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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.

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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 12.5mg of Ivacaftor working standard into a 10ml clean dry volumetric flask add about 7mL 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 0.75ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent. (150ppm of Lumacaftor and 93.75ppm of Ivacaftor)

Sample Solution Preparation

Accurately weigh 10 tablets crush in mortor and pestle and transfer equivalent to 20mg of Lumacaftor and 12.5mg Ivacaftor sample into a 10mL clean dry volumetric flask add about 7ml 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 0.45µ Injection filter. (Stock solution).

EXPERIMENTAL METHODS

Wave length selection

UV spectrum of 10µg/ml Lumacaftor and 10µg/ml Ivacaftor in diluents (mobile phase composition) was recorded by scanning in the range of 1000nm to 400nm. From the UV spectrum wavelength selected as 259nm. 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 250mm, 5µm) 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 250mm, 5µm)
Buffer: 3.4g of KH_2PO_4 in 1000ml of HPLC water Ph was adjusted with OPA up to 3.0.
pH : 3.0
Mobile phase: 80% buffer 20% Acetonitrile
Flow rate : 1ml per min
Wavelength : 259nm

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 Ivacatfor 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	Afcoset 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	KH_2PO_4	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	29672
2	100	525756	62.5	68336
3	150	856654	93.75	113345
4	200	1150925	125	159680
5	250	1435608	156.25	204473

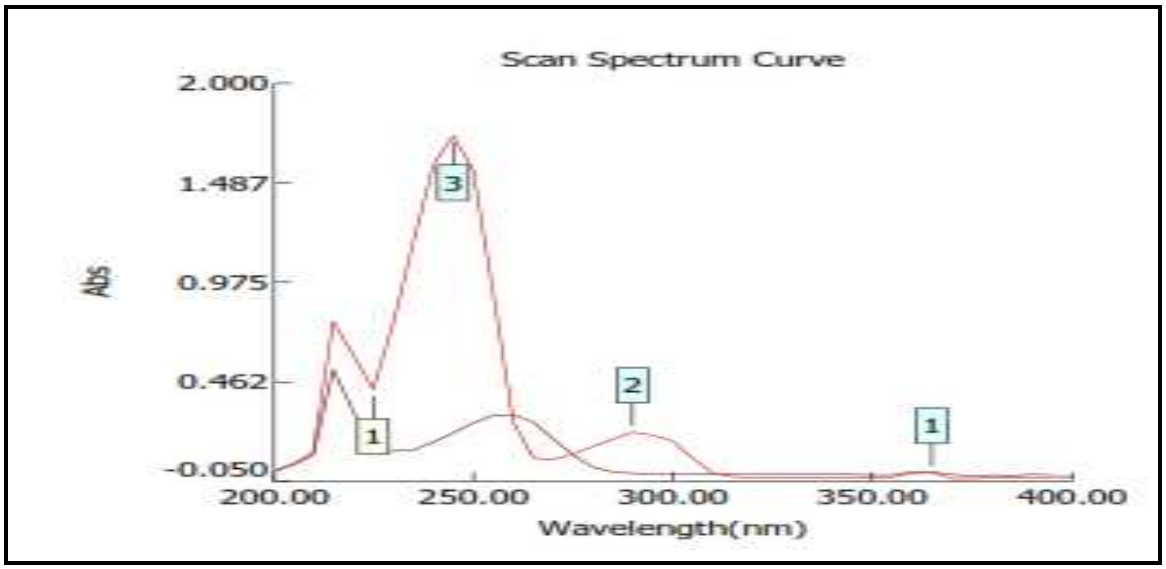

Figure No.1: Isobestic point of Lumacaftor and Ivacaftor

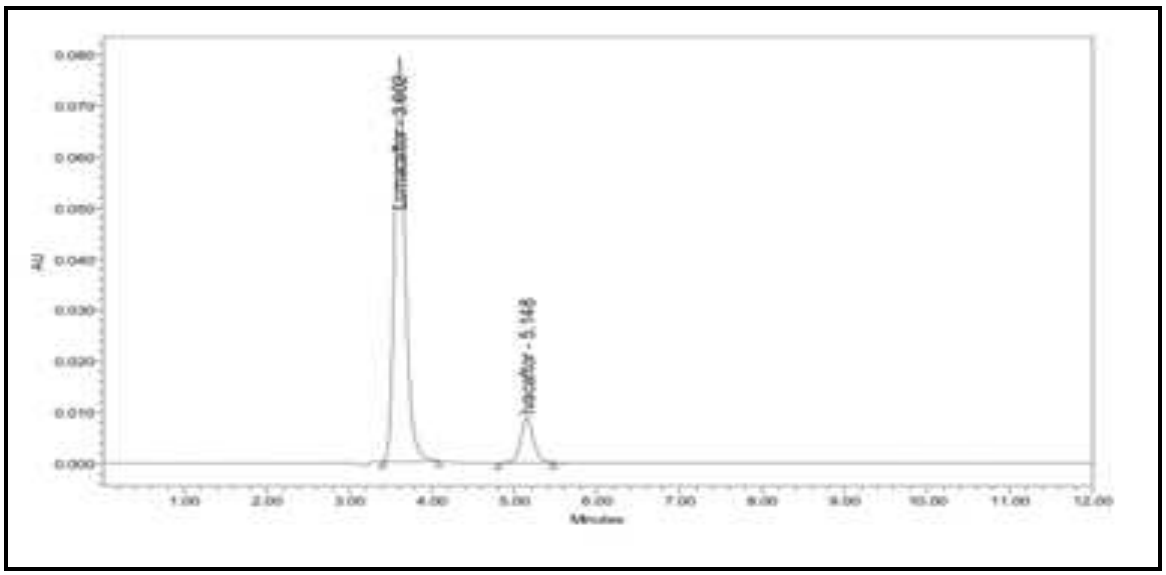

Figure No.2: Optimized chromatogram; Peaks are separated and peak shapes are also good

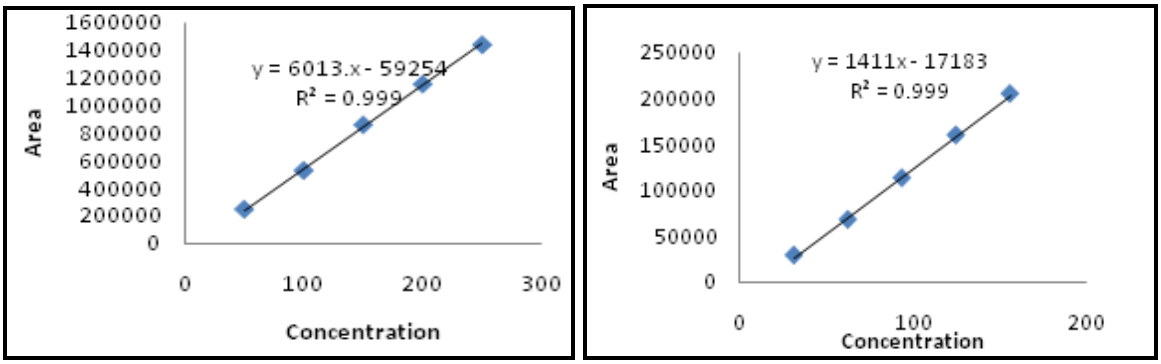

Figure No.3: Calibration graph for Lumacaftor and Ivacaftor

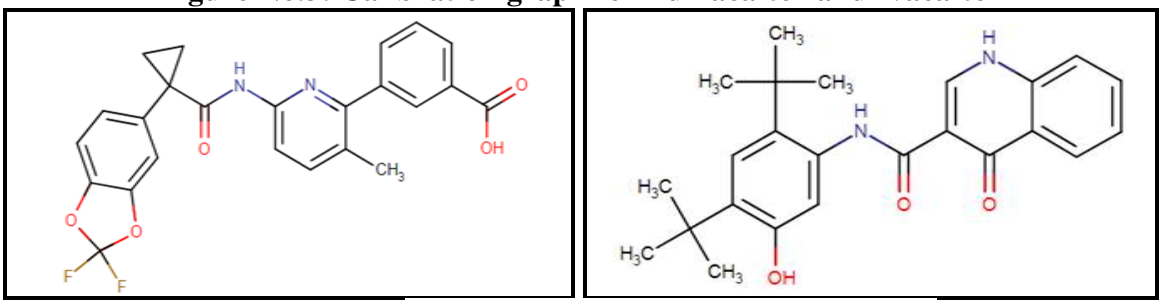

Figure No.4: Structure of Lumacaftor and Ivacaftor

CONCLUSION

The linearity of Lumacaftor and Ivacaftor is found to be linear with a correlation coefficient is 0.999 and 0.999 respectively, which shows that the method is capable of producing good sensitivity. The validation of 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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