METHOD DEVELOPMENT AND VALIDATION OF LUMACAFTOR AND IVACAFTOR IN PHARMACEUTICAL DOSAGE FORMS IN RP-HPLC

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ABSTRACT
A reverse phase high performance liquid chromatographic method was developed for the determination of Lumacaftor and Ivacaftor in bulk and Pharmaceutical dosage form. The separation was effected on a C18 column (250mm x 4.6mm; 5μm) using a mobile phase mixture 50 volumes of Acetonitrile and 50 volumes of phosphate buffer in a ratio of 80:20v/v with a flow rate of 1ml/min. The detection was made at 259nm. Calibration curve was linear over the concentration range of 50-250μg/ml of Lumacaftor and 31.25-156.25µg/ml of Ivacaftor. The proposed method is validated as per the ICH guidelines. The method is accurate, precise, specific and rapid and found to be suitable for the quantitative analysis of the drug and Pharmaceutical dosage forms.

KEYWORDS
Buffer, Acetonitrile, Lumacaftor and Ivacaftor and RPHPLC.

INTRODUCTION
Lumacaftor improves Cystic fibrosis symptoms and underlying disease pathology by aiding the conformational stability of F508 del-mutated Cystic fibrosis transmembrane conductance regulator, resulting in increased processing and trafficking of mature protein to the cell surface. More specifically, Lumacaftor acts as a protein-folding chaperone, preventing misfolding of Cystic fibrosis transmembrane conductance regulator ion channels and consequent destruction during processing in the endoplasmic reticulum.
Half Life
The apparent terminal half-life was approximately 26 hours following a single dose. Ivacaftor exerts its effect by acting as a potentiator of the Cystic fibrosis transmembrane conductance regulator protein, an ion channel involved in the transport of chloride and sodium ions across cell membranes of the lungs, pancreas, and other organs. Alterations in the Cystic fibrosis transmembrane conductance regulator gene result in altered production, misfolding, or function of the protein and consequently abnormal fluid and ion transport across cell membranes. Ivacaftor improves Cystic fibrosis symptoms and underlying disease pathology by potentiating the channel open probability (or gating) of Cystic fibrosis transmembrane conductance regulator protein in patients with impaired Cystic fibrosis transmembrane conductance regulator gating mechanisms. The overall level of Ivacaftor-mediated Cystic fibrosis transmembrane conductance regulator chloride transport is dependent on the amount of Cystic fibrosis transmembrane conductance regulator protein at the cell surface and how responsive a particular mutant Cystic fibrosis transmembrane conductance regulator protein is to Ivacaftor Potentiation.

Half Life
The apparent terminal half-life was approximately 12 hours following a single dose.

MATERIAL AND METHODS
PREPARATION OF THE LUMACAFTOR AND IVACAFTOR WORKING SOLUTIONS
Standard Solution Preparation
Accurately weigh and transfer 20 mg of Lumacaftor and 12.5 mg of Ivacaftor working standard into a 10 mL clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make up the solution up to the mark with the same solvent. (Stock solution). Further pipette 0.75 mL of the above stock solutions into a 10 mL volumetric flask and dilute up to the mark with diluent. (150 ppm of Lumacaftor and 93.75 ppm of Ivacaftor)

Sample Solution Preparation
Accurately weigh 10 tablets crush in mortor and pestle and transfer equivalent to 20 mg of Lumacaftor 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 15 mins to dissolve it completely and make up the volume up to the mark with the same solvent. Then it is filtered through 0.45 µ Injection filter. (Stock solution).

EXPERIMENTAL METHODS
Wave length selection
UV spectrum of 10 µg/mL Lumacaftor and 10 µg/mL Ivacaftor in diluents (mobile phase composition) was recorded by scanning in the range of 1000 nm to 400 nm. From the UV spectrum wavelength selected as 259 nm. At this wavelength both the drugs show good absorbance.

Mobile Phase Optimization
Initially the mobile phase tried was methanol: Ortho phosphoric acid buffer and Methanol: phosphate buffer, Acetonitrile: methanol with various combinations of pH as well as varying proportions. Finally, the mobile phase was optimized to Phosphate buffer (pH 3.0), Acetonitrile in proportion 80: 20 v/v respectively.

Optimization of Column
The method was performed with various columns like C18 column Phenomenex column, YMC, and Inertsil ODS column. Inertsil ODS (4.6 x 250 mm, 5 µm) was found to be ideal as it gave good peak shape and resolution at 1.0 mL/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS
Instrument used: HPLC with Auto sampler and UV detector (WATERS)
Temperature: Ambient
Column: Inertsil ODS (4.6 x 250 mm, 5 µm)
Buffer: 3.4 g of KH₂PO₄ in 1000 mL of HPLC water
Ph was adjusted with OPA up to 3.0.
pH: 3.0
Mobile phase: 80% buffer 20% Acetonitrile
Flow rate: 1 mL per min
Wavelength: 259 nm
January – February
Injection volume : 20µl
Run time : 12min.

RESULTS AND DISCUSSION
The estimation of Lumacaftor and Ivacaftor was performed by RP-HPLC. The assay of Lumacaftor and Ivacaftor was performed with tablets and the % assay was found to be 100.09 and 100.76 which shows that the method is useful for routine analysis. The acceptance criteria of precision is RSD should not be more than 2.0% and the method show precision 0.4 and 0.8 for Lumacaftor and Ivacaftor which states that the method is precise. The acceptance criteria of intermediate precision is RSD should not be more than 2.0% and the method show precision 0.1 and 0.7 for Lumacaftor and Ivacaftor which shows that the method is repeatable when Performed in different days.

The accuracy limit of the percentage recovery should be in the range of 97.0% - 103.0%. The total recovery was found to be 99.86% and 99.96% for Lumacaftor and Ivacaftor. The robustness limit of the mobile phase variation and flow rate variation are well and within the limit, the % degradation results also within the limits. Which states that the method is having good system suitability and precision under given set of conditions.

The isopiestic point of Lumacaftor and Ivacaftor is 259nm. The assay % of Lumacaftor and Ivacaftor is 99.97 and 100.64 and found the system suitability 3.607 and 5.141 respectively. The Validation parameters such as.

Table No.1: Instruments used

| S.No | Instrument        | Model                                      |
|------|-------------------|--------------------------------------------|
| 1    | HPLC              | WATERS, software: Empower, 2695 separation module, UV detector |
| 2    | UV/VIS spectrophotometer | LABINDIA UV 12.500+                        |
| 3    | pH meter          | Adwa – AD 10100                            |
| 4    | Weighing machine | Afcozet ER-1000A                           |
| 5    | Pipettes and Burettes | Borosil                                   |
| 6    | Beakers           | Borosil                                    |

Table No.2: Chemicals used

| S.No | Chemical                          | Company Name                      |
|------|-----------------------------------|-----------------------------------|
| 1    | Lumacaftor                        | PHARMATRAIN                       |
| 2    | Ivacaftor                         | PHARMATRAIN                       |
| 3    | KH₂PO₄                             | FINER chemical LTD                |
| 4    | Water and Methanol for HPLC       | LICHROSOLV (MERCK)               |
| 5    | Acetonitrile for HPLC             | MOLYCHEM                          |
| 6    | Ortho phosphoric Acid             | MERCK                             |

Table No.3: Parameters used

| S.No | Parameters | Lumacaftor | Ivacaftor |
|------|------------|------------|-----------|
| 1    | Accuracy   | 99.86      | 99.96     |
| 2    | Precision  | 0.4        | 0.8       |
| 3    | LOD        | 3.00       | 3.02      |
| 4    | LOQ        | 9.98       | 10        |
| 5    | Robustness | AC         | AC        |
Table No.4: Calibration of drugs used

| S.No | Lumacaftor | Ivacaftor |
|------|------------|-----------|
|      | Concentration (µg/ml) | Area      | Concentration (µg/ml) | Area      |
| 1    | 50         | 244841    | 31.25      | 31672     |
| 2    | 100        | 525756    | 62.5       | 68336     |
| 3    | 150        | 856654    | 93.75      | 113345    |
| 4    | 200        | 1150925   | 125        | 159680    |
| 5    | 250        | 1435608   | 156.25     | 204473    |

Figure No.1: Isobestic point of Lumacaftor and Ivacaftor

Figure No.2: Optimized chromatogram; Peaks are separated and peak shapes are also good
CONCLUSION
The linearity of Lumacaftor and Ivacaftor is found to be linear with a correlation coefficient of 0.999 and 0.999 respectively, which shows that the method is capable of producing good sensitivity. The validation of the developed method states that the accuracy is well and within the limit, which states that the method is capable of showing good accuracy and Reproducibility.

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CONFLICT OF INTEREST
We declare that we have no conflict of interest.

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