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

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ABSTRACT

A simple and selective LC method is described for the determination of Ivacaftor and Lumacaftor intablet dosage forms. Chromatographic separation was achieved on a c_{18} column using mobile phase consisting of a mixture of 45 volumes of acetonitrile and 55 volumes of mixed phosphate buffer with detection of 225 nm. Linearity was observed in the range 30-70 µg /ml for Ivacaftor(r^2 =0.997) and 40-80µg /ml for Lumacaftor (r^2 =0.998) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim.

The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. 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.

Keywords: Lumacaftor and Ivacaftor**,** Reverse phase HPLC.

INTRODUCTION

A drug includes all medicines intended for internal or external use for or in the diagnosis, treatment, mitigation or prevention of disease or disorder in human beings or animals, and manufactured exclusively in accordance with the formulae mentioned in authoritative books [1- 5].

Pharmaceutical analysis is a branch of chemistry involving a process of identification, determination, quantification, purification and separation of components in a mixture or determination of chemical structure of compounds [6-10]. There are two main types of analysis – Qualitative and Quantitative analysis [15-21].

AIM AND PLAN OF WORK

Aim

To develop new RP HPLC method for the estimation of **LUMACAFTOR AND IVACAFTOR** in pharmaceutical dosage form.

Plan of work

- Solubility determination of **LUMACAFTOR AND IVACAFTOR** various solvents and buffers.
- Determine the absorption maxima of the drug in UV–Visible region in different solvents/buffers and selecting the solvents for HPLC method development.

- Optimize the mobile phase and flow rates for proper resolution and retention times.
- Validate the developed method as per ICH guidelines.

METHODOLOGY

Mobile Phase

A mixture of 45 volumes of acetonitrile and 55 volumes of mixed phosphate buffer were prepared. The mobile phase was sonicated for 10min to remove gases and filtered through 0.45µ membrane filter for degassing of mobile phase.

Determination of Working Wavelength (λmax)

In estimation of drug wavelength maxima is used.. So this wavelength is used in estimation to estimate drug accurately.

Preparation of standard stock solution of IVACAFTOR

10 mg of IVACAFTORwas weighed and transferred in to 100ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

Preparation of standard stock solution of LUMACAFTOR

10mg of LUMACAFTORwas weighed in to 100ml volumetric flask and dissolved in Methanol and then dilute up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol

RESULTS AND DISCUSSIONS

Solubility studies

These studies are carried out at 25 ^{0}C

Ivacaftor

Sparingly soluble in acetonitrile and in ethanol, very slightly soluble in water and phosphate buffer .

Lumacaftor

Soluble in water, ACN, and slightly soluble in methanol

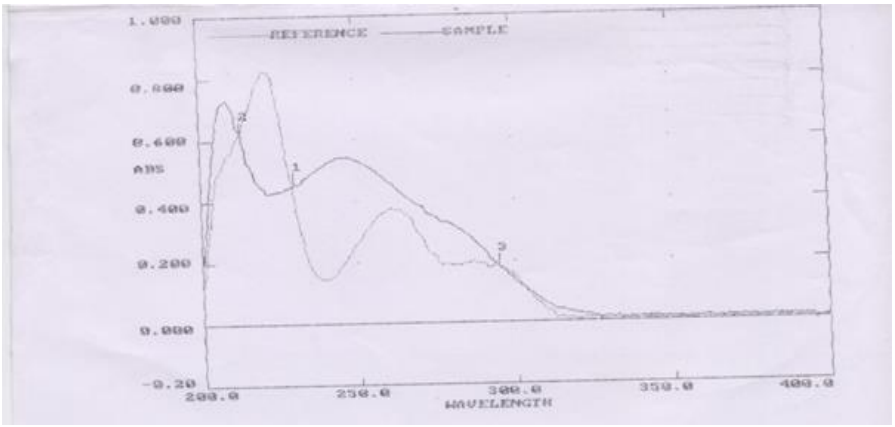
Wavelength determination

In simultaneous estimation of two drugs isobestic wavelength is used. Isobestic point is the wavelength where the molar absorptivity is the same for two substances that are interconvertible. So this wavelength is used in simultaneous estimation to estimate both drugs accurately.

RESULTS

The wavelength of maximum absorption (λ_{max}) of the drug, 10 µg/ml solution of the drugs in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. The resulting spectra and the absorption curve shows the isobestic point was found to be 225 nm for the combination.

Isobestic point of Lumacaftor and Ivacaftor



Method Development of Lumacaftor and Ivacaftor

Trial- 1

Preparation of mixed standard solution

Weigh accurately 60 mg of IVACAFTOR and 50 mg of LUMACAFTOR in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 60 µg/ml of IVACAFTOR and 50 µg/ml of LUMACAFTOR is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

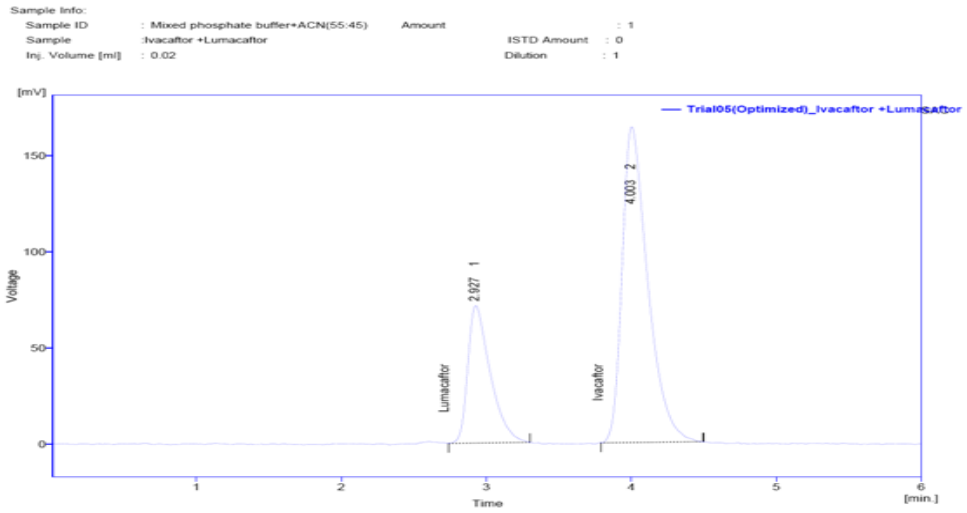


Fig. 8.3.5: Chromatogram of LUMACAFTOR and IVACAFTOR by using mobile phase

Observation

- All the system suitability requirements were met.
- The peak Asymmetry factor was less than 2 for both LUMACAFTOR and IVACAFTOR.
- The efficiency was more than 2000 LUMACAFTOR and IVACAFTOR.
- Resolution between two peaks >1.5.
- Thedetails are given in the table 8.3.8 and figure 8.3.8, hence this method was for optimized.

Table 8.3.8: Optimized chromatographic conditions

Mobile phase	Mixed phosphate buffer +CAN
Ph	-
Column	Inertsil ODS 3V column,C18(150x4.6 ID) 5µm
Flow rate	1.0 ml/min
Column temperature	Room temperature(20-25^{o}C)
Sample temperature	Room temperature(20-25^{o}C)
Wavelength	225
Injection volume	20 µl
Run time	6 min
Retention time	About 4.003min for IVACAFTOR and 2.927min for LUMACAFTOR.

ASSAY

Preparation of samples for Assay

Preparation of mixed standard solution

Weigh accurately 60 mg of IVACAFTOR and 50 mg of LUMACAFTOR in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 60 µg/ml of IVACAFTOR and 50 µg/ml of LUMACAFTOR is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Tablet sample

10 tablets (each tablet contains LUMACAFTOR-500 mg. IVACAFTOR-600 mg) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of LUMACAFTOR and IVACAFTOR (µg/ml) were prepared by dissolving weight equivalent to 500 mg of LUMACAFTOR and 600 mg of IVACAFTOR and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 50ml with mobile phase. Further dilutions are prepared in 5 replicates of 50µg/ml of LUMACAFTOR and 60µg/ml of IVACAFTOR was made by adding 1 ml of stock solution to 10 ml of mobile phase.

Calculation

The amount of LUMACAFTOR and IVACAFTOR present in the formulation by using the formula given below, and results shown in above table:

$$\% \text{ Assay} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{AW}{LC} \times 100$$

Where,

AS: Average peak area due to standard preparation

AT: Peak area due to assay preparation

WS: Weight of LUMACAFTOR /IVACAFTORin mg

WT: Weight of sample in assay preparation

DT: Dilution of assay preparation

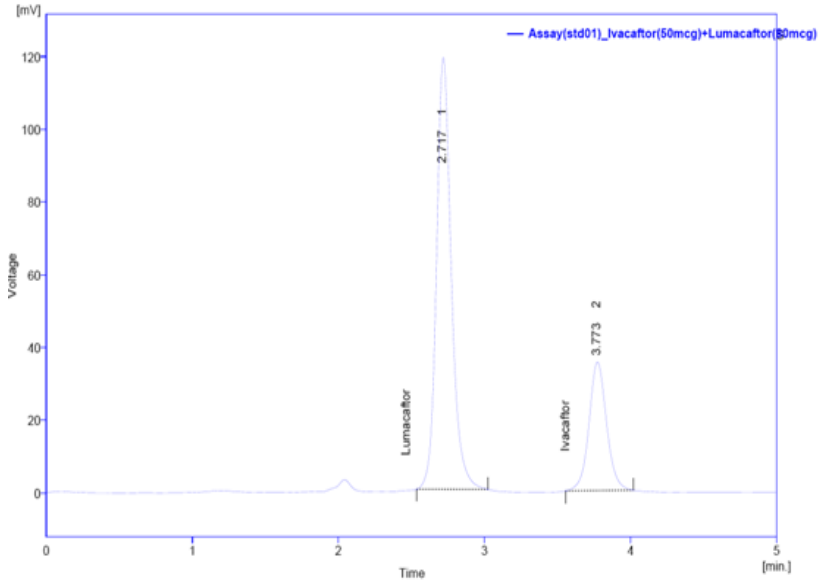


Fig: Chromatogram of Assay standard preparation-1'

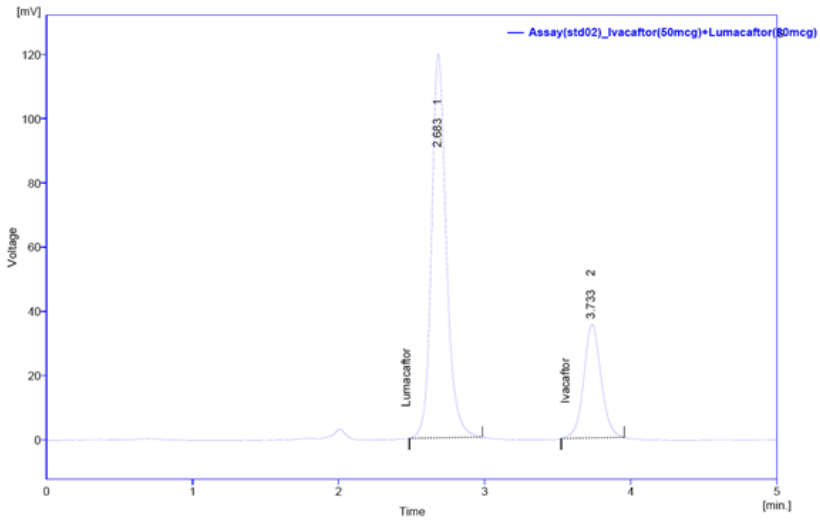


Fig: Chromatogram of Assay standard preparation-2

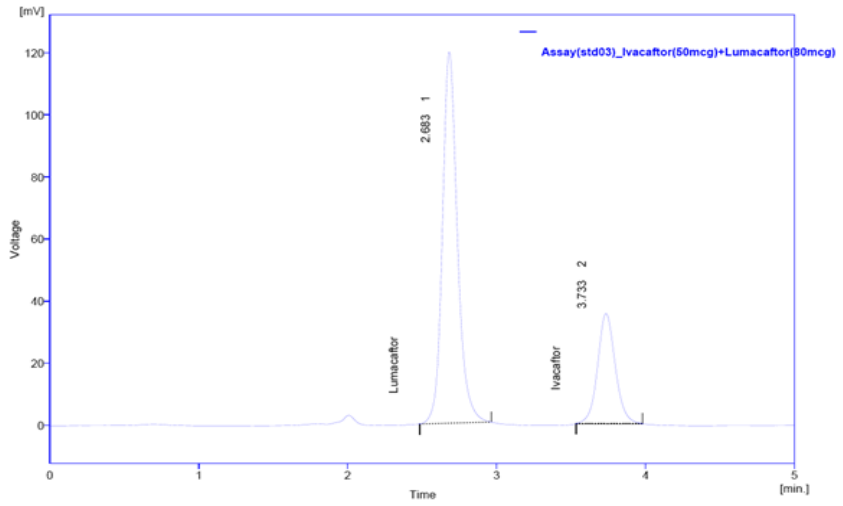


Fig: Chromatogram of Assay standard preparation-3

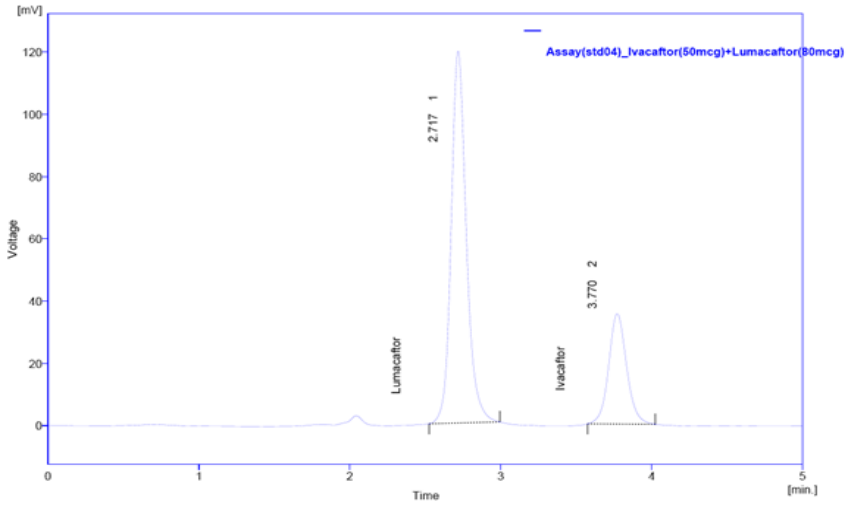


Fig: Chromatogram of Assay standard preparation-4

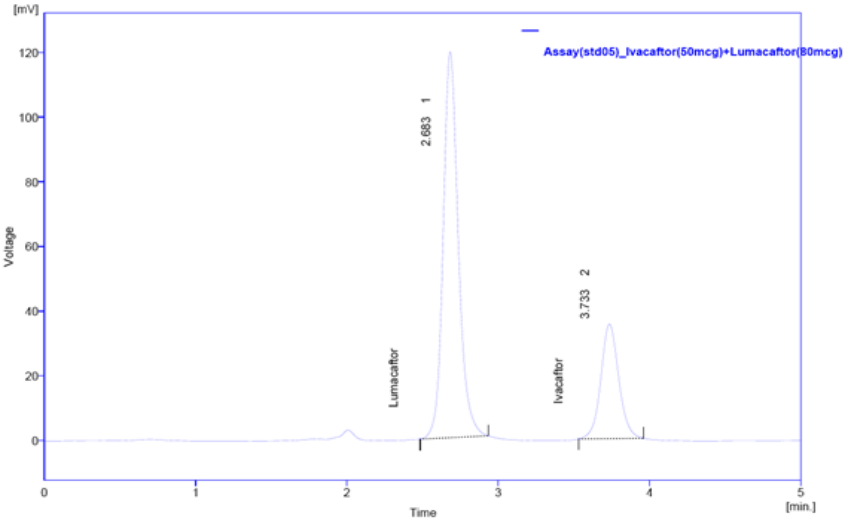


Fig: Chromatogram of Assay standard preparation-5

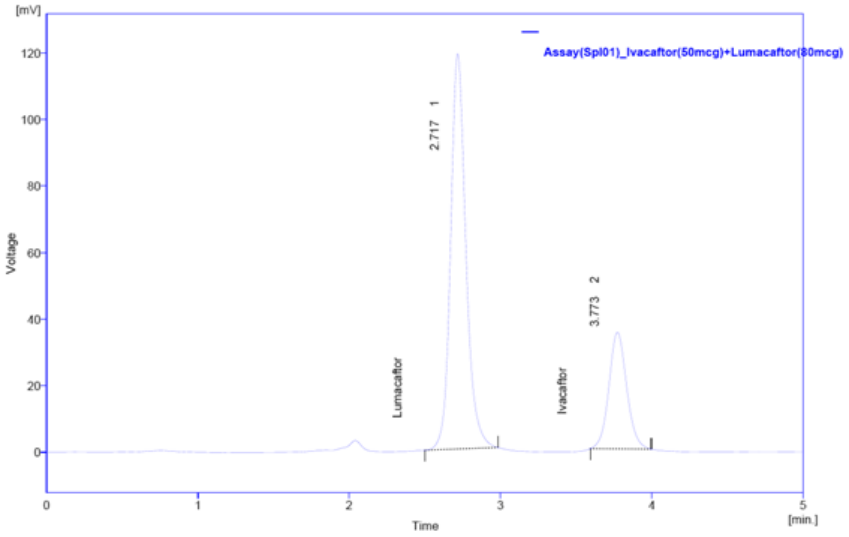


Fig: Chromatogram of Assay sample preparation-1

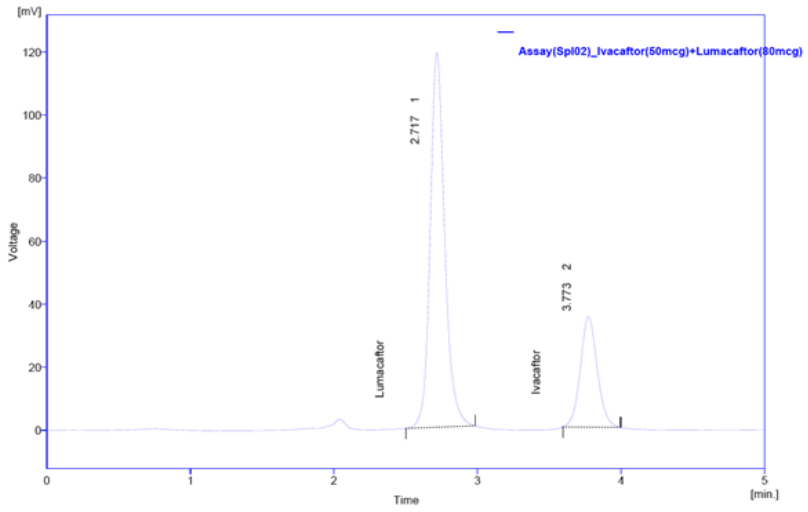


Fig: Chromatogram of Assay sample preparation-2

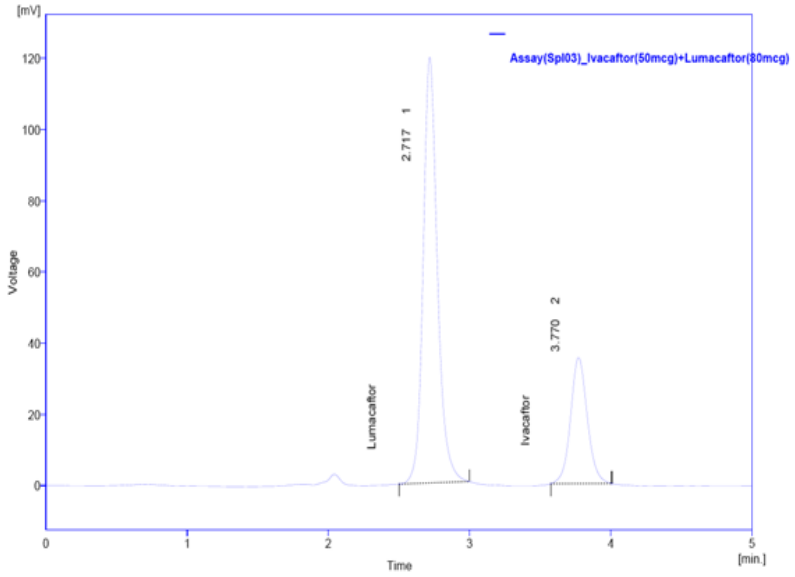


Fig: Chromatogram of Assay sample preparation-3

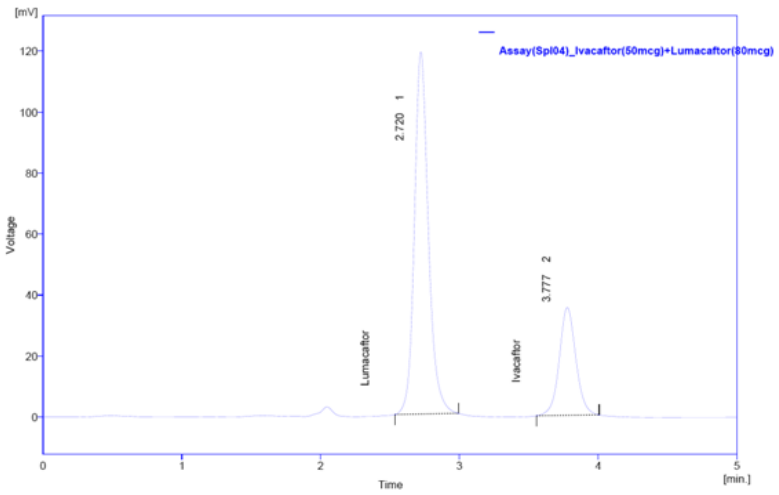


Fig: Chromatogram of Assay sample preparation-4

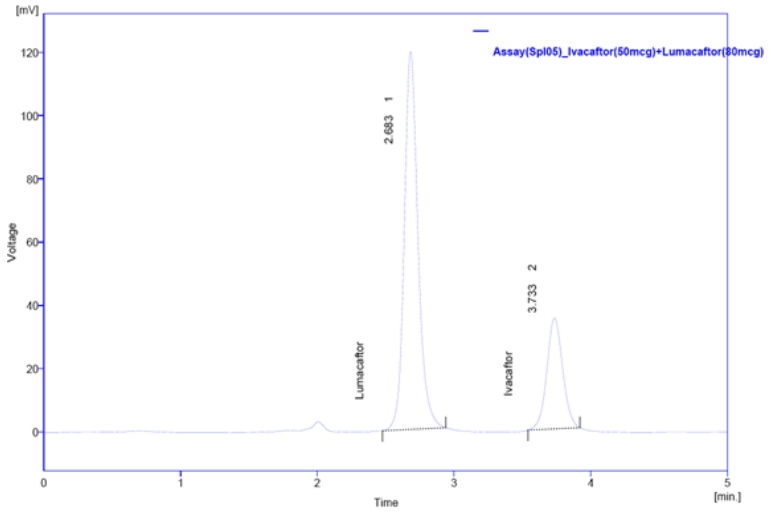


Fig: Chromatogram of Assay sample preparation-5

Table No. 8.4.9.2: Assay Results

IVACAFTOR			**LUMACAFTOR**	
	Standard Area	**Sample Area**	**Standard Area**	**Sample Area**
Injection-1	295.884	286.448	836.469	833.214
Injection-2	290.743	286.448	839.076	833.214
Injection-3	292.910	291.818	835.627	837.225
Injection-4	293.024	293.805	834.719	833.303
Injection-5	290.900	280.827	829.554	831.491
Average Area	292.692	287.869	835.089	833.689
Standard deviatuion	3.1102		2.1174	
%RSD	1.9		1.2	
Assay(%purity)	**99.8%**		**99.6%**	

Observation

The amount of IVACAFTOR and LUMACAFTOR present in the taken dosage form was found to be 99.8 % and 99.6% respectively.

VALIDATION

Specificity by Direct comparison method

There is no interference of mobile phase, solvent and placebo with the analyte peak and also the peak

purity of analyte peak which indicate that the method is specific for the analysis of analytes in their dosage form.

Preparation of mixed standard solution

Weigh accurately 60 mg of Ivacaftor and 50 mg of Lumacaftor in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 60 µg/ml of Ivacaftor and 50 µg/ml of Lumacaftor is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Tablet sample

10 tablets (each tablet contains LUMACAFTOR - 500 mg. IVACAFTOR -600 mg) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of LUMACAFTOR and IVACAFTOR (µg/ml) were prepared by dissolving weight equivalent to 500 mg of LUMACAFTOR and 600 mg of IVACAFTOR and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 50ml with mobile phase. Further dilutions are prepared in 5 replicates of 50 µg/ml of LUMACAFTOR and 60µg/ml of IVACAFTOR was made by adding 1 ml of stock solution to 10 ml of mobile phase.

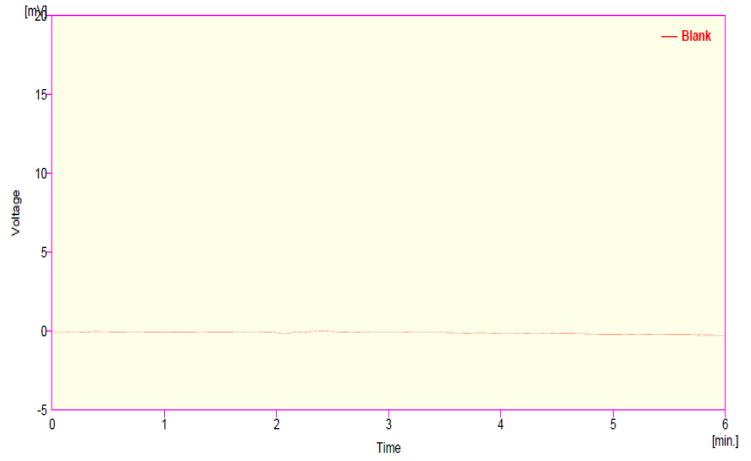


Fig: Blank chromatogram for specificity by using mobile phase

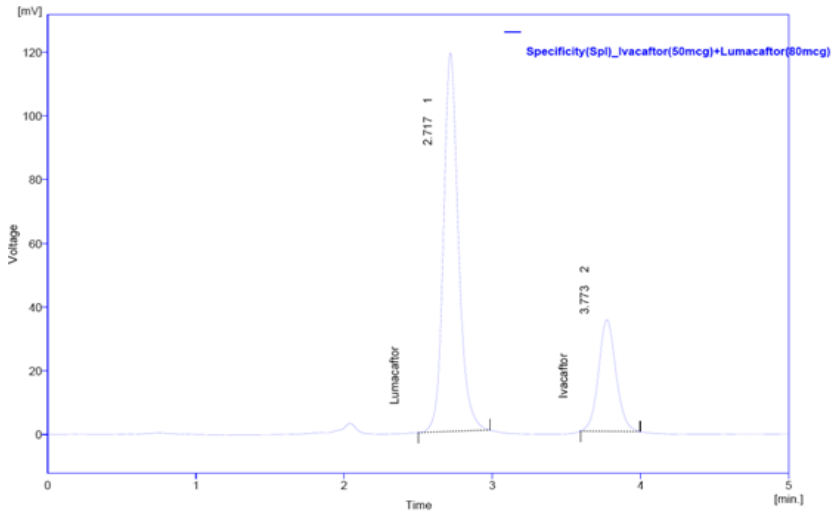


Fig: Chromatogram for specificity of Ivacaftor and Lumacaftor sample

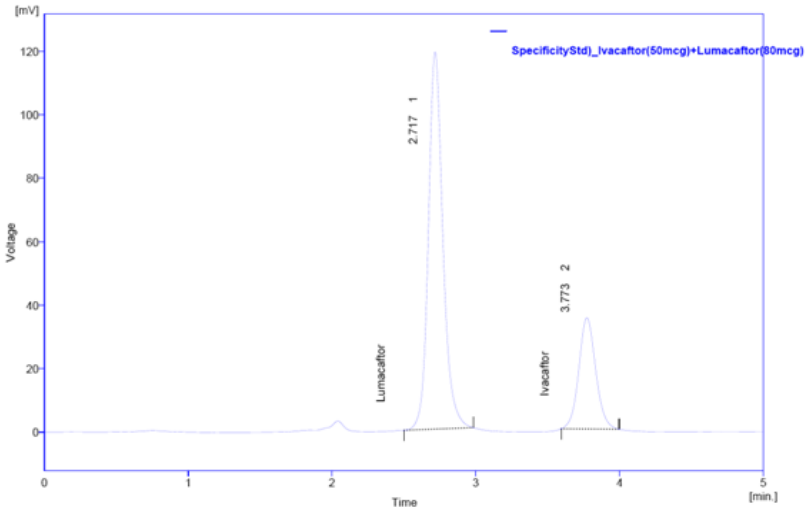


Fig: Chromatogram for Specificity of Ivacaftor and Lumacaftor standard

Observation

It is observed from the above data, diluent or excipient peaks are not interfering with the Ivacaftor and Lumacaftor peaks.

LINEARITY AND RANGE

Preparation of standard stock solution

Standard stock solutions of Ivacaftor and Lumacaftor (microgram/ml) were prepared by dissolving 60 mg of Ivacaftor and 50 mg of Lumacaftor dissolved in sufficient mobile phase and dilute to 100 ml with mobile phase. Further dilutions were given in the table No 8.3.1

Table 9.3 .1: Linearity Preparations

Preparations	Volume from standard stock transferred in ml		Volume made up in ml (with mobile phase)	Concentration of solution(µg /ml)	
				IVACAFTOR	LUMACAFTOR
Preparation 1	0.3	0.4	10	30	40
Preparation 2	0.4	0.5	10	40	50
Preparation 3	0.5	0.6	10	50	60
Preparation 4	0.6	0.7	10	60	70
Preparation 5	0.7	0.8	10	70	80

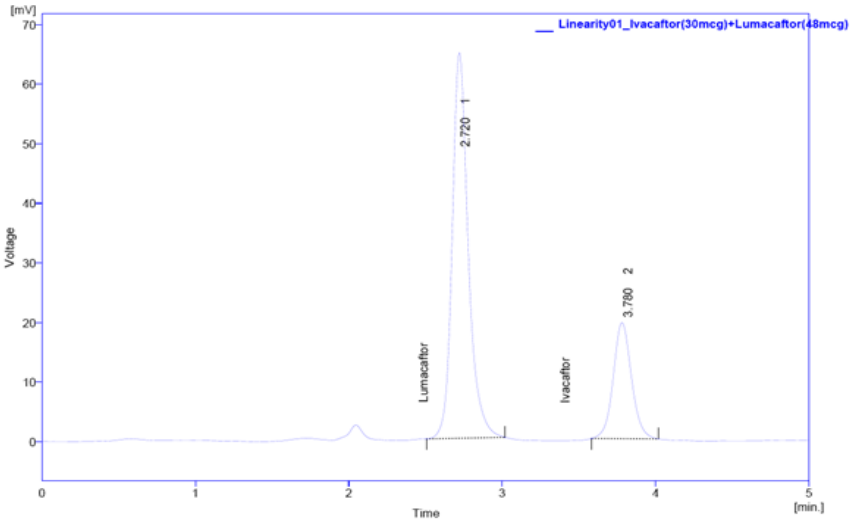


Fig: Chromatogram of LUMACAFTOR AND IVACAFTOR preparation-1

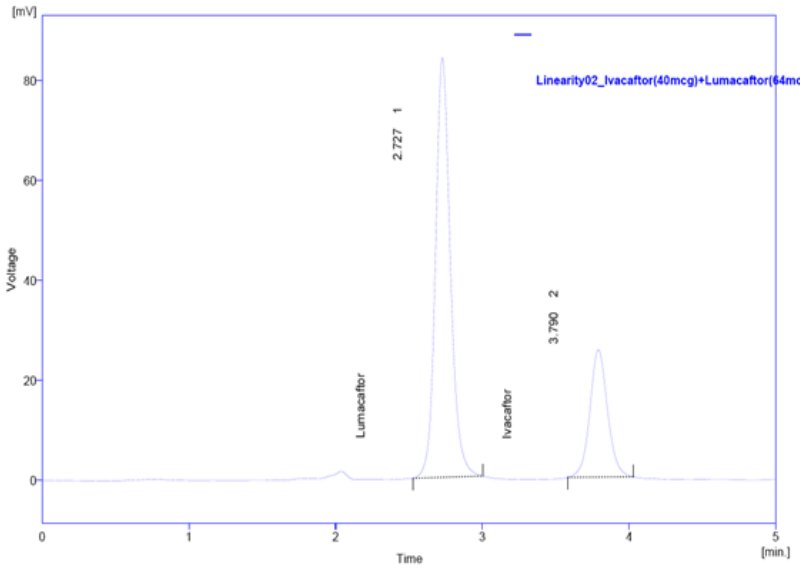


Fig: Chromatogram of LUMACAFTOR AND IVACAFTOR preparation-2

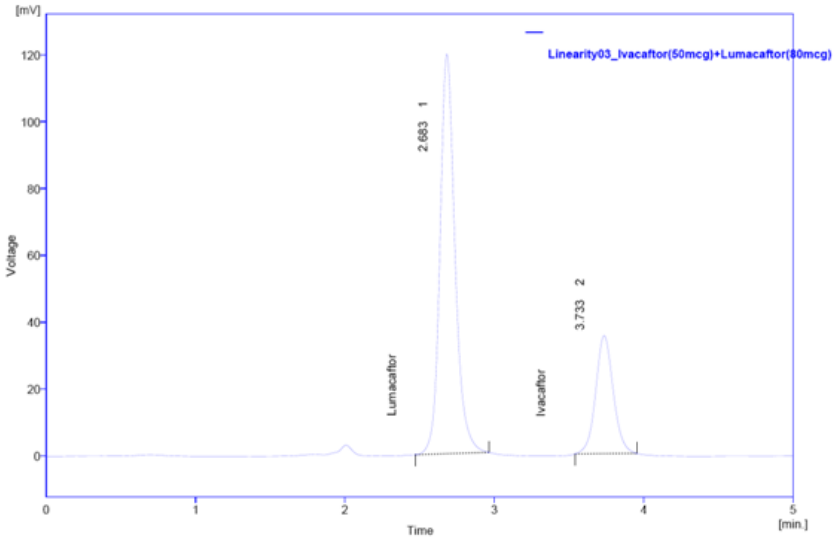


Fig: Chromatogram of LUMACAFTOR AND IVACAFTOR preparation-3

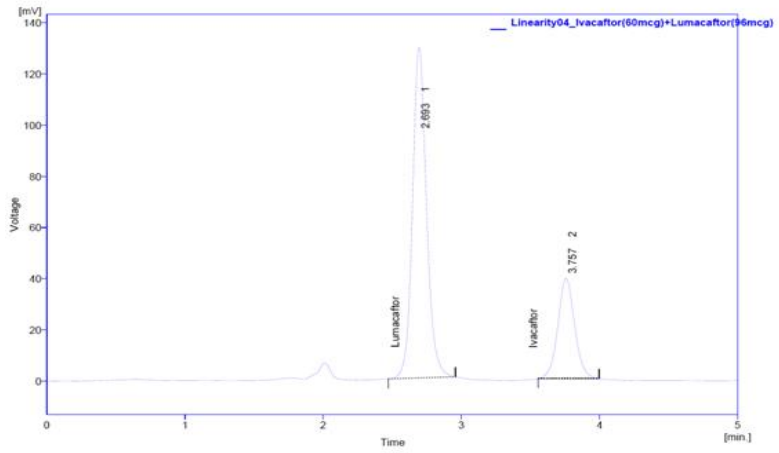


Fig: Chromatogram of LUMACAFTOR AND IVACAFTORpreparation-4

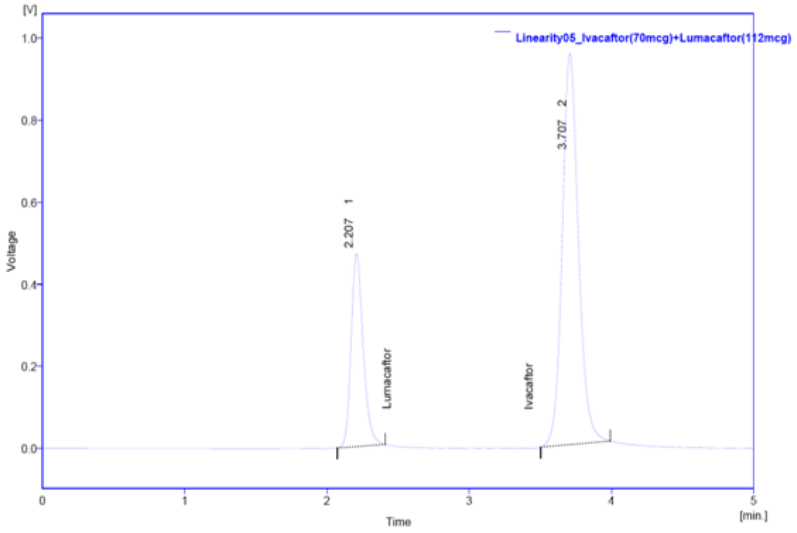


Fig: Chromatogram of LUMACAFTOR AND IVACAFTORfor preparation-5

Table 9.3.7: linearity of IVACAFTOR

S.No.	Conc.(µg/ml)	Area
1	30	161.404
2	40	213.356
3	50	288.207
4	60	330.037
5	70	7541.702

Table 9.3.8: linearity of LUMACAFTOR

S.No.	Conc.(µg/ml)	Area
1	40	471.68
2	50	593.037
3	60	836.360
4	70	913.252
5	80	2763.590

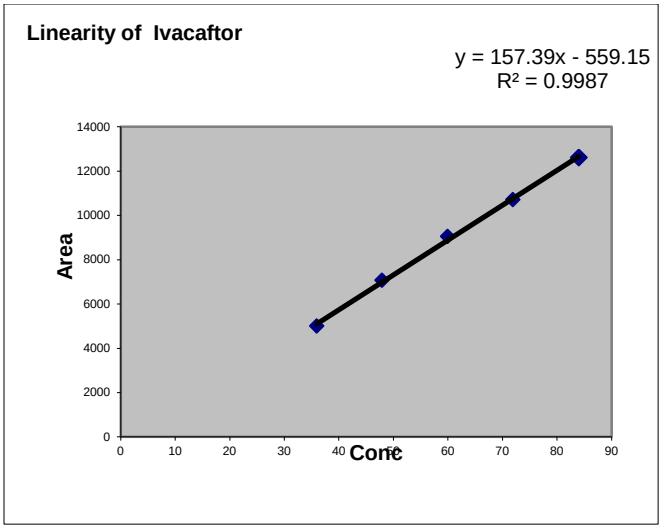


Fig. 9.3.9: Linearity graph of IVACAFTOR

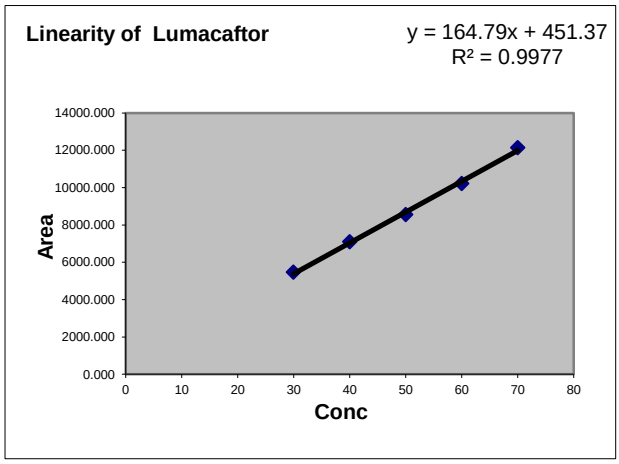


Fig. 9.3.9.1: Linearity graph of LUMACAFTOR

Acceptance criteria

The relationship between the concentration of Ivacaftor and Lumacaftor and area of Ivacaftor and Lumacaftor should be linear in the specified range and the correlation should not be less than 0.99.

Observation

The correlation coefficient for linear curve obtained between concentration vs. Area for standard preparations of Ivacaftor and Lumacaftoris 0.998 and 0.997. The relationship between the concentration of Ivacaftor and Lumacaftor and area of Ivacaftor and Lumacaftoris linear in the range examined since all points lie in a straight line and the correlation coefficient is well within limits.

Accuracy

Accuracy of the method was determined by Recovery studies. To the formulation (pre analyzed sample), the reference standards of the drugs were added at the level of 50%, 100%, 150%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug is shown in table. To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 50%, 100%, 150%.

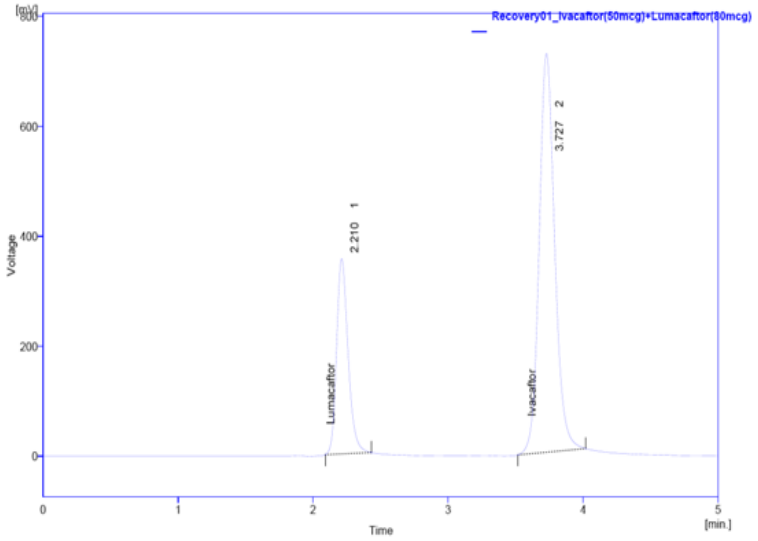


Fig: Chromatogram of 50% recovery (injection 1)

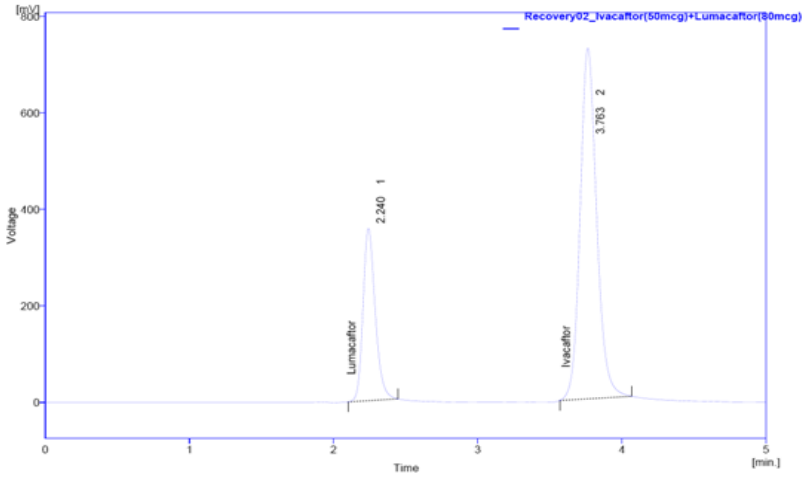


Fig: Chromatogram of 100% recovery (injection 2)

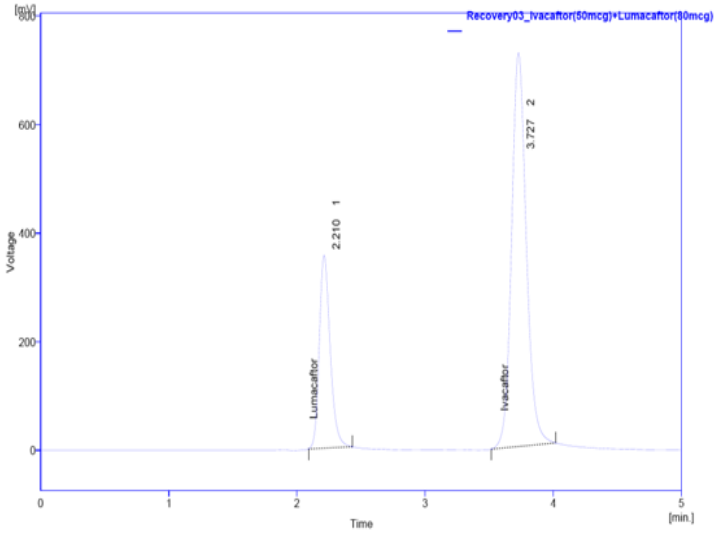


Fig: Chromatogram of 150% recovery (injection 3)

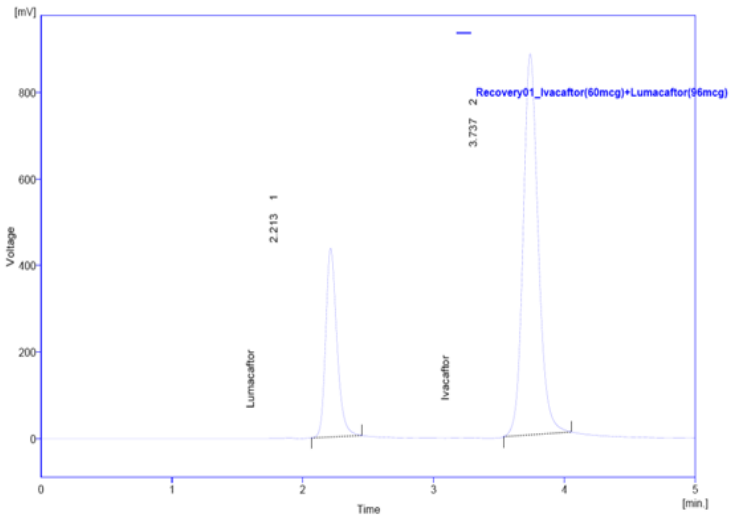


Fig: Chromatogram of 50% recovery (injection 1)

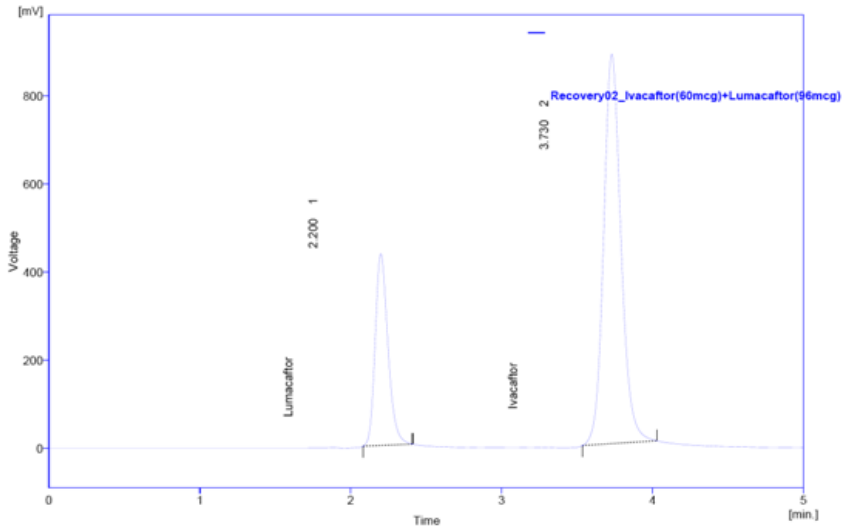


Fig: Chromatogram of 100% recovery (injection 2)

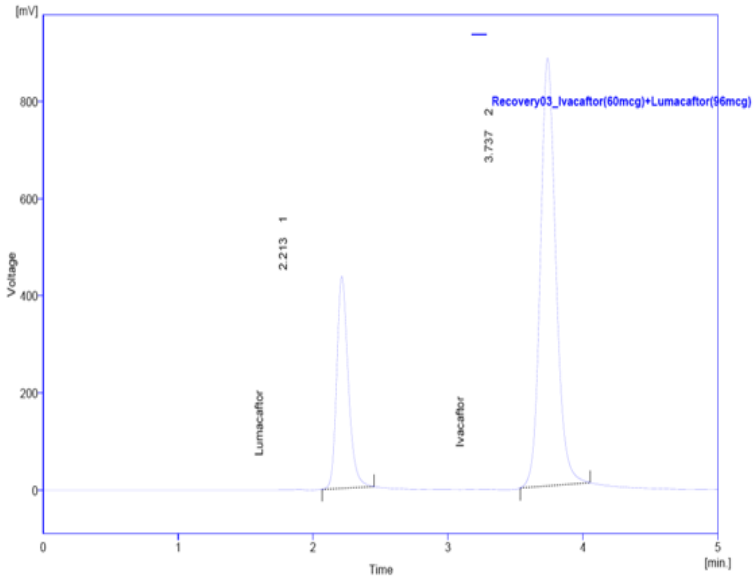


Fig: Chromatogram of 150% recovery (injection 3)

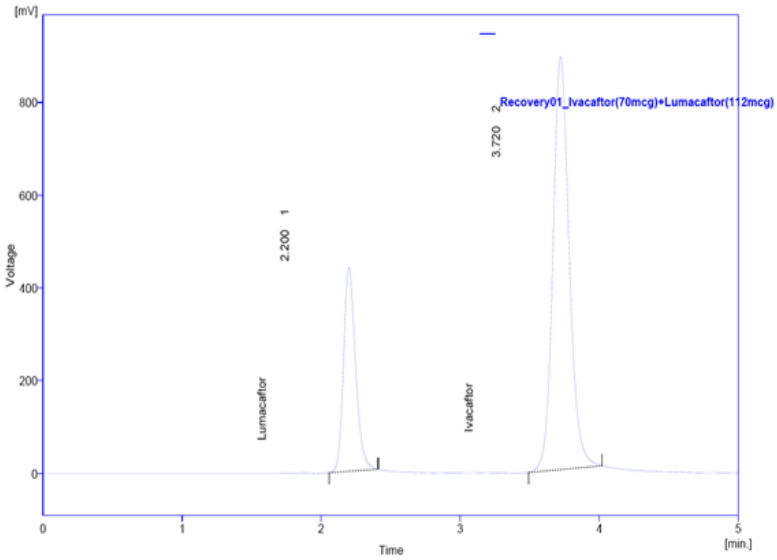


Fig: Chromatogram of 50% recovery (injection 1)

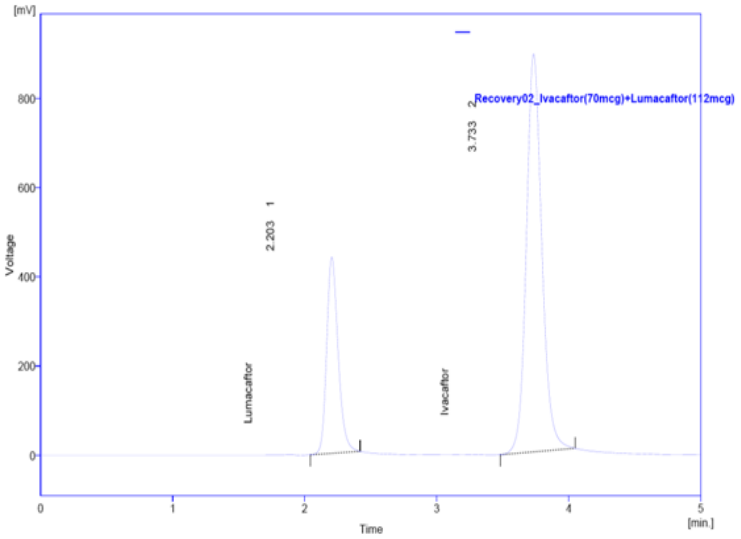


Fig: Chromatogram of 100% recovery (injection 2)

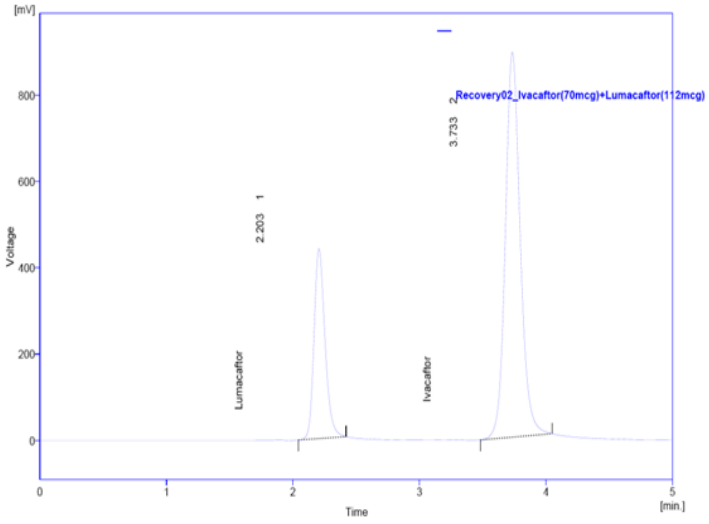


Fig: Chromatogram of 150% recovery (injection 3)

Acceptance criteria

The % recovery of LUMACAFTOR AND IVACAFTOR should lie between 98% and 110%.

Table 9.4.9.1: Recovery results for IVACAFTOR

Recovery level	Accuracy IVACAFTOR					Average % Recovery
	Amount taken(mcg/ml)	Area	Average area	Amount recoverd	%Recovery	
50%	50	5789.751	5789.898	54.03	102.18	
	50	5790.192				
	50	5789.751				
100%	60	7070.222	7071.38	32.99	98.99	
	60	7073.715				100.93%
	60	7070.222				
150%	70	7242.895	7258.66	101.62	101.62	
	70	7290.219				
	70	7242.895				

Table: Recovery results for LUMACAFTOR, Recovery results for LUMACAFTOR

Recovery level	Accuracy LUMACAFTOR					Average % Recovery
	Amount taken(mcg/ml)	Area	Average area	Amount recovered(mcg/ml)	%Recovery	
50%	80	2099.428	2100.890	19.594	126.70	108.93
	80	2103.816				
	80	2099.428				
100%	96	2602.209	2590.043	33.15	98.999	
	96	2565.673				
	96	2602.249				
150%	112	2642.187	2645.416		101.11	
	112	2651.875		101.11		
	112	2642.187				

Observation

The percentage mean recovery of Ivacaftor and Lumacaftor is 100.93% and 108.93% respectively.

PRECISION

Method precision

Method precision

Prepared sample preparations of LUMACAFTOR and IVACAFTOR as per test method and injected 6 times in to the column.

Acceptance criteria

The % Relative standard deviation of Assay preparations of LUMACAFTOR and IVACAFTOR should be not more than 2.0%.

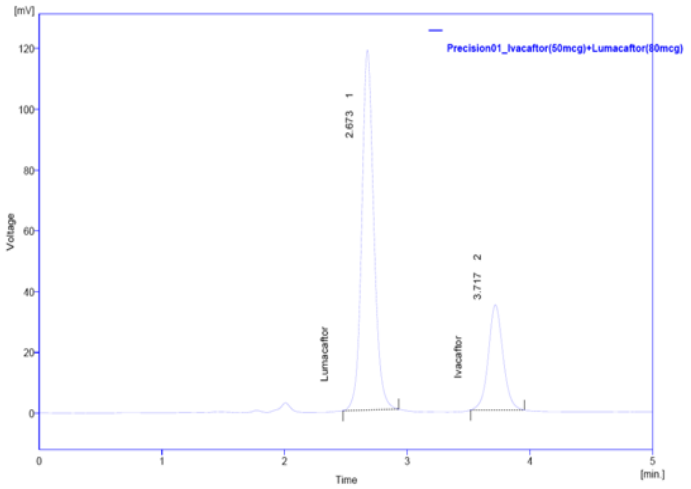


Fig. 9.5. 1: Chromatogram of precision injection 1

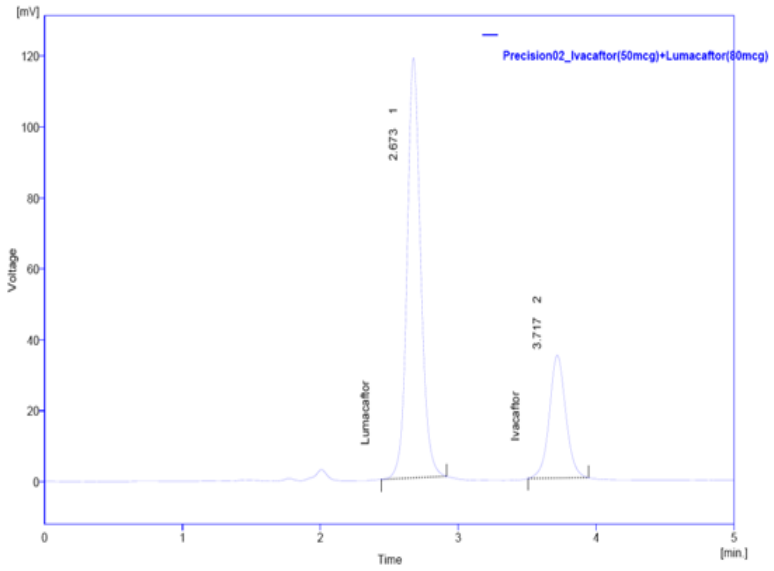


Fig. 9.5.2: Chromatogram of precision injection 2

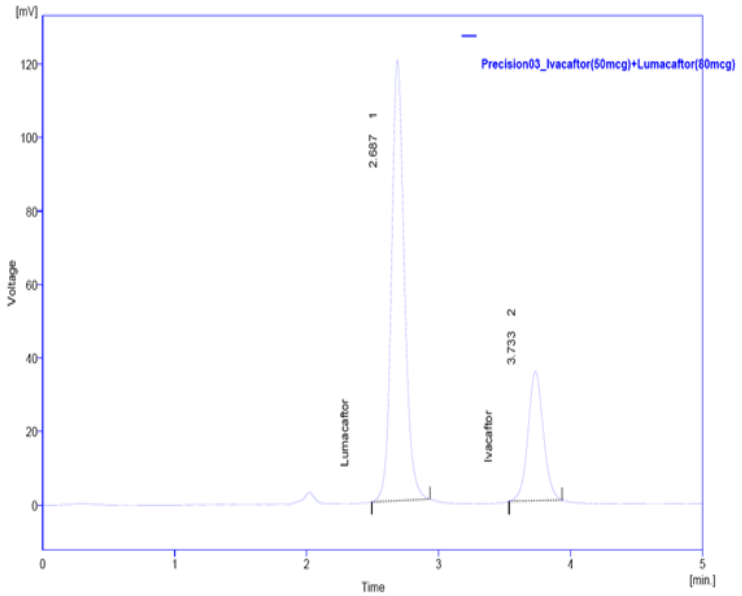


Fig. 9.5.3: Chromatogram of precision injection 3

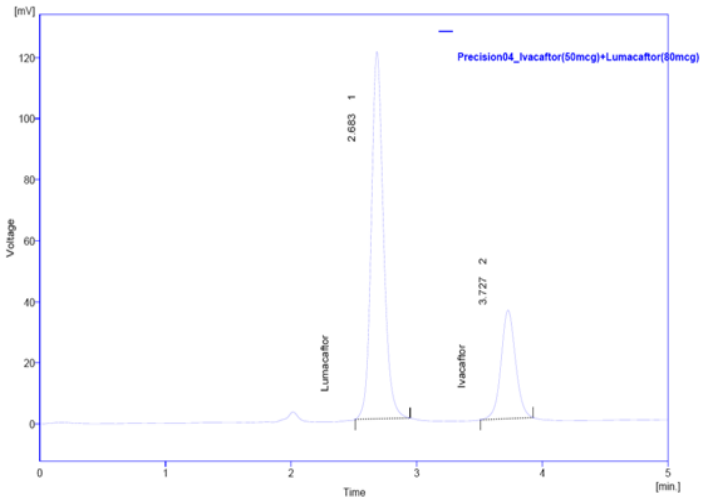


Fig. 9.5.4: Chromatogram of precision injection 4

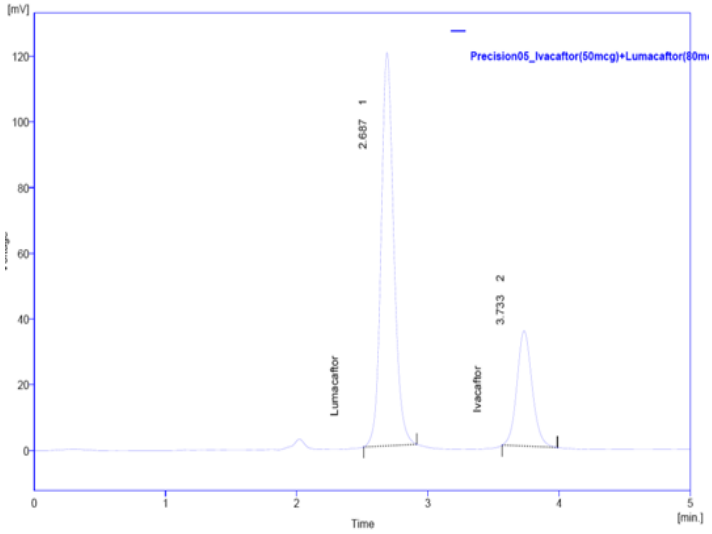


Fig. 9.5.5: Chromatogram of precision injection 5

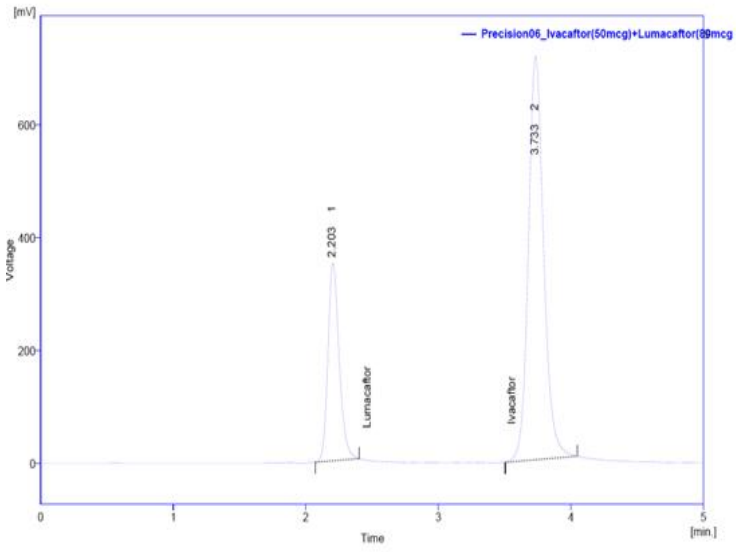


Fig. 9.5.6: Chromatogram of precision injection 6

Results for Method precision of IVACAFTOR and LUMACAFTOR

IVACAFTOR			LUMACAFTOR		
S.No.	**Rt**	**Area**	**S.No.**	**Rt**	**Area**
1	3.717	286.770	1	2.673	811.336
2	3.717	287.146	2	2.673	810.062
3	3.733	283.647	3	2.687	811.956
4	3.727	285.277	4	2.683	810.151
5	3.733	281.675	5	2.687	806.248
6	3.733	5740.309	6	2.203	2058.026
avg	3.726667		avg	2.601	
stdev	0.00784		stdev	0.195084	
%RSD	**0.0053**		**%RSD**	**0.1184**	

Observation

Test results for LUMACAFTOR and IVACAFTOR are showing that the %RSD of Assay results are within limits. The results were shown in table

ROBUSTNESS

Chromatographic conditions variation

To demonstrate the robustness of the method, prepared solution as per test method and injected at different variable conditions like using different conditions like flow rate and wavelength. System suitability parameters were compared with that of method precision.

Acceptance criteria

The system suitability should pass as per the test method at variable conditions.

Chromatographic conditions variation

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Acceptance criteria

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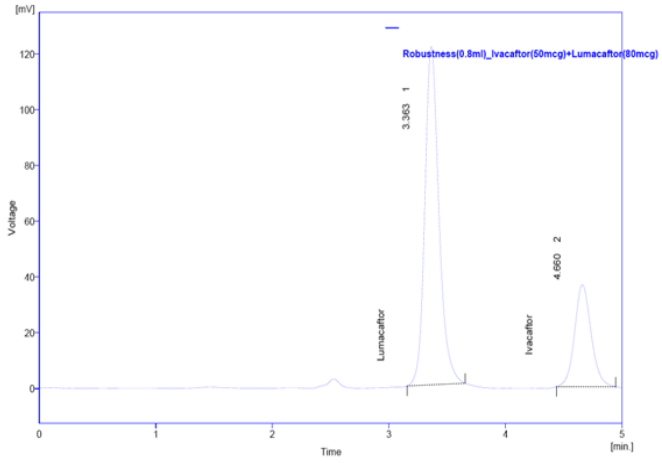

Fig. 9.8.1: Chromatogram of Ivacaftor and Lumacaftor Robustness (0.8 ml/min)

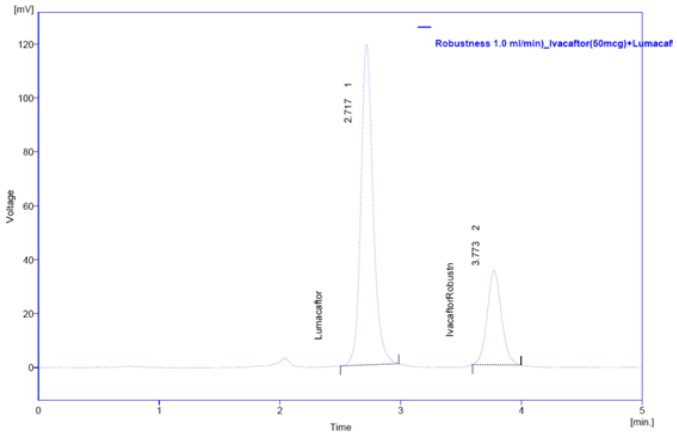

Fig. 9.8.2: Chromatogram of Ivacaftor and Lumacaftor Robustness (1.0 ml/min)

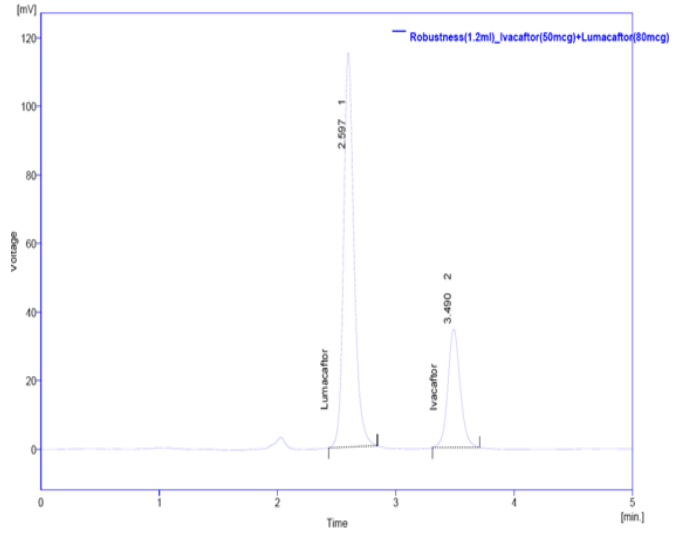

Fig. 9.8.3: Chromatogram of Ivacaftor and Lumacaftor for Robustness (1.2 ml/min)

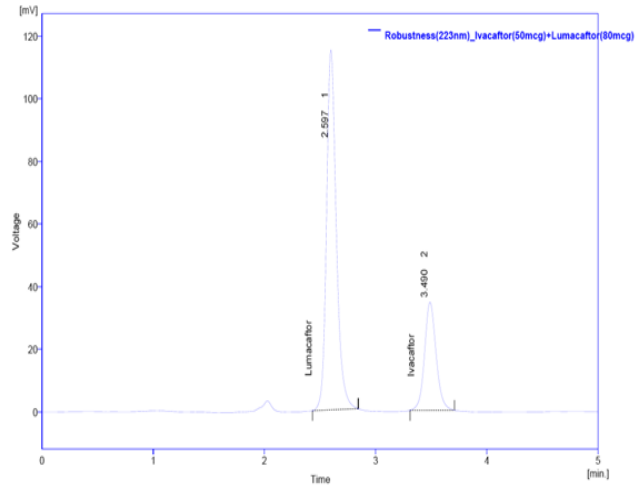

Fig. 9.8.4: Chromatogram of Ivacaftorfor Robustness (223nm)

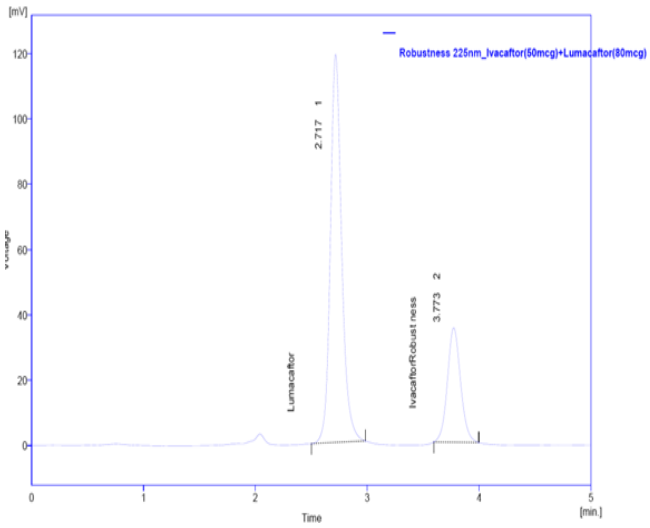

Fig. 9.8.5: Chromatogram of Ivacaftor and Lumacaftorfor Robustness (225nm)

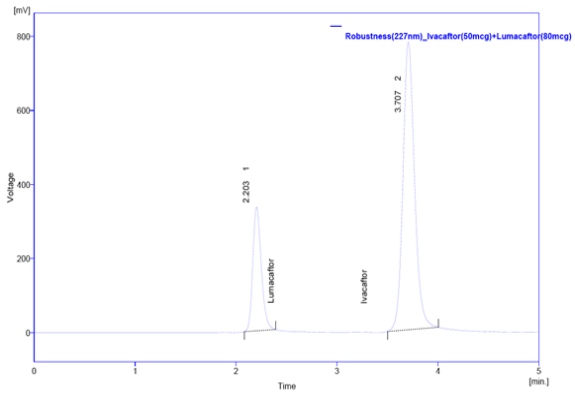

Fig. 9.8.6: Chromatogram of IVACAFTOR and LUMACAFTOR for Robustness (227nm)

Table 9.8.5: Result of Robustness study

Parameter	IVACAFTOR		LUMACAFTOR	
	Retention time(min)	**Tailing factor**	**Retention time(min)**	**Tailing factor**
Flow Rate				
0.8 ml/min	4.660	1.171	3.363	1.333
1.0ml/min	3.773	1.114	2.717	1.286
1.2 ml/min	3.490	1.167	2.597	1.391
Wavelength				
223nm	3.490	1.167	2.597	1.391
225nm	3.773	1.114	2.717	1.286
227nm	3.707	1.219	2.203	1.409

Observation

From the observation it was found that the system suitability parameters were within limit at all variable conditions.

Ruggedness

The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the Assay by two different analysts

Acceptance criteria

The % Relative standard deviation of Assay values between two analysts should be not more than 2.0%.

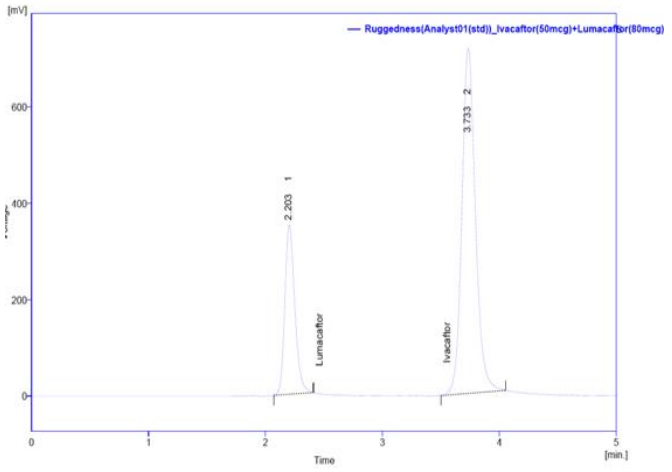

Fig. 9.9.1: Chromatogram of Analyst 01 standard preparation

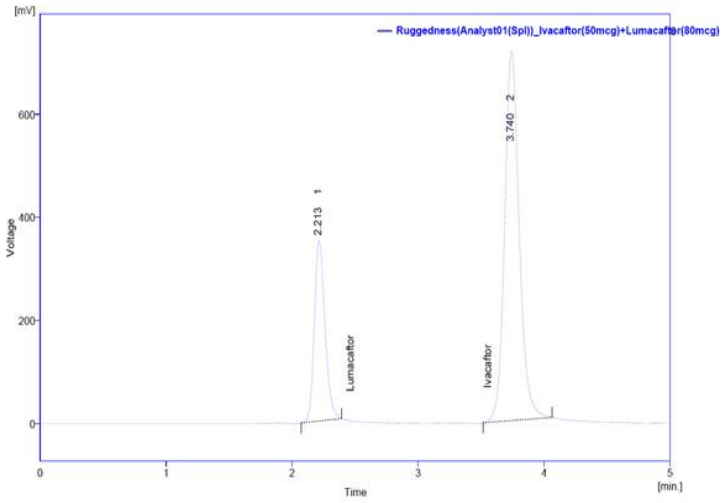

Fig. 9.9.2: Chromatogram of Analyst 01 sample preparation

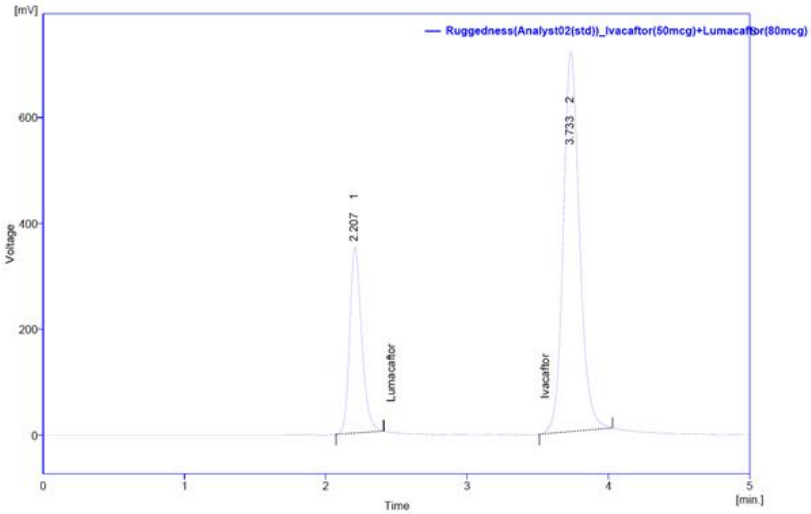

Fig. 9.9.3: Chromatogram of Analyst 02 standard preparation

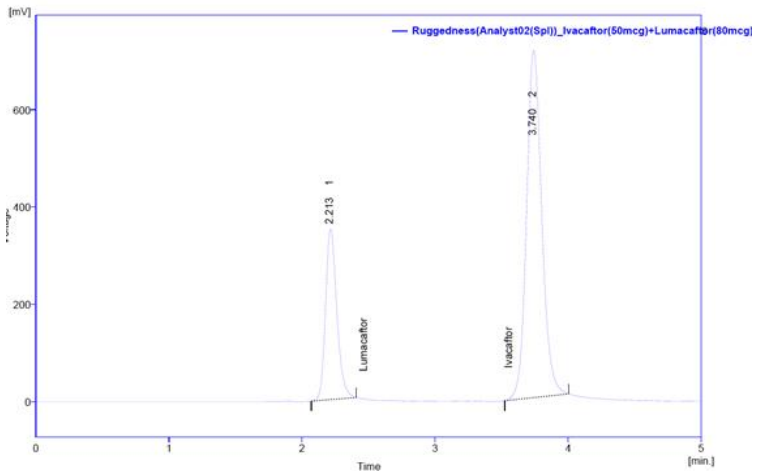

Fig. 9.9.4: Chromatogram of Analyst 02 sample preparation

Table 9.9.5: Results for Ruggedness

IVACAFTOR	%Assay	LUMACAFTOR	%Assay
Analyst 01	99.92%	**Analyst 01**	98.64%
Anaylst 02	98,36%	**Anaylst 02**	99.60%

OBSERVATION

From the observation the between two analysts Assay values not greater than 2.0%, hence the method was rugged

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