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A NEW RP-UPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF IVACAFTOR AND LUMACAFTOR

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ABSTRACT

The different analytical performance parameters such as linearity, precision, accuracy, and specificity were determined according to International Conference on Harmonization ICH Q2B guidelines. The calibration curve was obtained by plotting peak area versus the concentration over the range of 62.5-187.5 µg/ml For ICF and 100-300 µg/ml for LMF. From linearity the correlation coefficient R^2 value was found to be 0.999 for ICF and 0.999 for LMF. The proposed HPLC method was also validated for system suitability, system precision and method precision. The %RSD in the peak area of drug was found to be less than 2%. The number of theoretical plates was found to be more than 2000, which indicates efficient performance of the column. The percentage of recovery of ICF and LMF were found to be 99.9 and 99.8respectively shows that the proposed method is highly accurate. The optimum wavelength for the determination of ICF and LMF was selected at 265 nm on the basis of isobestic point. Various trials were performed with different mobile phases in different ratios, but finally Potassium di hydrogen orthophosphate Phosphate Buffer pH 4.5: Methanol (60:40) %v/v) was selected as good peak symmetry and resolution between the peaks was observed. The Retention time of ICF and LMF were found to be 1.180 and 2.547 min respectively. The Retention times for both the drugs were considerably less compared to the Retention time obtained for the drugs in the other mobile phase.

INTRODUCTION

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 Trans membrane Conductance Regulator (CFTR) protein, an ion channel involved in the transport of chloride and sodium ions across cell membranes. Chemically, it is N-(2,4-di-tert-butyl-5-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide.

Its molecular formula and molecular weight is $C_{24}H_{28}N_2O_3$ and 392.20 respectively.

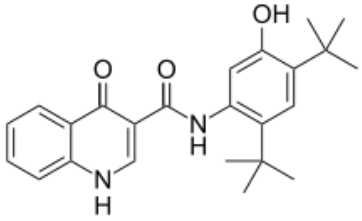

Fig 1: Chemical structure of Ivacaftor

Lumacaftor is a drug used in combination with Ivacaftor as the fixed dose combination product Orkambi for the management of Cystic Fibrosis (CF) in patients aged 6 years and older. Its molecular formula is $C_{24}H_{18}F_2N_2O_5$ and molecular weight is 452.118. It is used to treat people with cystic fibrosis who have the F508del mutation in the cystic fibrosis transmembrane conductance regulator(CFTR), the defective protein that causes the disease. Chemically, it is3-{6-[1-(2,2-difluoro-2H-1,3-benzodioxol-5-yl)cyclopropaneamido]-3-methylpyridin-2-yl}benzoic acid.

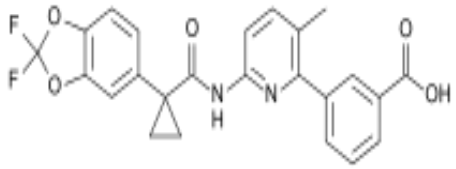

Fig 2: Chemical structure of Lumacaftor.

MATERIALS AND METHODS: Ivacaftor and Lumacaftor sample were obtained from Madras pharmaceuticals, Chennai, India. All reagents used were HPLC/AR grade, nylon membrane filters of 0.45µ pore size were used to filter the mobile phase and its components.

Instrumentation: Analysis was carried out in waters acquity with binary UPLC pump equipped with PDA detector. Separation has been carried out using Agilent 1290 Infinity C18(50x2.1mm ID), 1.8 µ column.

Method Development: Various analytical development trials has been performed by using different chemicals and reagents, organic solvents at different pH ranges and strengths in different proportions of buffer and Organic solvents to separate the three peaks with acceptable resolution and with good peak shape. Various stationary phases of multiple makes were used to check the chromatography with acceptable peak shape, tailing factor and plate count for reproducibility at 25^0C. Based on the observations and conclusions obtained from the number of chromatographic trials performed on UPLC, a particular set of chromatographic conditions were optimized to be suitable for estimation of the Ivacaftor and Lumacaftor in the tablets. The optimized chromatographic conditions which are found to be suitable for the estimation of the Ivacaftor and Lumacaftor are given below. Table No.1.

Preparation of Diluent: Prepared a mixture of water and Acetonitrile in the ration 75:25 (v/v). Mixed well.

Preparation of Phosphate buffer pH 3.5: 2.72 gm of Potassium Di hydrogen orthophosphate was weighed and dissolved in 1000ml of water. Adjust the pH to3.5±0.02 using diluted orthophosphoric acid. Buffer was filtered through 0.45µm filters to remove all fine particles and gases.

Preparation of Mobile Phase: Prepared a mixture of buffer and Acetonitrile in the ratio of 75:25 (v/v). Mixed well. Sonicated for 10mins.

Preparations for Methodology:

Preparation of Ivacaftor standard stock solution: About 125mg of Ivacaftor were weighed into a 100ml volumetric flask, to this 70ml of mobile phase was added, sonicated and the volume was made up with the mobile phase. Pipetted 5ml of the clear solution into 50ml volumetric flask and make up volume with mobile phase.

Preparation of Lumacaftor standard stock solution: About 100mg of LUMACAFTOR were weighed into a 100ml volumetric flask, to this 70ml of mobile phase was added, sonicated and the volume was made up with the mobile phase. Pipetted 5ml of the clear solution

into 50ml volumetric flask and make up volume with mobile phase.

Preparation of Standard solution: About 10mg of Ivacaftor and 10mg of Lumacaftor were weighed into a 50ml volumetric flask, to this 50ml of mobile phase was added, sonicated and the volume was made up to mark with the mobile phase.

Method Validation:

System suitability: It is assessed by injecting the six replicate into a system. Results are given in Table no.1 & 2.

Calibration curve: A linear relationship was evaluated across the range of the analytical procedure and demonstrated directly on the drug substance. The test results were evaluated by calculation of regression line by the method of least square. The respective component concentrations were given below.

vacaftor – 62.5, 100, 125, 150, 188.5 and umacaftor – 100, 160, 200, 240, 300

All the above prepared solutions of respective individual component were analysed to calculate the correlation coefficient of the individual components.

Accuracy: Accuracy of the method was determined by Recovery studies. To the formulation (preanalysed sample), the reference standards of the drugs were added at the level of 50%, 100%, 150%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated.

Sample stock preparation: Crush more than 20 tablets then weigh a quantity of powder equivalent to 125mg of Ivacaftor and 100mg of Lumacaftor in 100ml volumetric flask and add 70ml of mobile phase then sonicated it for 30min intermittent shacking after 30min make up volume with mobile phase. Pipetted 5ml of the clear solution in to 50ml volumetric flask and make up volume with mobile phase. Filter the solution through 0.45µm filter paper.

Method Precision: The Precision of the method was determined by sample preparation. Calculated % of assay using formula

$$\%\,\text{Assay} = \frac{AT}{AS} \times \frac{\text{WS}}{DS} \times \frac{\text{DT}}{WT} \times \frac{\text{P}}{100} \times \frac{\text{AW}}{LC} \times 100$$

Where,

AS: Average peak area due to standard preparation

AT: peak area due to assay preparation, ws: standard weight of ivacaftor/ lumacaftor in mg

WT: Weight of sample in assay preparation

DT: Dilution of assay preparation

ds: dilution of standard preparation

p: purity of ivacaftor /lumacaftor

av: average weight of tablets in mg

lc: labelled claim of ivacaftor/lumacaftor

RESULTS AND DISCUSSIONS:

Method Optimization: The developed method was optimized after many trials. The optimized method developed on C18(50mm x 2.1mm ID), 1.8µm as stationary phase. Using phosphate buffer and Acetonitrile in the ratio 75:25 %v/v as mobile phase. The column temperature was maintained constantly at 35ºC. Mobile phase pumped with a flow rate of 0.5ml/min and injection volume is 10µl.

Table no. 1: Chromatographic conditions

Mobile phase	**Phosphate Buffer pH 3.5: Acetonitrile (75:25) %v/v**
Column	Waters AcquityC18(50mm x2.1 mm ID) 1.8µm
Flow rate	0.5ml/min
Column temperature	35°C
Sample temperature	15°C
Wavelength	265 nm
Injection volume	10µl
Run time	5 min
Retention time	1.827min for IVACAFTOR and 3.577 min for LUMACAFTOR

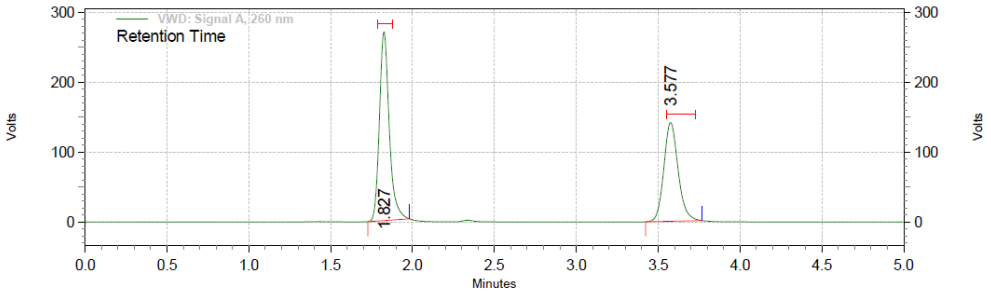

Fig no.3: chromatogram of optimized condition

Table no. 2: System Suitability

S.NO	Name of the component	Retention time	Area	Theoretical plates	Theoretical factor	Resolution
1	IVACAFTOR	1.827	18924601	4707	1.24	-
2	LUMACAFTOR	3.577	14353057	8200	1.14	4.5

Table no. 3: Linearity

S.No	**Parameter**	**IVACAFTOR**	**LUMACAFTOR**
1	Correlation coefficient	0.9995	0.9997
2	Slope	311744	168573
3	Intercept	65251	469211

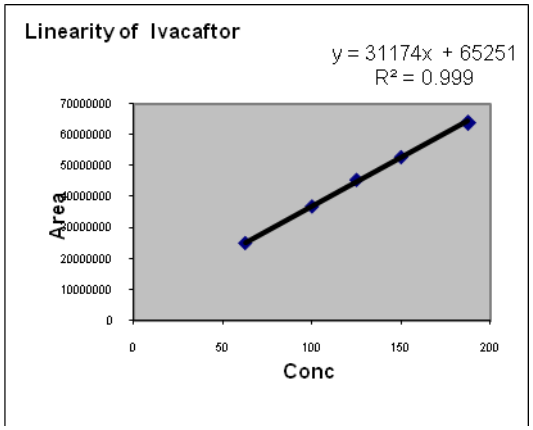

Fig no.4 a :Linearity graph of Ivacaftor

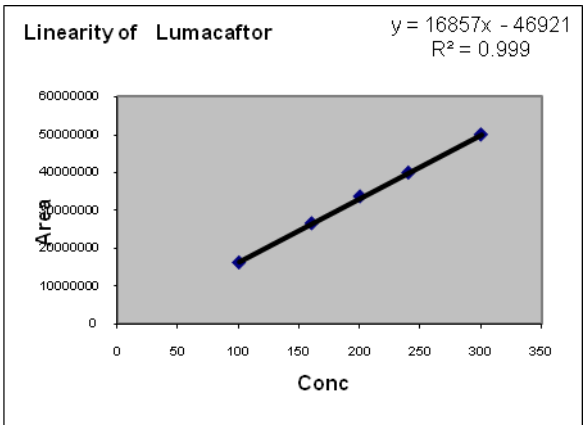

Fig no. 4 b: Linearity graph of Lumacaftor

Table no. 4: Accuracy

Table no.4 (a): Results for Recovery of ivacaftor.

%Recovery	Amount present (µg/ml)	Amount found (µg/ml)*	Percent Recovery *	% Mean Recovery
50%	62.50	62.66	100.3	99.9
100%	125.0	123.75	99.4	
150%	188.5	188.17	100.4	

***** Mean of three observations

Table no. 4 (b): Results for recovery of Lumacaftor

%Recovery	Amount present (µg/ml)	Amount found (µg/ml)*	Percent Recovery *	% Mean Recovery
50%	100	100.94	99.1	99.8
100%	200	198.58	100.7	
150%	300	300.95	99.7	

***** Mean of three observations

Specificity

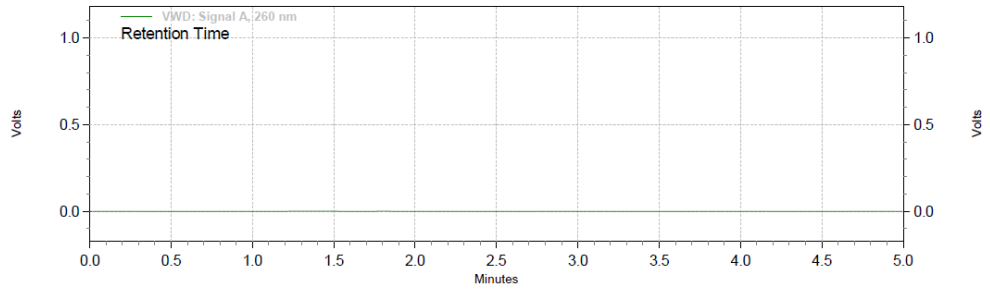

Fig no. 5 (a) : Chromatogram of Ivacaftor and Lumacaftor Blank

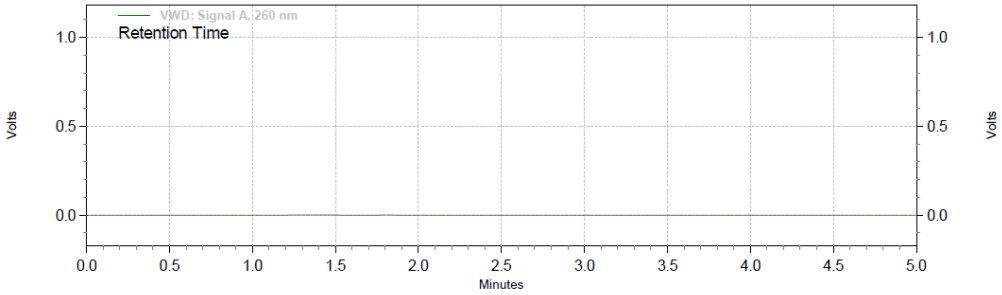

Fig no.5(b): Chromatogram of Placebo

Table no. 5: Precision

Injection	IVACAFTOR		LUMACAFTOR	
	Area	%Assay	Area	%Assay
1	45741313	98.8	33365477	98.9
2	45787695	98.9	33411360	99.1
3	46080749	99.6	33638194	99.7
4	45802928	99.0	33304993	98.7
5	45286836	98.8	32828676	98.3
6	45786681	98.9	33039426	98.0
Average	-	98.8	-	98.6
SD	-	0.6	-	0.9
%RSD	-	0.6	-	0.9

System suitability: All system suitability parameters were passed which include the theoretical plates, tailing factor, resolution for Ivacaftor and Lumacaftor respectively. Table No.02.

Linearity: The best fit line was obtained with regression coefficient between the peak area vs concentration. Results are given below .Table No.03 and Fig No.4a and 4b.

Specificity: It was evaluated by injecting blank and placebo along with drug product, no interference was found at the components respective retention timings.

Chromatogram depicted below Fig No.5a and 5b.

Method Precision: The Precision of the method was determined by injecting Ivacaftor and Lumacaftor with sample solution 6 times respectively. Method precision was expressed in terms of % RSD. Results are given in table no.05.

Accuracy: Prepared accuracy at 3 levels in triplicate at 50% , 100% and 150% with matrix and achieved satisfactory results and at each level of recovery was calculated. Results are given below table No.04.

CONCLUSION:

A new precise, accurate, rapid method has been developed for the simultaneous estimation of Ivacaftor and Lumacaftor in pharmaceutical dosage form by RP-HPLC. The optimum wavelength for the determination of ICF and LMF was selected at 260 nm on the basis of isobestic point. Various trials were performed with different mobile phases in different ratios, but Phosphate Buffer pH 3.5: Acetonitrile(75:25) %v/v) was selected as good peak symmetry and resolution between the peaks was observed. The Retention time of ICF and LMF were found to be 1.827 and 3.577 min respectively. The retention times for both the drugs were considerably less compared to the retention time obtained for the drugs in the other mobile phase. The different analytical performance parameters such as linearity, precision, accuracy, and specificity were determined according to International Conference on Harmonization ICH Q2B guidelines. The calibration curve was obtained by plotting peak area versus the concentration over the range of 62.5-187.5 µg/ml For ICF and 100-300 µg/ml for LMF. From linearity the correlation coefficient R^2 value was found to be 0.999for ICF and 0.999 for LMF. The proposed HPLC method was also validated for system suitability, system precision and method precision. The %RSD in the peak area of drug was found to be less than 2%. The number of theoretical plates was found to be more than 2000, which indicates efficient performance of the column. The percentage of recovery of ICF and LMF were found to be 99.9 and 99.8respectivelyshows that the proposed method is highly accurate. Hence the proposed method is highly sensitive, precise and accurate and it successfully applied for the quantification of API content in the commercial formulations of Ivacaftor and Lumacaftor in Educational institutions and Quality control laboratories.

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