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R E S E A E C H A R T I C L E

Stability Indicating RP-HPLC Method for Simultaneous Estimation of Lumacaftor and Ivacaftor in Bulk and Pharmaceutical Dosage Forms

Rameeja Pattan*, V. Haribaskar, Pasala Sandhya Mounika, Ramesh Dhani, B. Prathap

Department of Pharmaceutical Analysis, Ratnam Institute of Pharmacy, Pidathapolur, Muthukur, Nellore

A B S T R A C T

A simple, fast, accurate, precise, reproducible Stability indicating Reverse phase High performance liquid chromatography (RP-HPLC) method was developed and validated for the determination of Lumacaftor and Ivacaftor in Bulk and pharmaceutical dosage form. Chromatographic separation was done by using Agilent Eclipse XDB-C8 column having dimension of(4.6×150mm,5µm).Mobile phase containing 0.1% O.P.A and methanol in the ratio of 30:70 was pumped through column at a flow rate of 1ml/min. Temperature was maintained at 25°c.Optimized wavelength for Lumacaftor and Ivacaftor was 280 nm. Retention time of Lumacaftor and Ivacaftor were found to be 1.8&2.6 min. Percentage purity of Lumacaftor and Ivacaftor was found to be 100.19% and 101.45% respectively. System suitability parameters for Lumacaftor and Ivacaftor such as Theoretical plates are 4725.92&6256.39, Tailing factor are 1.46&1.29, resolution was found to be 3.18. The proposed method has been validated for accuracy, precision, linearity; robustness and range were within the acceptance limit according to ICH guidelines (ICH, Q2 (R1)). Mean recovery was found to be 100.39% &100.39% respectively. Correlation coefficient (r2) was found to be 0.999&0.999, % RSD for Precision are 0.2 and 0.7 respectively. LOD, LOQ values of Lumacaftor are 3.07&10.09, Ivacaftor are 2.95 &9.93 respectively. Lumacaftor and Ivacaftor were subjected to stress conditions like Acidic, Alkaline, Oxidation, Photolysis and Thermal degradation. Hence the developed method can be successfully employed for the routine analysis of Lumacaftor and Ivacaftor in bulk and pharmaceutical dosage forms.

Key words: Lumacaftor, Ivacaftor, RP-HPLC, Method development, Validation.

A R T I C L E I N F O

CORRESPONDING AUTHOR
Rameeja Pattan
Department of Pharmaceutical analysis,
Ratnam Institute of Pharmacy,
Pidathapolur, Muthukur, Nellore
MS-ID: IJCPS3700

PAPER-QRCODE

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C O N T E N T S

1. Introduction

Lumacaftor1is chemically known as 3-[6-[[1-(2, 2-difluoro-1, 3-benzodioxol-5-yl) cyclopropanecarbonyl] amino]-3-methylpyridin-2-yl] benzoic acid. It is a novel CFTR (Cystic fibrosis transmembrane regulator) Potentiator. This drug is used in treatment of Cystic Fibrosis and improves breathing[2].

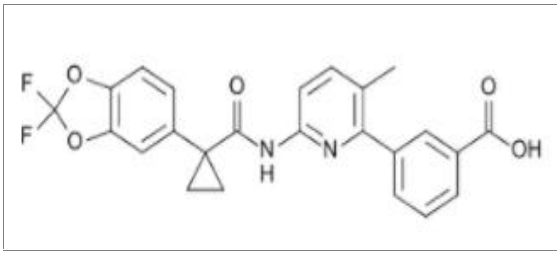

Fig 1: Structure of Lumacaftor

Ivacaftor[3] chemically known as N-(2, 4-di-tert-butyl-5-hydroxyphenyl)-4-oxo-1, 4- dihydroquinoline-3-carboxamide.Ivacaftor in combination with other agents, is indicated for the treatment of cystic fibrosis[4].

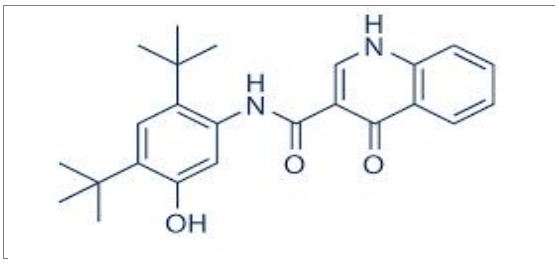

Fig 2: Structure of Ivacaftor

The literature review shows very few methods for Lumacaftor and Ivacaftor by simultaneous estimation by RP-HPLC. Hence, it was felt that, there is a need of new, precise and much efficient analytical method development for the simultaneous estimation of Lumacaftor and Ivacaftor in pharmaceutical dosage form[5]. Present work is aimed to develop a new, simple, fast, rapid, accurate, and reproducible RP-HPLC method for the simultaneous analysis of Lumacaftor and Ivacaftor. The developed method will be validated according to ICH guidelines. Degradation studies were also performed[6-10].

2. Materials and Methods

Table 1: Instruments used

Instrument	Model
HPLC	WATERS, software: Empower, 2695 separation module,uv detector.
UV/VIS spectrophotometer	LABINDIA UV 3000$^+$
pH meter	Adwa – AD 1020
Weighing machine	Afcoset ER-200A
Pipettes and Burettes	Borosil
Beakers	Borosil

Table 2: Details of drug

Drugs	Lumacaftor, Ivacaftor
LABEL CLAIM	Lumacaftor-200mg&Ivacaftor-125mg

Table 3: Chemicals used

Chemical	Company Name
Lumacaftor	PHARMATRAIN
Ivacaftor	PHARMATRAIN
KH_2PO_4	FINER chemical LTD
Water and Methanol for HPLC	LICHROSOLV (MERCK)
Acetonitrile for HPLC	MOLYCHEM
Ortho phosphoric Acid	MERCK

Wave length selection:

UV spectrum of 10µg/ml Lumacaftor and 10 µg/ml Ivacaftor in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 280 nm. At this wavelength both the drugs show good absorbance. The chromatographic method development for the simultaneous estimation of Lumacaftor and Ivacaftor were optimized by several trials for various parameters as different column, flow rate and mobile phase, finally the following chromatographic method was selected for the separation and quantification of Lumacaftor and Ivacaftor in API and pharmaceutical dosage form by RP-HPLC method.

Optimized Chromatographic Conditions:

Instrument used : Waters HPLC with auto sampler and UV detector.
Temperature : Ambient (25° C)
Mode of separation : Isocratic mode
Column : Agilent Eclipse column (4.6 x150mm, 5µm)
Mobile phase : 0.1% OPA: Methanol (30: 70)
Flow rate : 1 ml per min
Wavelength : 280 nm
Injection volume : 10 µl
Run time : 10 min.

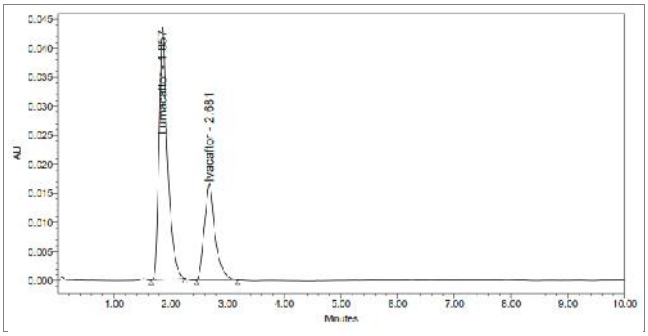

Fig 1: Optimized Chromatogram of Lumacaftor and Ivacaftor

Standard Solution Preparation:

Accurately weigh and transfer 40 mg of Lumacaftor and 25 mg of Ivacaftor working standard into a 100 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 and transfer equivalent to 40 mg of Lumacaftor and 25 mg of Ivacaftor sample into a 100 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.

Preparation of 0.1% OPA:

Take 1ml Orthophosphoric acid in 1000ml volumetric flask and make up with HPLC water and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Preparation of mobile phase:

Accurately measured 300 ml (30%) of 0.1% OPA Buffer and 700 ml (60%) of Methanol were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation: The Mobile phase was used as the diluent

3. Results and Discussions

Method Development

RP- HPLC method was developed by considering the system suitability parameters i.e. resolution between peaks, tailing factor (T), number of theoretical plates (N), runtime and the cost effectiveness. The optimized method developed resulted in the elution of Lumacaftor at 1.857 min and Ivacaftor at 2.681 min. The total run time is 10 minutes with all system suitability parameters as ideal for the mixture of standard solutions.

Tailing factor for the peaks due to Lumacaftor and Ivacaftor in Standard solution should not be more than 2.0. Theoretical plates for the Lumacaftor and Ivacaftor peaks in Standard solution should not be less than 2000. Resolution for the Lumacaftor and Ivacaftor peaks in standard solution should not be less than 2. Results were shown in table 4.

Method Validation

Validation of the analytical method is the process that establishes by laboratory studies in which the performance characteristics of the method meet the requirements for the intended analytical application. The RP-HPLC method developed was validated according to International Conference on Harmonization guidelines for validation of analytical procedures. The method was validated for the parameters in terms of system suitability, selectivity, linearity, accuracy, precision, ruggedness, robustness, limit of detection (LOD) and limit of quantitation (LOQ).

Specificity: For Specificity Blank and Standard are injected into system. There is no any interference of any peak in blank with the retention time of the analytical peaks.

The specificity test was performed for Lumacaftor and Ivacaftor. It was found that there was no interference of impurities in retention time of analytical peak.

Accuracy:

The accuracy study was performed for 50%, 100% and 150 % for Lumacaftor and Ivacaftor. Each level was injected in triplicate into chromatographic system. T he area of each level was used for calculation of % recovery. The results are tabulated in Table.No5 &6

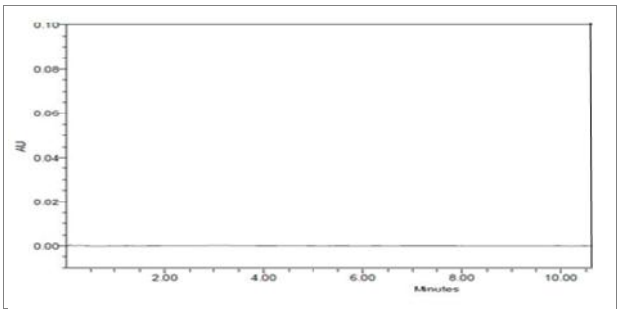

Fig 4: Chromatogram for blank

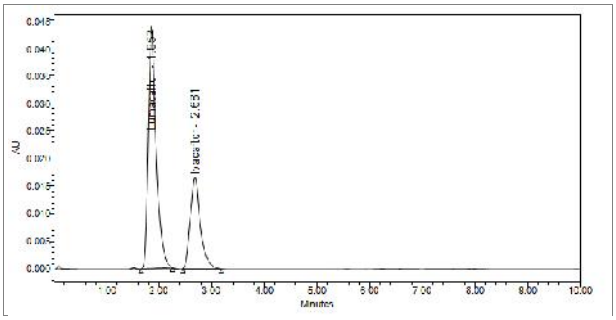

Fig 5: Chromatogram for Standard

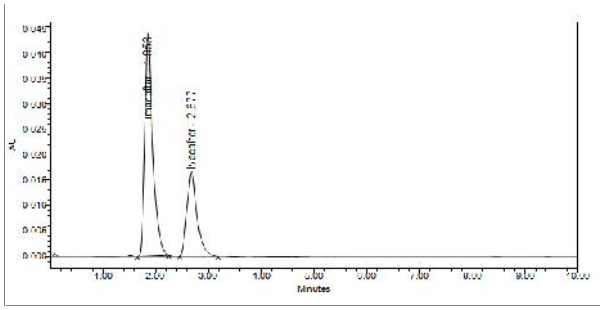

Fig 6: Chromatogram for Sample

Precision:

- Repeatability
- Intermediate Precision/ Ruggedness

Repeatability: The precision study was performed for five injections of Lumacaftor and Ivacaftor. Each standard injection was injected into chromatographic system. The area of each Standard injection was used for calculation of % RSD. The results are tabulated in Table no 7.

Intermediate Precision/Ruggedness:

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within thespecified limits.The results are tabulated in Table no 8.

Linearity: The linearity study was performed for the concentration of 20 ppm to 100 ppm Lumacaftor and 12.5ppm to 62.5ppm Ivacaftor level. Each level was

injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. The results are tabulated in table no-9.

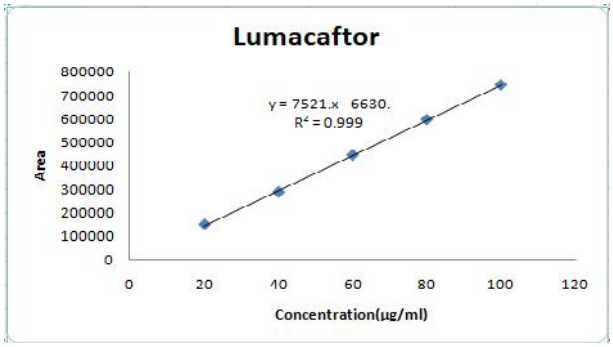

Fig 7: Calibration graph of lumacaftor

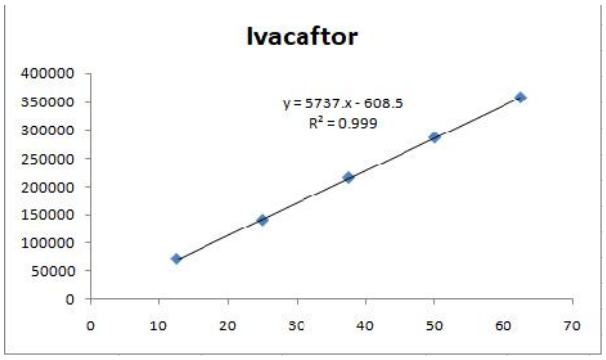

Fig 8: Calibration graph of Ivacaftor

The linearity study was performed for concentration range of 20µg -100µg Lumacaftor and 12.5µg – 62.5 µg Ivacaftor and the correlation coefficient was found to be 0.999 and 0.999.(NLT 0.999)respectively.

Limit of Detection:

The detection limit of Lumacaftor was found to be 3.07

The detection limit of Ivacaftor was found to be 2.95

Acceptance Criteria:

S/N Ratio value shall be 3 for LOD solution

Limit of Quantification: The quantification limit of Lumacaftor was found to be 10.09. The quantification limit of Ivacaftor was found to be 9.93

Acceptance Criteria:

S/N Ratio value shall be 10 for LOQ solution

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 60 ppm of Lumacaftor & 37.5 ppm of Ivacaftor was prepared and analyzed using the varied flow rates along with method flow rate. On evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±10%.

B. The Organic composition in the Mobile phase was varied from ±10%. Standard solution 60 ppm of Lumacaftor & 37.5 ppm of Ivacaftor was prepared and analyzed using the varied Mobile phase composition along with the actual mobile phase composition in the method. On evaluation of the above results, it can be concluded that the variation in 10%. Organic composition in the mobile phase affected the method significantly. Hence it Indicates that the method is robust even by change in the Mobile phase ±10%.

Degradation Studies for Lumacaftor and Ivacaftor:

Preparation of stock:-Accurately weigh and transfer 40 mg of Lumacaftor and 25 mg of Ivacaftor working standard into a 100 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)

Hydrolytic degradation under acidic condition:

Pipette 3 ml of above solution into a 10ml volumetric flask and 3 ml of 0.1N HCl was added. Then, the volumetric flask was kept at 60ºC for 24 hours and then neutralized with 0.1 N NaOH and make up to 10ml with diluent. Filter the solution with 0.44 microns syringe filters and place in vials.

Hydrolytic degradation under alkaline condition:

Pipette 3 ml of above solution into a 10ml volumetric and add 3ml of 0.1N NaOH was added in 10ml of volumetric flask. Then, the volumetric flask was kept at 60ºC for 24 hours and then neutralized with 0.1N HCl and make up to 10ml with diluent. Filter the solution with 0.44 microns syringe filters and place in vials.

Thermal induced degradation:

Lumacaftor and Ivacaftor sample was taken in Petri dish and kept in Hot air oven at 1100 C for 3 hours. Then the sample was taken and diluted with diluents and injected into HPLC and analyzed.

Oxidative degradation:

Pipette 3 ml above stock solution into a 10ml volumetric flask and 1ml of 30% w/v of hydrogen peroxide added in 10 ml of volumetric flask and the volume was made up to the mark with diluent. The volumetric flask was then kept at room temperature for 15 min. Filter the solution with 0.45 microns syringe filters and place in vials.

Photo degradation:

Pipette 3 ml above stock solution into a 10ml volumetric flask and expose to sunlight for 24hrs and the volume was made up to the mark with diluent. Filter the solution with 0.45 microns syringe filters and place in vials.

4. Conclusion

A simple precise and selective RP-HPLC method was developed for the determination of Lumacaftor and Ivacaftor. Chromatographic separation was achieved by using mobile phase consisting of a mixture of 30 volumes 0.1% OPA, 70 volumes of Methanol (30: 70) on Agilent Eclipse XDB-C8, column (4.6 x 150mm, 5 m,) column, with detection limit of 280 nm. Linearity was observed in the range 20-100 µg /ml for Lumacaftor and 12.5-62.5µg /ml for Ivacaftor the amount of drugs estimated by the proposed methods was in good agreement with the label claim. The proposed method was validated. The accuracy of the methods was assessed by recovery studies at three different levels.. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. Method was validated as per ICH guidelines like

system suitability, accuracy, precision, linearity, specificity, forced degradation studies, ruggedness, robustness, therefore, this HPLC method can be used as a routine analysis of these drugs in bulk, pharmaceutical formulations and also for stability studies.

Table 4: System suitability results of Lumacaftor and Ivacaftor

S.No	Sample Name	Ret. Time	Area	Theoretical Plates	Tailing Factor
1.	Lumacaftor	1.857	446832	4725.92	1.46
2.	Ivacaftor	2.681	218536	6256.39	1.29

Table 5: Accuracy results for Lumacaftor

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	225703.3	20	20.14	100.69	100.39
100%	448469.7	40	40.01	100.04	
150%	675482.7	60	60.27	100.45	

Table 6: Accuracy results for Ivacaftor

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	109553.3	12.5	12.56	100.44	100.39
100%	219228.7	25	25.12	100.50	
150%	327988.3	37.5	37.59	100.24	

Table 7: Summarized precision results for Lumacaftor and Ivacaftor

Injection	RT (Lumacaftor)	Area for Lumacaftor	RT (Ivacaftor)	Area for Ivacaftor
Injection-1	1.853	448662	2.677	218753
Injection-2	1.854	446873	2.678	214829
Injection-3	1.855	446352	2.680	216426
Injection-4	1.859	447562	2.683	218452
Injection-5	1.863	447529	2.687	216468
Injection-6	1.864	446244	2.688	217567
Average		447203.7		217082.5
Standard Deviation		907.4		1468.9
%RSD		0.2		0.7

Table 8: Summarized ID precision results for Lumacaftor and Ivacaftor

Injection	RT (Lumacaftor)	Area for Lumacaftor	RT (Ivacaftor)	Area for Ivacaftor
Injection-1	1.853	448776	2.677	218573
Injection-2	1.857	445735	2.681	218562
Injection-3	1.859	447673	2.683	214652
Injection-4	1.861	448673	2.685	215354
Injection-5	1.863	445876	2.687	216454
Injection-6	1.865	448676	2.689	216457
Average		447568.2		216675.3
Standard Deviation		1424.2		1618.5
%RSD		0.3		0.7

Acceptance Criteria: The % RSD for the area of six standard injections results should not be more than 2%.

Table 9: Linearity Results of Lumacaftor & Ivacaftor

	Lumacaftor		Ivacaftor	
Linearity Level	**Concentration**	**Area**	**Concentration**	**Area**
I	20	148475	12.5	71914
II	40	286753	25	140828
III	60	445725	37.5	215732
IV	80	596836	50	286753
V	100	745622	62.5	357562
Correlation Coefficient			0.999	

Table 10: Robustness results for variation flow

S. No	Flow rate	System Suitability Results				
		Lumacaftor		Ivacaftor		
		USP Plate Count	USP Tailing	USP Plate Count	USP Tailing	USP Resolution
1	0.9	4626.92	1.46	6132.29	1.29	3.31
2	1.0	4725.92	1.46	6256.39	1.29	3.18
3	1.1	4865.39	1.46	6352.29	1.29	3.02

Table 11: Robustness results for change in mobile phase composition

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results				
		Lumacaftor		Ivacaftor		
		USP Plate Count	USP Tailing	USP Plate Count	USP Tailing	USP Resolution
1	10% less	1.46	4762.23	6214.27	1.29	3.37
2	*Actual	1.46	4725.92	6256.39	1.29	3.18
3	10% more	1.46	4767.76	6232.23	1.29	2.96

* Results for actual Mobile phase composition (30:70) Buffer: Methanol has been considered from Accuracy stand.

Table 12: Degradation results for Lumacaftor and Ivacaftor

	Lumacaftor		Ivacaftor	
Sample Name	**Area**	**% Degraded**	**Area**	**% Degraded**
Standard	447408.3	-	217707	-
Acid	436522	2.43	207853	4.53
Base	428673	4.19	196762	9.62
Peroxide	439657	1.73	206752	5.03
Thermal	430876	3.70	199672	8.28
Photo	421862	5.71	195534	10.18

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