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# A New Validated RP-HPLC Method for the Determination of Lumacaftor and Ivacaftor in its Bulk and Pharmaceutical Dosage Forms

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**ABSTRACT**

A New method was established for simultaneous estimation of Lumacaftor and Ivacaftor by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Lumacaftor and Ivacaftorby using Ineertsil ODS column (4.6×250 mm) 5µ, flow rate was 1ml/min, mobile phase ratio (30:10:60v/v) ACN,Methanol,1 ml of OPA in 1000 ml water pH 3 (pH adjusted with triethylamine), detection wavelength used by WATERS HPLC Auto Sampler, Separation module 2695, UV detector 2489, Empower-software version-2. The retention times were found to be 3.101 min. and 4.205mins. The % purity of Ivacaftor and Lumacaftor were found to be 100.17 and100.39 respectively. The present analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Ivacaftor and Lumacaftor was found in the concentration range 62.5µg/ml-312.5µg/ml and 100µg/ml-500µg/ml and correlation coefficient ($R^2$) be 0.999 and 0.999, % recovery was found to be 100.13 and 100.53, % RSD for repeatability 0.8 and 0.8, % RSD for intermediate precision was 0.7 and 0.6respectively. The precision study was precision, robustness and repeatability. It is a convenient, simple and quick method for the determination of Ivacaftor and Lumacaftorin its bulk and pharmaceutical dosage forms.

**keywords:** Ivacaftor, Lumacaftor, HPLC, Methanol, Acn.

## INTRODUCTION

Ivacaftor is Cystic fibrosis is caused by any one of several defects in a protein, cystic fibrosis trans membrane 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.

Lumacaftor I 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. Ivacaftor is currently approved for use in combination with Lumacaftor (as the combination product Orkambi) for the treatment of chronic cysticfibrosis[1-4]

Literature review reveals that there few HPLC[7-13] and HPTLC[14-15] methods are available for the determination of Lumacaftor and Ivacaftor in different dosage forms.

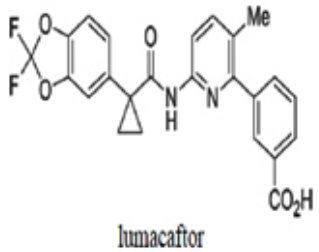
For Lumacaftor and Ivacaftor there are several HPLC[17-21] methods available in combined dosage forms.

The structures of Lumacaftor and Ivacaftor were shown in figures 1 and 2.

## MATERIALS AND METHODS

### Instrumentation

The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, UV detector and Empower 2 software. The analysis was carried out at 254 nm with an inertsil ODS (4.6 x 250mm, 5mm) dimensions at ambient temperature(25$^0$c).



lumacaftor

**Fig. 1: Structure of Lumacaftor**

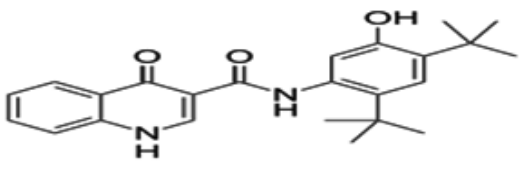



**Fig. 2: Structure of Ivacaftor**

### Chemicals and reagents

Ivacaftor and Lumacaftor were supplied fromMylon laboratories, Hyderabad. Ortho phosphoric acid (OPA) (Merck), Methanol(MERCK HPLC grade) Acetonitrile (Molychem, HPLC grade) and Water for HPLC (LICHROSOLV (MERCK). were employed in the present work.

### Preparation of solutions

### Preparation of buffer

1ml of Orthophosphoric acidin1000 ml of HPLC water. The P$^H$ is adjusted to 3.0 with TEA. The final solution is filtered through 0.45μm membrane filter and sonicate it for 10 mins.

### Preparation of mobile phase

Accurately measured 600 ml (60%) of P$^H$=3.0 buffer and 300 ml(30%) of Acetonitrile and 100ml(10%) of Methanol. mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µm membrane filter under vacuum filtration. Figure 4 represents the Chromatograms of mobile phase (blank solution).

### Diluent Preparation

The Mobile phase was used as the diluent.

### Preparation of standard stock solution

20 mg of Lumacaftor and 12.5 mg of Ivacaftor were accurately weighed and transferred into a 10 ml clean dry volumetric flask. Add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further, 1.5 ml of the above prepared stock solution is pipetted into a 10ml volumetric flask and dilute up to the mark with diluent.

### Preparation of Sample Solution

Accurately weigh the samples of 10 tablets. It is crushed in mortor and pestle. Transfer equivalent to 20 mg o fLumacaftor and 12.5 mg Ivacaftor sample into a 10 mL clean dry volumetric flask. Add about 7 mL of Diluent and sonicate it up to 30 mins to dissolve it completely and make volume up to the

mark with the same solvent. Then, it is Filtered through 0.44 micron Injection filter.

Further, pipette 1.5 ml of Lumacaftor and Ivacaftor from the above sample solution into a 10 ml volumetric flask and dilute up to the mark with diluent. The standard solutions were prepared on daily basis from which stock solutions were prepared.

**Procedure**

20 µL of the standard, stock and sample solution are injected into the chromatographic system. The areas are measured for Lumacaftor and Ivacaftor peaks are calculated. The % Assay by using the standard formula.

**Method development and selection of wavelength**

UV spectrum of 10 µg/ml Lumacaftor and 10 µg/ml Ivacaftor in diluents (mobile phase composition) are recorded by scanning in the range of 200nm to 400nm. The UV Spectrum obtain for From the UV spectrum Lumacaftor and Ivacaftor is shown in the figure.1.Form the UV spectrum , the wavelength is selected as 254 nm. At this wavelength both the drugs show good absorbance.

**Construction of calibration curve**

Aliquots of different concentrations of standard solution were prepared and their chromatograms were recorded at the optimized chromatographic conditions. The mean peak areas at different concentration levels were calculated from the chromatograms. Then the linearity plot was constructed using the mean peak areas at their respective concentrations. (Figures 8 &9)

**Method Of validation**

The developed method was validated for linearity, accuracy, precision, and limit of detection, limit of quantitation, robustness and system suitability parameters as described in ICH guidelines.

**Linearity**

From the stock solution, 100, 200, 300, 400, 500µg/ml solutions for Lumacaftor and 62.5, 125, 187.5,250, 312.5ìg/ml solutions for Ivacaftor were made and their chromatograms were recorded. From the recorded chromatograms, their respective mean peak areas were calculated and the linearity plot was constructed using the mean peak areas at their respective concentrations. The correlation coefficient was found to be 0.999. The linearity data of Lumacaftor and Ivacaftorare shown in the Tables 1 & 2.The calibration plots, are given in the figures 4&5.

**Table 1: Showing assay results**

| S. No | Name of compound | Amount taken (mg) | %purity |
|---|---|---|---|
| 1 | Lumacaftor | 200mg | 100.39 |
| 2 | Ivacaftor | 125mg | 100.17 |

**Table 2: Linearity results for Lumacaftor**

| S. No | Linearity Level | Concentration | Area (mm/ml) |
|---|---|---|---|
| 1 | I | 100 | 65792 |
| 2 | II | 200 | 98696 |
| 3 | III | 300 | 131638 |
| 4 | IV | 400 | 162911 |
| 5 | V | 500 | 200063 |
| | Correlation Coefficient | | 0.999 |

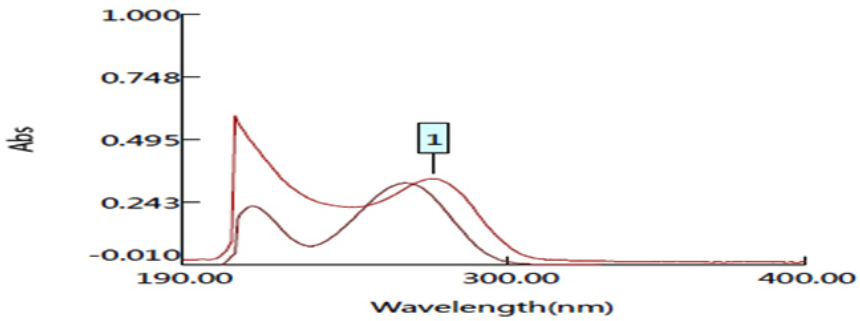



**Fig. 3: UV Spectra of Lumacaftor and Ivacaftor for Selection of Wavelength**

## RESULTS AND DISCUSSION

The present investigation reported by the authors are to develop a new validated method for the simultaneous estimation of Lumacaftor and Ivacaftor by RP-HPLC method. Mobile phase contains the mixture of 60% pH 3 Buffer(1ml OPA in 1000ml water) and 30% of acetonitrile and 10% of methanol.

**Table 3: Linearity results for Ivacaftor**

| S. No | Linearity Level | Concentration | Area (mm/ml) |
|---|---|---|---|
| 1 | I | 62.5 | 71267 |
| 2 | II | 125 | 99725 |
| 3 | III | 187.5 | 127369 |
| 4 | IV | 250 | 155275 |
| 5 | V | 312.5 | 179461 |
| Correlation Coefficient | | | 0.999 |

**Table 4: Linearity results for Ivacaftor**

| S. No | Linearity Level | Concentration | Area (mm/ml) |
|---|---|---|---|
| 1 | I | 62.5 | 71267 |
| 2 | II | 125 | 99725 |
| 3 | III | 187.5 | 127369 |
| 4 | IV | 250 | 155275 |
| 5 | V | 312.5 | 179461 |
| Correlation Coefficient | | | 0.999 |

**Table5: Showing accuracy results for Lumacaftor**

| %Concentration (at specification Level) | Area | Amount Added (mg) | Amount Found (mg) | % Recovery | Mean Recovery |
|---|---|---|---|---|---|
| 50% | 67838.3 | 10 | 10.00 | 100.02 | 100.53 |
| 100% | 136568.7 | 20 | 20.13 | 100.67 | |
| 150% | 205309.3 | 30 | 30.27 | 100.90 | |

**Table 6: Showing accuracy results for Ivacaftor**

| %Concentration (at specification Level) | Area | Amount Added (mg) | Amount Found (mg) | % Recovery | Mean Recovery |
|---|---|---|---|---|---|
| 50% | 60620.7 | 6.25 | 6.27 | 100.37 | 100.13 |
| 100% | 121845 | 12.5 | 12.61 | 100.87 | |
| 150% | 179676 | 18.75 | 18.59 | 99.16 | |

**Precision**

**Table 7: Showing% RSD results for Lumacaftor and Ivacaftor**

| Injection | Area for Lumacaftor | Area for Ivacaftor |
|---|---|---|
| Injection-1 | 141368 | 128876 |
| Injection-2 | 140717 | 127224 |
| Injection-3 | 142655 | 129055 |
| Injection-4 | 143939 | 128739 |
| Injection-5 | 143013 | 126699 |
| Injection-6 | 142282 | 129220 |
| Average | 14239 | 128302.2 |
| Standard Deviation | 1156.8 | 1064.1 |
| %RSD | 0.8 | 0.8 |

**Intermediate precision/Ruggedness**

**Table 8: Showing results for intermediate precision of Lumacaftor and Ivacaftor**

| Injection | Area for Lumacaftor | Area for Ivacaftor |
|---|---|---|
| Injection-1 | 139453 | 122535 |
| Injection-2 | 137162 | 121224 |
| Injection-3 | 139458 | 122915 |
| Injection-4 | 138377 | 123391 |
| Injection-5 | 138482 | 123108 |
| Injection-6 | 139771 | 122959 |
| Average | 138783.8 | 122688.7 |
| Standard Deviation | 976.1 | 769.7 |
| %RSD | 0.7 | 0.6 |

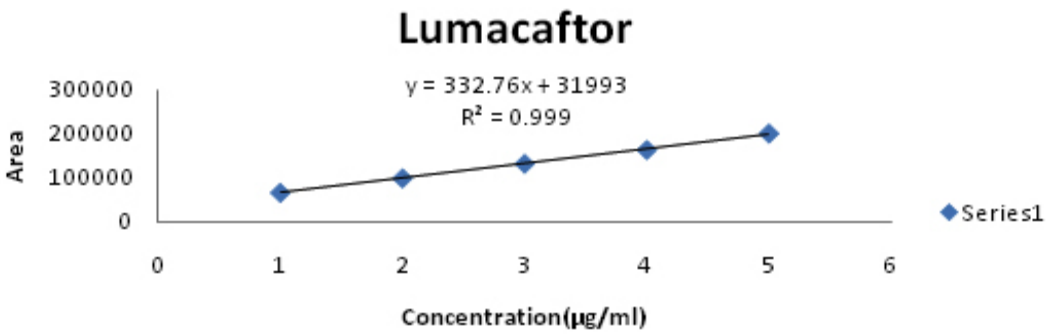



**Fig. 4: Showing calibration graph for Lumacaftor**

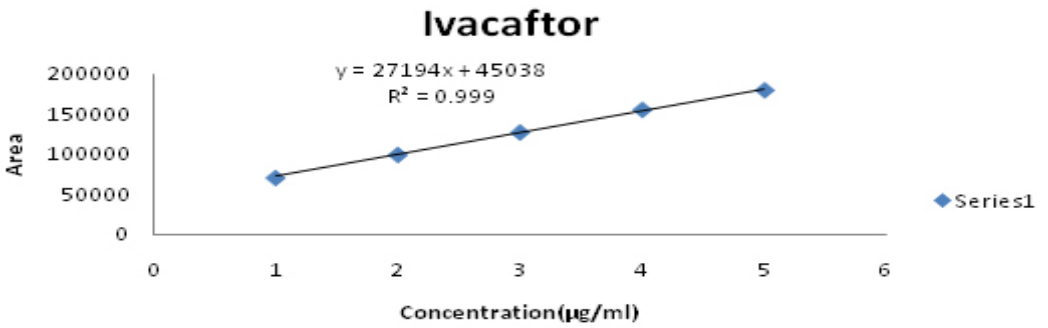



**Fig. 5: Showing calibration graph for Ivacaftor**

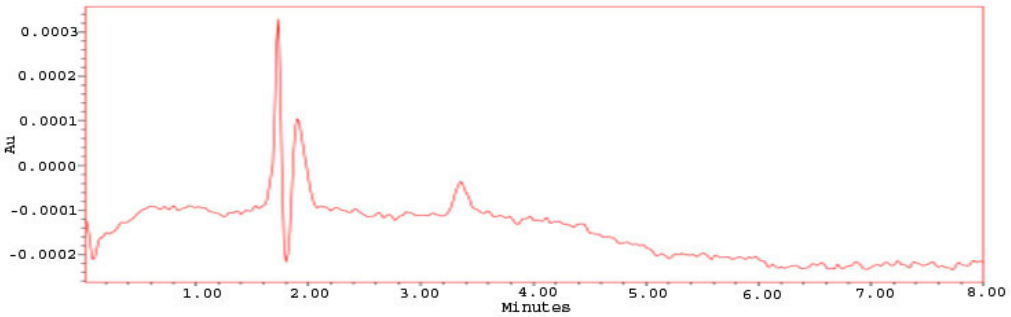



**Fig. 6: Chromatogram showing blank Solution (mobile phase)**

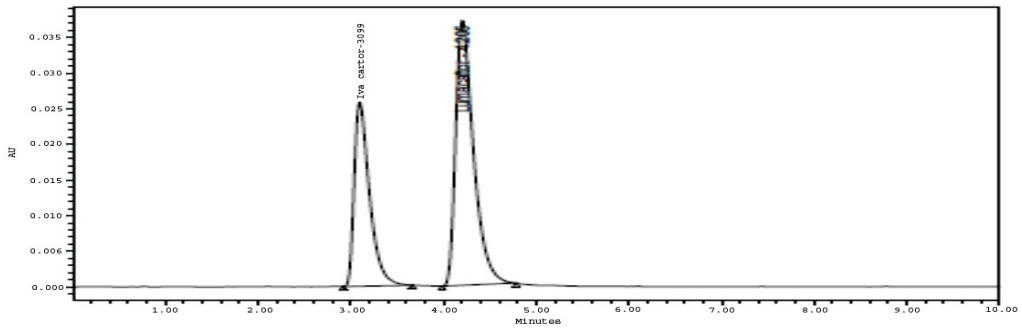



**Fig. 7: Chromatogram showing assay of sample injection**

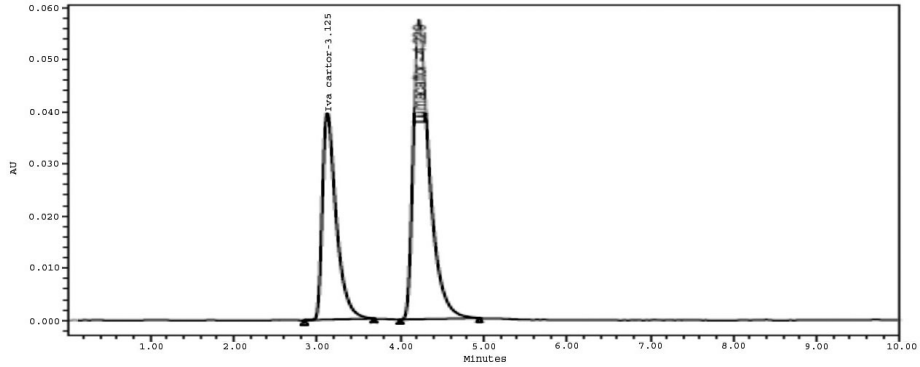



**Fig. 8: Chromatogram showing standard of sample injection -**

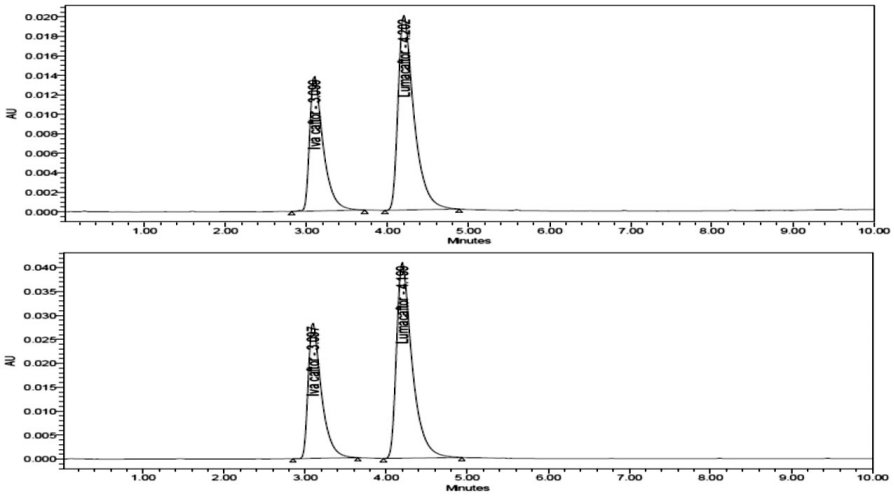



**Fig. 9.a,b Level 1,2 Chromatograms showing Linearity of Lumacaftor and Ivacaftor**

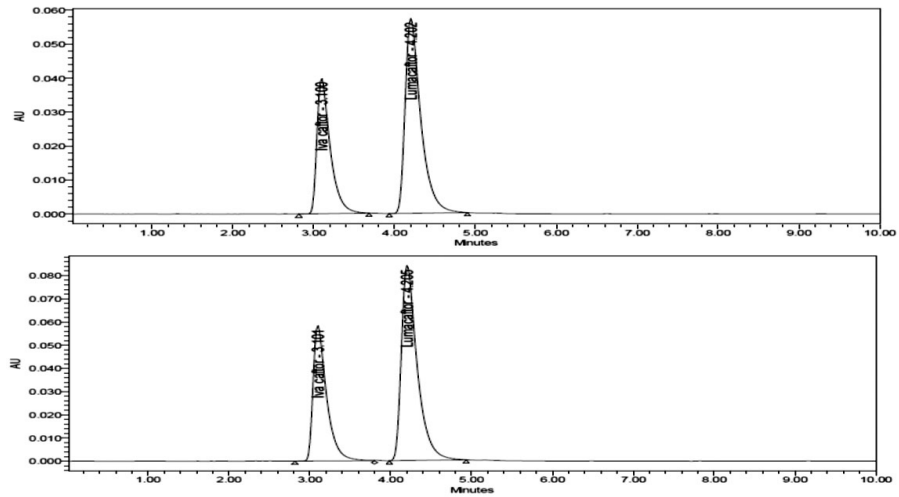



**Fig 9c,d. Level 3,4Chromatograms showing Linearity of Lumacaftor and Ivacaftor**

It is used as diluent in the present study. An intersil ODS column of 5μ (4.6X250mm) is employed for the simultaneous determination of Lumacaftor and Ivacaftor by RP-HPLC method. A flow rate of 1ml for minute is used in this method. UV detection wavelength at 254 mm and temperature of 25°C were maintained. Two sharp peaks were absorbed at 3.101mts and 4.025 mts for Ivacaftor and Lumacaftor respectively. The representative chromatograms of blank solution, Lumacaftor and Ivacaftor shown in this figure.4 Chromatograms of assay of sample injection and standard of sample injection are shown

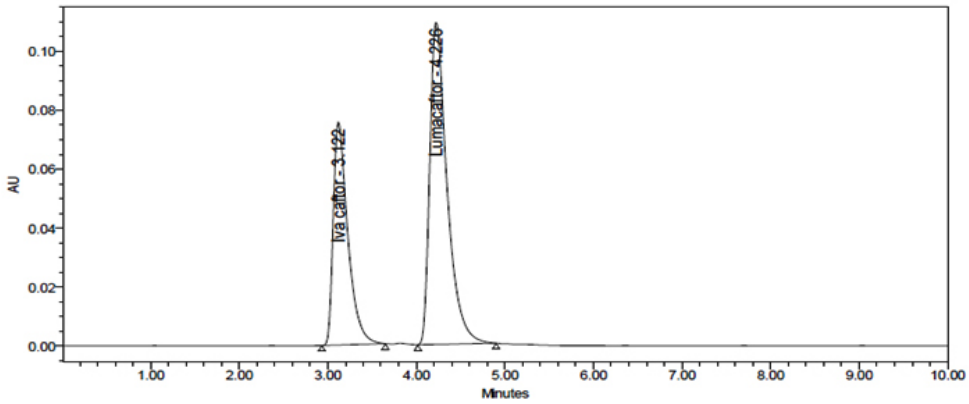



**Fig 9.e. Level 5 Chromatograms showing Linearity of Lumacaftor and Ivacaftor**

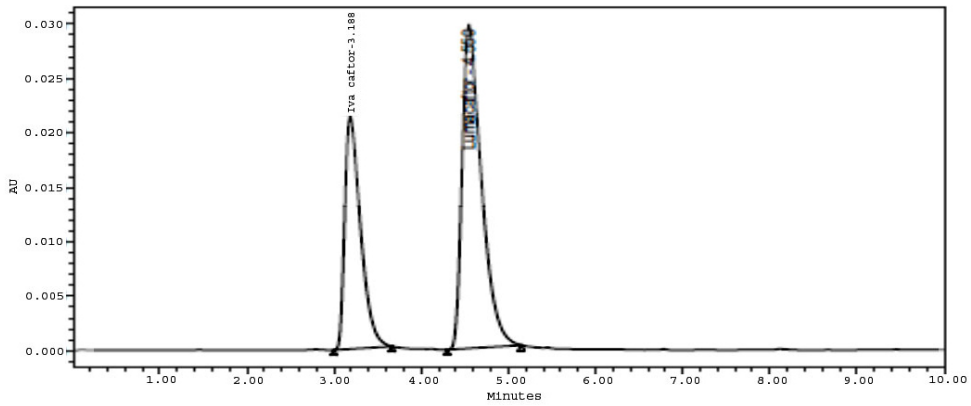



**Fig. 10: Chromatogram showing less flow rate**

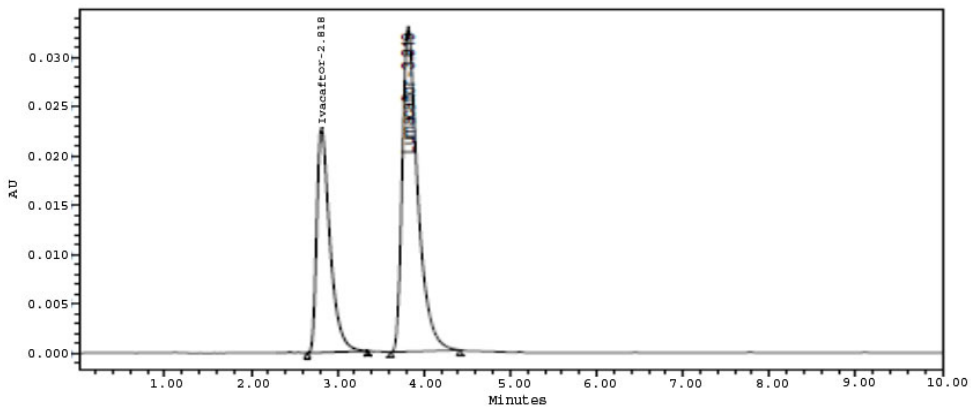



**Fig. 11: Chromatogram showing more flow rate.**

in the figures 5 & 6 and assay results of purity in the table 1. The % purity of Lumacaftor and Ivacaftor were found to be 100.39 and 100.17 respectively.

**Linearity**

Figures 7a to 7e represent the chromatograms showing different linearity levels with different concentrations of Lumacaftor and Ivacaftor and results of given in the tables 2 & 3. Both Lumacaftor and Ivacaftor obey Beer Lamberts Law in the range of concentrations of 100µg /ml to 500 µg /ml and 62.5µg /ml to 312.5µg/ml respectively with regression equations Y= 332.76 X + 31993(correlation and coefficient) $R^2$= 0.999 for Lumacaftor and Y= 27194 X + 45038 , $R^2$= 0.999. for Ivacaftor.

**Table 9: System suitability results for Lumacaftor**

| S. No. | Flow Rate (ml/min) | System Suitability Results | |
|---|---|---|---|
| | | USP Plate Count | USP Tailing |
| 1 | 0.9 | 2910 | 1.16 |
| 2 | 1.0 | 2310.88 | 1.58 |
| 3 | 1.1 | 2245.12 | 1.13 |

**Precision**

This validated method is more precise and the percentage of relative standardization (%RSD) and intermediate precision / Ruggedness were found to be 0.8 and 0.7 for Lumacaftor and 0.8 and 0.6 for Ivacaftor . The results are given the in the tables 6 and 7.

**System suitability**

The results for Lumacaftor and Ivacaftor are given in the tables 473. It was performed to ensure that complete testing system was suitable for the intended application. The USP tailing factor

**Table 10: System suitability results for Ivacaftor**

| S. No. | Flow Rate (ml/min) | System Suitability Results | | |
|---|---|---|---|---|
| | | USP Plate Count | USP Tailing | USP Resolution |
| 1 | 0.9 | 3425.70 | 1.19 | 3.62 |
| 2 | 1.0 | 2693.11 | 1.16 | 3.43 |
| 3 | 1.1 | 2675.84 | 1.17 | 3.35 |

***** Results for actual flow (1.0ml/min) have been considered from Assay standard.

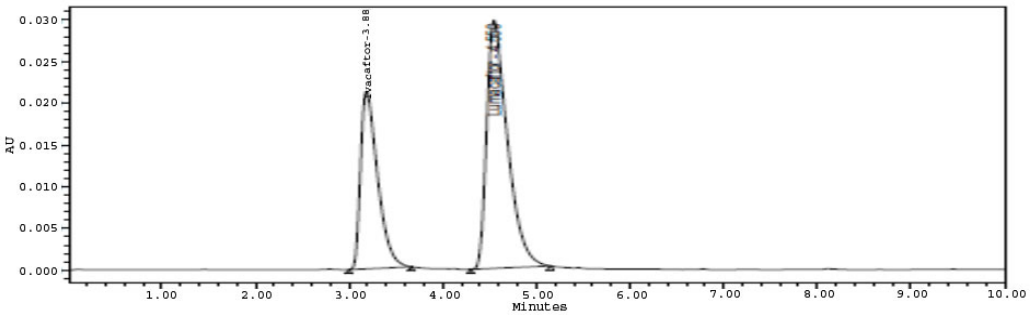



**Fig. 12: Chromatogram showing less organic composition in the mobile phase**

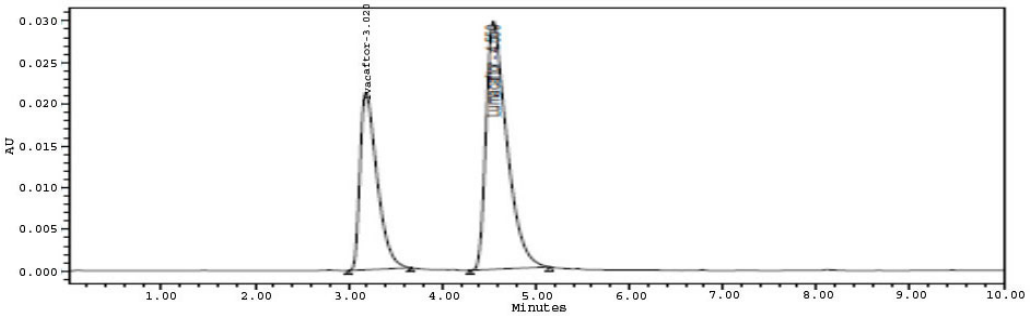



**Fig. 13: Chromatogram showing more organic composition in the mobile phase**

for Lumacaftor and Ivacaftor were 1.58 and 1.15 which is <2 and the USP plate found were 2693.56 and 2310.88 which is >2000 the results for actual flow of 1.0 ml/min is considered from assay standard. Tablets for all shows system suitability results with change in the organic composition in the mobile phase for Lumacaftor and Ivacaftor chromatograms of and Lumacaftor and Ivacaftor are show in the figures 12 and 13.

**Table 11: Showing system suitability results for Lumacaftor**

| S. No. | Change in Organic Composition in the Mobile Phase | System Suitability Results | |
|---|---|---|---|
| | | USP Plate Count | USP Tailing |
| 1 | 10% less | 2425.70 | 1.21 |
| 2 | *Actual | 2310.88 | 1.58 |
| 3 | 10% more | 2705.45 | 1.12 |

**Accuracy**

The accuracy study was performed for 50%, 100% and 150 % for Lumacaftor and Ivacaftor. Each level was injected in triplicate into chromatographic system. The area of each level was used for calculation of % recovery. These results were given in the tables 4 & 5. The Mean % of recovery is 100.53 for Lumacaftor and 100.13 for Ivacaftor. (NLT 98% and NMT 102%)

**Accuracy**

The accuracy study was performed for 50%, 100% and 150 % for Lumacaftor and Ivacaftor. Each

**Table 12: Showing system suitability results for Ivacaftor**

| S. No | Change in Organic Composition in the Mobile Phase | System Suitability Results | | |
|---|---|---|---|---|
| | | USP Plate Count | USP Tailing | USP Resolution |
| 1 | 10% less | 2910.66 | 1.16 | 3.61 |
| 2 | *Actual | 2693.11 | 1.16 | 3.43 |
| 3 | 10% more | 2248.50 | 1.12 | 2.96 |

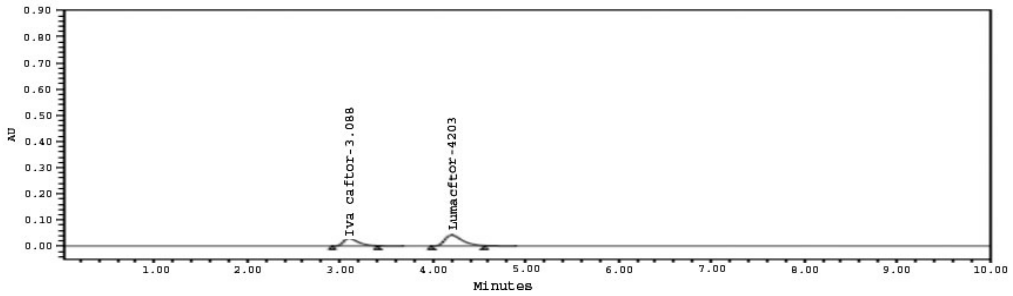



**Fig. 14: Chromatogram showing LOD**

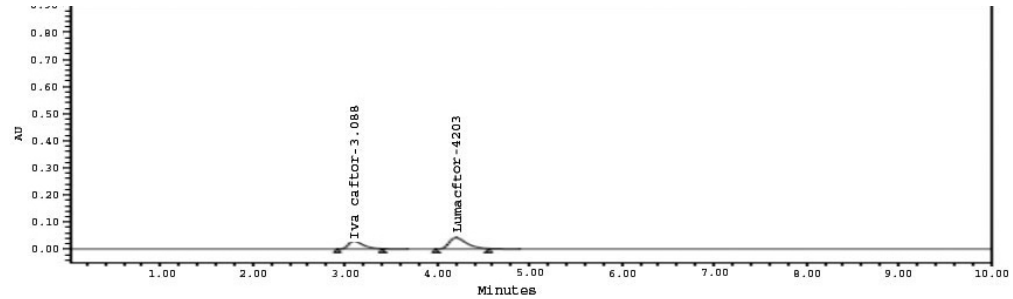



**Fig. 15: Chromatogram showing LOQ**

level was injected in triplicate into chromatographic system. The area of each level was used for calculation of % recovery.

**The accuracy results for Lumacaftor Detection limit**

As per ICH guidelience S/N Ratio value shall be 3 for LOD solution.

As per ICH guidelience S/N Ratio value shall be 10 for LOQ solution.

## CONCLUSION

The proposed HPLC method was found to be simple, precise, accurate and sensitive for the simultaneous estimation of Lumacaftor and Ivacaftorin pharmaceutical dosage forms. The results are accordance with ICH guidelines. Hence, this method can easily and conveniently adopt for routine quality control analysis of Lumacaftor and Ivacaftor in pure and its pharmaceutical dosage forms.

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