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RESEARCH ARTICLE

Simultaneous estimation and validation of Lumacaftor and Ivacaftor in the tablet dosage form using RP-HPLC method

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ABSTRACT

The aim present research work to development and validation of RP-HPLC method for the simultaneous estimation of Lumacaftor and Ivacaftor. Chromatographic separation was evaluated by Phenomenex C18 column (250 X 4.6 mm, 5 µ) using the mobile phase consisting of Phosphate buffer and Acetonitrile in the ratio of 40:60%v/v (pH was adjusted to 4.5 with O-phosphoric acid). The mobile phase was pumped at a flow rate of 1.0 mL/min and detection was done by UV detector at 255 nm. The retention time of Lumacaftor and Ivacaftor were found to be 2.857min and 6.329 min. The linearity was obtained in the range of 100-500µg/ml for lumacaftor and 62.5-312.5 µg/ml for Ivacaftor with correlation coefficient was 0.999. The proposed method was found to be simple, accurate, precise, robust and cost effective. It can be applied for routine quality control analysis for simultaneous estimation of Lumacaftor and Ivacaftor in pharmaceutical dosage forms.

Keywords: Lumacaftor, Ivacaftor, RP-HPLC, Mobile phase, Acetonitrile, Retention time

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CONTENTS

1. Introduction

Ivacaftor is a drug used to treat cystic fibrosis in people with certain mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, who account for 4–5% cases of cystic fibrosis. 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. It 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[1,2].

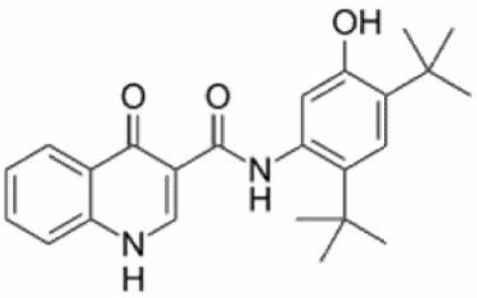

Fig 1: Chemical Structure of Ivacaftor

Lumacaftor/ivacaftor (brand name Orkambi) is a combination drug available a single pill that is used for the treatment of cystic fibrosis in people who have the F508del mutation in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) protein. It is a combination drug that consists of lumacaftor and ivacaftor. Ivacaftor increases the activity of the CFTR protein at the surface of epithelial cell, while lumacaftor acts as a chaperone during protein folding and increases the number of CFTR proteins that are trafficked to the cell surface[1,2]. It was approved by the US FDA in July 2015.

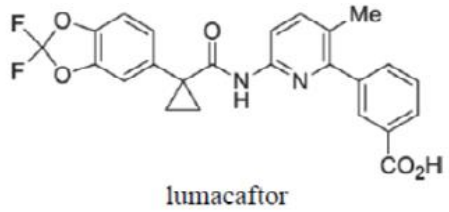

Fig 2: Chemical Structure of Lumacaftor

Literature reveals different methods for their analysis in their formulations. But our present plan is to develop a new, simple, precise& accurate method for its analysis in formulation after a detailed study a new RP-HPLC method was decided to be developed and validated[3,4,5,6].

2. Materials and Methods

Instruments used:

The following instruments are used to determination of Lumacaftor and Ivacaftor.

Table 1: List of Instruments

S. No	Instrument	Model
1	HPLC	WATERS, software: Empower, 2695 separation module.2487 UV detector.
2	UV/VIS spectrophotometer	LABINDIA UV 3000$^+$
3	pH meter	Adwa – AD 1020
4	Weighing machine	Afcoset ER-200A

Chemicals used:

The following chemicals are used to determination of Lumacaftor and Ivacaftor.

Table 2: List of Chemicals

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

HPLC Method development

Mobile Phase Optimization:

Initially the mobile phase tried was methanol: Ammonium acetate buffer and Methanol: phosphate buffer with various combinations of pH as well as varying proportions. Finally, the mobile phase was optimized to orthophosphoric acid with buffer (pH 4.5), Acetonitrile in proportion 40: 60 v/v respectively.

Wave length selection:

UV spectrum of 10 µg/ml Lumacaftor and Ivacaftor in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 255nm. At this wavelength both the drugs show good absorbance.

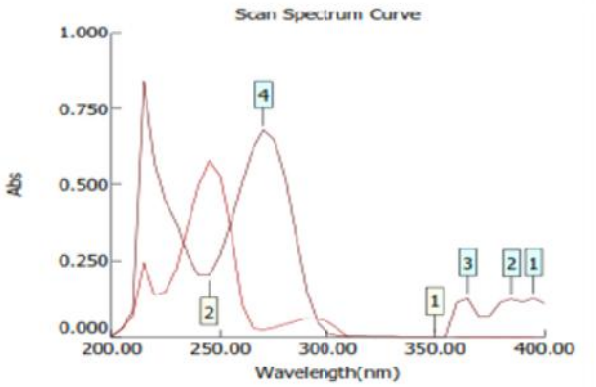

Fig 3: UV Spectra of Lumacaftor and Ivacaftor

Optimized Chromatographic Conditions:

Instrument used **:**Waters HPLC with auto sampler and 2487 UV detector.
Temperature **:**Ambient
Column **:** Phenominex (4.6 x 250mm, 5µm)
Buffer **:**1ml of orthophosphoric acid in 1000ml water, pH adjusted with NaOH.
pH **:** 4.5
Mobile phase **:**40% buffer 60% Acetonitrile
Flow rate **:**1.0 ml/min
Wavelength **:**255 nm
Injection volume **:**10 µl
Run time **:** 10 min
.

Preparation of mobile phase:

Accurately measured 400 ml (40%) of above buffer and 600 ml of Acetonitrile HPLC (60%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

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 volume up to the mark with the same solvent (Stock solution). Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Sample Solution Preparation:

Accurately weigh 10 tablets crush in mortor and pestle and transfer equivalent to 1000 mg of Lumacaftor and 10mg Ivacaftor (marketed formulation=1250.08 mg of tablet Powder) sample into a 10mL 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 (Stock solution). Further pipette 1.5 ml of Lumacaftor and Ivacaftor from the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

System Suitability Parameter:

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. Following system suitability test parameters were established.

Acceptance criteria:

- Resolution between two drugs must be not less than 2.
- Theoretical plates must be not less than 2000.
- Tailing factor must be not more than 2.

Method Validation

The developed method was statically validated according to ICH guidelines Q2(R1). The validation parameters like specificity, linearity, accuracy, precision, LOD & LOQ and robustness[10,11].

Linearity:

The linearity was determined for Lumacaftor and Ivacaftor six different concentrations were analyzed and calibration curve was constructed by plotting mean response factor against the respective concentration. The method was evaluated by determination of the correlation coefficient and intercept value. Linearity concentrations are made from in the range of 100-500µg/ml for Lumacaftor and 62.5-312.5µg/ml for Ivacaftor.

Precision:

The standard solution was injected for six times and measured the area for all six Injections in HPLC. It was done for the within the day and between the days with same chromatographic conditions.The %RSD for the area of six replicate injections was found to be within the specified limits.

Accuracy:

Percentage mean recovery was obtained by using standard addition technique which was by adding known quantities of pure standards at three different levels such as 50%, 100% and 150% to the pre analysed sample formulation. From the amount of drug found, amount of drug recovered and percentage recovery were calculated which sense to conformation that the proposed method was accurate.

LOD & LOQ:

The sensitivity of the proposed method for measurement of Lumacaftor and Ivacaftor were estimated in terms of Limit of Detection (LOD) and Limit of Quantification (LOQ).The LOD and LOQ were calculated by using the slope and SD of response (intercept).The mean slope value and SD of response were obtained after plotting six calibration curves.

Robustness:

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

a) The flow rate was varied at 0.9 ml/min to 1.1ml/min:

Standard solution 300 ppm of Lumacaftor & 187.5 ppm of Ivacaftor was prepared and analysed using the varied flow rates along with method flow rate.

b) The Organic composition in the Mobile phase was varied from 50% to 50%:

Standard solution 300 ppm of Lumacaftor & 187.5 ppm of Ivacaftor was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method.

Forced degradation studies:

The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. In present research work the standard solutions of Lumacaftor and Ivacaftor were placed in different stress conditions like acid, base, peroxide, thermal and photolytic condtions. Then observe the solutions in some period of time and calculate the percentage amount of drug degraded in above stress conditions.

3. Results and discussion

System suitability: The specificity of this method was determined by complete separation of Lumacaftor and Ivacaftor. The tailing factor was less than 2% and resolution was satisfactory. The peaks obtained for sharp and have clear baseline separation. The system suitability parameters are given in Table 3.

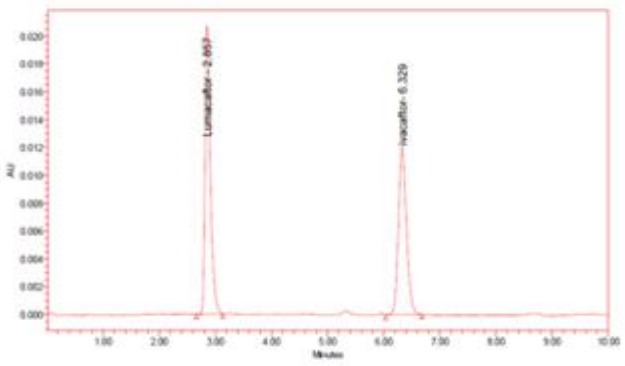

Fig 4: Chromatogram for system suitability

Assay:

Standard and sample solution injected as described under experimental work. The corresponding chromatograms and results are shown in table 4.

Linearity:

The linearity range was found to lie from 100µg/ml to 500µg/ml of Lumacaftor, 62.5µg/ml to 312.5µg/ml of Ivacaftor and then plotting the graph concentration Vs peak area. The correlation coefficient was found to be 0.999 for both. The results were reported in table 5 and fig 5&6.

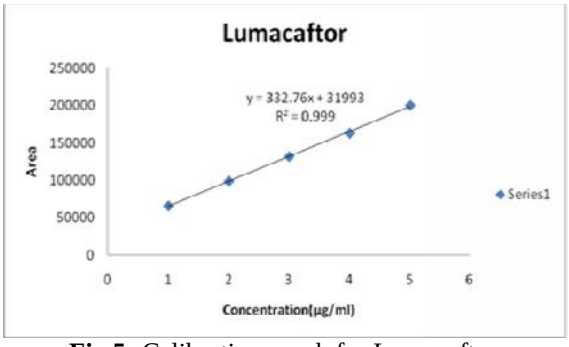

Fig 5: Calibration graph for Lumacaftor

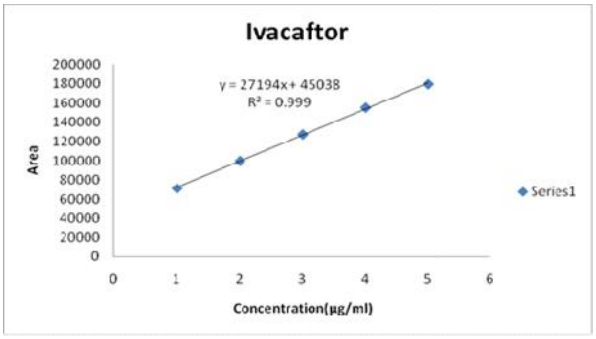

Fig 6: Calibration graph for Ivacaftor

Precision: Precision of the method was carried out for both sample solutions as described under experimental work. The % RSD was found to be less than 2%. The data was shown in table 6.

Accuracy:

Sample solutions at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated. The mean percentage recovery was found to be 100.53 for lumacaftor and 100.13 for ivacaftor. It was present in within the limit. The data was shown in table 7&8.

Robustness: The standard and samples of Lumacaftor and Ivacaftor were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count. All the paremeters are present in between the limit. The results were shown in table 10&11.

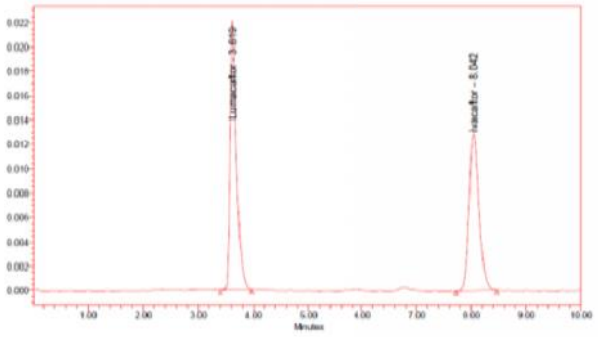

Fig 7: Chromatogram showing less flow

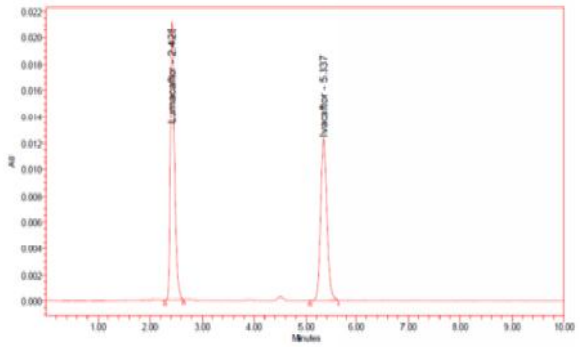

Fig 8: Chromatogram showing more flow

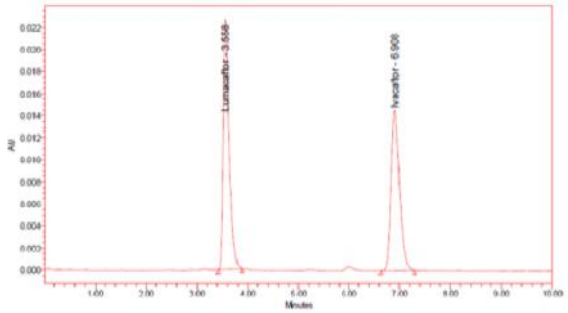

Fig 9: Chromatogram showing less organic composition

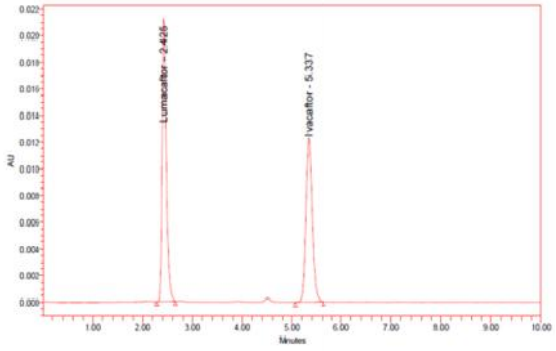

Fig 10: Chromatogram showing more organic composition

Table 3: Results of system suitability parameters

S.No	Name	RT(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Lumacaftor	2.857	134796	20824		1.47	4509.57
2	Ivacaftor	6.329	120104	12053	16.02	1.15	9239.89

Table 4: Results of Assay for Lumacaftor and Ivacaftor

Drug	Label Claim (mg)	% Assay
Lumacaftor	200	100.39
Ivacaftor	125	100.17

Table 5: Area of different concentration of Lumacaftor and Ivacaftor

S. No	Lumacaftor		Ivacaftor	
	Concentration (µg/ml)	Area	Concentration (µg/ml)	Area
1	100	65792	62.5	71267
2	200	98696	125	99725
3	300	131638	187.5	127369
4	400	162911	250	155275
5	500	200063	312.5	179461

Table 6: Results for intraday and inter day precision

Injection	Intraday precision		Intermediate precision	
	Peak area of Lumacaftor	Peak area of Ivacaftor	Peak area of Lumacaftor	Peak area of Ivacaftor
Injection-1	141368	128876	139453	122535
Injection-2	140717	127224	137162	121224
Injection-3	142655	129055	139458	122915
Injection-4	143939	128739	138377	123391
Injection-5	143013	126699	138482	123108
Injection-6	142282	129220	139771	122959
Average	142329.0	128302.2	138783.8	122688.7
Std Dev	1156.8	1064.1	976.1	769.7
%RSD	0.8	0.8	0.7	0.6

Table 7: 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	

*Average of three determinations

Table 8: Accuracy results for Ivacafttor

%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	12.6	100.87	
150%	179676.0	18.75	18.59	99.16	

*Average of three determinations

Table 9: Results for LOD & LOQ

Parameter	Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
LOD	Lumacaftor	66	198	3.00
	Ivacaftor	66	199	3.02
LOQ	Lumacaftor	66	659	9.98
	Ivacaftor	66	660	10.00

Table 10: Results for variation in flow for Lumacaftor and Ivacaftor

S. No	Flow Rate (ml/min)	System Suitability Results			
		Lumacaftor		Ivacaftor	
		USP Plate Count	USP Tailing	USP Plate Count	USP Tailing

1	0.9	4685.09	1.12	4731.46	1.21
2	1.0	4509.7	1.47	4509.7	1.47
3	1.1	4065.51	1.40	4549.3	1.12

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

Table 11: Results for variation in mobile phase composition

S. No	Variation in mobile phase ratio	System Suitability Results			
		Lumacaftor		Ivacaftor	
		USP Plate Count	USP Tailing	USP Plate Count	USP Tailing
1	10% less	4382.7	1.12	4643.64	1.26
2	*Actual	4509.7	1.47	4509.7	1.47
3	10% more	4982.7	1.17	4987.28	0.95

***** Results for actual Mobile phase composition have been consideredfrom Accuracy standard.

Table 12: Degradation results for Lumacaftor and Ivacaftor

Sample Name	Lumacaftor		Ivacaftor	
	Area	% Degraded	Area	% Degraded
Standard	135383.3	-	121004.3	-
Acid	125453	7.33	115289	4.72
Base	127849	5.57	117420	2.96
Peroxide	125131	7.57	113076	6.55
Thermal	128347	5.20	113704	6.03
Photo	129359	4.45	116820	3.46

4. Conclusions

The proposed method was simple, specific, precise and accurate can be used for simultaneous analysis Lumacaftor and Ivacaftor in bulk samples and its dosage form. The result of the study follows the protocol of ICH guidelines and it can be successfully applied for the simultaneous estimation of the marketed products of Lumacaftor and Ivacaftor in bulk samples and its combined dosage form.

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