A NEW RP-HPLC METHOD DEVELOPMENT VALIDATION AND DEGRADATION STUDIES FOR THE SIMULTANEOUS ESTIMATION OF IVACAFTOR AND LUMACAFTOR

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A new simple, precise, accurate RP-HPLC method was developed and validated for the simultaneous estimation of Ivacaftor and Lumacaftor. Chromatographic separation was achieved isocratically on Dikma (250 mm × 4.6 mm) column using a mobile phase, Acetonitrile : OPA Phosphate buffer pH adjusted to 4.5 in the ratio of 40:60%v/v. The flow rate was 1ml/min and effluent was detected at 255nm.The values of RSD were less than 2% indicating accuracy and precision method. Both drugs were subjected to stress conditions including acidic, basic, peroxide, themal and photolytic degradation. This method can be used to analyze commercial and solid dosage forms containing Ivacaftor and Lumacaftor with good recoveries for routine analysis. The results obtained on validation, parameters met the ICH and USP requirements. The method was found to have suitable applications in routine laboratory analysis with high degree of accuracy and precision.

KEYWORDS : Ivacaftor, Lumacaftor, Validation, RP-HPLC, Degradation.

INTRODUCTION [5-8, 13-14]

Ivacaftor chemically N-(2, 4-Di-tert-butyl-hydroxy phenyl)-4-oxo-1, 4-dihydroquinno-line-3-carboxamide with molecular formula $C_{24}H_{28}N_2O_3$ and brand name is Kalydeco. Ivacaftor category is Cystic fibrosis and is extensively metabolized in humans. Invitro and clinical studies indicate that Ivacaftor is primarily metabolized by CYP3A. M1 and M6 are the two major metabolites of Ivacaftor in humans. The structure of Ivacaftor shown in figure 1. Lumacaftor it is chemically 3-{6-{[1-(2, 2-Difluoro-1, 3-benzoddioxal-5-yl) cyclopropane carbonyl] amino}-3-methyl pyridine-2-yl} benzoic acid practically insoluble in water and molecular formula $C_{24}H_{18}F_2N_2O_5$ of category cystic fibrosis with brand name Afatinib. The structure of Lumacaftor is shown in figure 2.

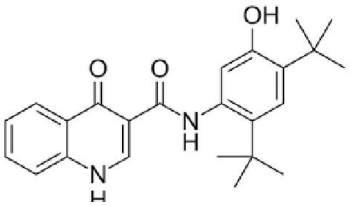


Fig. 1. Structure of Ivacaftor

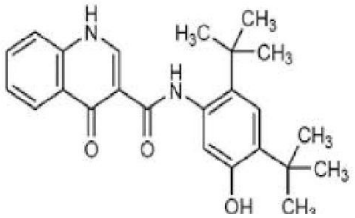


Fig. 2. Structure of Lumacaftor

Literature review

Literature survey reveals that some analytical methods have been reported for the estimation of Ivacaftor and Lumacaftor. The present work reports simple sensitive accurate, precise and economical methods for the determination of both drugs using same mobile phase by HPLC. The method was validated by parameters such as linearity, precision, accuracy, LOD, LOQ, Robustness and system suitability as per ICH guidelines and USP requirements.

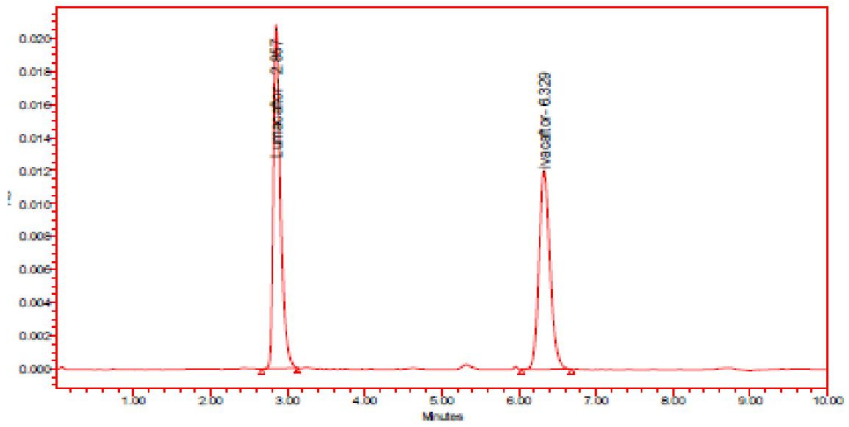


Fig. 3. Chromatogram of Lumacaftor and Ivacaftor

MATERIALS AND METHODS

INSTRUMENTATION [1-4]

The analysis of the drug was carried out on a waters, software: empower 2695 separation module PDA detector. Analytical balance Afcoset ER-200A was used.Adwa-AD-1020P^{H} meter was used to adjust the pH of the buffer. Degassing of the mobile phase was done by sonication using Dolphin ultra sonicator. Filteration was done by using millipore vaccum filter.

Drugs and chemicals

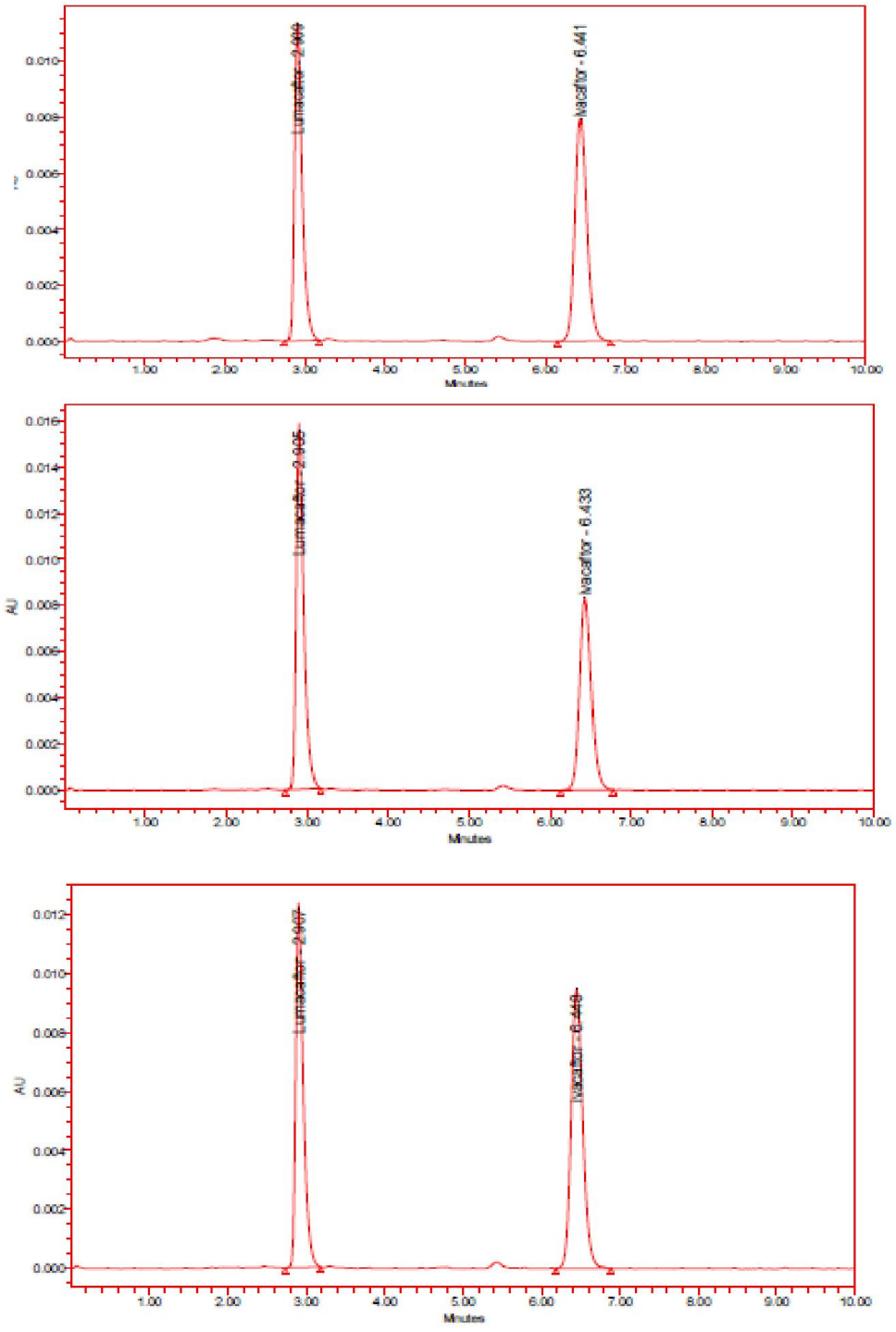
Pure standards of Ivacaftor and Lumacaftor were kindly gifted from HIQ laboratories. The HPLC grade methanol, Acetonitrile were purchased from merck.

PREPARATION OF BUFFER AND MOBILE PHASE:

Preparation of 0.1% OPA buffer:

Pipette out 1 ml of Ortho Phosphoric Acid was taken in a 1000 ml volumetric flask, dissolved and diluted to 1000ml with HPLC water and the volume was adjusted to pH 4.5 with NaOH.

Fig. 4-6. Chromatograms showing accuracy-50% injection-1, 2, 3.



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.

Diluent Preparation:

The Mobile phase was used as the diluent.

PREPARATION OF THE LUMACAFTOR & IVACAFTOR STANDARD & SAMPLE SOLUTION:

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 10 mg 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.

Chromatographic conditions [8]

Separation of Ivacaftor and Lumacaftor was achieved on Dikma C_{18} (250 mm × 4.6 mm) 5µ analytical column as the stationary phase, using mobile phase consists of OPA buffer P^H4.5 and Acetonitrile in the ratio of 60 : 40 v/v. Isocratic elution was achieved at a flow rate 1.2 ml/min with a ambient temperature. The injection volume was 10 ml. This chromatograms were recorded at 255nm using photo diode array detector.

Method validation [11-12]

The analytical method was validated for various parameters as per ICH guidelines.

Accuracy

Accuracy was evaluated in triplicate, at three different concentration levels equivalent to 50, 100, and 150% of the target concentration of active ingredient, by adding a known amount of each of the placebo to a pre-analyzed concentration of both drugs and calculating the % of recovery, and the results obtained were shown in Table 1 and 2.

Table 1. Showing 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	

Table 2. Showing accuracy results for ivacaftor

% 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	12.61	100.87	
150%	179676.0	18.75	18.59	99.16	

PRECISION

Repeatability

Intermediate precision

Repeatability

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of five replicate injections was found to be with in the specified limits, and the results obtained were shown in table 3.

Table 3. Showing precision results for Lumacaftor and Ivacaftor

Injection	Area for Lumacaftor	Area for Ivacaftor
Injection-1	141368	128876
Injection-2	140717	127224
Injection-3	142655	129055
Injection-4	143939	128739
Injection-5	143013	126699
Injection-6	142282	129220
Average	142329.0	128302.2
Standard Deviation	1156.8	1064.1
%RSD	0.8	0.8

Intermediate precision/Ruggedness

The standard solution was injected for six times and measured the area for all injections in HPLC. The % RSD for the area of five replicate injections was found to be within specified limits, and the results obtained were shown in table 4.

Table. 4. Showing Intermediate Precision results for Lumacaftor and Ivacaftor

Injection	Area for Lumacaftor	Area for Ivacaftor
Injection-1	139453	122535
Injection-2	137162	121224
Injection-3	139458	122915
Injection-4	138377	123391
Injection-5	138482	123108
Injection-6	139771	122959
Average	138783.8	122688.7
Standard Deviation	976.1	769.7
%RSD	0.7	0.6

Fig. 7, 8, 9. Chromatograms showing accuracy -100%injection-1, 2, 3

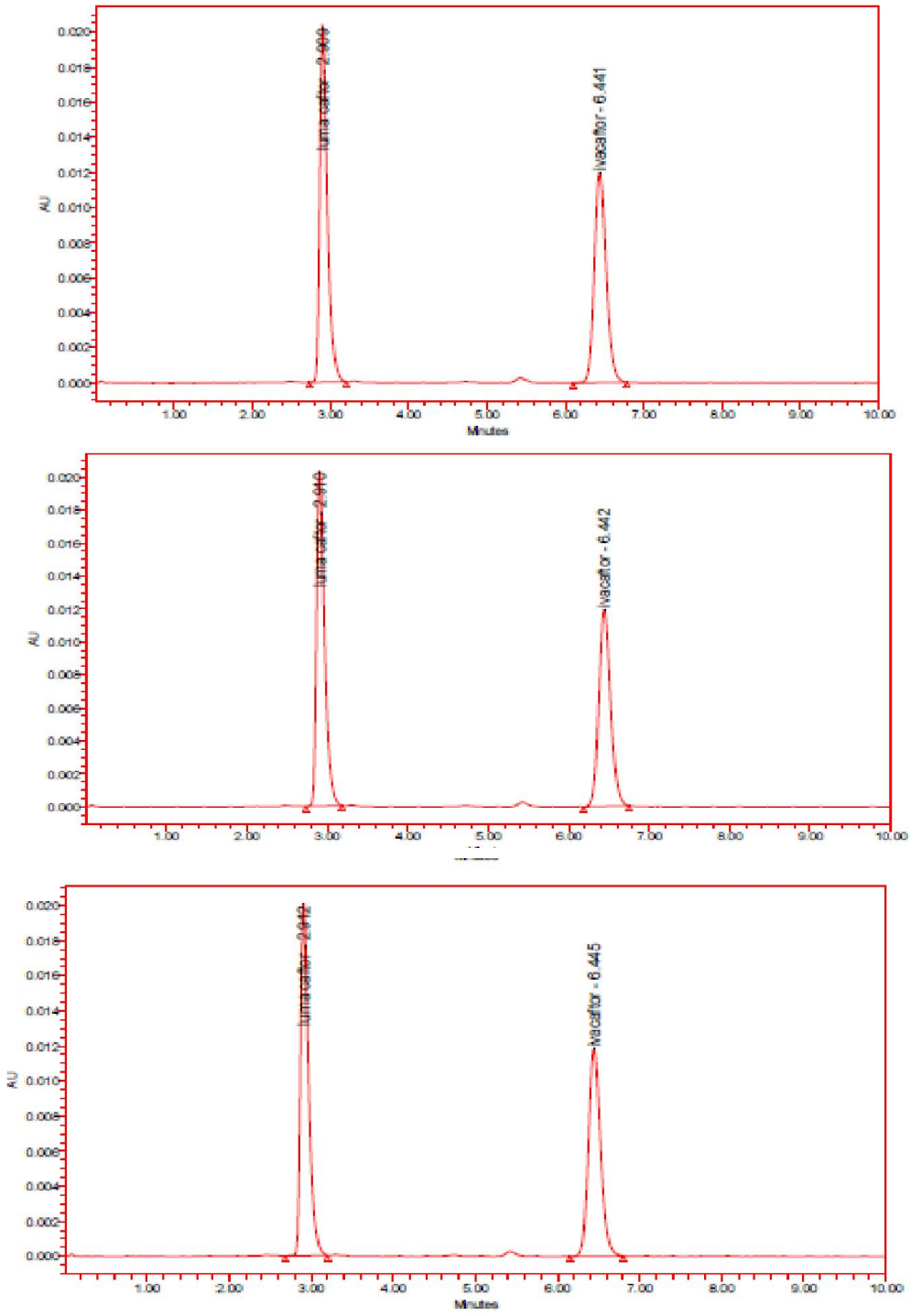


Fig. 10, 11, 12. Chromatograms showing accuracy-150%injection-1, 2, 3

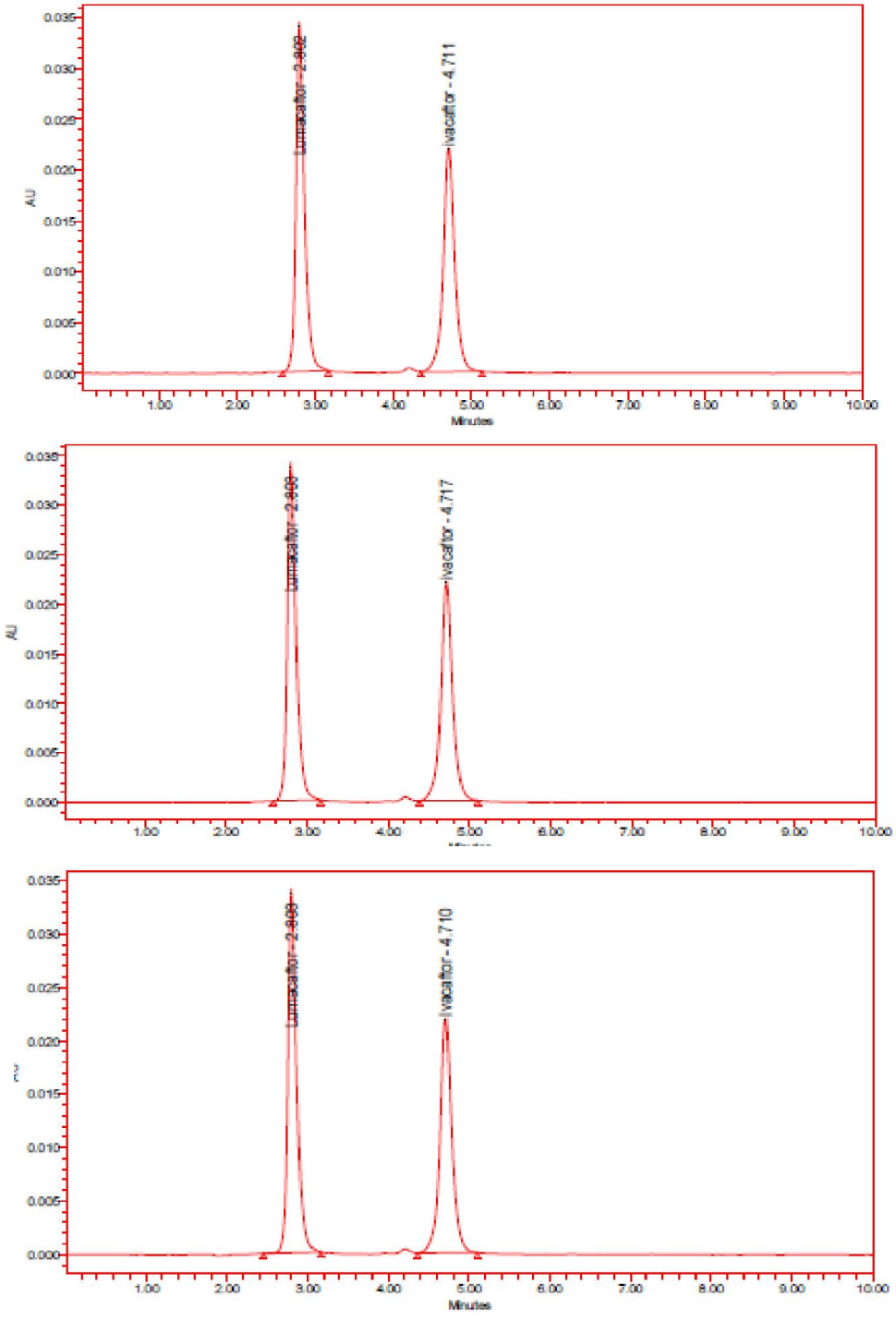
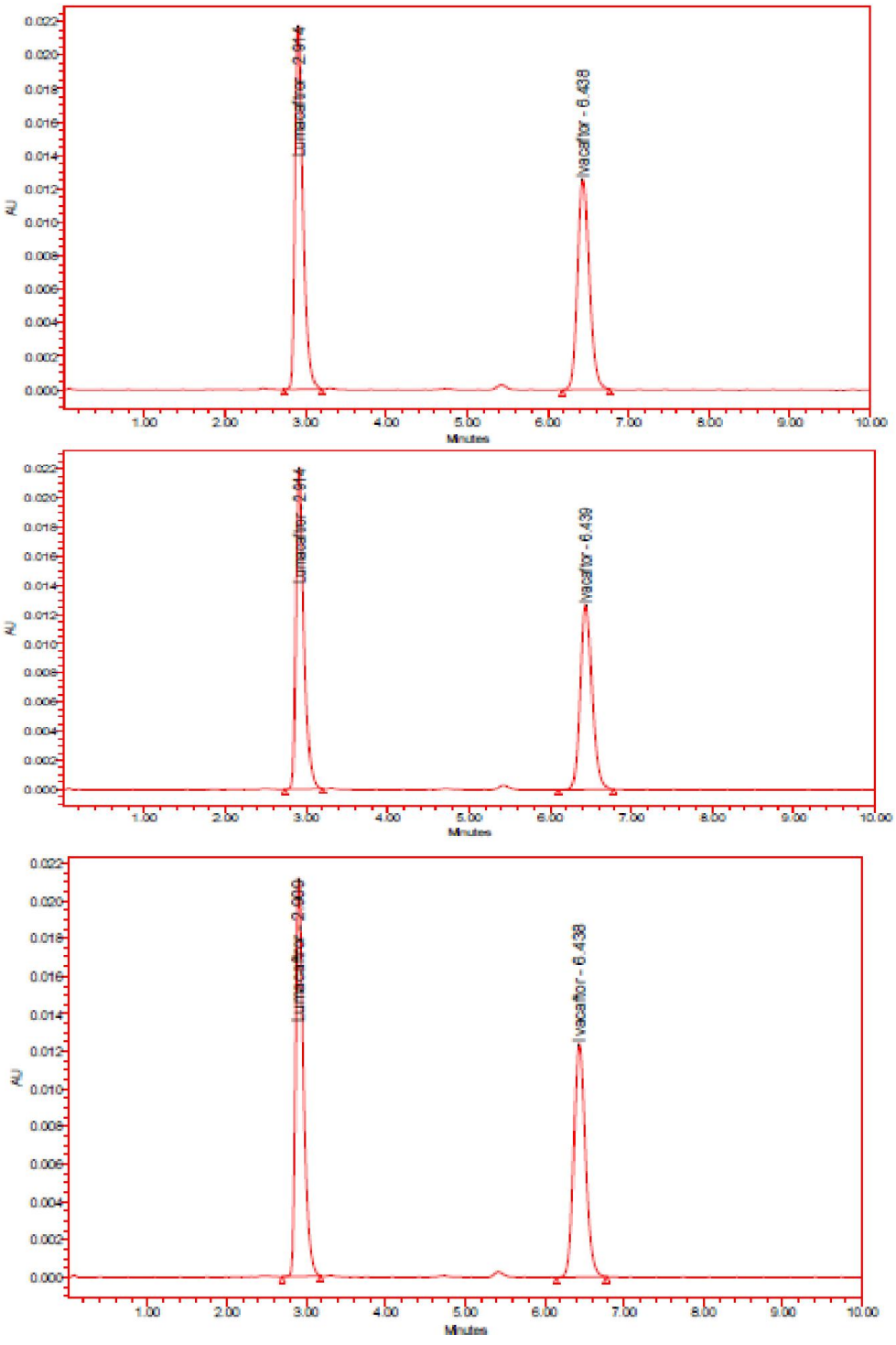
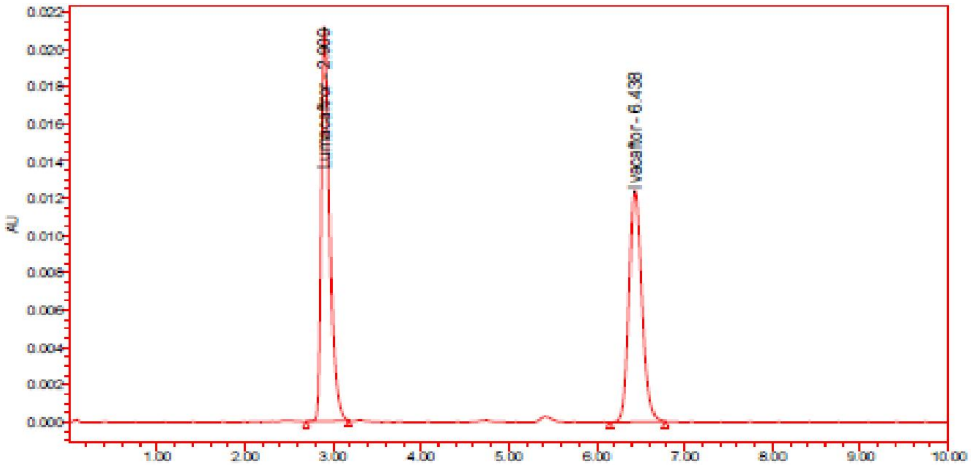


Fig. 13, 14, 15, 16, 17, 18. Chromatograms showing precision injections 1-6




Lumacaftor - 2.909
Ivacaftor - 6.438
AU
0.022
0.020
0.018
0.016
0.014
0.012
0.010
0.008
0.006
0.004
0.002
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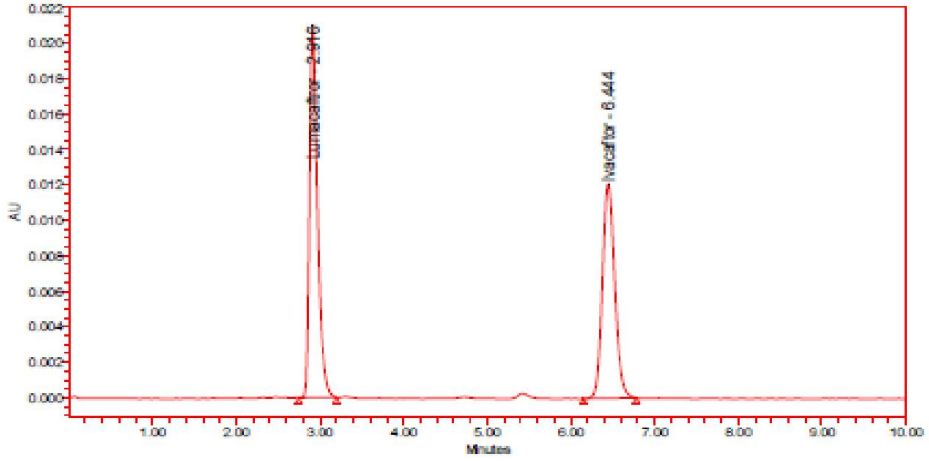
Lumacaftor - 2.916
Ivacaftor - 6.444
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0.020
0.018
0.016
0.014
0.012
0.010
0.008
0.006
0.004
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1.00
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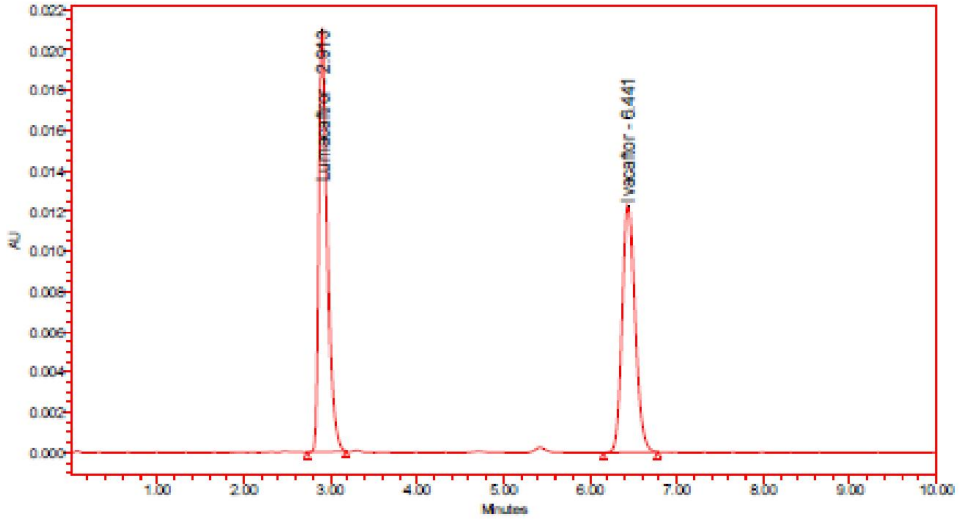
Minutes


Lumacaftor - 2.913
Ivacaftor - 6.441
AU
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0.020
0.018
0.016
0.014
0.012
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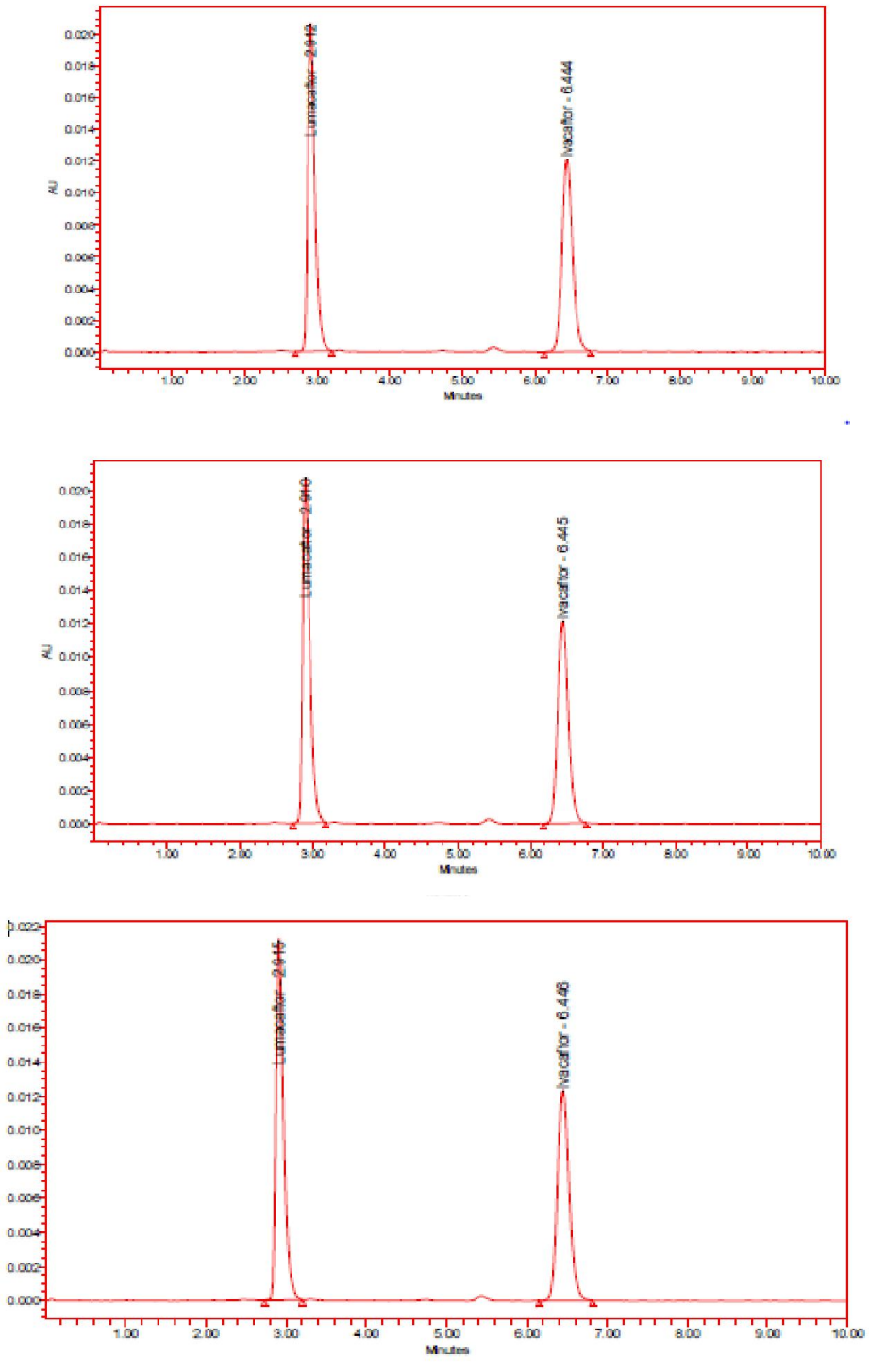
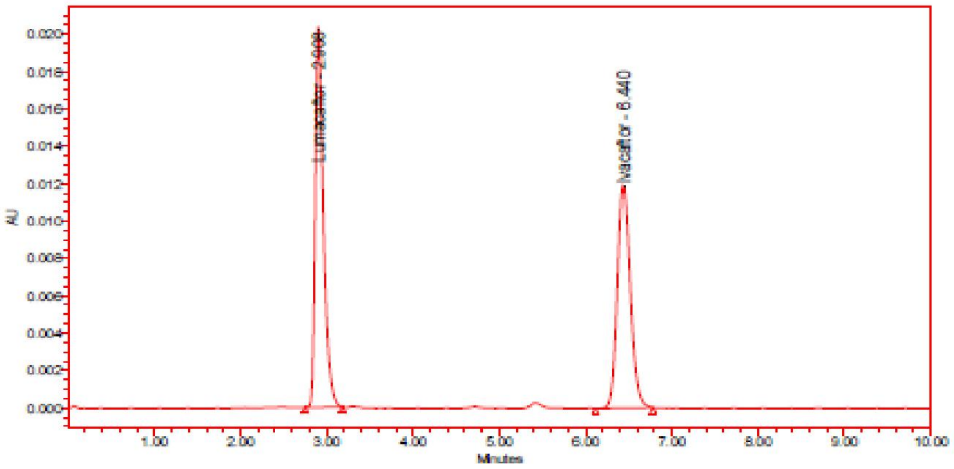
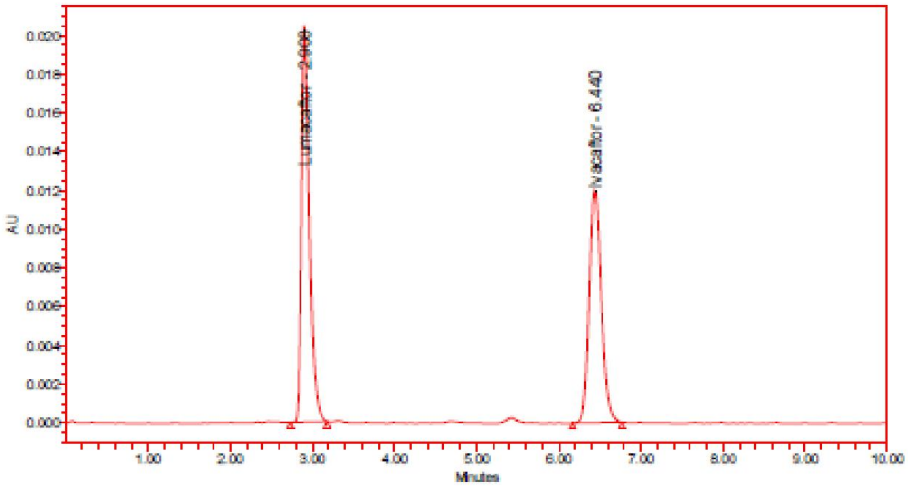
0.008
0.006
0.004
0.002
0.000
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
9.00
10.00
Minutes

Fig. 19, 20, 21, 22, 23, 24. Chromatograms showing intermediate precison injection 1-6




Lumacaftor - 2.998
Ivacaftor - 6.440
AU
Minutes


Lumacaftor - 2.998
Ivacaftor - 6.440
AU
Minutes

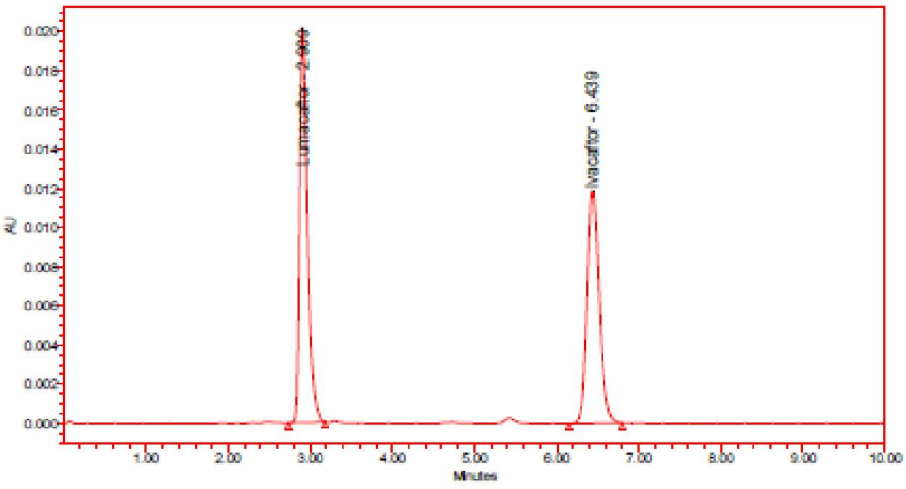
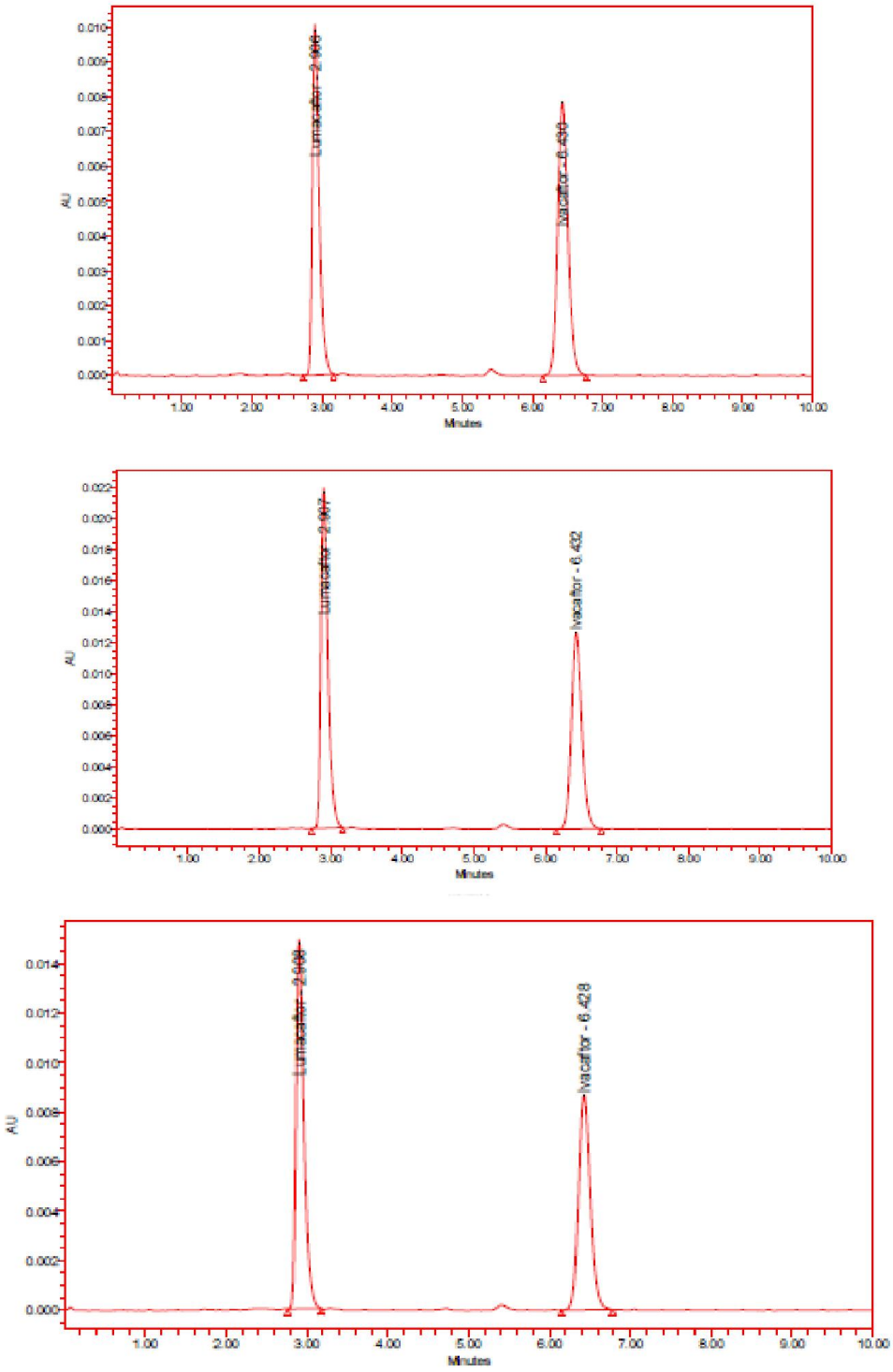
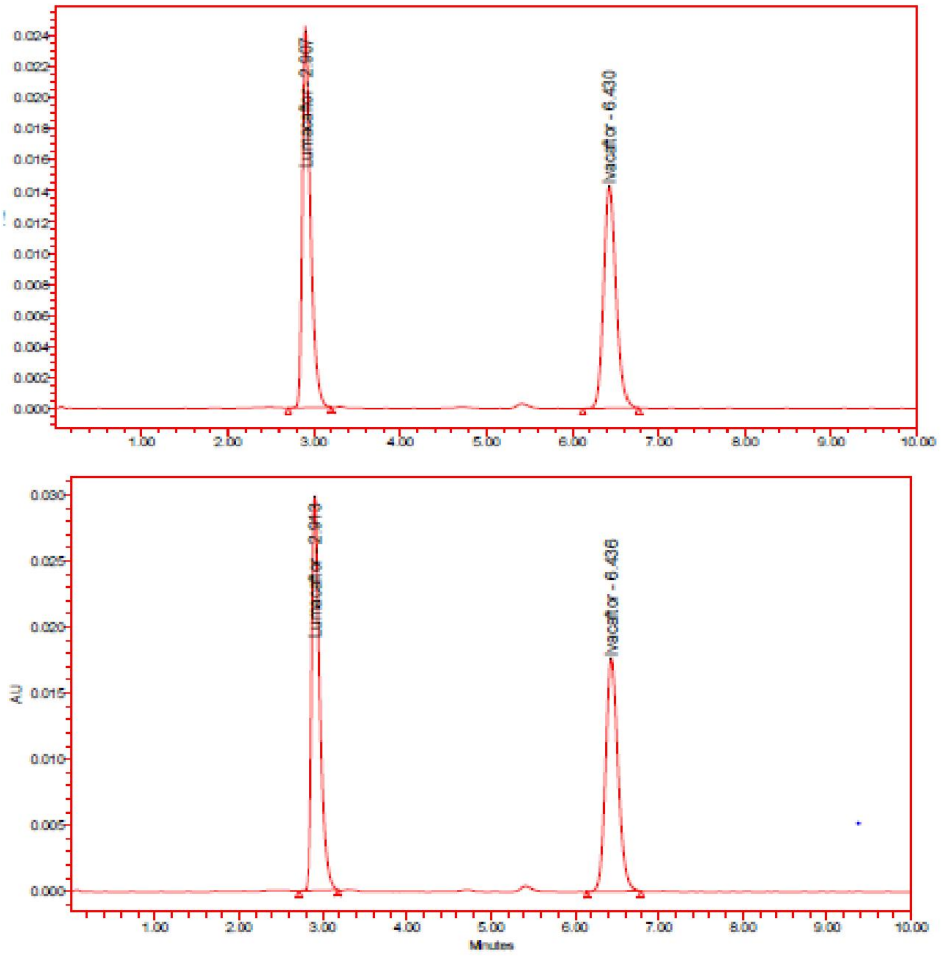

Lumacaftor - 2.997
Ivacaftor - 6.439
AU
Minutes

Fig. 25, 26, 27, 28, 29. Chromatograms showing linearity level-1 to level-5





Linearity

The linearity of the method was determined in the concentration range of 100-500 µg/ml for Lumacaftor and 62.5-312.5 µg/ml for Ivacaftor. Each solution was injected in triplicate. The average peak area versus concentration data of both drugs was treated by least squares linear regression analysis and the results obtained as shown in Table 5 and 6.

Table 5. Showing linearity results for Lumacaftor

S. No	Linearity Level	Concentration	Area
1	I	100	65792
2	II	200	98696
3	III	300	131638
4	IV	400	162911
5	V	500	200063
Correlation Coefficient			0.999

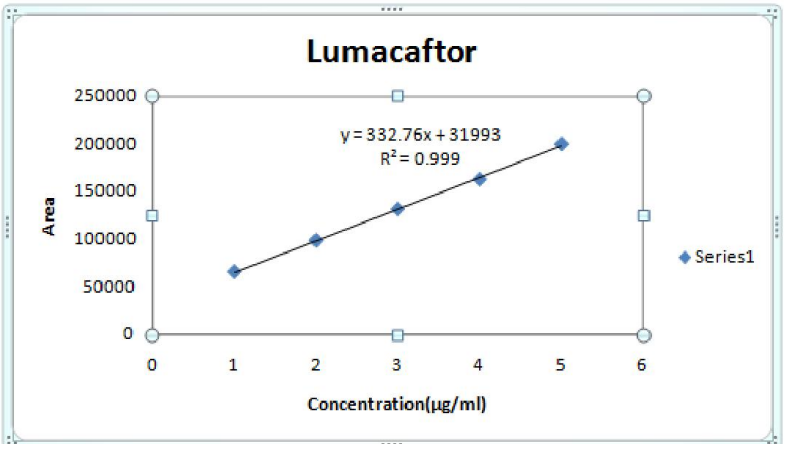


Fig. 30. Showing caliberation curve for Lumacaftor

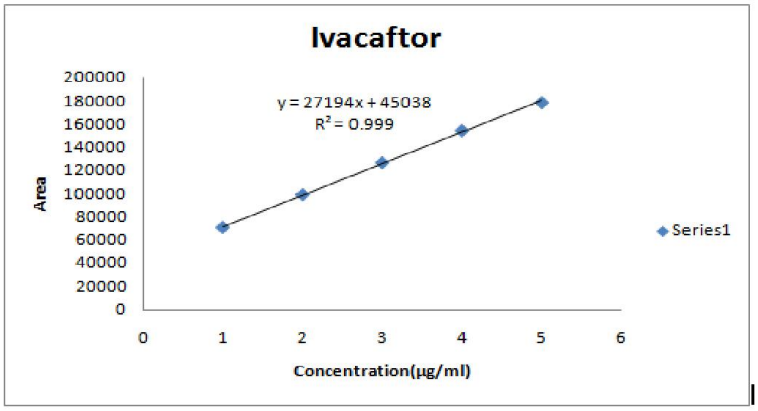


Fig. 31. Showing caliberation curve for Ivacaftor

Table 6. Linearity results for ivacaftor

S. No	Linearity Level	Concentration	Area
1	I	62.5	71267
2	II	125	99725
3	III	187.5	127369
4	IV	250	155275
5	V	312.5	179461
Correlation Coefficient			0.999

Limit of detection and limit of quantification

The LOD can be defined as the smallest level of analytes that gives a measurable response and LOQ was determined as the lowest amount of analytes that was reproducibly quantified. These to parameters were calculated using the formula based on the standard deviation of the response and the slope. LOD and LOQ were calculated using equation

LOD = 3.3 × s/S and LOQ = 10 × s/S, where s = standard deviation of Y-intercept, S = average slope of calibration curve, and results obtained shown in figure 32-33.

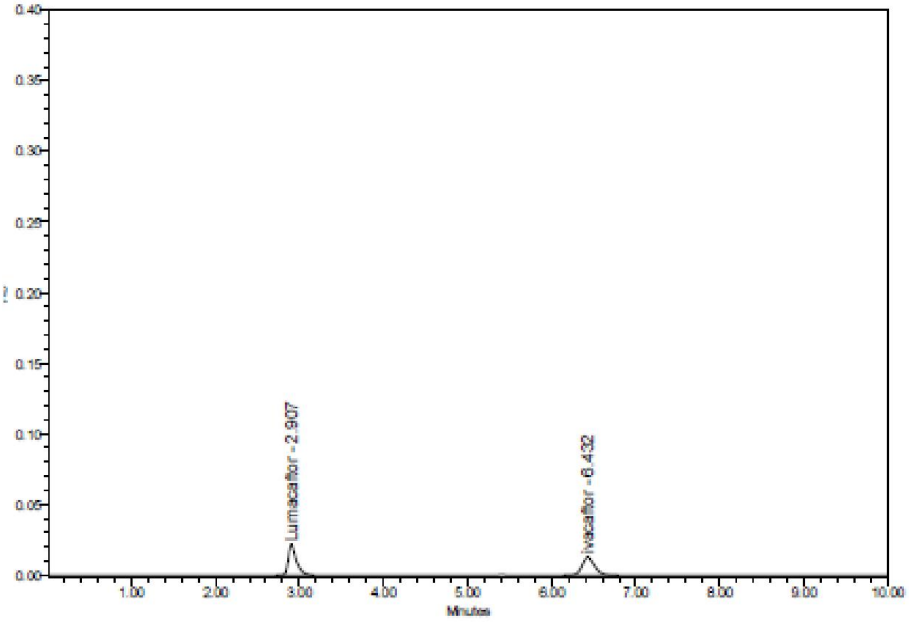


Fig. 32. Chromatogram showing limit of detection

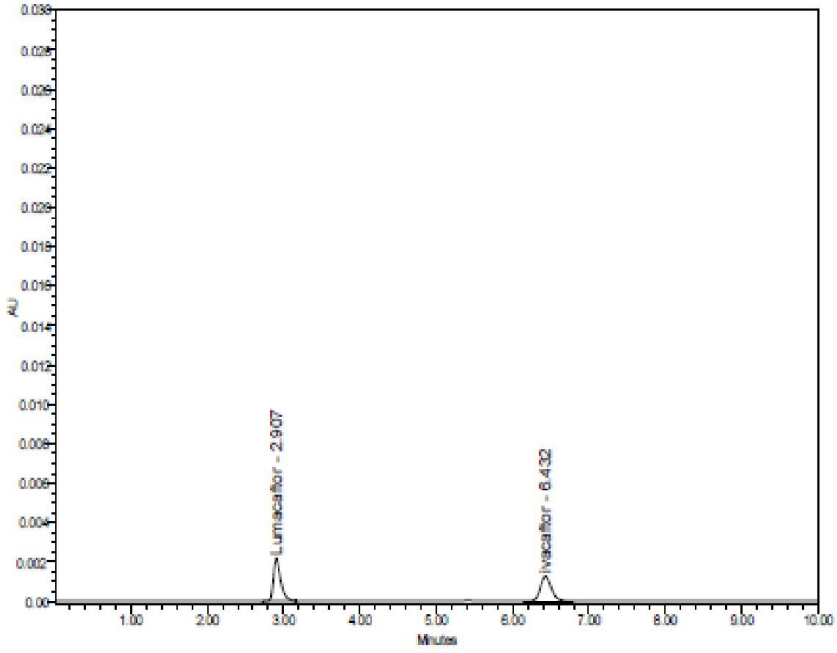


Fig. 33. Chromatogram showing limit of quantification

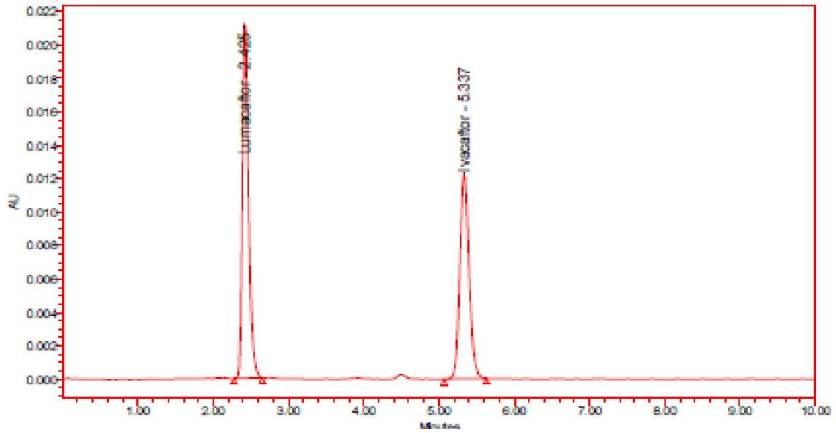


Fig. 34. Chromatogram showing more flow rate 1.1 ml/min

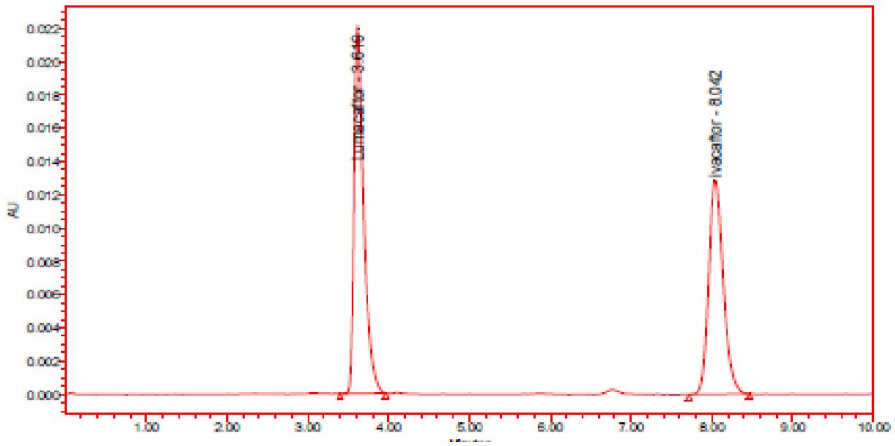


Fig. 35. Chromatogram showing less flow rate 0.9 ml/min

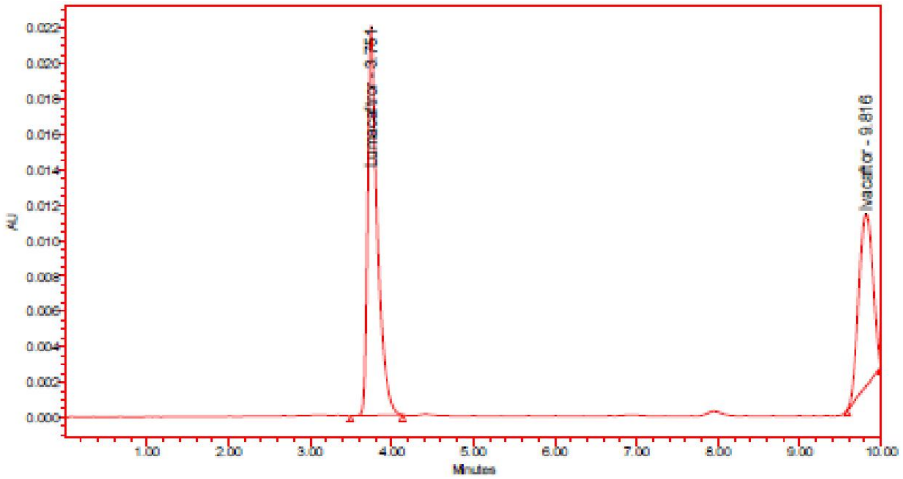


Fig. 36. Chromatogram showing more organic phase ratio

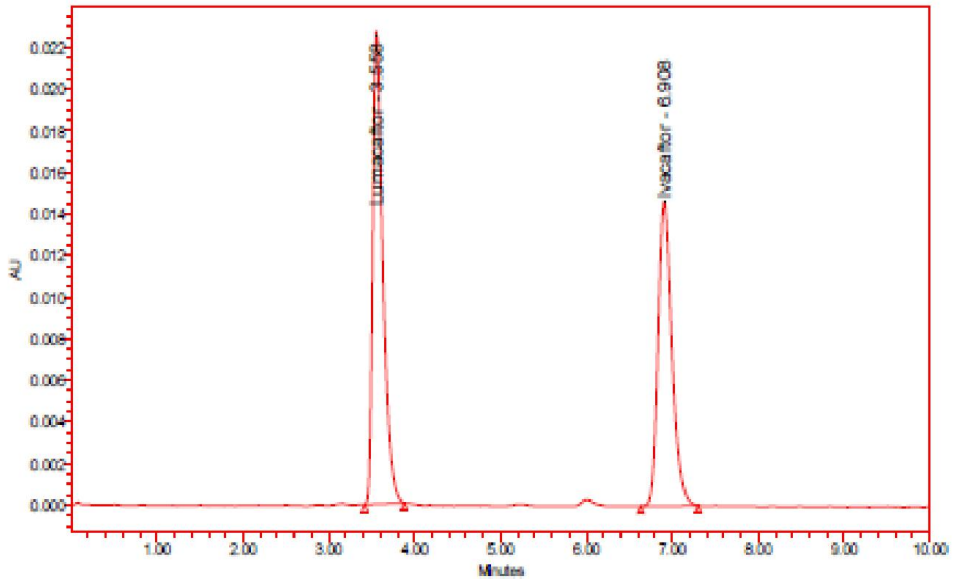


Fig. 37. Chromatogram showing less organic phase ratio

Degradation studies

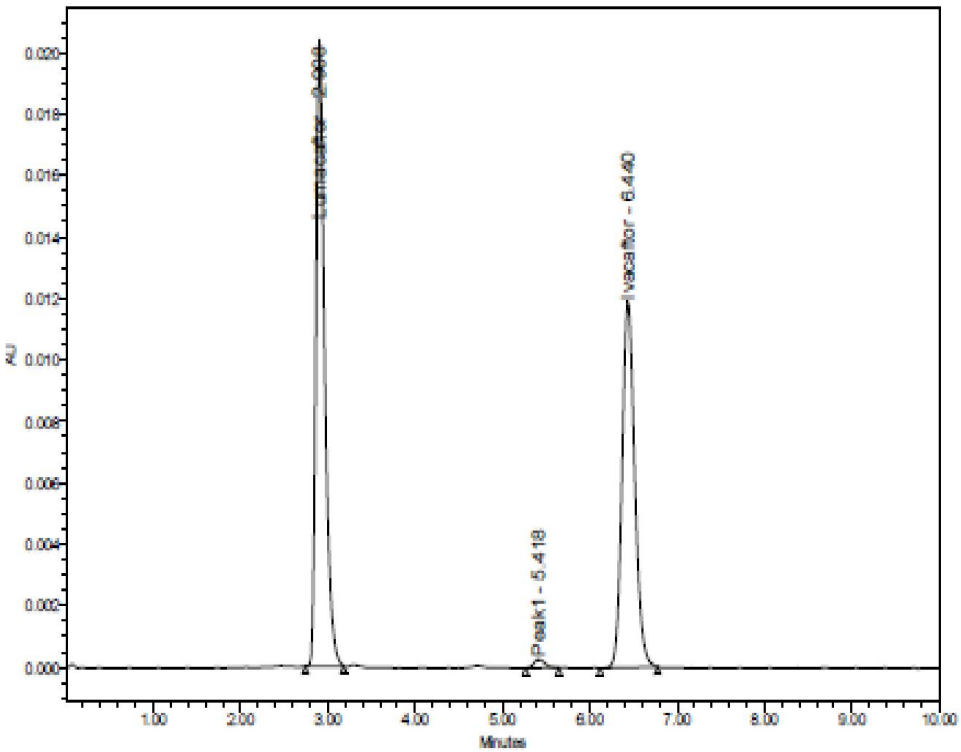


Fig. 38. Chromatogram showing acidic degradation

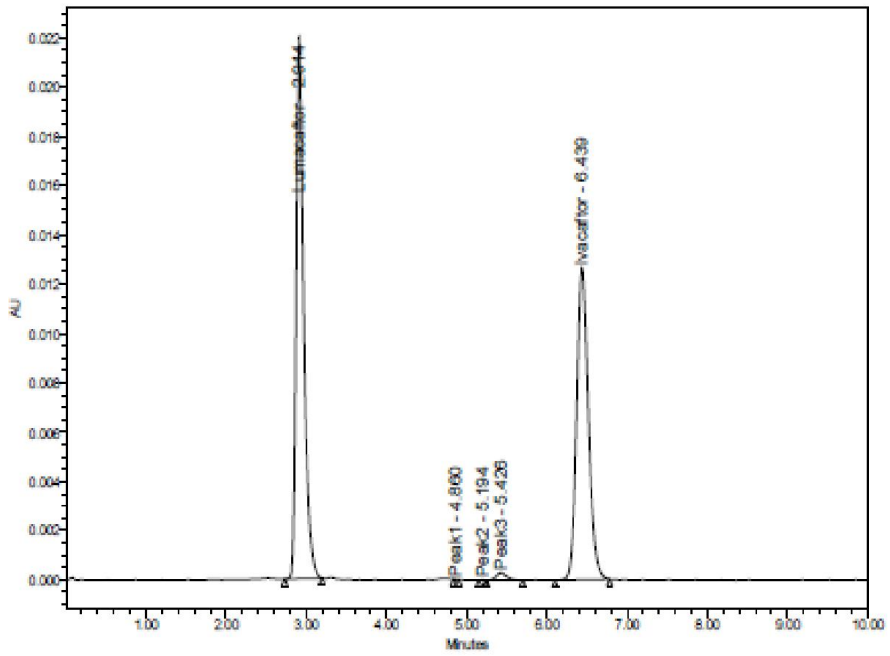


Fig. 39. Chromatogram showing basic degradation

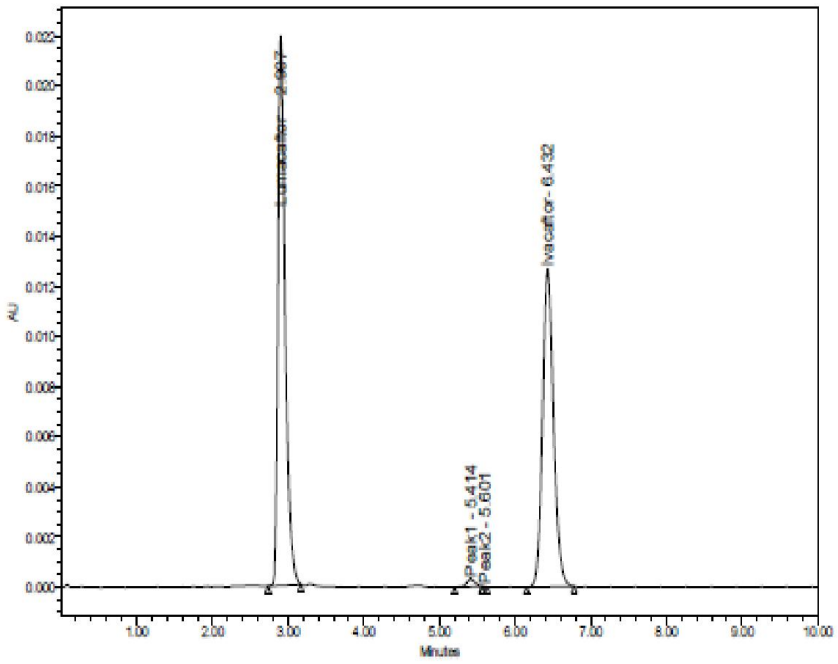


Fig. 40. Chromatogram showing peroxide degradation

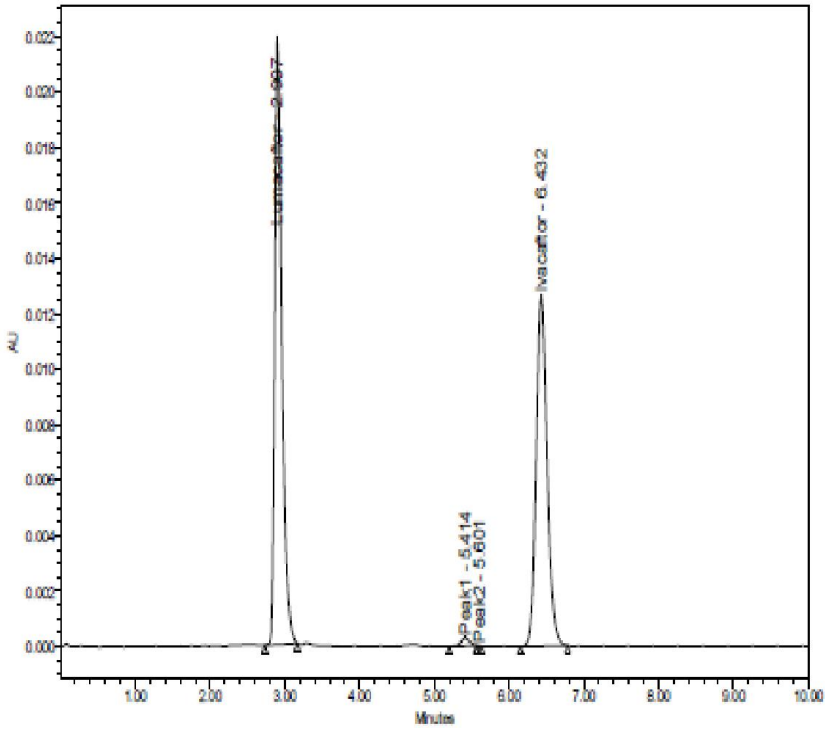


Fig. 41. Chromatogram showing Thermal degradation

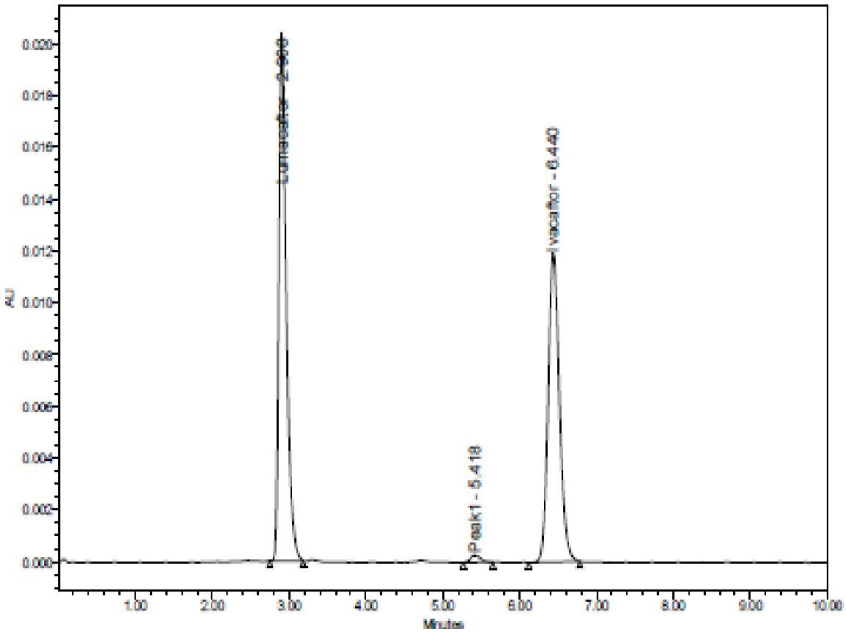


Fig. 42. Chromatogram showing photolytic degradation

Robustness : Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines, and the results obtained shown in table no. 7-10.

Table 7. Showing System suitability results for Lumacaftor:

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	4685.09	1.12
2	1.0	4509.7	1.47
3	1.1	4065.51	1.40

Table 8. Showing System suitability results for Ivacaftor:

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	4731.46	1.21
2	1.0	4509.7	1.47
3	1.1	4549.3	1.12

Table. 9. Showing System suitability results for Lumacaftor:

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	4382.7	1.12
2	*Actual	4509.7	1.47
3	10% more	4982.7	1.17

Table 10. Showing System suitability results for Ivacaftor:

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	4643.64	1.26
2	*Actual	4509.7	1.47
3	10% more	4987.28	0.95

DEGRADATION STUDIES [9, 10] :

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. The aim of this work was to perform the stress degradation studies on the Lumacaftor and Ivacaftor using the proposed method.

Preparation of stock:

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), results obtained shown in table 11-12.

Table 11. Showing degradation results of Lumacaftor

Sample Name	Lumacaftor				
	Area	**% Degraded**	**Purity Angle**	**Purity Threshold**	**Peak purity**
Standard	135383.3	100			
Acid	125453	92.67	7.33	90.00	Passes
Base	127849	94.43	5.57	90.00	Passes
Peroxide	125131	92.43	7.57	90.00	Passes
Thermal	128347	94.80	5.20	90.00	Passes
Photo	129359	95.55	4.45	90.00	Passes

Table 12. Showing degradation results for Ivacaftor

Sample Name	Ivacaftor				
	Area	**% Degraded**	**Purity Angle**	**Purity Threshold**	**Peak purity**
Standard	121004.3	100			
Acid	115289	95.28	4.72	90.00	Passes
Base	117420	97.04	2.96	90.00	Passes
Peroxide	113076	93.45	6.55	90.00	Passes
Thermal	113704	93.97	6.03	90.00	Passes
Photo	116820	96.54	3.46	90.00	Passes

Hydrolytic degradation under acidic condition

Pipette 1.5 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 6 hours and then neutralized with 0.1 N NaOH and make up to 10ml with diluent. Filter the solution with 0.22 microns syringe filters and place in vials.

Hydrolytic degradation under alkaline condition

Pipette 1.5ml 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 6 hours and then neutralized with 0.1N HCl and make up to 10ml with diluent. Filter the solution with 0.22 microns syringe filters and place in vials.

Thermal induced degradation

Lumacaftor and Ivacaftore sample was taken in petridish and kept in Hot air oven at 110^0 C for 24 hours. Then the sample was taken and diluted with diluents and injected into HPLC and analysed.

Oxidative degradation

Pipette 1.5ml above stock solution into a 10ml volumetric flask and 1 ml of 3% 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 1.5 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.

Conclusion

A new method was established for method validation and degradation studies by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Lumacaftor and Ivacaftor by using dikma (250 mm × 4.6 mm) flow rate was 1 ml/min, mobile phase ratio was (40:60 v/v) OPA buffer P^H 4.5 : ACN, detection wavelength was 255 mm. The instrument used was waters HPLC autosampler, separation module 2695, photodiode array detector, empower software version 2.The retention time were found to be 2.579 and 3.877 mins. The % purity of Lumacaftor and Ivacaftor was found to be 100.53% and 100.13%. The system suitability parameters for Lumacaftor and Ivacaftor such as theoretical plates and tailing factor were found to be 2320.05, 1.59 and 4005.65, 1.49.The resolution was found to be 4.71. The analytical method was validated according to ICH guidelines(ICH, Q2{R1}).The linearity study of Lumacaftor and Ivacaftor was found in concentration range of 100µg-500 µg and 62.5 µg-312.5 µg and correlation coefficient was found to be 0.999 and 0.999%. percentage recovery was found to be 100.53% and 100.13%. percentage repeatability was 0.8 and 0.8, % RSD for intermediate precision was 0.7 and 0.6 respectively. The precision study was precision, robustness and repeatability. LOD value was 3.00 and LOQ value was 10.00 respectively hence the suggested RP-HPLC method can be used for routine analysis of Lumacaftor and Ivacaftor in API and pharmaceutical dosage form.

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