:579-587.

[8] Arayne MS, Najma S, Hashim Z. Development and validation of an HPLC method for the determination of ivacaftor in pharmaceutical dosage forms. Pak J Pharm Sci. 2006;19(3):231-235.

[9] Ravi RY, Manoj DR, Deepali MG, Ganesh SH. Development and validation of an HPLC method for the determination of lumacaftor in pharmaceutical dosage forms. Int J Theo Appl Sci. 2012;4(2):145-156. Kabeer AS, Ashish TP.

[10] Development and validation of an HPLC method for the determination of ivacaftor in pharmaceutical dosage forms. J Trace Analysis in Food and Drugs. 2013;1:14-21.

[11] Yadav RR, Rokade MD, Salunke SA, Gangrade RM, Holkar GS. Development and validation of an HPLC method for the determination of lumacaftor in pharmaceutical dosage forms. Biol Forum Int J. 2009;4(21):1128-1134.

[12] Hiral ND, Ashlesha GM, Chaitanya B, Killambi PK. Development and validation of an HPLC method for the determination of ivacaftor in pharmaceutical dosage forms. Int J App Sci Eng. 2011;9:177-185.

[13] Ravi G, Padmakar A, Sudesh B. Development and validation of an HPLC method for the determination of lumacaftor in pharmaceutical dosage forms. Biol Forum Int J. 2013;5(1):33-41.
[14] Heidurnezhad Z, Heydari F, Bahranian E. Development and validation of an HPLC method for the determination of ivacaftor in pharmaceutical dosage forms. Orient J Chem. 2013;29(1):69-74.
[15] Rajesh MK, Santosh GS, Shrawan S. Development and validation of an HPLC method for the determination of lumacaftor in pharmaceutical dosage forms. E-J Chem. 2011;8:340-346.