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

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Abstract

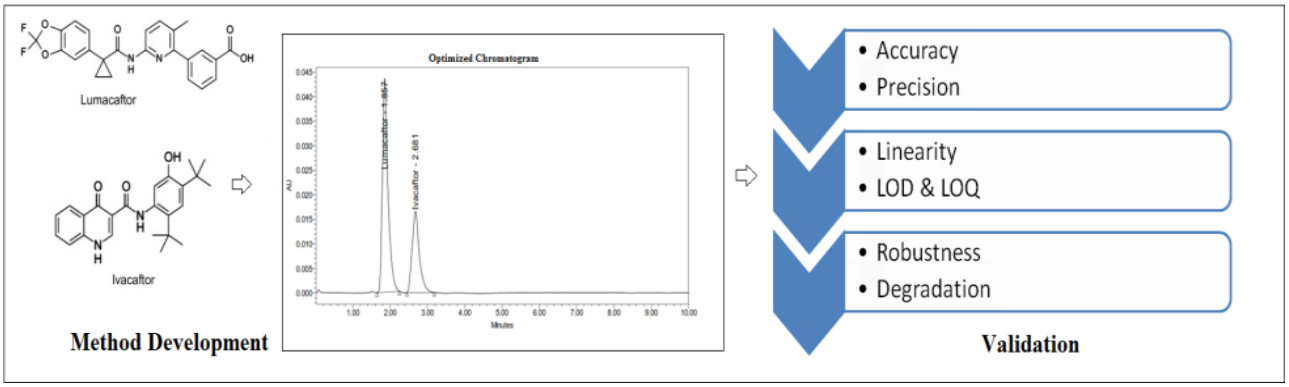
Objective: The present work was designed to develop a simple, fast, accurate, precise, reproducible stability indicating reverse phase high performance liquid chromatography (RP-HPLC) method developed and validated for the determination of lumacaftor and ivacaftor in bulk and pharmaceutical dosage form.

Methods: 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 acetonitrile in the ratio of 40:60 was pumped through column at a flow rate of 1ml/min. Temperature was maintained at 25°C.Optimized wavelength for Lumacaftor and Ivacaftor was 290 nm. Retention time of Lumacaftor and Ivacaftor were found to be 1.8 & 2.6 min.

Results: 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 was 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. Mean recovery was found to be 100.39% &100.39% respectively. Correlation coefficient (R^2) was found to be 0.999 & 0.999; % RSD for Precision was 0.2 and 0.7 respectively. LOD, LOQ values of Lumacaftor was 3.07&10.09; Ivacaftor was 2.95 &9.93 respectively.

***Conclusion*:** 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.



Schematic representation of method development and validation for Lumacaftor and Ivacaftor

INTRODUCTION:

The present work was designed to develop a simple, fast, accurate, precise, reproducible stability indicating reverse phase high performance liquid chromatography (RP-HPLC) method developed and validated for the determination of lumacaftor and ivacaftor in bulk and pharmaceutical dosage form. Lumacaftor and ivacaftor combination (brand name Orkambi) is used for the treatment for cystic fibrosis, a disease of the lungs. Lumacaftor is a strong inducer of CYP3A, and ivacaftor is a substrate of CYP3A. The fixed-dose combination of lumacaftor / ivacaftor (trade name: Orkambi) has been approved in Germany since November 2015 for the patients over the age of 12 years. Since February 2018, it has also been approved for children between the ages of 6 and 11 years. This medication is an option for people with cystic fibrosis who have a certain mutation (F508del) in their CFTR (cystic fibrosis transmembrane conductance regulator) gene. [1]

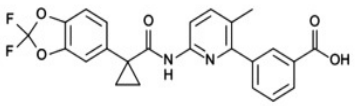


Fig-1 Structure of Lumacaftor

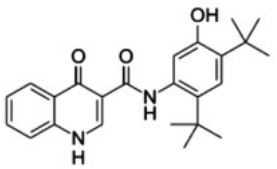


Fig-2: Structure of Ivacaftor

Cystic fibrosis, also called mucoviscidosis, is a genetic metabolic disease. It is caused by a defect in the CFTR (cystic fibrosis transmembrane conductance regulator) gene. This regulator influences the balance of salt and water in the mucus-producing gland cells, for example in the pancreas, bronchi and small intestine. The defect makes the mucus very thick and sticky. In the lungs, this thick mucus can't be coughed up, which makes it hard to breathe and may cause a chronic cough. Bacteria can also collect in the mucus, repeatedly causing respiratory infections. Thick and sticky digestive juices damage the pancreas and reduce the body's absorption of important nutrients in the bowel, increasing the risk of malnutrition and being underweight. The symptoms of cystic fibrosis already appear in children. [2]

There is no cure for cystic fibrosis. The fixed-dose combination of lumacaftor / ivacaftor aims to improve the function of the CFTR, so that the mucus becomes less thick and sticky, and the symptoms improve. The initial corrector compound for clinical development was lumacaftor (Figure 1), which has the chemical name 3-[6-({[1-(2, 2-difluoro-1, 3-benzodioxol-5-yl) cyclopropyl] carbonyl} amino)-3-methylpyridin-2-yl] benzoic acid.

Ivacaftor, (Figure 2) which has the chemical name N-(2, 4-di-tertbutyl-5-hydroxyphenyl)-1, 4-dihydro-4-oxoquinoline-3-carboxamide. Ivacaftor is a hydrophobic molecule and most of it (approximately 99%) is bound to plasma proteins, mainly alpha 1-acid glycoprotein and albumin. Literature review reveals that there are very few HPLC and HPTLC methods available for the determination of Lumacaftor and Ivacaftor in different dosage forms. For Lumacaftor and Ivacaftor there are several HPLC methods available in combined dosage forms. [2]

MATERIALS AND METHODS

HPLC waters, software: Empower, 2695 separation module, UV detector, UV/VIS spectrophotometer Labindia UV 3000^{+}, pH meter Adwa – AD 1020, Digital weighing balance Afcoset ER-200A, Lumacaftor and Ivacaftor was obtained from Ra Chem Pharma Ltd, KH_2PO_4 was obtained from Finer chemical Ltd, water and methanol for HPLC was obtained from Lichrosolv (Merck), Acetonitrile for HPLC was obtained from Molychem, Ortho phosphoric Acid was obtained from Merck

Chromatographic Trials

Trials	Column	Mobile phase ratio	Detection wavelength	Flow rate	Column Temperature	Injection volume	Run time
Trial 1	Symmetry, C18 4.6x150mm, 5µm	Methanol: Water (60:40)	272 nm	1ml/min	Ambient	20µl	10min
Trial 2	Symmetry, C18 4.6x150mm, 5µm	pH 3.5 phosphate buffer: methanol (40:60)	272 nm	1ml/min	Ambient	20µl	10min
Trial 3	Symmetry, C18 4.6x150mm, 5µm	Water: Acetonitrile (20:80)	272 nm	1ml/min	Ambient	20µl	10min
Trial 4	Symmetry, C18 4.6x150mm, 5µm	pH 3.5 phosphate buffer: Acetonitrile (35:65)	272 nm	1ml/min	Ambient	20µl	10min
Trial 5	Agilent Eclipse column (4.6 x 150mm, 5µm)	30% OPA buffer: 70% Methanol (30:70)	290 nm	1ml/min	Ambient	10µl	10min

Results of chromatographic conditions

Trials	Observation
Trial 1	Peaks are not eluted clearly and some impurities were observed
Trial 2	Peaks are not eluted clearly and some impurities were observed.
Trial 3	Peaks eluted clearly and some impurities were observed
Trial 4	Metformin and Linagliptin peaks were sharp, but extra peak was observed So further trail was carried out.
Trial 5	Resolution between three analytes was good. No peak asymmetry was observed. No other impurity interference was seen. All the results were found to be within the acceptance criteria. Hence the method was considered to be optimized.

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 x 150mm, 5µm)
Mobile phase	:	0.1% OPA: Acetonitrile (40: 60)
Flow rate	:	1 ml per min
Wavelength	:	290 nm
Injection volume	:	10 µl
Run time	:	10 min.

Observation: Resolution between three analytes was good. No peak asymmetry was observed. No other impurity interference was seen. All the results were found to be within the acceptance criteria. Hence the method was considered to be optimized.

Preparation of buffer and mobile phase:

Preparation of 0.1% OPA:

1ml Orthophosphoric acid was taken in a 1000ml volumetric flask and the volume made up with HPLC water degassed in an ultrasonic water bath for 10 minutes then filtered through 0.45 µ filter under vacuum filtration.

Preparation of mobile phase:

Accurately measured 400 ml (40%) of 0.1% OPA Buffer and 600 ml (60%) of Methanol were mixed degassed in an ultrasonic water bath for 10 minutes then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.

Preparation of the lumacaftor & ivacaftor standard & sample solution:

Standard Solution Preparation:

Accurately weighed 40 mg of Lumacaftor and 25 mg of Ivacaftor working standard was transferred into a 100 ml clean dry volumetric flask and about 7 ml of diluent was added and sonicated to dissolve it completely and the volume was made up to the mark with the same solvent. (Stock solution) Further 1.5 ml of the above stock solution was pippetted into a 10ml volumetric flask and diluted up to the mark with diluent.

Sample Solution Preparation:

Accurately weighed 40 mg of Lumacaftor and 25 mg of Ivacaftor sample was transferred into a 100 ml clean dry volumetric flask and about 7 mL of diluent and was added and sonicated to dissolve it completely and the volume was up made to the mark with the same solvent. (Stock solution) Further 1.5 ml of the above stock solution was pippetted into a 10ml volumetric flask and diluted up to the mark with diluent.

RESULTS AND DISCUSSION

Validation parameters:

System suitability Table-1Fig-6

Accuracy:

Sample solutions at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated. **Table-2**

Precision:

Precision of the method was carried out for both sample solutions as described under experimental work. The corresponding chromatograms and results are shown in Table 3 & Fig 7

Results of Precision

The results are summarized for Lumacaftor and Ivacaftor Table 3 & Fig 7

Intermediate Precision (Ruggedness)

There was no significant change in assay content and system suitability parameters at different conditions of ruggedness like day to day and system to system variation Table 4 & Fig 8

The results are summarized for Lumacaftor and Ivacaftor Table 4 & Fig 8

Linearity:

Linearity Results: (for Lumacaftor) Table 5 & 6 Fig 9& 10

Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

Limit of Detection

The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio Table 8

Limit of Quantification

The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio Table 9

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.

Robustness results for Lumacaftor: Table 10 Fig 11 less flow Fig 12 More flow

Robustness results for Ivacaftor: Table 11 Fig 13 Less organic Fig 14 More organic

Degradation Studies

Degradation results for Lumacaftor and Ivacaftor Table 12 Fig 15-19

Table-1:-System suitability results for Lumacaftor and Ivacaftor

S.No	Peak name	Retention time	Area	USP resolution	USP tailing	USP Plate count
1	Lumacaftor	1.857	446832		1.46	4725.92
2	Ivacaftor	2.681	218536	3.18	1.29	6256.39

Table-2: The 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%	448469.7	40	40.01	100.04	100.39
150%	675482.7	60	60.27	100.45	
		Accuracy results for Ivacaftor			
50%	109553.3	12.5	12.56	100.44	
100%	219228.7	25	25.12	100.50	100.39
150%	327988.3	37.5	37.59	100.24	

Table-3: Precision Results for Lumacaftor and Ivacaftor

Injection	Area for Lumacaftor	Area for Ivacaftor
Injection-1	448662	218753
Injection-2	446873	214829
Injection-3	446352	216426
Injection-4	447562	218452
Injection-5	447529	216468
Injection-6	446244	217567
Average	447203.7	217082.5
Standard Deviation	907.4	1468.9
%RSD	0.2	0.7

Table-4: ID Precision Results for Lumacaftor and Ivacaftor

Injection	Area for Lumacaftor	Area for Ivacaftor
Injection-1	448776	218573
Injection-2	445735	218562
Injection-3	447673	214652
Injection-4	448673	215354
Injection-5	445876	216454
Injection-6	448676	216457
Average	447568.2	216675.3
Standard Deviation	1424.2	1618.5
%RSD	0.3	0.7

Table-5: Linearity Results for Lumacaftor

S. No	Linearity Level	Concentration	Area
1	I	20	148475
2	II	40	286753
3	III	60	445725
4	IV	80	596836
5	V	100	745622
	Correlation Coefficient		0.999

Table-6: Linearity Results for Ivacaftor

S. No	Linearity Level	Concentration	Area
1	I	12.5	71914
2	II	25	140828
3	III	37.5	215732
4	IV	50	286753
5	V	62.5	357562
	Correlation Coefficient		0.999

Table-7: Analytical performance parameters of Ivacaftor and Lumacaftor

parameters	Lumacaftor	Ivacaftor
Slope(m)	7521	5737
Intercept(c)	6630	608.5
Correlation coefficient(R^2)	0.999	0.999

Table-8: Results of LOD

Drug name	Baseline noise (µV)	Signal obtained (µV)	S/N ratio
Ivacaftor	56	172	3.07
Lumacaftor	56	165	2.95

Table-9: Results of LOQ

Drug name	Baseline noise (µV)	Signal obtained (µV)	S/N ratio
Ivacaftor	56	565.1	10.09
Lumacaftor	56	556	9.93

Table-10: Robustness results For Lumacaftor in variation flow

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Tailing	USP Plate Count
1	0.9	1.46	4626.92
2	1.0	1.46	4725.92
3	1.1	1.46	4865.39

Table-11: Robustness results for Ivacaftor in variation flow

S. No	Flow Rate (ml/min)	System Suitability Results		
		USP Resolution	USP Tailing	USP Plate Count
1	0.9	3.31	1.29	6132.29
2	1.0	3.18	1.29	6256.39
3	1.1	3.02	1.29	6352.29

Table-12: Degradation results for Lumacaftor and Ivacaftor

Sample Name	Lumacaftor		Ivacaftor	
	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

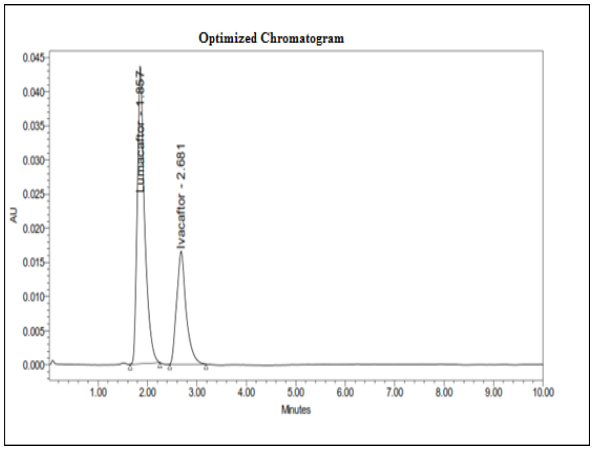


Fig-3: Optimized Chromatogram for Lumacaftor and Ivacaftor

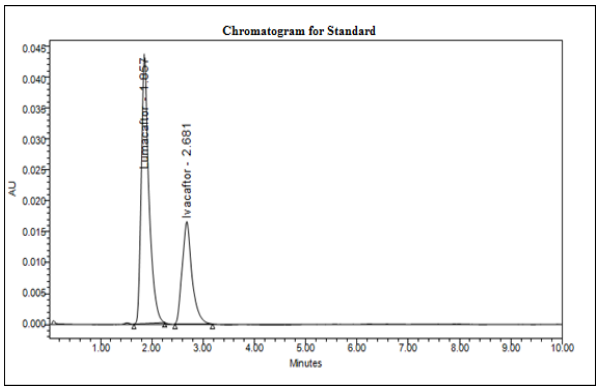


Fig 4: Chromatogram for Standard

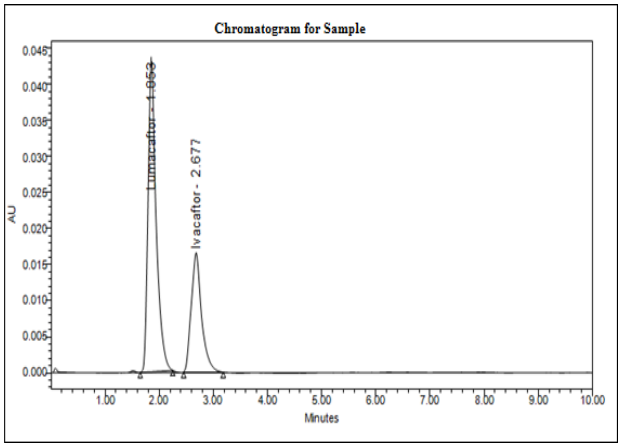


Fig 5: Chromatogram for Sample

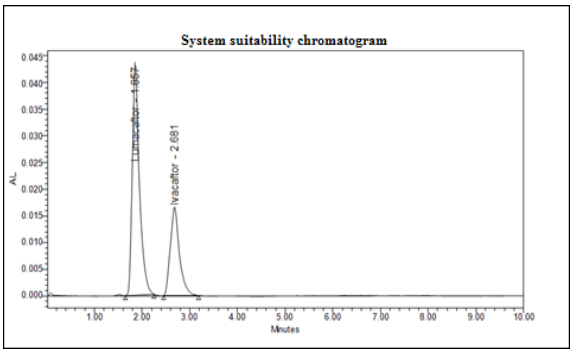


Fig-6: Chromatogram for system suitability for Lumacaftor and Ivacaftor

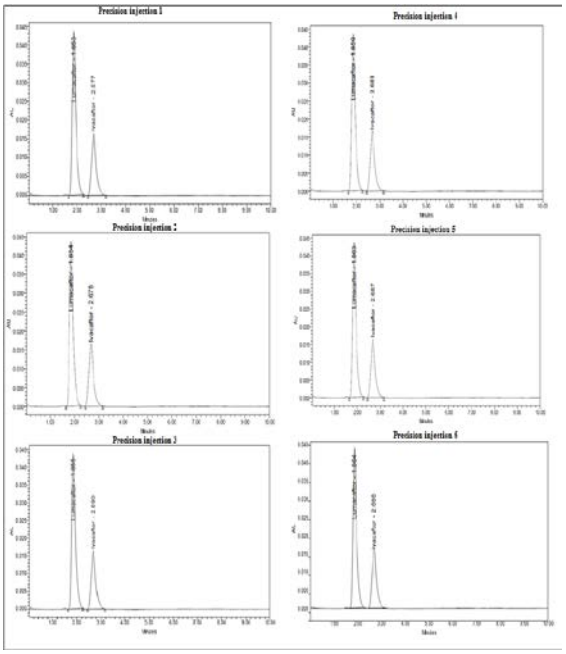


Fig 7 Precision Chromatograms for Lumacaftor and Ivacaftor

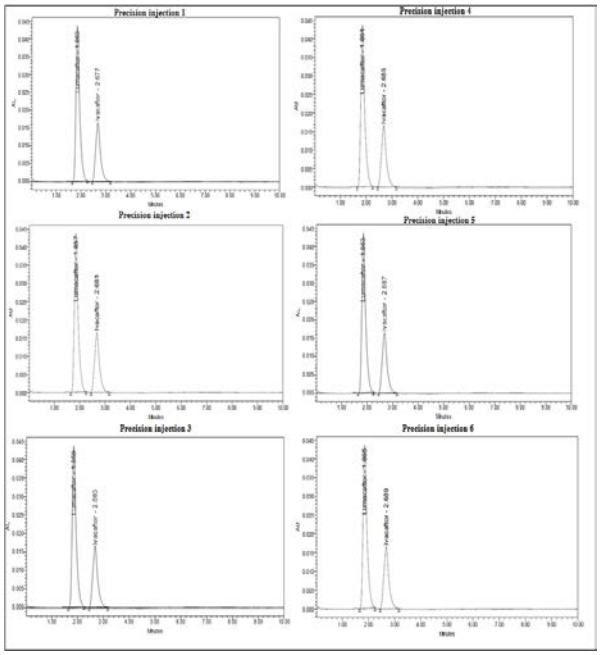


Fig 8 Intermediate Precision (Ruggedness)

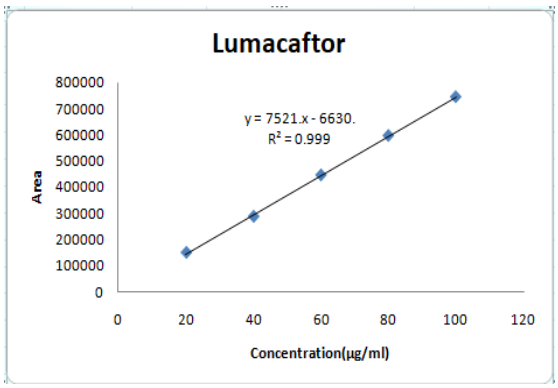


Fig-9: Calibration graph of lumacaftor

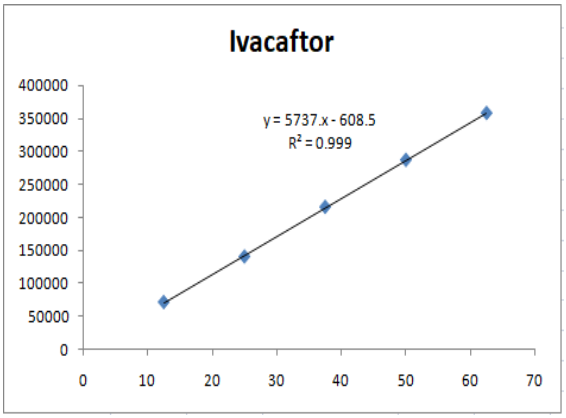


Fig-10: Calibration graph of Ivacaftor

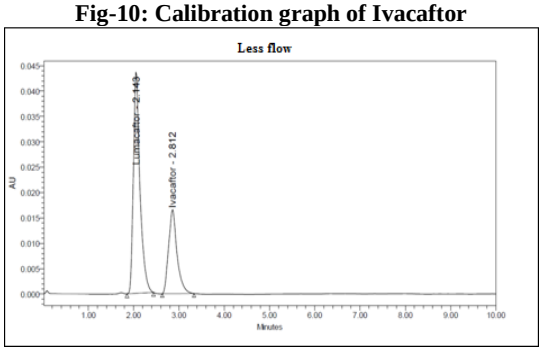


Fig 11 less flow

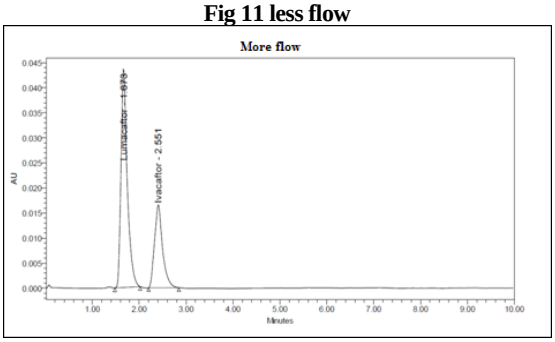


Fig 12 More flow

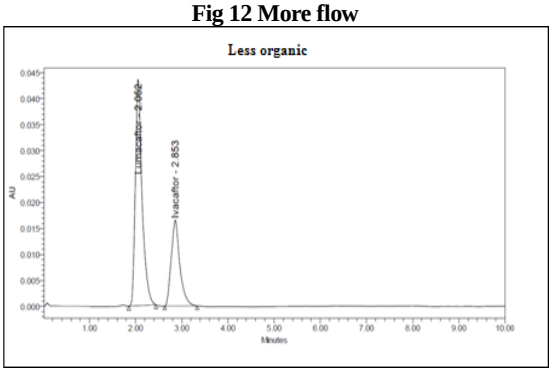


Fig 13 Less organic

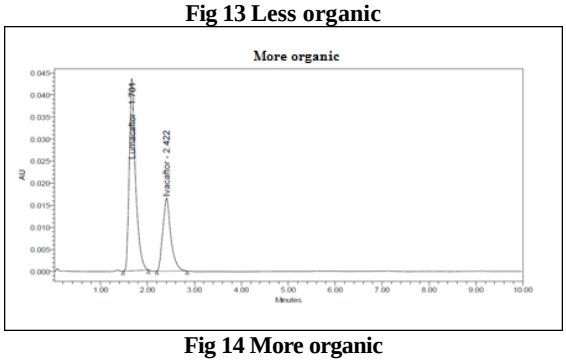


Fig 14 More organic

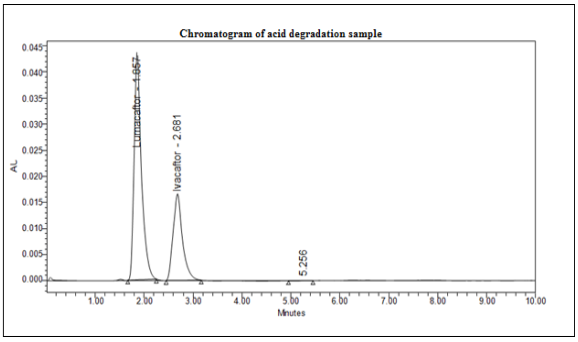


Fig 15 Chromatogram of acid degradation sample

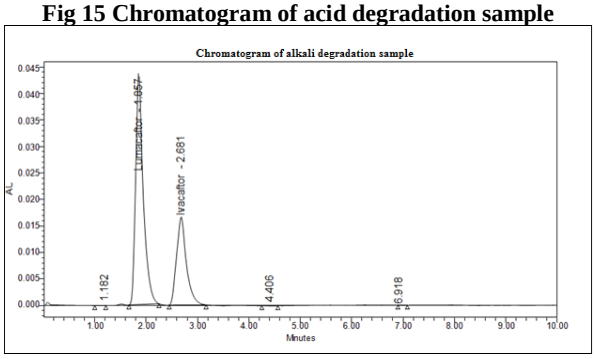


Fig 16 Chromatogram of alkali degradation sample

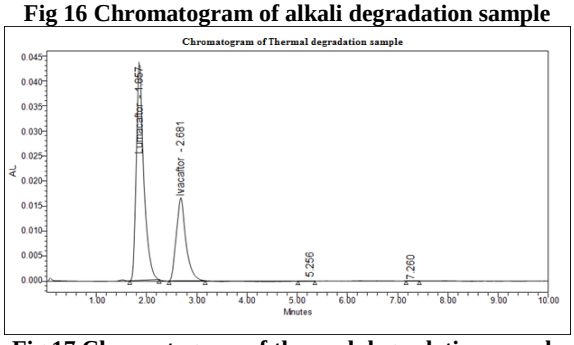


Fig 17 Chromatogram of thermal degradation sample

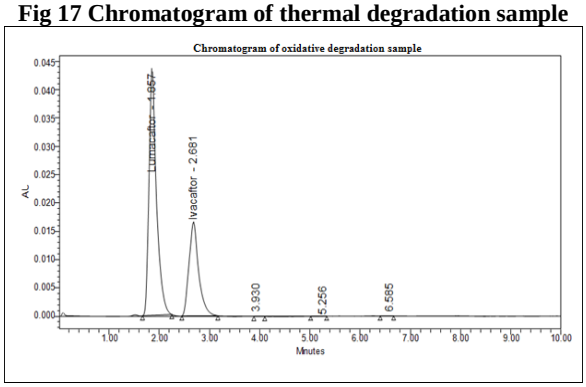


Fig 18 Chromatogram of oxidative degradation sample

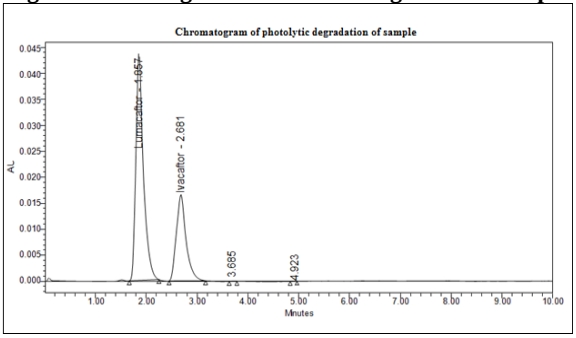


Fig 19 Chromatogram of photolytic degradation sample

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 40 volumes 0.1% OPA, 60 volumes of Methanol (30: 70) on Agilent Eclipse XDB-C_8, column (4.6 x 150mm, 5µm,) column, with detection limit of 290 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. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form. The proposed RP-HPLC (Reverse phase High Performance Liquid Chromatography) method has been evaluated for the accuracy, precision and linearity. The method was found to be precise, accurate and linear over the concentration range. The analytical method validation of Lumacaftor and Ivacaftor by RP-HPLC was found to be satisfactory and could be used for the routine pharmaceutical analysis of Lumacaftor & Ivacaftor. 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.

ABBREVIATIONS:

RP-HPLC: Reverse Phase High performance Liquid Chromatography; HPLC: High Performance Liquid Chromatography; GC-MS-Gas: Chromatography Mass Spectroscopy; LC-MS: Liquid Chromatography Mass Spectroscopy; LC: Liquid Chromatography; RSD: Relative Standard Deviation; SD: Standard Deviation; RT: Retention Time; UV: Ultraviolet Spectroscopy; T: Tailing factor; N: Theoretical Plates; nm: Nanometer; ppm: Parts Per Million; LOD: Limits of detection; LOQ: Limits of Quantification; R2: Correlation Coefficient

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Conflict of Interest

The authors do not declare any conflict of interest.

REFERENCES

1. Talamo Guevara M, McColley SAThe safety of lumacaftor and ivacaftor for the treatment of cystic fibrosis. Expert Opin Drug Saf. 2017; 16(11):1305-1311.
2. Deborah M. Cholon, Charles R. Esther, Jr, and Martina Gentzsch Efficacy of lumacaftor-ivacaftor for the treatment of cystic fibrosis patients homozygous for the F508del-CFTR mutation Expert Rev Precis Med Drug Dev. 2016; 1(3): 235–243.
3. N. Md. Akram and Dr. M. Uma Mahesh. A New Validated RP-HPLC Method for the Determination of Lumacaftor and Ivacaftor in its Bulk and Pharmaceutical Dosage Forms. Orient J Chem. 2017, 33 (3): 1492-1501.
4. Anne Marie Liddy, Gavin McLaughlin, Susanne Schmitz, Deirdre M. D'Arcy, Michael G. Barry The pharmacokinetic interaction between ivacaftor and ritonavir in healthy volunteers. British journal of clinical pharmacology 2017, 83, 10, -2235-2241.
5. Mrs. M. Suresh Babu, N. Spandhana, P. BabyRani, P. Jagadheesh, P.Akhil; Analytical method development and validation for the estimation of Lumacaftor and Ivacaftor using RP-HPLC. Journal of Pharmacreations. 2017, 4(1): 55-78.
6. Pawanjeet. J. Chhabda, M. Balaji, Srinivasarao .V Development and validation of a new and stability indicating RP-HPLC method for the determination of ivacaftor in presence of degrading products international journal of pharmacy and pharmaceutical sciences. 2013, 5(4): 607-613.
7. Schneider EK, Reyes-Ortega F, Wilson JW, Kotsimbos T, Keating D, Li J, Velkov T Development of HPLC and LC-MS/MS methods for the analysis of ivacaftor, its major metabolites and lumacaftor in plasma and sputum of cystic fibrosis patients treated with orkambi or kalydeco. J Vis Exp. 2017; 128.
8. Baki Sharon, Meruva Sathish Kumar, S. Marakatham, Kanduri Valli Kumari A New RP-UPLC Method Development And Validation For The Simultaneous Estimation Of Ivacaftor And Lumacaftor Global Trends Pharm Sci, 2018; 9(3): 5730- 5737.
9. B. Sravanthi; and m. divya; analytical method development and validation of ivacaftor and lumacaftor by RP -HPLC method. Indo American Journal of Pharmaceutical Sciences. 2016, 3(8): 900-904.
10. Satheesh, Dr. D. Naresh, P. Sowjanya, Dr. Gampa Vijaya Kumar Analytical Method Development and Validation for the Simultaneous Estimation of Ivacaftor and Lumacaftor in its Bulk and pharmaceutical dosage forms Pharma research library. 2016, 4(6): 331-335.