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Original Article

SIMULTANEOUS ESTIMATION OF IVACAFTOR AND LUMACAFTOR IN TABLET DOSAGE FORM BY RP-HPLC METHOD

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ABSTRACT

A *simple, accurate, and precise method was developed for the simultaneous estimation of Lumacaftor and Ivacaftor in tablet dosage form. The method uses a C18 column with a mobile phase of 0.1% triethylamine in acetonitrile. The retention times of Lumacaftor and Ivacaftor were 2.269 and 3.164 minutes, respectively. The %RSD for both drugs was less than 1%, and the %recovery was 100% for both drugs. The LOD and LOQ values were 0.56 and 1.71 µg/ml for Lumacaftor, and 0.07 and 0.11 µg/ml for Ivacaftor. The method was validated according to ICH guidelines, and it was found to be simple, economical, and suitable for routine quality control testing. The method was developed using a laboratory-grade HPLC system. However, it is likely that the method could be adapted for use with a commercial HPLC system. The method is simple and straightforward, and it does not require any specialized equipment. Therefore, it is a cost-effective method that can be used for routine quality control testing.*

Key words: *Lumacaftor, Evacaftor, stability indicating, RP- HPLC*

INTRODUCTION

Ivacaftor and lumacaftor are drugs that are used to treat cystic fibrosis (CF). CF is a genetic disorder that affects the lungs, pancreas, and other organs. Ivacaftor works by increasing the activity of the CFTR protein, which is a chloride channel that helps to transport chloride and sodium ions across cell membranes. Lumacaftor helps to stabilize the CFTR protein so that it can function properly. There are a number of HPLC methods that have been published for the estimation of ivacaftor and lumacaftor, both individually and in combination with other drugs. However, there are no methods published to date for the simultaneous estimation of ivacaftor and lumacaftor by HPLC. This study aimed to develop a simple, accurate, precise, sensitive, selective, reproducible, and rapid analytical technique for the simultaneous estimation of ivacaftor and lumacaftor in tablet dosage form. The method developed in this study uses a C18 column with a mobile phase of acetonitrile and 0.1% triethylamine. The retention times of Ivacaftor and Lumacaftor were 2.269 and 3.164 minutes, respectively. The %RSD for both drugs was less than 1%, and the %recovery was 100% for both drugs. The LOD and LOQ values were 0.56 and 1.71 µg/ml for lumacaftor, and 0.07 and 0.11 µg/ml for ivacaftor. The method was validated according to ICH guidelines, and it was found to be simple, economical, and suitable for routine quality control testing. The results of this study demonstrate that the developed method is a simple, accurate, and precise method for the simultaneous estimation of ivacaftor and lumacaftor in tablet dosage form. The method is suitable for routine quality control testing, and it could also be used for research purposes..

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2. MATERIALS AND METHODS

2.1. Ivacaftor and lumacaftor are pure drugs (APIs) that were received from Aurobindo Pharma Ltd. The combined form of ivacaftor and lumacaftor tablets (Orkambi) were also used in this study. Acetonitrile, phosphate buffer, methanol, triethylamine, potassium dihydrogen orthophosphate buffer, and ortho-phosphoric acid were all used in this study. All of these chemicals and solvents were obtained from Merck.

2.2. Solutions:

2.2.1. Stock solutions:

To prepare the standard stock solutions, accurately weigh 10 mg of ivacaftor and 10 mg of lumacaftor and transfer them to a 10-mL volumetric flask. Add diluent to the flask until it is ¾ full, then sonicate the solution for 5 minutes. Add diluent to the flask until the volume reaches the mark, then label the flask as the "standard stock solution". To prepare the working solutions, pipette 1 mL of the standard stock solution into a 10-mL volumetric flask. Add diluent to the flask until the volume reaches the mark, then label the flask as the "working solution". The working solution will contain 125 µg/mL of ivacaftor and 200 µg/mL of lumacaftor. The diluent used in this procedure can be any solvent that is compatible with ivacaftor and lumacaftor. The sonicator used in this procedure should be set to a power level that is sufficient to dissolve the powders but not so high that it causes the solutions to foam. The working solutions should be stored in a refrigerator and used within 1 week.

2.2.2. Samples Preparation

To prepare the sample solution, weigh 20 tablets and calculate the average weight of each tablet. Transfer the weight equivalent to 1 tablet into a 200-mL volumetric flask. Add 100 mL of diluent and sonicate the solution for 20 minutes. Add diluent to the flask until the volume reaches the mark, then filter the solution using HPLC filters. The sample solution will contain 1250 µg/mL of ivacaftor and 2000 µg/mL of lumacaftor. The diluent used in this procedure can be any solvent that is compatible with ivacaftor and lumacaftor. The sonicator used in this procedure should be set to a power level that is sufficient to dissolve the powders but not so high that it causes the solutions to foam. The sample solution should be filtered immediately before use to remove any particulate matter that may interfere with the analysis.

2.2.3. Cc standards:

To prepare the calibration curve standards, pipette suitable aliquots from the standard stock solutions (1250 µg/mL of ivacaftor and 2000 µg/mL of lumacaftor) into separate 10-mL volumetric flasks. Add diluent to each flask until the volume reaches the mark. Label the flasks accordingly. The calibration curve standards will contain 62.5 µg/mL to 187.5 µg/mL of ivacaftor and 100 µg/mL to 500 µg/mL of lumacaftor. The diluent used in this procedure can be any solvent that is compatible with ivacaftor and lumacaftor. The volumes of the standard stock solutions that are pipetted into the volumetric flasks should be adjusted to achieve the desired concentrations. The calibration curve standards should be stored in a refrigerator and used within 1 week.

2.3. Preparation of diluent: A diluent was selected based on the solubility of the drugs. Acetonitrile and water were mixed in a 50:50% v/v ratio to ensure that the drugs were completely dissolved. The solution was sonicated for 5 minutes to break down any large particles and to ensure that the drugs were evenly distributed. The prepared solution was stored under room temperature at 20 ± 5 °C and should be used within 7 days of preparation to ensure that the drugs are still effective..

2.4. Chromatographic conditions:

A new HPLC method was developed and validated for the estimation of ivacaftor and lumacaftor. The method uses a C18 column (Inertsil ODS 3V, 150x4.6 ID, 5µm) and a mobile phase consisting of 50% HPLC grade acetonitrile and 50% 0.1% triethylamine buffer (50:50% v/v). Separation was achieved through isocratic elution mode at a flow rate of 1.0 mL/min. The method was validated for linearity, accuracy, precision, robustness, and recovery. The linear range was found to be from 62.5 µg/mL to 187.5 µg/mL for ivacaftor and from 100 µg/mL to 500 µg/mL for lumacaftor. The accuracy and precision were within acceptable limits. The robustness of the method was also demonstrated. The recovery was found to be 99.8% for ivacaftor and 99.9% for lumacaftor. The developed method was found to be simple, accurate, precise, and robust. It can be used for the routine analysis of ivacaftor and lumacaftor in pharmaceutical formulations.

2.4. System suitability:

Standard solutions were prepared according to the test method and injected into the chromatographic system. The system suitability parameters, such as theoretical plates, resolution, and asymmetric factor, were evaluated.

2.5. Method validation

The method validation was performed in accordance with ICH guidelines

2.5.1. Linearity

The linearity of an analytical procedure is its ability (within given range) to obtain test results, which are directly proportional to the concentration (amount) of analyte in the sample. For linearity prepared series of solutions of different concentrations in given range (62.3µg/ml to 187.4µg/ml of Ivacaftor and 100µg/ml to 300µg/ml of Lumacaftor) were injected and chromatogram was recorded in duplicate

A calibration curve was constructed by taking concentration on X- axis and average peak area on Y- axis.

2.5.2. Accuracy

Accuracy was determined by the recovery studies of the analyte. The recovery studies were performed by the standard addition method. In this method, a test solution of known quantity is spiked with standard solutions at three levels, namely 50%, 100%, and 150%. The spiked solutions are then analyzed by HPLC. The mean percentage recoveries at all the levels were calculated. The recovery studies were performed to ensure that

the method was accurate. The accuracy of the method was expressed as the percentage recovery of the analyte. The percentage recovery should be within acceptable limits.

2.5.3. Precision

The precision of the method was established at two levels: system precision, method precision (repeatability), and reproducibility. System precision was assessed by taking six injections from a homogenous working standard solution. Method precision (repeatability) was demonstrated by preparing sample stock solution and six working sample solutions of the same concentrations. An injection was given from each working sample solution. Reproducibility was determined by injecting a single injection from each of six working sample solutions that were prepared. The average area, standard deviation, and % RSD were calculated for the two drugs. The limit for precision was less than 2%. The precision of the method was found to be within acceptable limits. This means that the results of the analysis are reproducible and reliable.

2.5.4. Robustness

The robustness of the method was demonstrated by making deliberate changes to the chromatographic conditions, such as flow rate and wavelength. The system suitability parameters were compared with those of the method precision. The results showed that the method was robust and that the changes in the chromatographic conditions did not affect the accuracy, precision, or reproducibility of the method. In other words, the method was not affected by small changes in the experimental conditions, and the results of the analysis are reliable..

2.5.5. Specificity:

The specificity of the method was established by examining a blank chromatogram for any interfering peaks. The specificity of the method was also evaluated with regard to interference due to the presence of any other excipient. The specificity of the method was found to be within acceptable limits. This means that the method is able to distinguish between the analyte of interest and other substances that may be present in the sample.

2.5.6. Limit of Detection (LOD)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value based on the Standard Deviation of the Response and the slope

LOD = (3.3 * σ) / S

σ = Standard deviation

S = Slope of the calibration curve of the analyte

2.5.7. Limit of Quantification (LOQ)

The quantification limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. From the linearity data calculate the limit of detection and quantification, using the following formula,

LOQ = (10 * σ) / S

σ = Standard deviation

S = Slope of the calibration curve of the analyte

3. RESULTS AND DISCUSSION

3.1 Assay of formulation:

The assay of the formulation was performed according to the given procedure. This was done in triplicate. The amount of drug present in the formulation was calculated from the standard graph. The % assay of ivacaftor and lumacaftor obtained was 99.83% and 99.84%, respectively. Representative chromatograms for the standard and test were shown in Figures 3 and 4. The results were summarized in Table 1.

3.2 System suitability

System suitability parameters were determined according to the ICH guidelines. The parameters include plate count, tailing factor, and resolution. The plate count should be more than 2000, the tailing factor should be less than 2, and the resolution must be more than 2. All the system suitability parameters were passed and were within the limits. The results showing system suitability parameters were given in Table 2.

3.3 Validation

3.3.1. Linearity

The linearity of the method was determined at five concentrations in the range of 62.3 µg/mL to 187.4 µg/mL for ivacaftor and 100 µg/mL to 300 µg/mL for lumacaftor. The peak areas were plotted against concentration and the calibration curve was constructed. The calibration curve was illustrated in Figure 3. The correlation coefficient (r^2) was greater than 0.99 within the concentration range for both drugs. The results for linearity were given in Table 3. The standard graphs are shown in Figures 5 and 6.

3.3.2. Accuracy

The accuracy of the method was established at three levels of concentrations using the standard addition method. Triplicate injections were given at each level of accuracy and percentage recoveries were calculated. The mean percentage recoveries were obtained as 98.98% and 100.48% for ivacaftor and lumacaftor, respectively. The results for accuracy were given in Table 4.

3.3.3. Precision:

The precision of the method was studied by considering system precision, method precision, and interday precision. The average area, standard deviation, and % RSD were calculated for both drugs. System precision was determined by injecting the same solution six times. The % RSD obtained was 0.5% and 0.4% for ivacaftor and lumacaftor, respectively. Method precision was determined by preparing six working standard solutions and injecting each one six times. The % RSD obtained was 0.3% and 0.3% for ivacaftor and lumacaftor, respectively. Interday precision was determined by preparing six working sample solutions and injecting each one on different days. The % RSD obtained was 0.17% and 0.1% for ivacaftor and lumacaftor, respectively. The results for system precision, method precision, and interday precision were given in Tables 5, 6, and 7, respectively..

3.3.4. Robustness:

The robustness of the method was studied by making deliberate changes in the flow rate and wavelength. After making each change, chromatograms were recorded by injecting the standard solutions in six replicates. System suitability parameters were checked at each level. The system suitability parameters were not much affected and all the parameters were passed. The % RSD was within the limit. The results were given in Table 7.

3.3.5. Specificity:

The specificity of the method was evaluated by examining the blank chromatogram for any interfering peaks. The blank chromatogram showed no extra peaks, which indicates that the method is specific.The blank chromatogram showed no extra peaks, which means that there were no interfering peaks present in the solution. This indicates that the method is specific and can distinguish between the analyte of interest and other substances that may be present in the sample.

3.3.6. Limit of detection

The limit of detection (LOD) for ivacaftor and lumacaftor was found to be 0.79 µg/mL and 2.95 µg/mL, respectively.

3.3.7. Limit of Quantification

LOQ of for Ivacaftor and Lumacaftor was found to be 0.98 µg/ml and 3.97 µg/ml respectively.

4. CONCLUSION

The developed HPLC method was found to be simple, precise, accurate, and sensitive for the simultaneous estimation of lumacaftor and ivacaftor in pharmaceutical dosage form. The results are in accordance with ICH guidelines. Hence, this method can be easily and conveniently adopted for routine analysis of lumacaftor and ivacaftor in pure and pharmaceutical dosage form. From the results, it was concluded that this newly developed method for the simultaneous estimation of lumacaftor and ivacaftor was found to be simple, precise, and accurate. The high resolution and shorter retention time make this method more acceptable and cost-effective, and it can be effectively applied for routine analysis in research institutions, quality control departments in industries, and approved testing laboratories in the near future.

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Table 1. Assay Data of Ivacaftor

S.no	Average Peak area of Ivacaftor	Average Peak area of Lumacaftor
Avg	3907667	2516.461
Regression equation	y = 10513x + 7556.9	
% Assay	99.83 %	99.84 %

Table 2: System suitability parameters for Ivacaftor and Lumacaftor

S no	Ivacaftor			Lumacaftor			
Inj	RT (min)	USP Plate Count	Tailing	RT (min)	USP Plate Count	Tailing	Resolution
1	2.700	2877	1.441	3.947	2476	1.500	4.45
2	2.700	2966	1.343	3.937	2554	1.477	4.41
3	2.697	2961	1.455	3.933	2550	1.512	4.39
4	2.707	2976	1.485	3.953	2576	1.477	4.35
5	2.703	2971	1.485	3.947	2567	1.512	4.44
6	2.7014	2959	1.468	3.9434	2548	1.462	4.29

Table 3: Linearity data of Ivacaftor and Lumacaftor

Ivacaftor		Lumacaftor	
Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area
0	0	0	0
62.3	495227	100	1052124
93.5	745541	150	1607304
125	965117	200	2115072
156.2	119046	250	2629514
187.4	1470799	300	3154098

Table 4: Accuracy table of Lumacaftor

% Level N=3	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery
Ivacaftor			
50 %	62.5	61.9	99.04
100%	125	124.6	99.68
150 %	187.5	185.3	98.82
Lumacaftor			
50 %	100	99.65	99.65
100%	200	198.89	99.44
150 %	300	297.63	99.21

Table 5: Precision data of Ivacaftor and Lumacaftor

	System precision		Method precision	
S. No	Area of Ivacaftor	Area of Lumacaftor	Area of Ivacaftor	Area of Lumacaftor
1.	266078	1109066	345890	2518.891
2.	265667	1110202	323900	2515.559
3.	256680	1113271	346917	2514.373
4.	234683	1112450	387903	2512.866
5.	267680	1108599	345913	2517.609
6.	254690	1109570	321923	2519.468
Mean	2676667	1110526	3907667	2516.461
S.D	0011057	1902663	0012193	2.621535
%RSD	0.412278	0.17133	0.3	0.10

Table no 6: Intermediate precision for Ivacaftor and Lumacaftor

S. No	Area of Ivacaftor	Area of Lumacaftor
1.	3845245	1185658
2.	3803714	1194699
3.	3845972	1183867
4.	3838125	1186147
5.	3824099	1185646
6.	3808939	1195454
Mean	3827682	1188579
S.D	18388.3	5098.5
%RSD	0.5	0.4

Table 7: Robustness data for Ivacaftor and Lumacaftor

Parameter	IVACAFTOR		LUMACAFTOR	
	Retention time (min)	Tailing factor	Retention time (min)	Tailing factor
Flow Rate				
0.8 ml/min	3.727	1.558	5.457	1.589
1.2 ml/min	2.127	1.464	3.113	1.421
Wavelength				
223nm	2.707	1.412	3.960	1.500
227nm	2.657	1.382	3.903	1.477

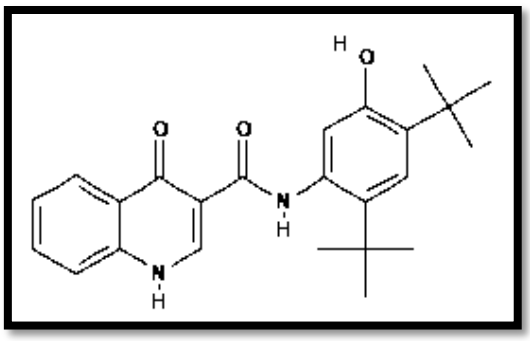

Fig 1: Structure of Ivacaftor

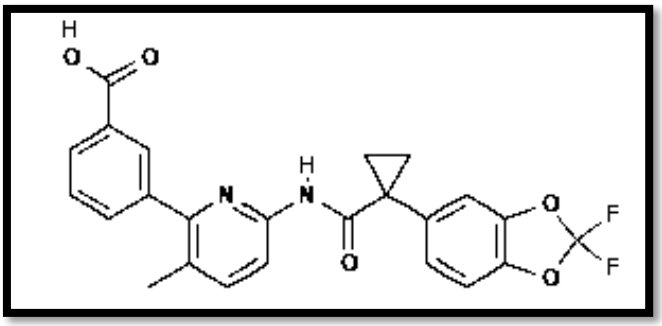

Fig. 2: Structure of Lumacaftor

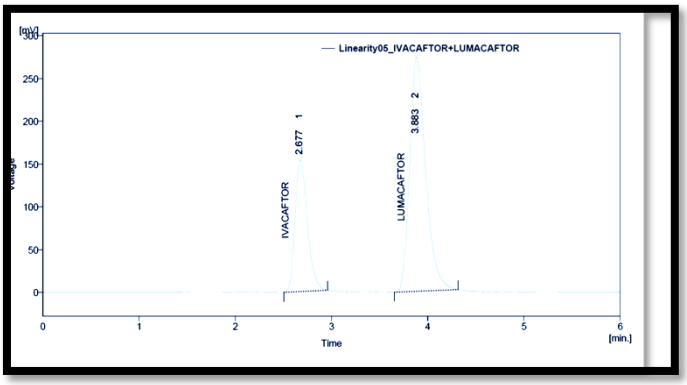

Fig 3: Representative Chromatogram of working standard solution

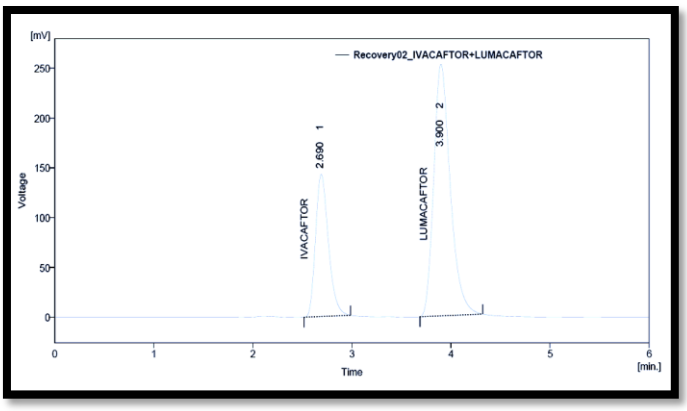

Fig 4: Representative Chromatogram of working sample solution

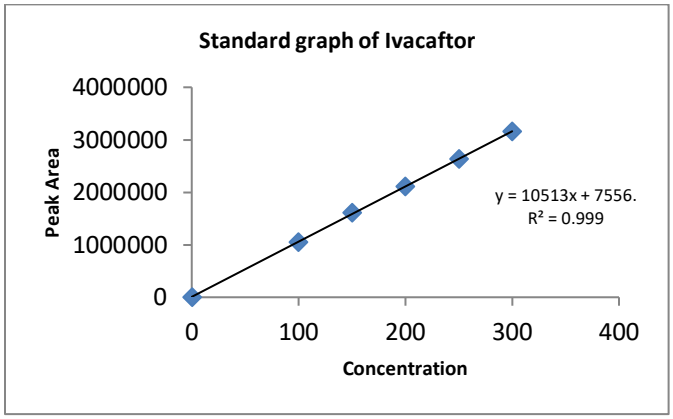

Fig 5: Calibration curve of Ivacaftor

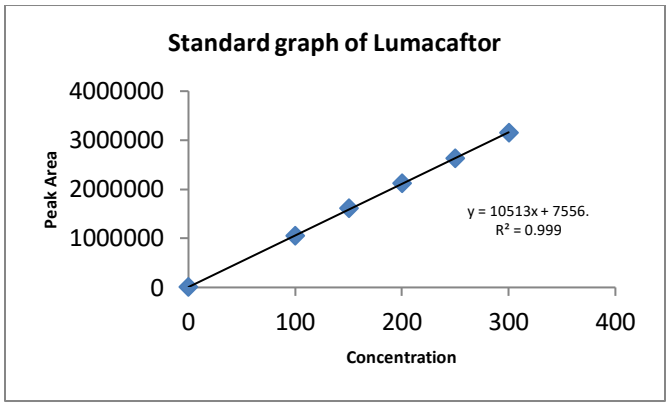

Fig 6: Calibration curve of Lumacaftor

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