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Analytical method development and validation for the simultaneous estimation of Ivacaftor and Lumacaftor by RP-HPLC method

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Abstract
Background: The developed method was validated in terms of accuracy, precision, linearity, robustness and ruggedness, and results will be validated statistically according to ICH guidelines. From literature review and solubility analysis initial chromatographic conditions Mobile phases ortho phosphate acid buffer: Methanol 65:35 were set (Buff P^H 3 adjusted with opa), symmetry C 18.1 (250×4.6mm, 5µ) Column, Flow rate 1.0 ml/min and temperature was ambient, eluent was scanned with PDA detector in system and it showed maximum absorbance at 254 nm. The retention times for Ivacftor and Lumacaftor was found to be 2.972 min and 3.548 min respectively.
Objective: The main objective of the simultaneous estimation of combined drug was to establish identity, purity, physical characteristics and potency of the drugs.
Materials and Methods: Symmetry C 18 column was used for the analysis and maintained buffer pH 3 with diluted OPA and Methanol in the ration of 65:35 was running through column at 1.0 ml flow rate at ambient temperature and absorption maxima was observed at 254 nm.
Results: All the results obtained were precise, accurate and robust as per international conference on Harmonization (ICH) guidelines.
Conclusion: The developed method was validated in terms of accuracy, precision, linearity, robustness and ruggedness, and results will be validated statistically according to ICH guidelines. From literature review and solubility analysis initial chromatographic conditions Mobile phase ortho phosph acid buff: Metha 65:35 were set (Buff Ph 3 adjusted with opa), symmetry C 18.1 (250×4.6mm, 5µ) Column, Flow rate 1.0 ml/min and temperature was ambient, eluent was scanned with PDA detector in system and it showed maximum absorbance at 254 nm. As the metha content was increased Ivacftor and Lumaca got eluted with good peak symmetric properties. The retention times for Ivacftor and Lumaca was found to be 2.972 min and 3.548 min respectively.

Keywords: HPLC, symmetry C 18, Ivacaftor and Lumacaftor

Introduction
Ivacaftor is an aromatic amide mostly obtained by formal condensation process of the carboxy group of 4-oxo-1, 4-dihydroquinoline-3-carboxylic acid with the amino group of 5-amino-2, 4-ditert-butylphenol 1-3. It is used for the treatment of cystic fibrosis. It has a role as a CFTR potentiator and an orphan drug. It is a quinolone which is a member of phenols, an aromatic amide and a monocarboxylic acid amide.
Lumacaftor is an aromatic amide which is obtained by formal condensation process of the carboxy group of 1-(2, 2-difluoro-1, 3-benzodioxol-5-yl) cyclopropane-1-carboxylic acid with the aromatic amino group of 3-(6-amino-3-methylpyridin-2-yl) benzoic acid 19-22. It is used for the 3 treatment of cystic fibrosis. It has a role as a CFTR potentiator and an orphan drug. It is a member of benzoic acids, a member of pyridines, an aromatic amide, and a member of cyclopropanes, a member of benzo dioxoles and an organo fluorine compound

Chemicals and Reagents
HPLC grade Acetonitrile, Methanol, Sodium Perchlorate, Orthophosphoric Acid and water are purched near market. Ivacaftor and Lumacaftor (working standard) are from KP Labs Pvt. Ltd.

Instrumentation
HPLC-waters, software, empower, 2695 separation Module with 296 PDA detector, pH meter from Lab India, Sonicator, Constant Water bath from Themolab GMP

Preparation of Mobile Phase
Take. 6.8 gm of KH_2PO_4 into 1000ml volumetric flask dissolved in Hplc grated water and adjust Ph upto 3 with ortho phospho acid. From the above prepared buff take 300 ml (30%) and 700ml Metha (70%) HPLC were mixed and degassed in ultrasonic water bath for 5 minutes and was filtered through 0.45 µ filter under vacuum filtration.

Preparation of Standard stock Solution
10 mg of Ivaca and 10mg of Lumaca accurately weighed and transferred into a 10 ml clean dry volumetric flask, about 7 ml of diluent was added, sonicate to dissolve it completely and volume was made up to the mark with the same solvent to give the concentration of 1000 µg/ml.

Preparation of Sample Solutions
About 20 mg of Ivaca and 10 mg of Lumaca samples was weighed in to 10ml volumetric flask, it was dissolved with diluent and the volume was made up to the mark with same diluent (2000µg/ml of Ivaca and 1000µg/ml of Lumaca). From Above solution, required concentration of sample can get with proper dilution.

Results
Validation Report
Specificity
The system suitability for specificity was carried out to evaluate whether there is any interference of any impurities in retention time of analytical peak.

Linearity
The linearity study was performed for the concentrations of Ivacafor is 20µg/ml to 100µg/ml and 10µg/ml to 30 µg/ml for Lumocaftor it is shown in Table 1.

Accuracy
The accuracy study was performed for 50%, 100% and 150% Ivacaftor and Lumocaftor. The percentage retrieval was found to be 98.o to 102.0% as shown in Table 2.

Precision (Repeatability)
The precision evaluation was performed for five injections of Ivacaftor and Lumocaftor. Each standard injection was injected into chromatographic system. Which is depicted in Table 3.

Limit of Detection (LOD) and Limit of Quantification (LOQ)
The LOD was performed for Ivacaftor and Lumocaftor which was estimated to be 3.041 and 3.08, respectively. The LOQ was performed for Ivacaftor and Lumocaftor which was estimated to be 9.79 and 10.37, respectively.

Robustness
The robustness was performed for the flow rate variations from 0.6 mL/min to 1.0 mL/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Ivacaftor and Lumacaftor which indicated that the variation in flow rate affected the method significantly.

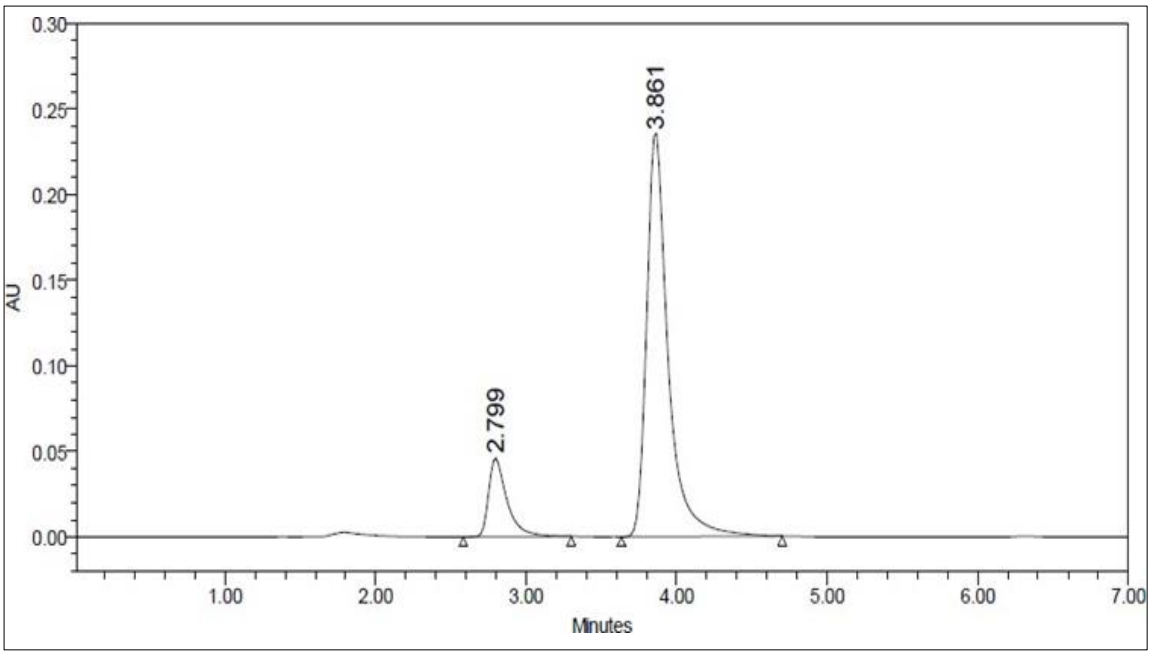

Fig 1: Spectrum showing overlapping spectrum of Ivacaftor and Lumacaftor

Table 1: Linearity results for Ivacaftor and Lumacaftor

S. No	Linearity Level	Concentration	Area
1	I	20 ppm	839286
2	II	40 ppm	1067774
3	III	60 ppm	1246474
4	IV	80 ppm	1439994
5	V	100 ppm	1639065
	Correlation Coefficient		0.999

S. No	Linearity Level	Concentration	Area
1	I	10 ppm	626221
2	II	15 ppm	778750
3	III	20 ppm	931447
4	IV	25 ppm	1070162
5	V	30 ppm	1196060
Correlation Coefficient			0.999

Table 2: Accuracy results of Ivacaftor and Lumacaftor

Sample No.	Level	Amount (µg/ml) added	Amount (µg/ml) found	% Recovery	Mean% Recovery
1	50%	5	4.9	98%	100%
		5	5.1	102%	
		5	5	100%	
2	100%	10	9.88	98.8%	99.13%
		10	9.91	99.1%	
		10	9.95	99.5%	
3	150%	15	14.89	99.2%	99.69%
		15	14.86	99.0%	
		15	14.82	99.79%	
Sample No.	**Spike Level**	**Amount (µg/ml) added**	**Amount (µg/ml) found**	**% Recovery**	**Mean% Recovery**
1	50%	5	4.9	98%	100%
		5	5.1	102%	
		5	5	100%	
2	100%	10	9.88	98.8%	99.31%
		10	9.91	99.1%	
		10	9.95	99.5%	
3	150%	15	14.89	99.2%	99.89%

Table 3: The method precision study was performed for the Relative Standard Deviation (%RSD) of Ivacaftor and Lumacaftor

Injection No	Peak Area of Ivacaftor	R_T
1	1231404	2.808
2	1233196	2.806
3	1231008	2.805
4	1238575	2.807
5	1232407	2.804
Mean	1233318	
SD	3061.06	
%RSD	0.2481	

Injection No	Peak Area of Lumacaftor	R_T
1	912412	3.882
2	913062	3.880
3	909642	3.801
4	916881	3.882
5	914005	3.880
Mean	913200.4	
SD	2621.886	
% RSD	0.287	

Table 4: Robustness results for Ivacaftor and Lumacaftor

S. No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5% less	3249	1.6
2	*Actual	3245	1.6
3	5% more	3829	1. 6

S. No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5% less	2249	1.4
2	*Actual	2245	1.4
3	5% more	2829	1.4

Conclusion

The developed method was validated in terms of accuracy, precision, linearity, robustness and ruggedness, and results will be validated statistically according to ICH guidelines. From literature review and solubility analysis initial chromatographic conditions Mobile phase ortho phosph acid buff: Metha 65:35 were set (Buff P^H 3 adjusted with opa), symmetry C 18.1 (250×4.6mm, 5µ) Column, Flow rate 1.0 ml/min and temperature was ambient, eluent was scanned with PDA detector in system and it showed maximum absorbance at 254 nm. As the Metha content was increased Ivacftor and Lumaca got eluted with good peak symmetric properties. The retention times for Ivacftor and Lumacaftor was found to be 2.972 min and 3.548 min respectively.

Discussion

The analysis method developed for separation of Ivacaftor and Lumacaftor has shown good resolved peaks. Since the RT is short, it indicates that in a shorter duration more samples could be completed and developed method will be easy for analyzing larger samples. The values of LOD and LOQ for these both drugs were significantly low; hence, this method is appropriate for detecting and quantifying the fairly low concentrations of these drugs. Results of statistical analysis, lower% RSD values confirm the ability of the analytical assay. The analytical to ICH guidelines (ICH, Q2 (R1)). Results is simple, reliable, précised, accurate, linear and reproducible, hence can be applied for drug delivery analysis. This method could be even suitable in active pharmaceutical preparations for quality control analysis.

Consent and ethical approval

It is not applicable.

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Competing interest

Authors have declared that no completing interests exists

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