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VALIDATED STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF LUMACAFTOR AND IVACAFTOR IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Lumacaftor and Ivacaftor in Tablet dosage form. Chromatogram was run through AltimaC18 (150 x 4.6 mm, 5µ). Mobile phase containing Buffer:Acetonitrile taken in the ratio 55:45v/v was pumped through column at a flow rate of 0.9 ml/min. Buffer used in this method was KH_2PO_4. Temperature was maintained at 30°C. Optimized wavelength selected was 220 nm. Retention time of Lumacaftor and Ivacaftor were found to be 2.283 min and 2.759. %Recovery was obtained as 100.57% and 99.56% for Lumacaftor and Ivacaftor respectively. LOD, LOQ values obtained from regression equations of Lumacaftor and Ivacaftor were 1.06, 3.22 and 0.16, 0.48µg/ml respectively. Regression equation of Lumacaftor is y = 11244x + 12930, and y = 8284x + 7794.4of Ivacaftor. The developed method was simple and economical that can be adopted in regular Quality control test in Industries.

KEYWORDS: Ivacaftor, Lumacaftor, RP-HPLC.

INTRODUCTION

Lumacaftor is a drug used in combination with Ivacaftor as the fixed dose combination product for the management of Cystic Fibrosis (CF) in patients aged 6 years and older. Cystic Fibrosis is an autosomal recessive disorder caused by one of several different mutations in the gene for the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) protein, a transmembrane ion channel involved in the transport of chloride and sodium ions across cell membranes of the lungs, pancreas, and other organs. Mutations in the CFTR gene result in altered production, misfolding, or function of the CFTR protein and consequently abnormal fluid and ion transport across cell membranes. As a result, CF patients produce thick, sticky mucus that clogs the ducts of organs where it is produced making patients more susceptible to infections, lung damage, pancreatic insufficiency, and malnutrition. Lumacaftor improves CF symptoms and underlying disease pathology by aiding the conformational stability of F508del-mutated CFTR proteins, preventing misfolding and resulting in increased processing and trafficking of mature protein to the cell surface.[1,2]

Ivacaftor (also known as Kalydeco or VX-770) is a drug used for the management of Cystic Fibrosis (CF) in patients aged 2 years and older. Cystic Fibrosis is an autosomal recessive disorder caused by one of several different mutations in the gene for the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) protein, an ion channel involved in the transport of chloride and sodium ions across cell membranes. CFTR is active in epithelial cells of organs such as of the lungs, pancreas, liver, digestive system, and reproductive tract. Alterations in the CFTR gene result in altered production, misfolding, or function of the protein and consequently abnormal fluid and ion transport across cell membranes. As a result, CF patients produce a thick, sticky mucus that clogs the ducts of organs where it is produced making patients more susceptible to complications such as infections, lung damage, pancreatic insufficiency, and malnutrition.[3,4]

The stability indicating method is defined as validated quantitative analytical method that can detect the change with time in the chemical, physical or microbiological properties of the drug substance and the drug product, that are specific so that the content of active ingredient, degradation can be accurately measured without interference. Stability testing provides information about degradation mechanisms, potential degradation products, possible degradation pathways of the drug as well as interaction between the drug and the excipients in drug product.

Literature survey revealed few analytical methods were reported for both the drugs in alone and in combination HPLC[5-8] methods. The aim of the present study was to develop a simple, precise, reliable, sensitive and selective stability indicating HPLC method with UV

detection for the analysis of Lumacaftor and Ivacaftor in bulk samples and in combined dosage formulation.

EXPERIMENTAL

Chemicals and reagents

- Ivacaftor and Lumacaftor pure drugs (API), Combination Lumacaftor and Ivacaftor (Orkambi) capsule, Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dehydrogenate ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem Electronics Balance-Denver
- p^H meter -BVK enterprises, India
- Ultrasonicator-BVK enterprises
- WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and Auto sampler integrated with Empower 2 Software.
- UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2 mm and 10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbances of Ivacaftor and Lumacaftor solutions.

Apparatus and chromatographic condition

The chromatographic separation was performed on a HPLC system (WATERS) Series HPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector. The analytical columns Altima C18 (4.6 x 150mm, 5µm) was used for the separation. The mobile phase consisted of ACN, Potassium dihydrogen phosphate in the ratio of 45:55 (v/v). The mobile phase was prepared freshly, filtered, sonicated before use and delivered at a flow rate of 0.9mL/min and the detector wavelength was set at 220nm. The injection volume was 10 µL. Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50

Preparation of Ivacaftor and Lumacaftor standard & sample solution

Standard solution preparation: Accurately weighed 50 mg of Lumacaftor, 31.25mg of Ivacaftor and transferred to 50ml volumetric flask and 3/4 th of diluents was added to these flask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (1000µg/ml of Lumacaftor and 625µg/ml of Ivacaftor)

Sample Solution Preparation

5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to one tablet was transferred into a 100 ml volumetric flask, 5ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (100µg/ml of Lumacaftor and 62.5µg/ml of Ivacaftor)

Preparation of Sample working solutions (100% solution): 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (100µg/ml of Lumacaftor and 62.5µg/ml of Ivacaftor)

Procedure

Inject 10µL of the standard, Ivacaftor and Lumacaftor sample solution into the chromatographic system and measure the peak areas for and calculate the % assay value.

RESULTS AND DISCUSSION

All of the analytical validation parameters for this proposed method were determined according to ICH guidelines. Obtained validation parameters are presented in Table 1.

Linearity Accurately weighed 50 mg of Lumacaftor, 31.25mg of Ivacaftor and transferred to 50ml volumetric flask and 3/4 th of diluents was added to these flask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (100µg/ml of Lumacaftor and 62.5µg/ml of Ivacaftor)

The linearity of this method was evaluated by linear regression analysis.

Recovery

Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean % Recovery for each level should be between 98.0 to 102 for Ivacaftor and Lumacaftor respectively. The obtained results are presented in Table 2.

Sensitivity

The limit of detection (LOD) was determined as lowest concentration giving response and limit of quantification (LOQ) was determined as the lowest concentration analyzed with accuracy of the proposed RP-HPLC method. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 1.06 and 3.22 for Lumacaftor and 0.16µg/ml and 0.48µg/ml for Ivacaftor. The LOD and LOQ showed that the method is sensitive for Ivacaftor and Lumacaftor

System suitability test

The specificity of this method was determined by complete separation of Ivacaftor and Lumacaftor with parameters like retention time, resolution and tailing factor. Here tailing. Retention time of Ivacaftor and Lumacaftor were found to be 2.283min and 2.759min factor for peaks of Ivacaftor and Lumacaftor was less than 2% and resolution was satisfactory. The peaks obtained for Ivacaftor and Lumacaftor were sharp and have clear baseline separation. Analysis was also performed for active Simvastatin and Ezetimibe, placebo sample (All the ingredients except active Simvastatin and Ezetimibe) both at stressed and unstressed condition. After analysis it was found that there is no interference of peak in the amlodipine and metoprolol region for the

stressed, placebo & active sample. Hence the developed method was specific for the analysis of this product**.**

Precision

The method precision study was performed for five sample preparations of marketed formulations. A study was carried out for intermediate precision with the same analyst on the different day for five sample preparations of marketed formulations. Robustness of the method was determined by small deliberate changes in flow rate, mobile phase P^H and mobile phase ratio. The content of the drug was not adversely affected by these changes as evident from the low value of relative standard deviation indicating that the method was rugged and robust. The Intra-day and Inter-day precision results are presented in Table 3. The assay results of tablet dosage formulation by the proposed method are presented in Table 4.

Stability

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 24 hr at room temperature. The results show that for both solutions, the retention time and peak area of Ivacaftor and Lumacaftor remained almost similar (% R.S.D. less than 2.0) and no significant degradation within the indicated period, thus indicated that both solutions were stable for at least 24 hr, which was sufficient to complete the whole analytical process. Further forced degradation studies were conducted indicating the stability of the method developed. The results of the degradation studies are presented in Table 5.

Control sample

Weigh and finely powder not fewer than 2 tablets. Accurately weigh and transfer sample equivalent to 10 mg of lumacaftor and 10 mg Ivacaftor into a 100 mL clean dry volumetric flask, add about 75 mL of methanol and sonicate to dissolve it completely and make volume up to the mark with the diluent. Filter the solution through 0.45µm membrane filter. Further pipette 2 mL of the above stock solution into a 10 mL volumetric flask and dilute up to the mark with diluent.

Acid degradation sample

Weigh and finely powder not fewer than 2 tablets. Accurately weigh and transfer sample equivalent to 173 mg into a 100 mL clean dry volumetric flask, add about 10 mL of 0.1N acid (Hydrochloric acid), refluxed for 30 minutes at 60°C, then cooled to room temperature, neutralize with 10 ml of 0.1N base (Sodium hydroxide) and make volume up to the mark with methanol and mix. Filter the solution through 0.45 µm membrane filter. Further pipette 2 mL of the above stock solution into a 10 mL volumetric flask and dilute up to the mark with methanol. The typical chromatogram of acid degradation was given in Fig. 6.

Base degradation sample

Weigh and finely powder not fewer than 2 tablets. Accurately weigh and transfer sample equivalent to 173 mg into a 100 mL clean dry volumetric flask add 10 ml of 0.1N base (Sodium hydroxide), refluxed for 30 minutes at 60°C, then cooled to room temperature, neutralize with 10 ml of 0.1N acid (hydrochloric acid) and make volume up to the mark with methanol and mix. Filter the solution through 0.45 µm membrane filter. Further pipette 2 mL of the above stock solution into a 10 mL volumetric flask and dilute up to the mark with methanol. The typical chromatogram of base degradation was given in Fig. 7.

Peroxide degradation sample

Weigh and finely powder not fewer than 2 tablets. Accurately weigh and transfer sample equivalent to 173 mg into a 100 mL clean dry volumetric flask add 10 ml of 1% H_2O_2, refluxed for 30minutes at 60°C, then cooled to room temperature, make volume up to the mark with methanol and mix. Filter the solution through 0.45 µm membrane filter. Further pipette 2 mL of the above stock solution into a 10 mL volumetric flask and dilute up to the mark with methanol. The typical chromatogram of oxidative degradation was given in Fig. 8.

Water degradation sample

Weigh and finely powder not fewer than 2 tablets. Accurately weigh and transfer sample equivalent to 173 mg into a 100 mL clean dry volumetric flask add 10 ml of H_2O, refluxed for 30minutes at 60°C, then cooled to room temperature, make volume up to the mark with methanol and mix. Filter the solution through 0.45 µm membrane filter. Further pipette 2 mL of the above stock solution into a 10 mL volumetric flask and dilute up to the mark with methanol. The typical chromatogram of oxidative degradation was given in Fig. 9.

Thermal degradation sample

Weigh and finely powder not fewer than 2 tablets, this powder is exposed to heat at 105°C for about 2 days. Accurately weigh and transfer sample equivalent to 173 mg into a 100 mL clean dry volumetric flask. Add about 75 mL of methanol and sonicate to dissolve it for about 30minutes with intermittent shaking at controlled temperature. Then make volume up to the mark with methanol and mix. Filter the solution through 0.45 µm membrane filter. Further pipette 2 mL of the above stock solution into a 10 mL volumetric flask and dilute up to the mark with methanol. The typical chromatogram of thermal degradation was given in Fig. 10.

Fig. 1: Chemical structure of Ivacaftor.

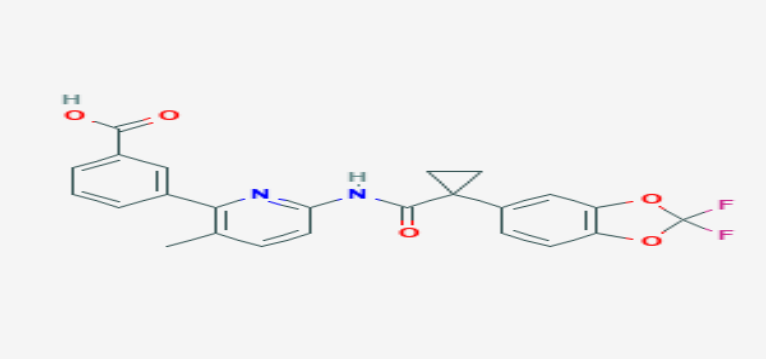

Fig. 2: Chemical structure of Lumacaftor.

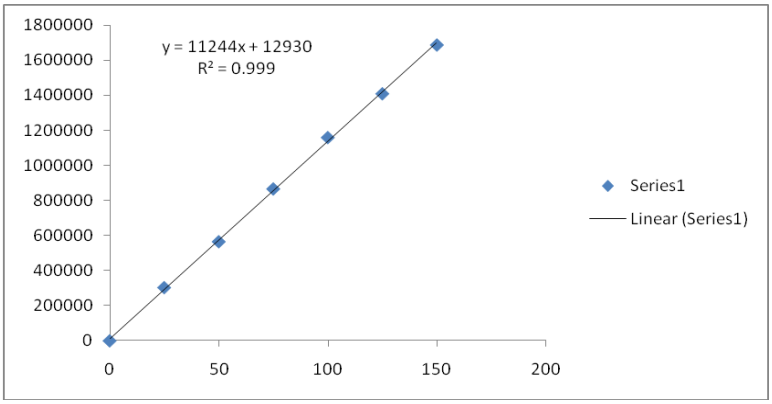

Fig. 3: Calibration curve for Lumacaftor.

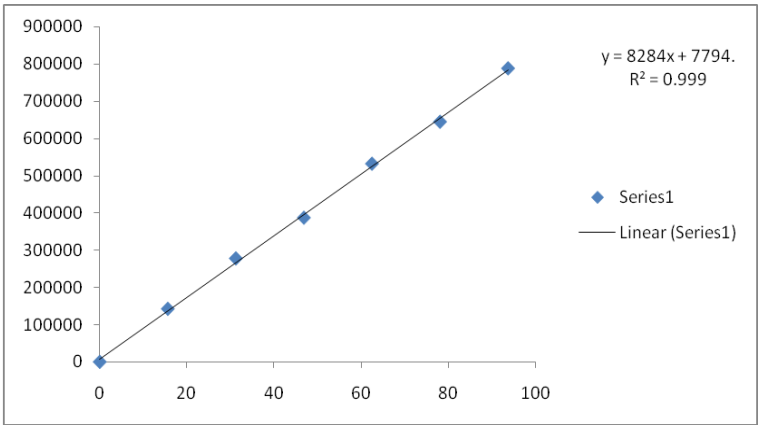

Fig. 4: Calibration curve for Ivacaftor.

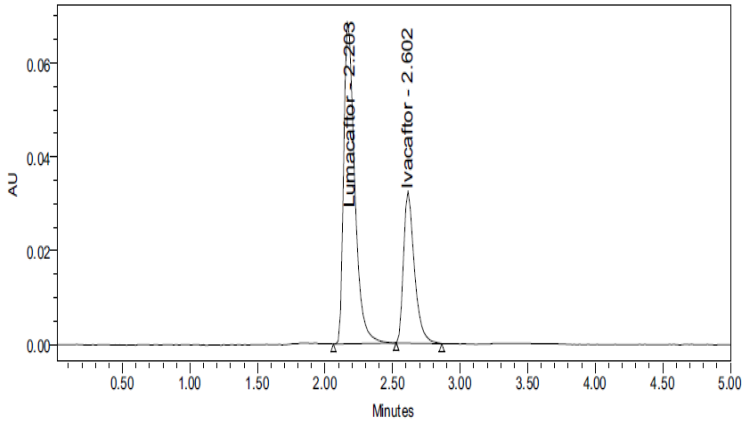

Fig. 5: Typical chromatogram of Lumacaftor and Ivacaftor.

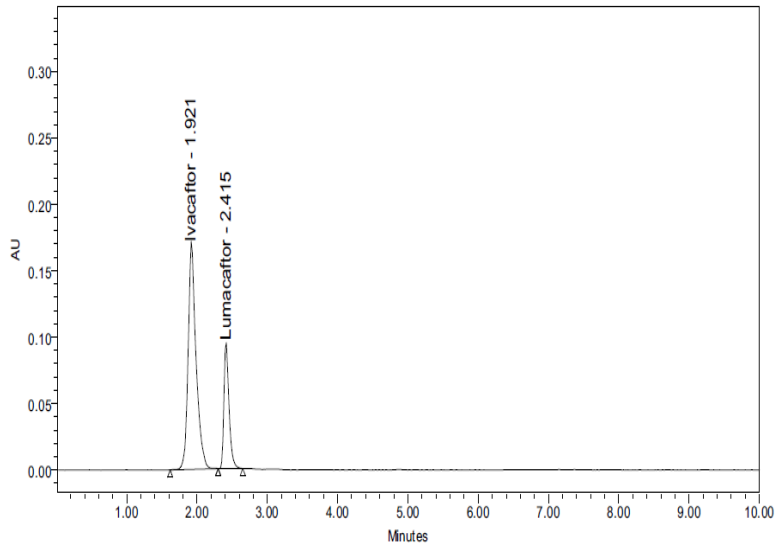

Fig. 6: Acid degradation chromatogram of Lumacaftor and Ivacaftor.

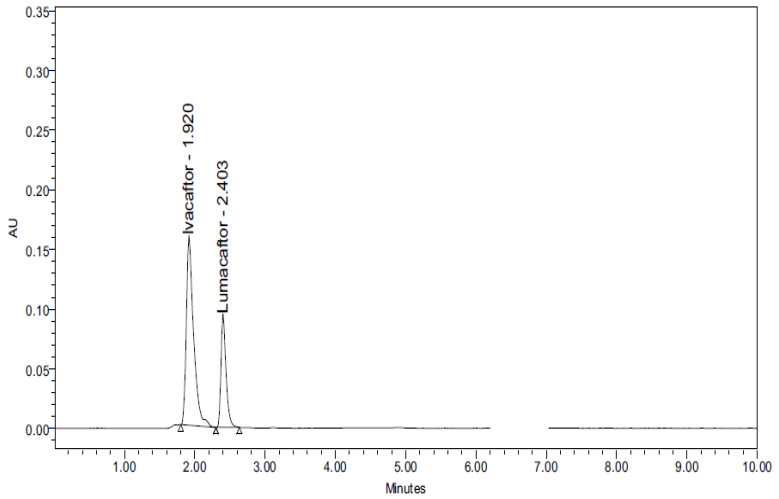

Fig 7: Base degradation chromatogram of Lumacaftor and Ivacaftor.

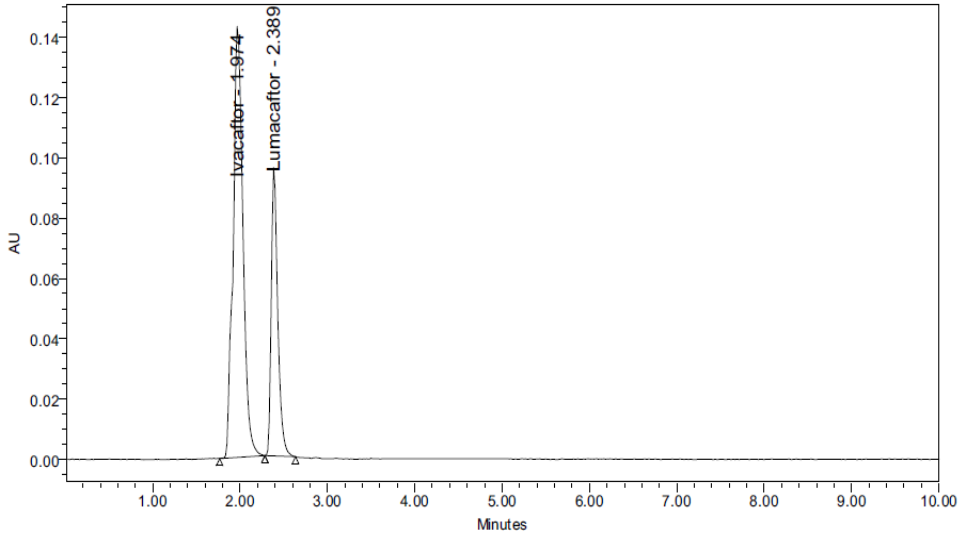

Fig. 8: Peroxide degradation chromatogram of Lumacaftor and Ivacaftor.

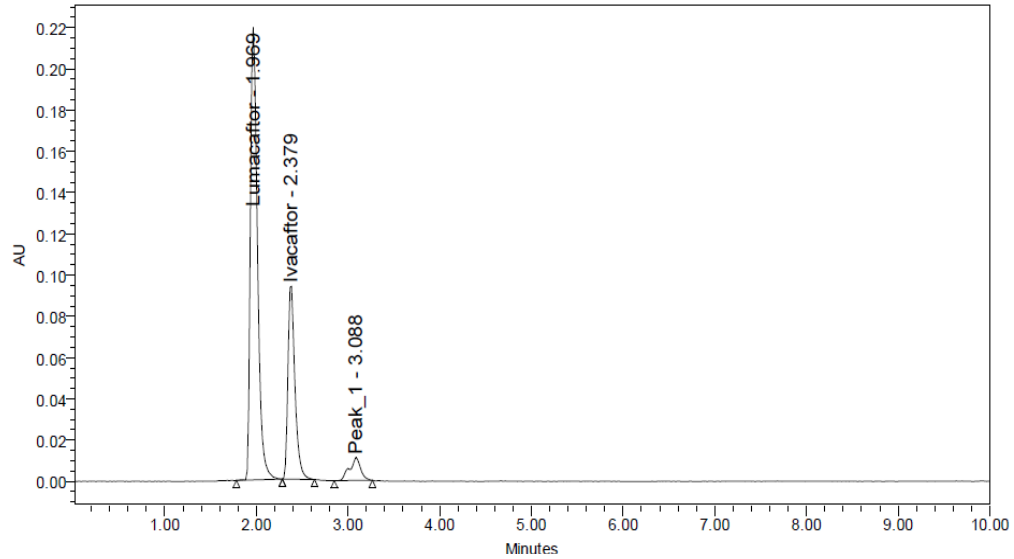

Fig 9: Water degradation chromatogram of Lumacaftor and Ivacaftor.

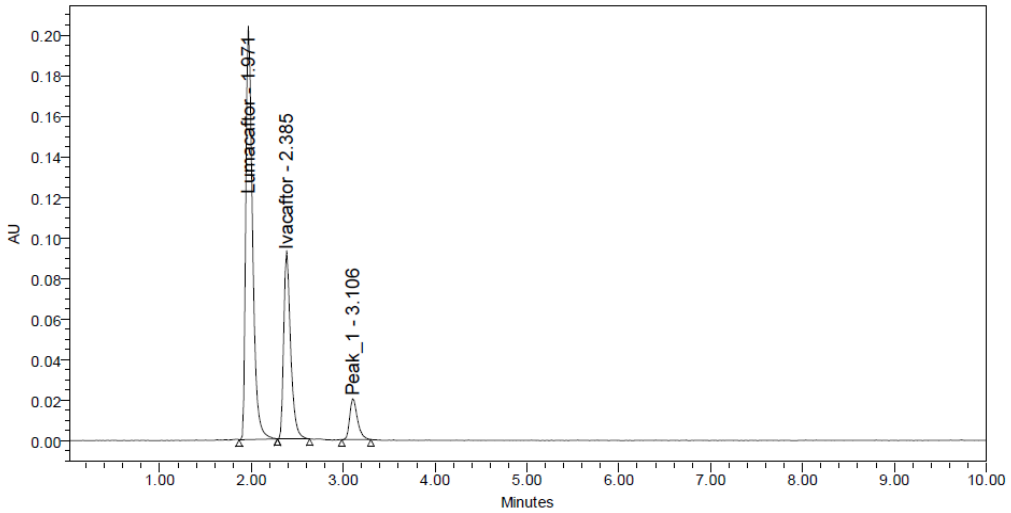

Fig 10: Thermal degradation chromatogram of Lumacaftor and Ivacaftor

Table 1: Analytical validation parameters (System suitability and Linearity).

Parameter	Lumacaftor	Ivacaftor
Linearity	25-150 µg/ml	15.625-78.125µg/ml
Slope	11244	8284
Intercept	12930	7794
R^2	0.9995	0.999
LOD	1.06	0.16
LOQ	3.22	0.48
Theoretical Plates	3669	5080
Tailing Factor	1.49	1.5
Retention Time (min)	2.283	2.759

Table 2: Recovery studies of Lumacaftor and Ivacaftor.

Recovery data of Lumacaftor					
Concentration (at specification level)	**Peak Area**	**Amount Added (mg)**	**Amount Found (mg)**	**% Recovery**	**Mean % Recovery**
50%	1693457	49.500	50.08	101.2	100.57
100%	1704585	99	98.18	101.6	
150%	1706375	148.500	146.78	100	

Recovery data of Ivacaftor					
Concentration (at specification level)	**Peak Area**	**Amount Added (mg)**	**Amount Found (mg)**	**% Recovery**	**Mean % Recovery**
50%	781059	150	151.6	100	99.56
100%	782146	300	98.02	99.34	
150%	780868	450	148.90	99.99	

Table 3: Intra-day and Inter-day precision of Lumacaftor and Ivacaftor.

Drug	Sample Weight(mg)	Intra-day precision		Inter-day precision	
		SD	%RSD	SD	%RSD
Lumacaftor	50mg	99.94	0.62	99.92	1.4
Ivacaftor	31.5mg	98.87	0.81	98.97	1.3

Table 4: Assay result of tablet dosage formulation.

Drug	Label strength (mg)	Amount found (mg)	% Assay
Lumacaftor	50	9.947	99.26
Ivacaftor	31.5	9.99	99.36

Table 5: Forced degradation studies of Lumacaftor and Ivacaftor.

Stress Conditions	Degradation Time	Peak Area		% Degradation		% of Active drug present after degradation	
		Lumacaftor	Ivacaftor	Lumacaftor	Ivacaftor	Lumacaftor	Ivacaftor
Acid	-	1097763	512589	4.97	4.33	95.03	95.67
Base	1 hour	1106608	515951	4.21	3.71	95.79	96.29
Peroxide	1 hour	1120320	516682	3.02	3.57	96.98	96.43
Thermal	1 hour	1138939	523085	1.41	2.37	98.59	97.63
Uv		1140902	526230	1.24	1.79	98.76	98.21
Water	48 hours	1146903	530351	0.72	1.02	99.28	98.98

CONCLUSION

The findings of the present investigation are summarized as follows

1) A suitable chromatographic method was developed through optimization by changing various parameters such as the mobile phase, injection volume, flow rate etc

2) In the present method a column, Altima C18 (4.6 x 150mm, 5µm) has been used for Ivacaftor and Lumacaftor drugs respectively.

3) Mobile phase used was Acetonitrile: KH_2PO_4 (55:45v/v) for drugs Ivacaftor and Lumacaftor respectively, Retention of Ivacaftor and Lumacaftor has more dependence on the mobile phase. The separation of the two peaks was also dependent on the buffer and the percentage of mobile phases. Ivacaftor and Lumacaftor were eluted at acceptable retention times and got good resolution.

Several assay methods has been developed for the determination of Ivacaftor and Lumacaftor in formulations and biological fluids but this method is most economic and accurate so this method is very useful for the determination of Ivacaftor and Lumacaftor in tablet formulations. This method was validated as per ICH-Q2 (R1) guidelines and met the regulatory requirements for selectivity, accuracy and stability. Considering the obtained data, it was possible to affirm that the proposed method was fast, simple and suitable for the accurate determination of drug Ivacaftor and Lumacaftor in tablet formulation.

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