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Research Article

Stability indicating validated method development for the simultaneous estimation of tezacaftor, ivacaftor & elexacaftor in API and pharmaceutical dosage form

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ABSTRACT

A Simple accurate, precise method was developed for the simultaneous estimation of the Tezacaftor, Ivacaftor and Elexacaftor in bulk and tablet dosage form. Chromatogram was run through Kromasil C18 150×4.6 mm, 5µ. Mobile phase containing 0.1% OPA and acetonitrile in the ratio of 60: 40 v/v was pumped through column at a flow rate of 1ml / min. Temperature was maintained at 30º c. Optimized wavelength for Tezacaftor, Ivacaftor and Elexacaftor was 260.0 nm. Retention time of Tezacaftor, Ivacaftor and Elexacaftor were found to be 3.468 min, 2.322 min and 2.855 min. % RSD of system precision for Tezacaftor, Ivacaftor and Elexacaftor were found to be 1.0, 0.6 and 1.4 respectively. % RSD of method precision for Tezacaftor, Ivacaftor and Elexacaftor were found to be 1.0, 0.4 and 1.2 respectively. % Recovery was obtained as 99.44%, 100.03% and 100.15% for Tezacaftor, Ivacaftor and Elexacaftor respectively. LOD, LOQ values obtained from regression equations of Tezacaftor, Ivacaftor and Elexacaftor were 0.07 ppm and 0.21ppm, 0.29 ppm and 0.88 ppm ,0.40 ppm and 1.20 ppm respectively. Regression equation of Tezacaftor was y=10491x + 863.8, Ivacaftor was y= 18876x + 7683 and Elexacaftor was y= 33896x + 19526. Retention times are decreased, so; the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

Key Words: Tezacaftor, Ivacaftor, Elexacaftor, RP-HPLC

INTRODUCTION

Tezacaftor is a small molecule that can be used as a corrector of the cystic fibrosis trans membrane conductance regulator (CFTR) gene function. It was developed by Vertex Pharmaceuticals and FDA approved in combination with Ivacaftor; a CFTR potentiator that allows the proteins at the cell surface to open longer and improve nutrient transport.

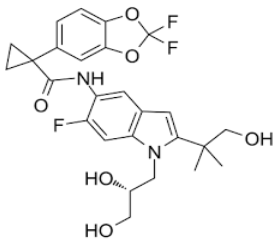

Fig- 1: Chemical structure of Tezacaftor

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Ivacaftor: Ivacaftor is a drug used for the management of cystic fibrosis (CF) in patients aged 2 years and older. Cystic fibrosis is an autosomal recessive disorder caused by one of several different mutations in the gene for the cystic fibrosis transmembrane conductance regulator (CFTR) protein, an ion channel involved in the transport of chloride and sodium ions across the cell membranes. CFTR is active in epithelial cells of organs such as lungs, pancreas, liver, digestive system and reproductive tract. Alterations in the CFTR gene result in altered production, misfolding, or function of the protein and consequently abnormal fluid and ion transport across cell membranes. As a result, CF patients produce a thick, sticky mucus that clogs the ducts of organs where it is produced making patients more susceptible to complications such as infections, lung damage, pancreatic insufficiency and malnutrition.

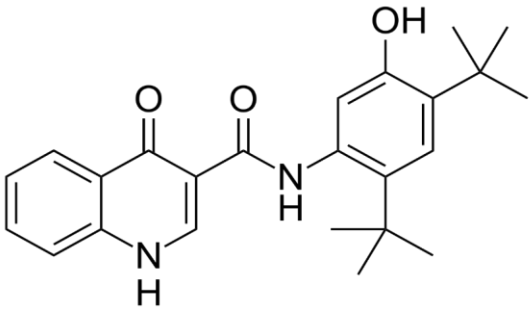

Fig-2: Chemical structure of Ivacaftor

Elexacaftor is a small molecule, next- generation corrector of the cystic fibrosis transmembrane conductance regulator (CFTR) Protein. Elexacaftor is considered a next- generation CFTR corrector as it possesses both a different structure and mechanism as compared to first generation correctors like tezacaftor. While dual corrector/ potentiator combination therapy has proven useful in the treatment of a subset of CF patients, their use is typically limited to patients who are homozygous for the F508del-CFTR gene. Elexacaftor, along with VX-659, was designed to fill the need for an efficacious CF therapy for patients who are heterozygous for F508del-CFTR and a gene that does not produce protein or produce proteins unresponsive to ivacaftor or tezacaftor. The triple combination product Trikafta TM, manufactured by Vertex Pharmaceuticals, is the first product approved for the treatment of CF in individuals who are either homo- or heterozygous for the F508del-CFTR gene.

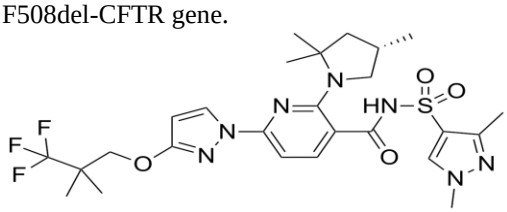

Fig-3 : Chemical structure of Elexacaftor

MATERIALS AND METHODS

AR Grade of materials were used as supplied by the manufacturer without further purification or investigation. Pure Drug samples were obtained from Spectrum labs, Hyderabad.

Chemicals & Solvents Used: All the below chemicals & solvents are from Ranchem.

- Water – HPLC grade
- Acetonitrile – HPLC grade
- Tri ethyl amine – AR grade
- Potassium dihydrogen orthophosphate – AR grade
- Orthophosphoric acid – AR grade

Instruments:

- Electronic Balance
- pH meter
- Waters HPLC2695 series with quaternary pumps
- Photo Diode array Detector
- Auto sampler integrated with empower software
- Ultrasonicator
- UV double beam spectrophotometer with UV win 5.

METHOD DEVELOPMENT:

Optimised method was developed by changing various Columns, Mobile phase ratios, Buffers and its pH etc….

- **Chromatographic conditions:**

Preparation of Buffer: 0.1% OPA Buffer
1ml of Conc. Ortho Phosphoric Acid was diluted to 1000ml with water.

Mobile phase :0.1% OPA: Acetonitrile (60:40 v/v)
Flow rate **:**1.0ml/min
Column **:**C_{18} (250× 4.6mm),5µ
Detector Wavelength **:**260nm
Column Temperature **:**30ºC
Injection Volume **:**10µL
Run time **:**6 Min
Diluent **:**Water: Acetonitrile (50:50 v/v)

Preparation of standard stock solutions: Precisely measured 12.5 mg of Tezacaftor, 18.75mg of Ivacaftor and 25mg of Elexacaftor and transferred to three 50ml volumetric flasks independently. 10 ml of methanol was added and sonicated for 15 mins. Volume was made up with water and acetonitrile (50:50) and marked as Standard stock arrangement 1,2 and 3.

Preparation of standard working solution: 1 ml from each stock solution was pipetted out and taken into a 10 ml volumetric flask and made up with water: acetonitrile.

Preparation of sample stock solution: 20 tablets were weighed and the average weight was calculated, then the weight equivalent to API was transferred into a 100ml volumetric flask, 25ml of diluent was added and sonicated for 50 min. Further the volume was made up with diluent and filtered.

Preparation of sample working solution: From the filtered solution 0.5 ml was pipetted out into a 10ml volumetric flask and made upto 10ml with diluent. Tezacaftor, Ivacaftor and Elexacaftor were eluted at 3.468 min, 2.322min and 2.855min respectively with good resolution. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated.

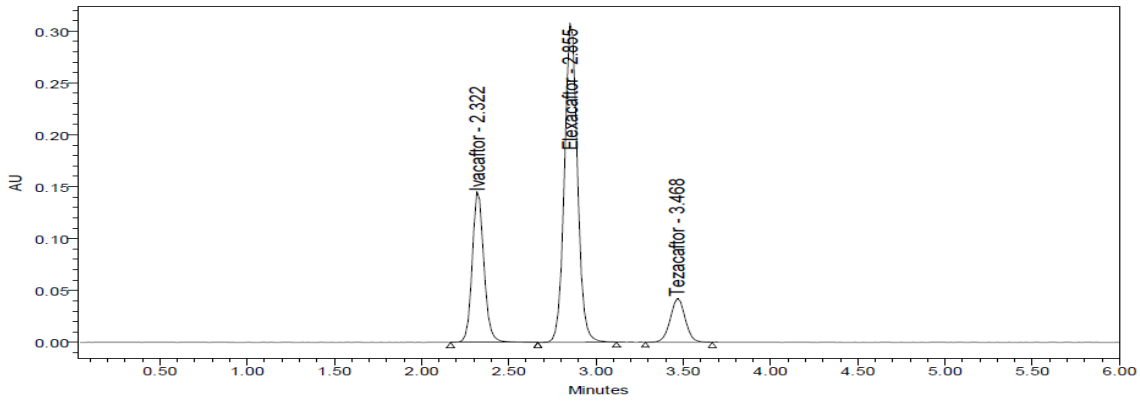

Fig 4: Typical chromatogram

Linearity: From the stock solutions aliquots of 0.25ml, 0.5ml, 0.75ml, 1.0ml, 1.25ml and 1.5ml of Tezacaftor, Ivacaftor and Elexacaftor were pipetted out in a 10ml volumetric flask and made up to the mark with diluent. The concentrations obtained were 6.25ppm, 12.5ppm, 18.75ppm, 25ppm, 31.25ppm, 37.5ppm for Tezacaftor & 9.375ppm, 18.75ppm, 28.125ppm, 37.5ppm, 46.875ppm, 56.25ppm for Ivacaftor & 12.5ppm, 25ppm, 37.5ppm, 50ppm, 62.5ppm, 75ppm for Elexacaftor.

Table 1: Linearity table for Tezacaftor, Ivacaftor and Elexacaftor

Tezacaftor		**Ivacaftor**		**Elexacaftor**	
Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area	Conc(µg/mL)	Peak area
6.25	71432	9.375	203034	12.5	464556
12.5	128943	18.75	350727	25	842060
18.75	196609	28.125	543909	37.5	1327190
25	259676	37.5	701470	50	1710770
31.25	330862	46.875	902412	62.5	2145538
37.5	395424	56.25	1068518	75	2544342

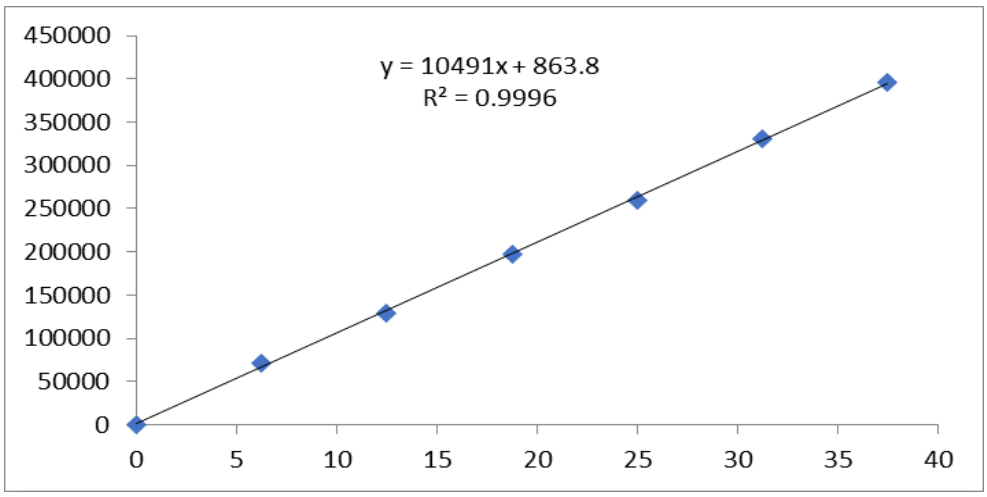

Fig5: Calibration curve of Tezacaftor.

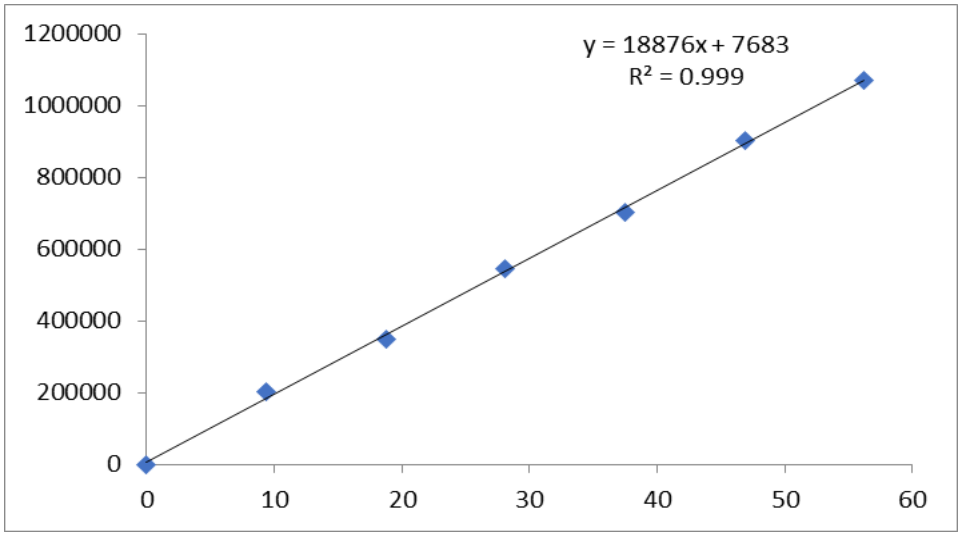

Fig 6: calibration curve of Ivacaftor

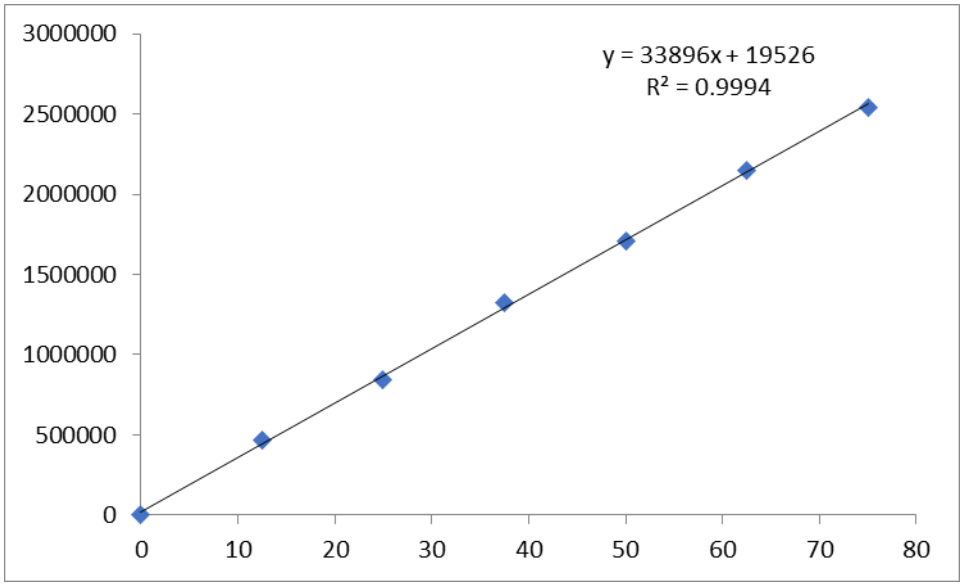

Fig 7: calibration curve of Elexacaftor

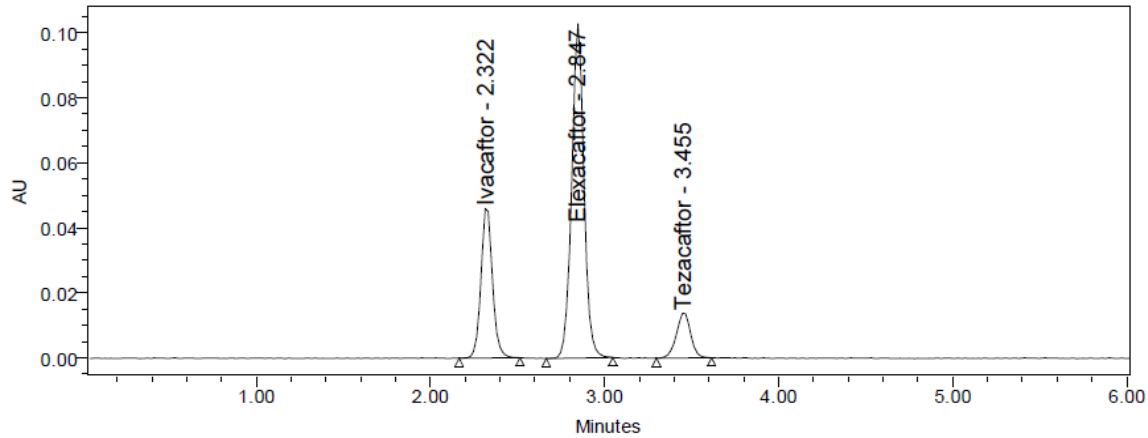

Fig 8: Linearity chromatogram at 6.25(µg/mL), 9.375(µg/mL),12.5(µg/mL) of Tezacaftor, Ivacaftor & Elexacaftor

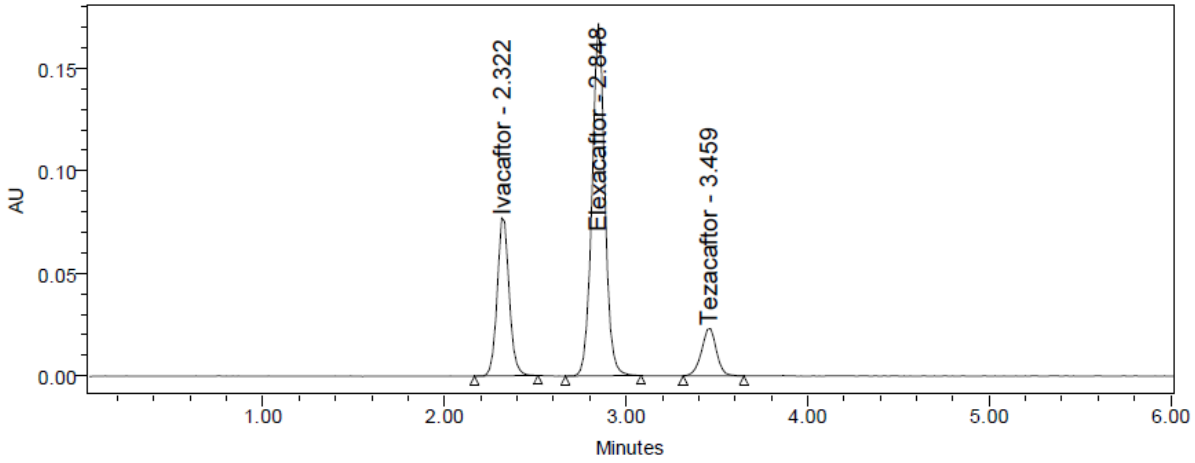

Fig9: Linearity chromatogram at 12.5(µg/mL), 18.75(µg/mL), 25(µg/mL) of Tezacaftor, Ivacaftor & Elexacaftor

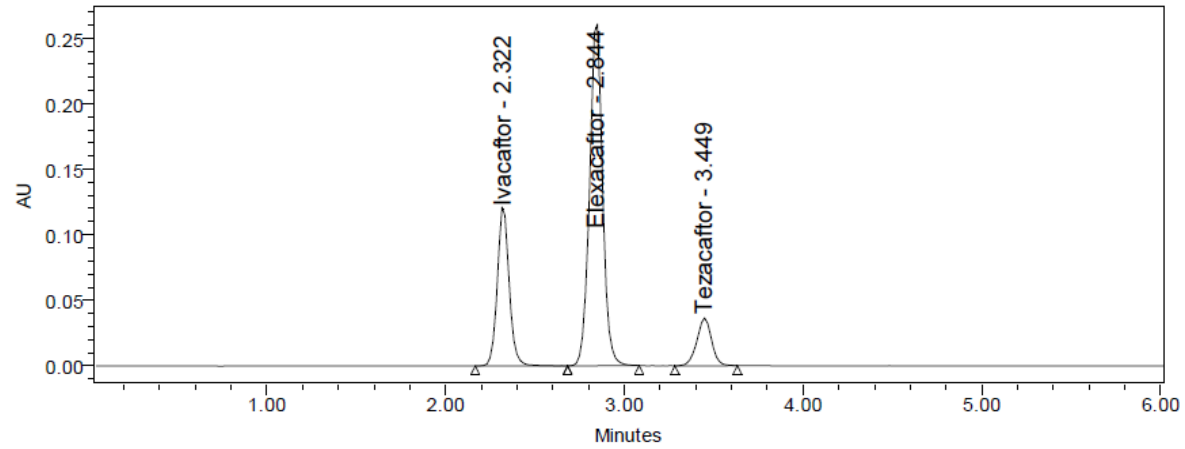

Fig 10: Linearity chromatogram at 18.75(µg/mL),28.125(µg/mL),37.5(µg/mL) of Tezacaftor, Ivacaftor & Elexacaftor

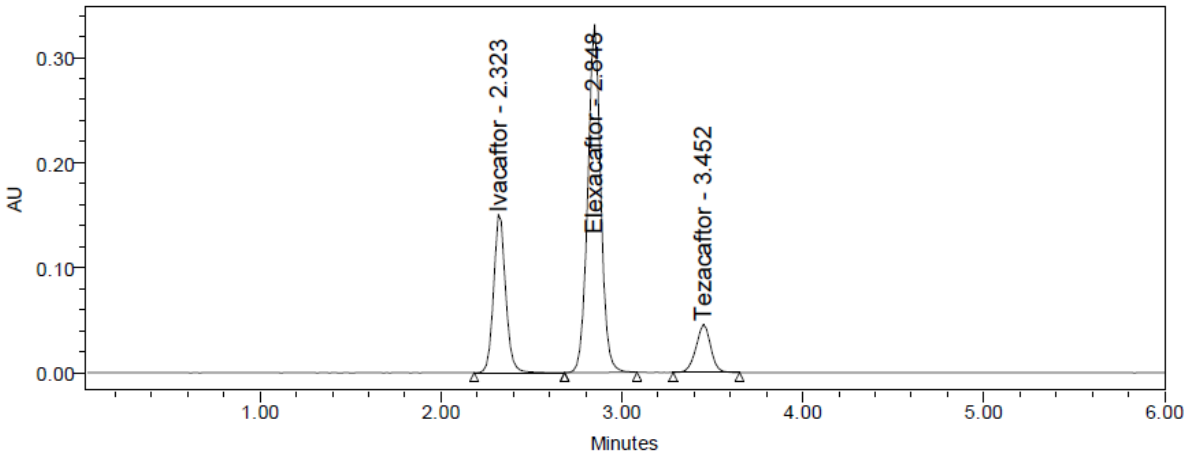

Fig 11: Linearity chromatogram at 25(µg/mL), 37.5(µg/mL), 50(µg/mL) of Tezacaftor, Ivacaftor & Elexacaftor

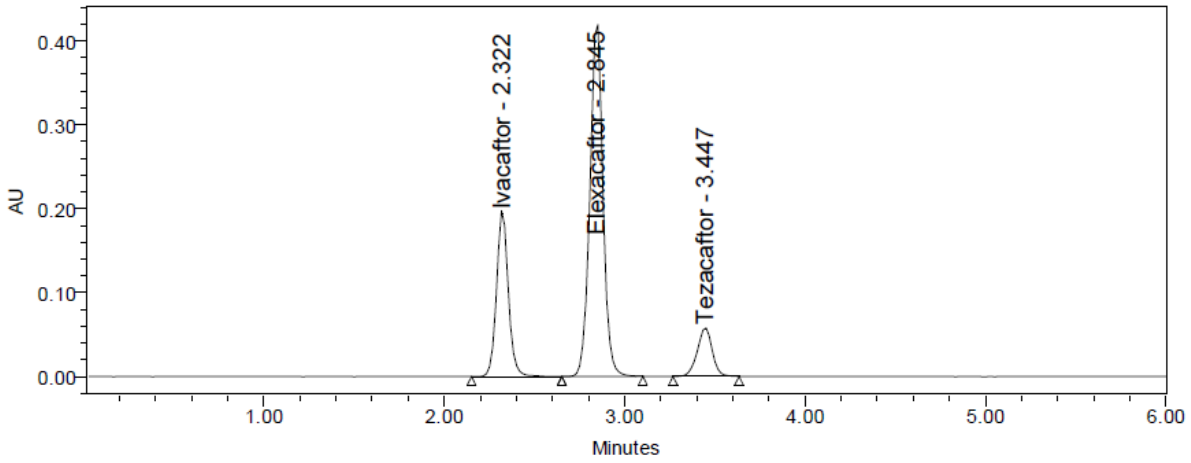

Fig 12: Linearity chromatogram at 31.25(µg/mL), 46.875(µg/mL), 62.5(µg/mL) of Tezacaftor, Ivacaftor & Elexacaftor

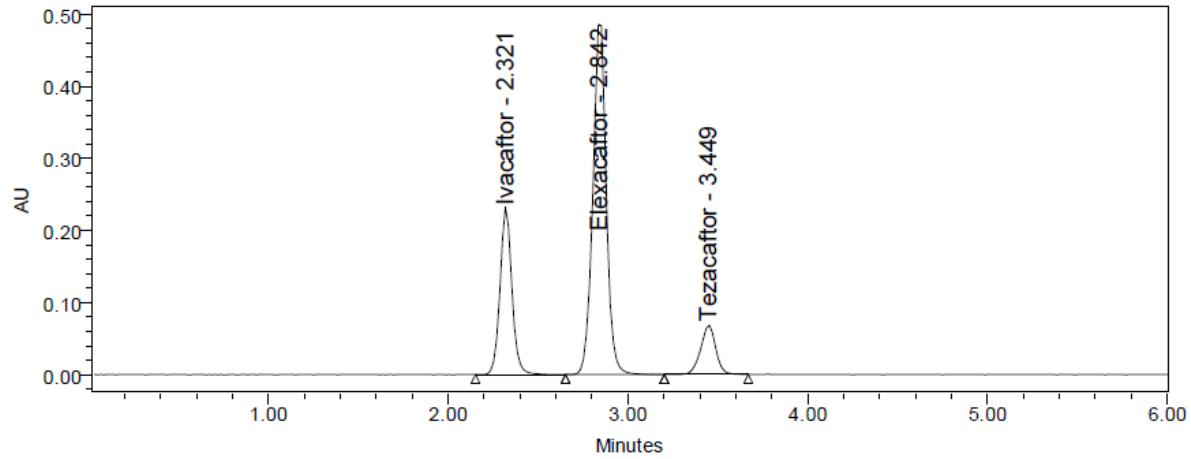

Fig 13: Linearity chromatogram at 37.5(µg/mL), 56.25(µg/mL),75(µg/mL) of Tezacaftor, Ivacaftor & Elexacaftor

RESULTS AND DISCUSSION

System suitability parameters: The system suitability parameters were determined by preparing standard solutions of Tezacaftor, Ivacaftor and Elexacaftor and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

Table 2: System suitability parameters for Ivacaftor, Elexacaftor & Tezacaftor

Ivacaftor			**Elexacaftor**				**Tezacaftor**			
RT(min) (min)	TP	Tailing	RT(min)	TP	Tailing	RS	RT(min)	TP	Tailing	RS
2.32	6463	1.12	2.84	7723	1.05	4.1	3.45	8806	1.0	4.2
2.32	6523	1.10	2.84	7825	1.05	4.2	3.45	9059	1.0	4.3
2.32	6504	1.09	2.84	7876	1.03	4.2	3.45	8518	0.9	4.4
2.32	6030	1.09	2.85	7628	1.05	4.2	3.45	8647	1.0	4.3
2.32	6141	1.12	2.85	7873	1.03	4.2	3.46	8700	1.0	4.2
2.32	6271	1.12	2.85	7547	1.05	4.1	3.46	8262	1.0	4.3

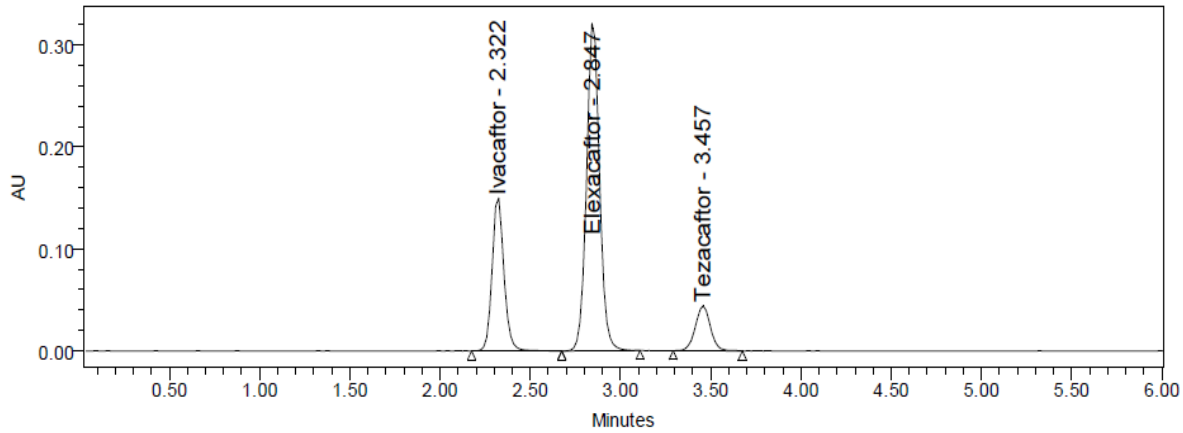

Fig 14: System suitability chromatogram

Specificity: Specificity is the ability of the analytical method to distinguish between the analyte(s) and the other components in the sample matrix.

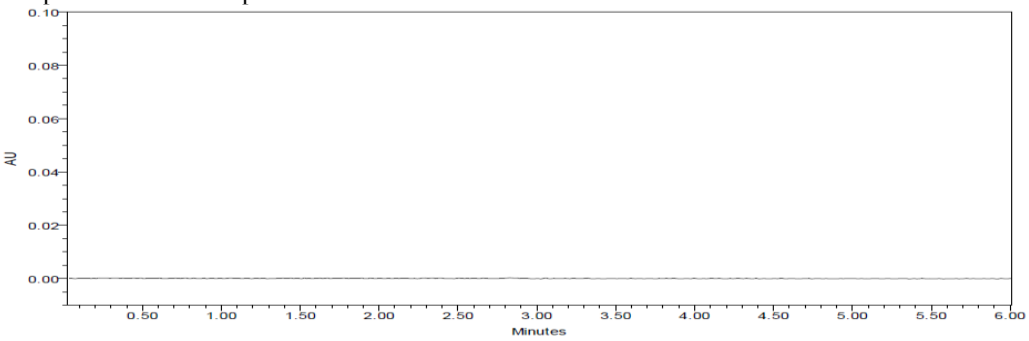

Fig 15: Blank Chromatogram

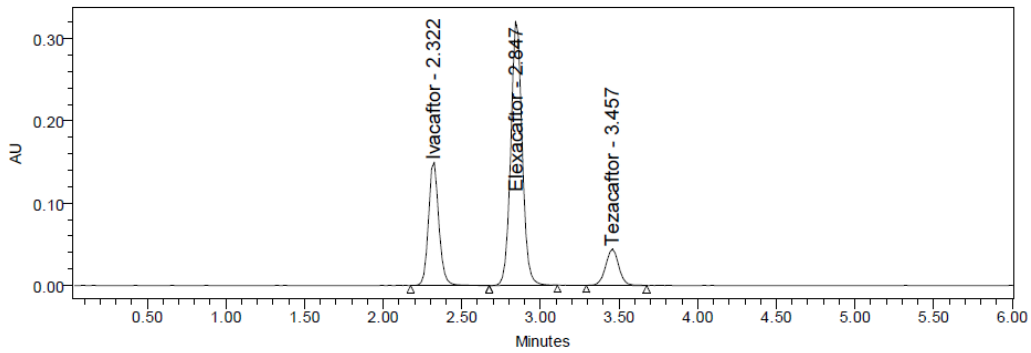

Fig 16: Optimised Chromatogram

Retention times of Tezacaftor, Ivacaftor and Elexacaftor were 3.457 min, 2.322min and 2.847min respectively. We did not find any interfering peaks in blank and placebo at retention times of these drugs in this method. So; this method was said to be specific.

Precision: Precision refers to the closeness of two or more measurements to each other.

Preparation of standard stock solutions: Precisely measured 12.5 mg of Tezacaftor, 18.75mg of Ivacaftor and 25mg of Elexacaftor and transferred to three 50ml volumetric flasks independently. 10 ml of methanol was added and sonicated for 15 mins. Volume was made up with water and acetonitrile (50:50) and marked as Standard stock arrangement 1,2 and 3.

Preparation of standard working solution:1 ml from each stock solution was pipetted out and taken into a 10 ml volumetric flask and made up with water: acetonitrile.

Preparation of sample stock solution: 20 tablets were weighed and the average weight was calculated, then the weight equivalent to API was transferred into a 100ml volumetric flask, 25ml of diluent was added and sonicated for 50 min.

Further the volume was made up with diluent and filtered.

Preparation of sample working solution: From the filtered solution 0.5 ml was pipetted out into a 10ml volumetric flask and made upto 10ml with diluent.

Table 3: System precision table of Tezacaftor, Ivacaftor & Elexacaftor

S.NO	Area of Tezacaftor	Area of Ivacaftor	Area of Elexacaftor
1	241552	696496	1649897
2	238037	695730	1662332
3	243849	693416	1666767
4	241828	685418	1716345
5	243505	694133	1687564
6	244992	689646	1687753
Mean	242294	692473	1678443
S.D	2451.7	4200.1	23736.9
% RSD	1.0	0.6	1.4

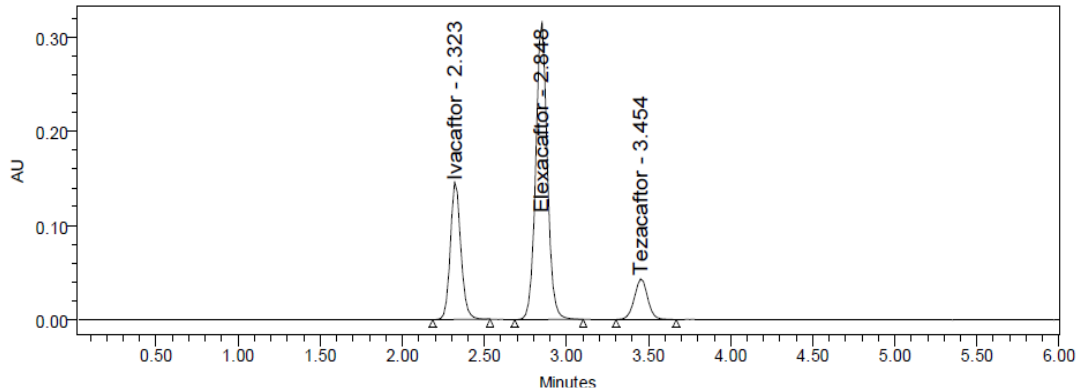

Fig 17: System precision chromatogram

From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned above. Average area, standard deviation and % RSD were calculated for three drugs and obtained as 1.0%, 0.6% and 1.4% respectively for Tezacaftor, Ivacaftor and Elexacaftor. As the limit of Precision was less than "2" the system precision was passed in this method.

Accuracy: The accuracy of an analytical method is the closeness of test results obtained by that method to the true value.

Preparation of Standard Stock solutions: Accurately weighed 12.5 mg of Tezacaftor, 18.75mg of Ivacaftor and 25mg of Elexacaftor and transferred to three 50ml volumetric flasks separately. 10ml of diluent was added to flasks and sonicated for 20mins. Flasks were made up with diluent and labelled as Standard stock solution 1,2 and 3.

Preparation of 50% Spiked solution: 0.1ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out and made up to the mark with diluent.

Preparation of 100% Spiked solution: 0.2ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out and made up to the mark with diluent.

Preparation of 150% Spiked solution: 0.3ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out and made up to the mark with diluent.

Table 4: Accuracy table for Tezacaftor

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	12.5	12.468	99.74	100.01%
	12.5	12.520	100.16	
	12.5	12.372	98.97	
100%	25	25.047	100.19	
	25	25.108	100.43	
	25	24.712	98.85	
150%	37.5	38.048	101.46	
	37.5	37.224	99.26	
	37.5	37.894	101.05	

Table 5: Accuracy table of Ivacaftor

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	18.75	18.752	100.01	99.69%
	18.75	18.797	100.25	
	18.75	18.872	100.65	
100%	37.5	37.195	99.19	
	37.5	36.920	98.45	
	37.5	37.359	99.62	
150%	56.25	55.909	99.39	
	56.25	56.142	99.81	
	56.25	56.167	99.85	

Table 6: Accuracy table of Elexacaftor

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	25	25.17	100.66	100.20%
	25	24.91	99.63	
	25	24.86	99.43	
100%	50	49.49	98.99	
	50	50.47	100.94	
	50	49.66	99.31	
150%	75	75.02	100.02	
	75	75.99	101.33	
	75	76.10	101.47	

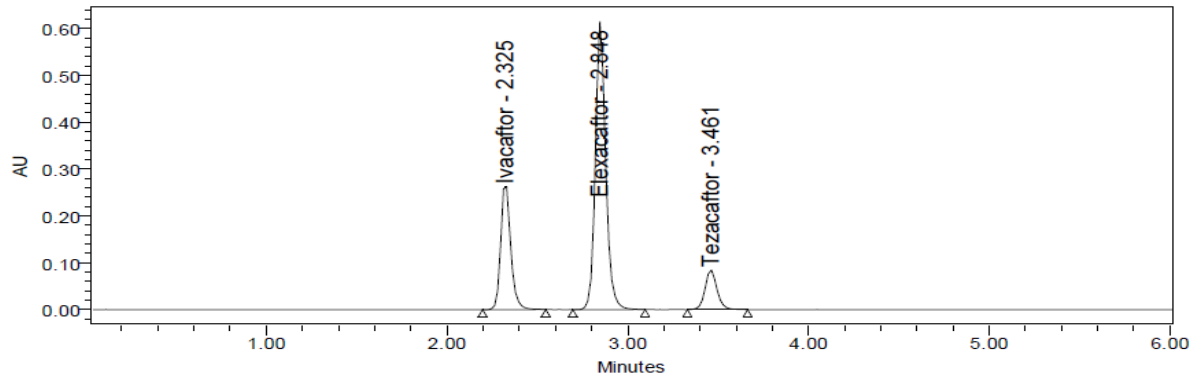

Fig 18: Accuracy 50% chromatogram

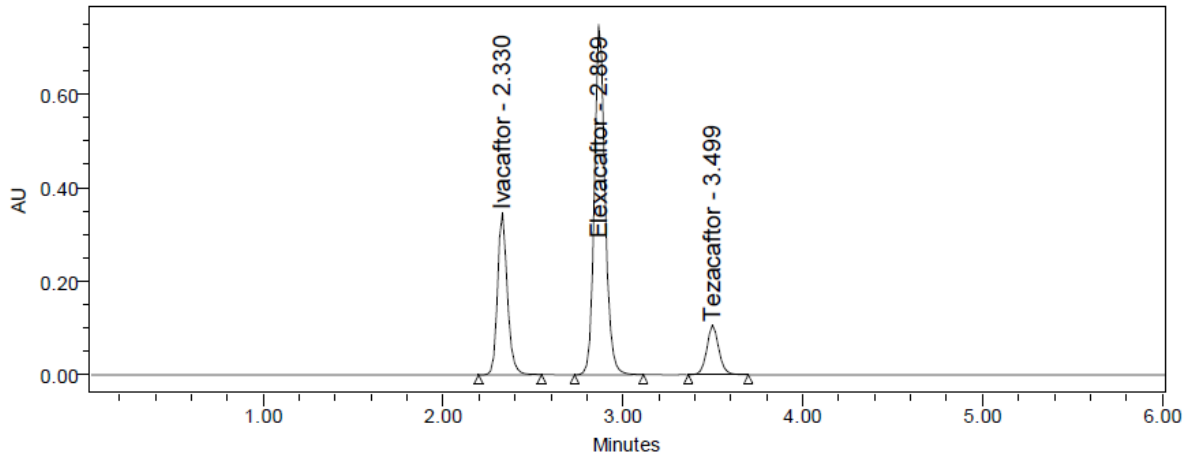

Fig 19: Accuracy 100% chromatogram

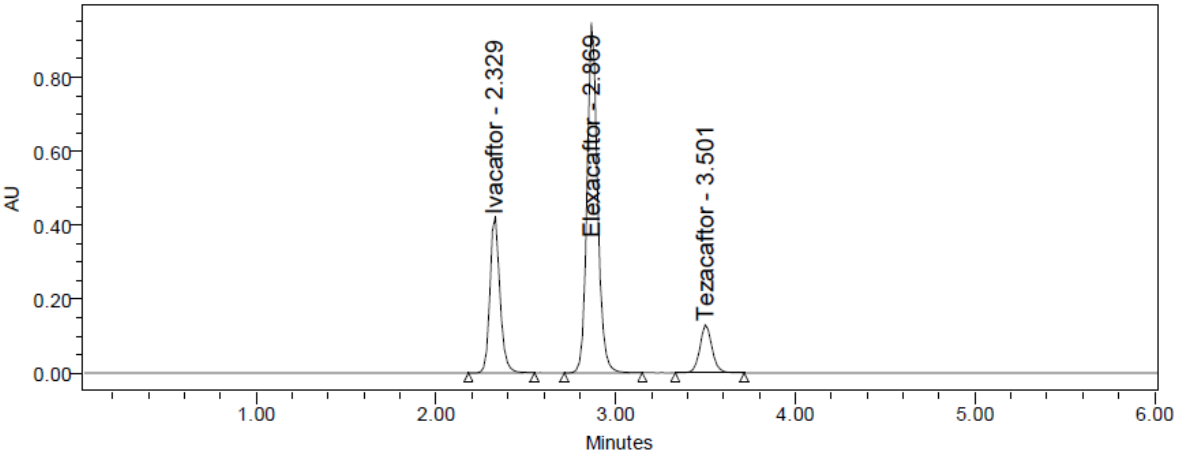

Fig 20: Accuracy 150% chromatogram

Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio and temperature are made but there was no recognized change in the result and are within range as per ICH guidelines. Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), Mobile phase minus, Mobile phase plus, Temperature minus (25ºC), Temperature plus (35ºC) was maintained and samples were injected in duplicate manner. System suitability parameters were not much effected and all the parameters were passed. % RSD was within the limit.

Table 7: Robustness data for Tezacaftor, Ivacaftor & Elexacaftor

S.no	Condition	%RSD of Tezacaftor	%RSD of Ivacaftor	%RSD of Elexacaftor
1	Flow rate (-) 0.9ml/min	1.5	0.7	1.0
2	Flow rate (+) 1.1ml/min	0.7	1.3	1.7
3	Mobile phase (-) 65B:35A	0.2	0.4	0.2
4	Mobile phase (+) 55B:45A	0.8	0.8	1.1
5	**Temperature (-) 25°C**	0.14	0.7	0.1
6	**Temperature (+) 35°C**	0.3	0.8	0.9

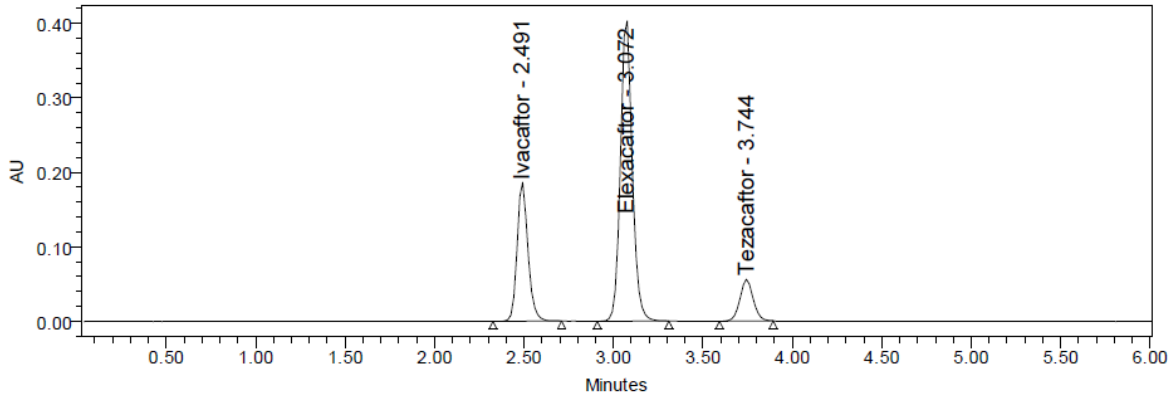

Fig 21: Flow minus chromatogram

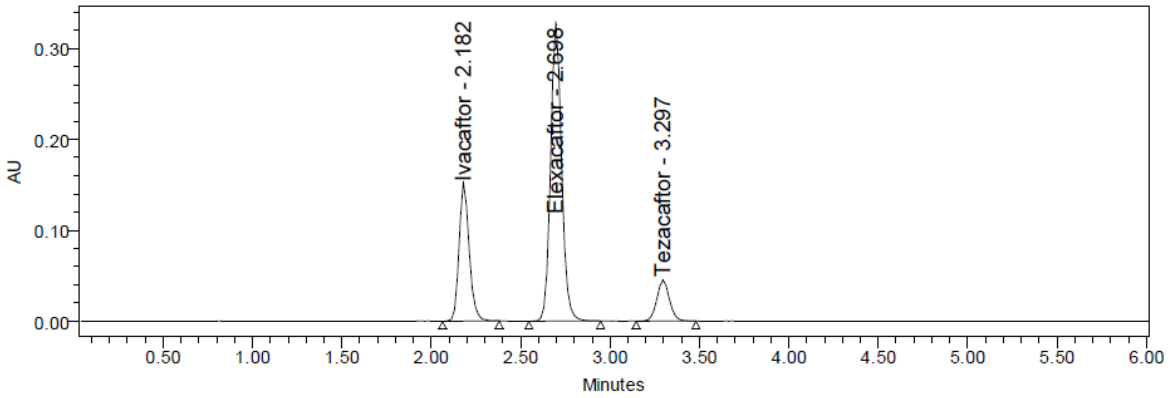

Fig 22: Flow plus chromatogram

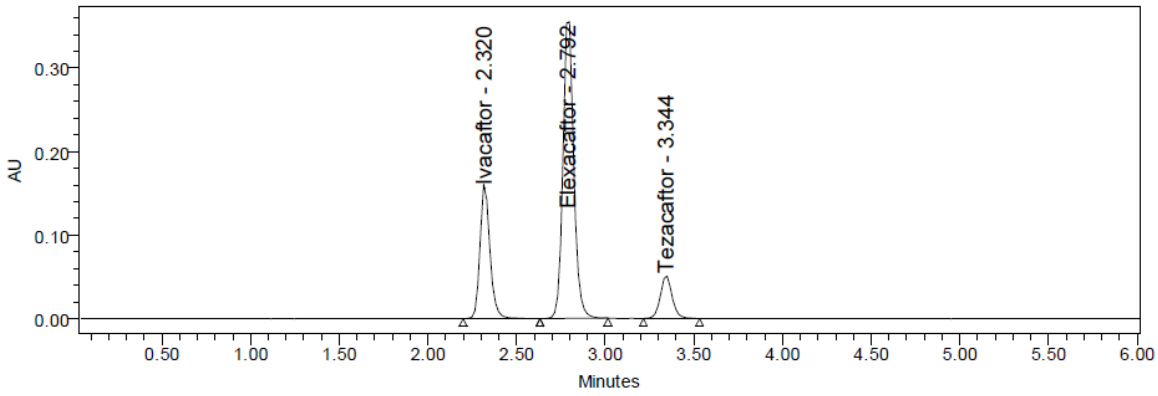

Fig 23: Mobile phase minus chromatogram

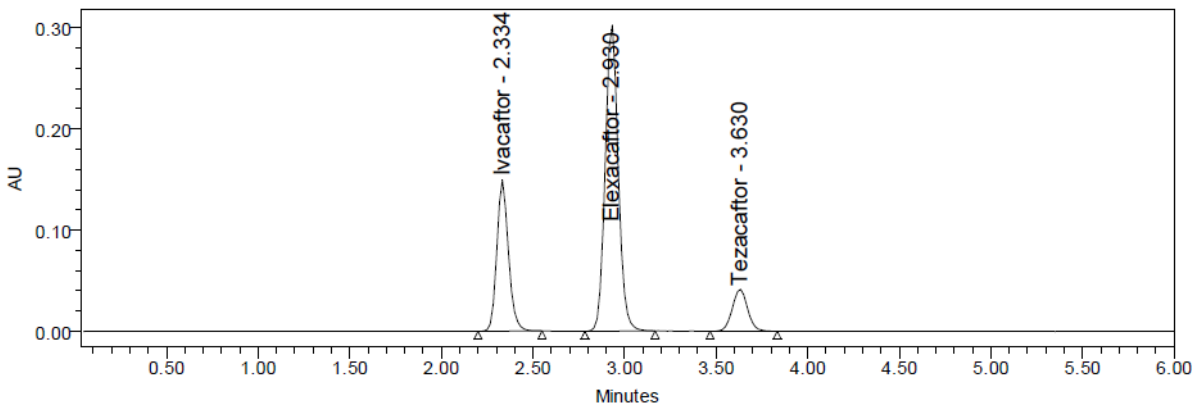

Fig 24: Mobile phase plus chromatogram

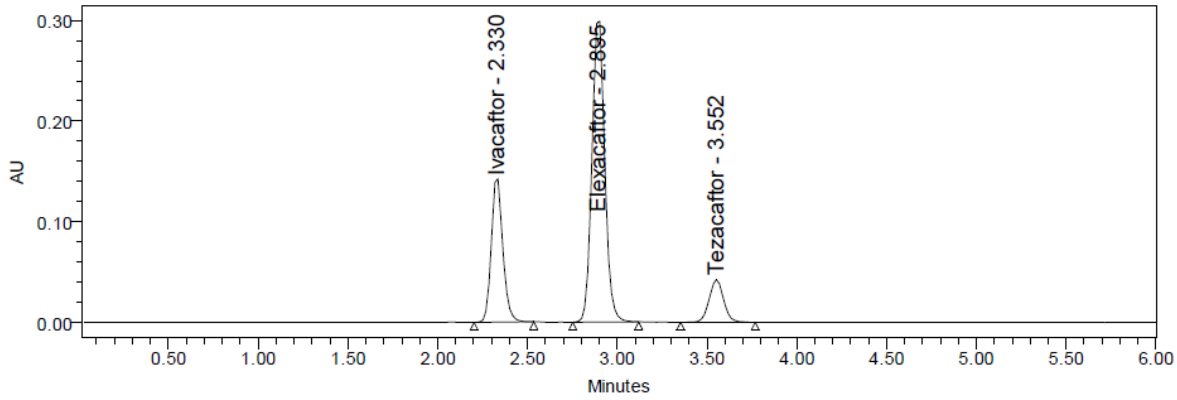

Fig 25: Temperature minus chromatogram

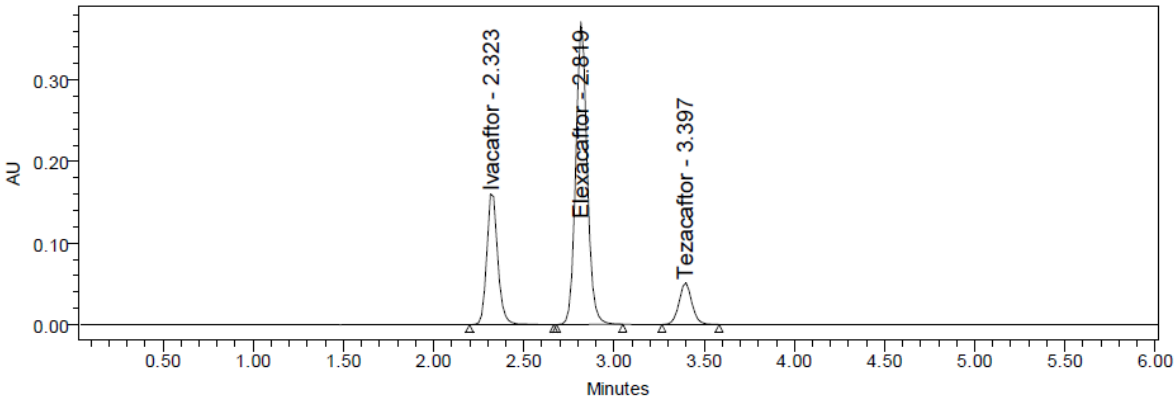

Fig 26: Temperature plus chromatogram

Limit of Detection: The limit of detection (LOD) is usually defined as the lowest quantity or concentration of a component that can be reliably detected with a given analytical method.

LOD = 3.3× Standard deviation of intercept / Average of slope

Sample preparation: 0.25ml each from three standard stock solutions was pipetted out and transferred to 3 separate 10ml volumetric flasks and made up with diluent. From the above solutions 0.1ml, 0.1ml, 0.1ml of Tezacaftor, Ivacaftor & Elexacaftor solutions were transferred to 10ml volumetric flasks and made up with the diluent.

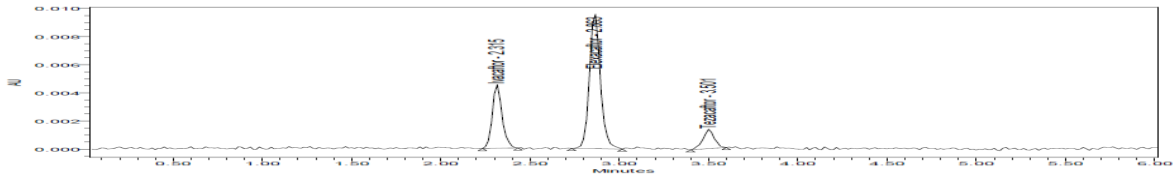

Fig 27: LOD chromatogram of standard

Limit of Quantification: Limit of quantification**,** *LOQ* stands for the smallest amount or the lowest concentration of a substance that is possible to be determined by means of a given analytical procedure with the established accuracy, precision, and uncertainty.

LOQ=10 × Standard deviation of intercept / Average of slope

Sample preparation: 0.25ml each from three standard stock solutions was pipetted out and transferred to 3 separate 10ml volumetric flasks and made up with diluent. From the above solutions 0.3ml, 0.3ml, 0.3ml of Tezacaftor, Ivacaftor & Elexacaftor solutions were transferred to 10ml volumetric flasks and made up with the diluent.

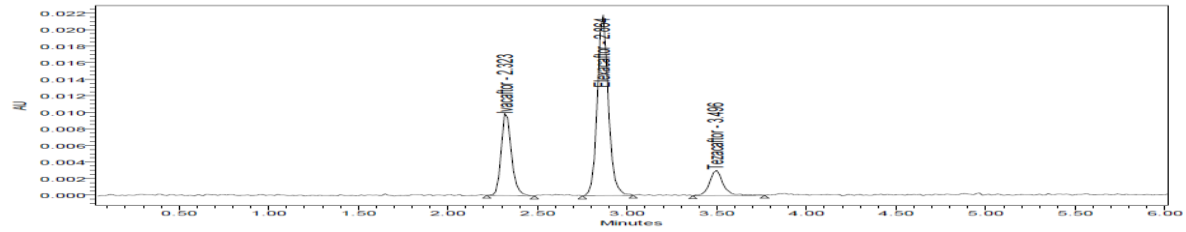

Fig 28: LOQ chromatogram of standard

Sensitivity:

Table 8: Sensitivity table of Tezacaftor, Ivacaftor & Elexacaftor

Molecule	LOD(µg/ml)	LOQ(µg/ml)
Tezacaftor	0.07 µg/ml	0.21 µg/ml
Ivacaftor	0.29 µg/ml	0.88 µg/ml
Elexacaftor	0.40 µg/ml	1.20 µg/ml

DEGRADATION STUDIES

Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation.

Oxidation: To 1 ml of stock solutions of Tezacaftor, Ivacaftor and Elexacaftor; 1ml of 20% hydrogen peroxide (H202) was added separately. The solutions were kept for 30min at 60ºC. For HPLC study, the resultant solution was diluted to obtain 25µg/ml, 37.5µg/ml, 50µg/ml of all the components and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

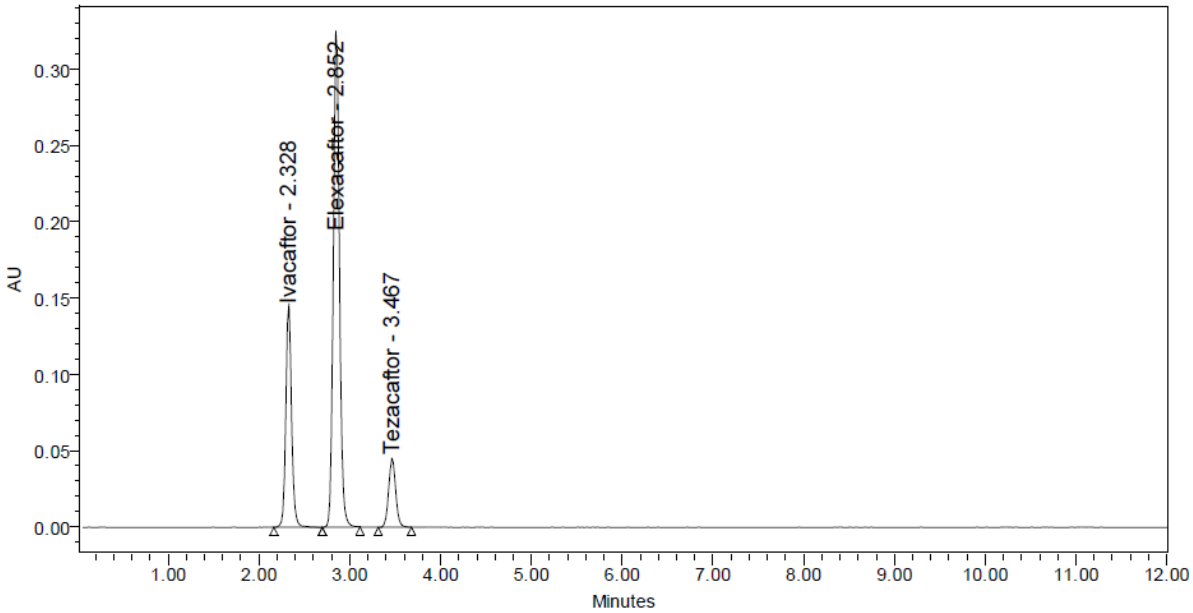

Fig 29: Peroxide chromatogram

Acid Degradation Studies: To 1ml of stock solution Tezacaftor, Ivacaftor &Elexacaftor ; 1ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60ºC. The resultant solution was diluted to obtain 25µg/ml, 37.5µg/ml, 50µg/ml of all the components and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

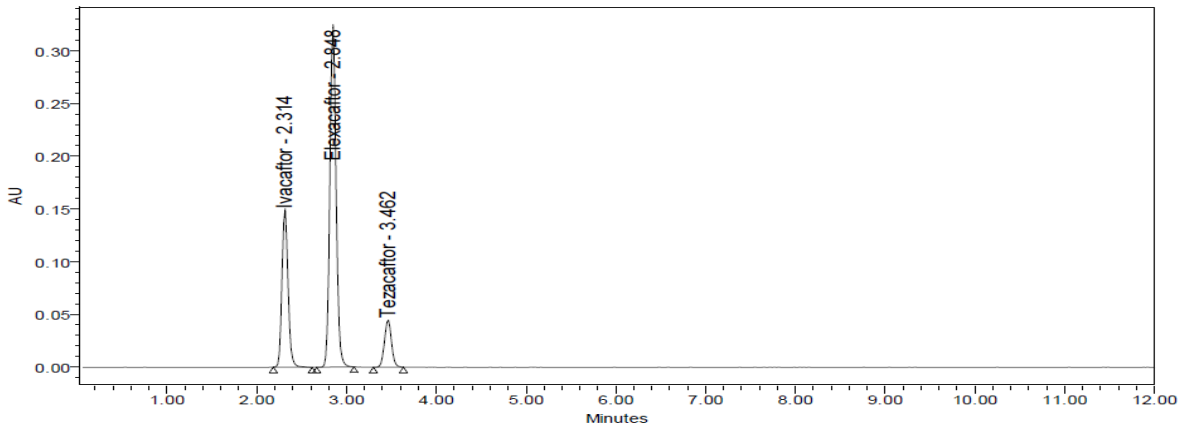

Fig 30: Acid chromatogram

Alkali Degradation Studies: To 1ml of of stock solution Tezacaftor, Ivacaftor &Elexacaftor ; 1ml of 2N Sodium Hydroxide was added and refluxed for 30mins at 60ºC. The resultant solution was diluted to obtain 25µg/ml, 37.5µg/ml, 50µg/ml of all the components and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

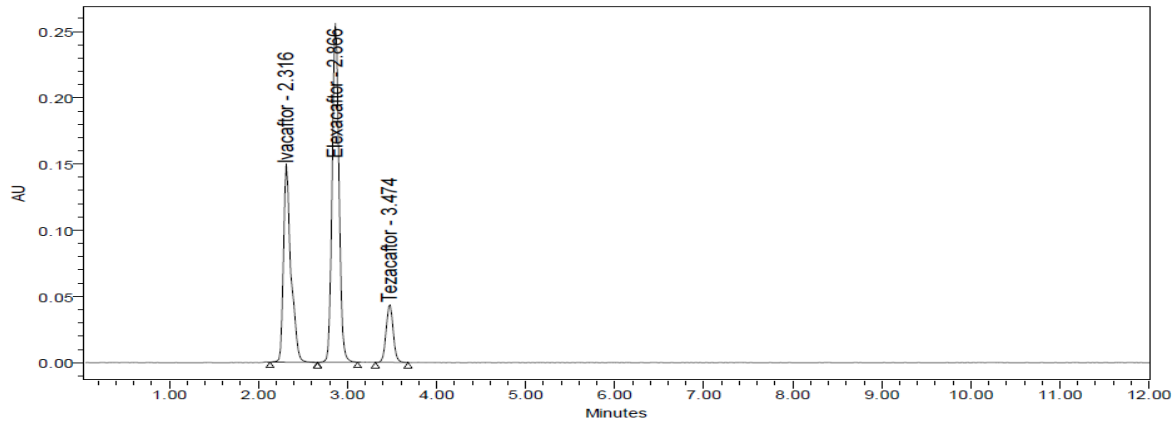

Fig 31: Base chromatogram

Dry heat Degradation Studies: The standard drug solution was placed in oven at 105ºC for 1hour to study dry heat degradation. For HPLC study, the resultant solution was diluted to obtain 25µg/ml, 37.5µg/ml, 50µg/ml of all the components and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

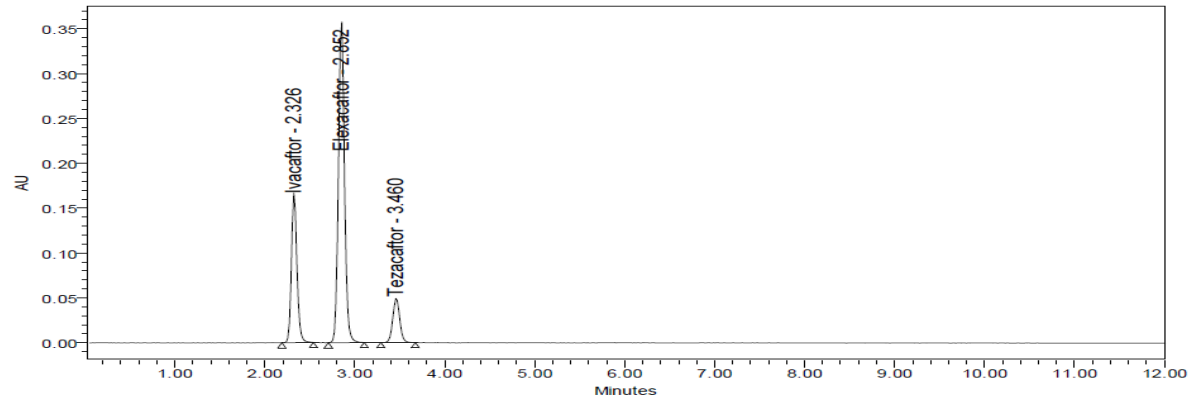

Fig 32: Thermal chromatogram

Photo Stability Studies: The photochemical stability of the drug was also studied by exposing the 250µg/ml, 375µg/ml, 500µg/ml solution to UV light by keeping the beaker in UV chamber for 1day or 200-watt hours/m2 in photo stability chamber. For HPLC study the resultant solution was diluted to obtain 25µg/ml, 37.5µg/ml, 50µg/ml of all the components and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

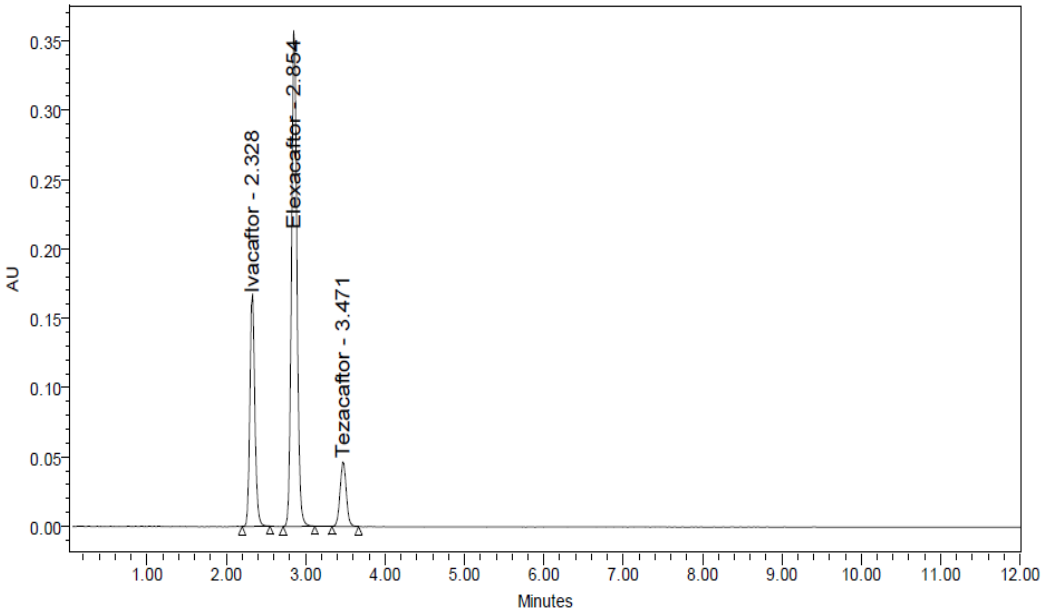

Fig 33: UV chromatogram

Neutral Degradation Studies: Stress testing under neutral conditions was studied by refluxing the drug in water for 6 hrs at a temperature of 60ºC. For HPLC study, the resultant solution was diluted to 25µg/ml, 37.5µg/ml, 50µg/ml of all the components and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

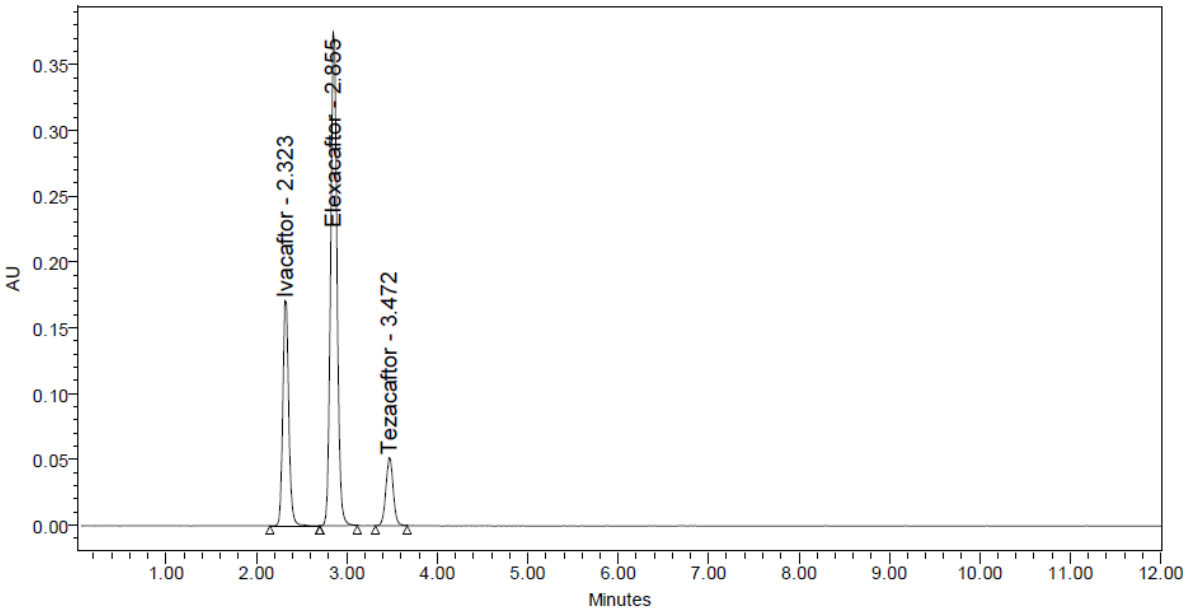

Fig 34: Water chromatogram

Table 9: Degradation Data of Tezacaftor

S.NO	Degradation Condition	Area	% Area Recovery	% Drug Degraded
1	Acid	231994	95.56	4.44
2	Alkali	230518	94.95	5.05
3	Oxidation	231055	95.17	4.83
4	Thermal	234276	96.50	3.50
5	UV	238075	98.06	1.94
6	Water	241204	99.35	0.65

Table 10: Degradation Data of Ivacaftor

S.NO	Degradation Condition	Area	% Area Recovery	% Drug Degraded
1	Acid	679644	97.95	2.05
2	Alkali	675568	97.36	2.64
3	Oxidation	667770	96.24	3.76
4	Thermal	662999	95.55	4.45
5	UV	677528	97.65	2.35
6	Water	688208	99.19	0.81

Table 11: Degradation Data of Elexacaftor

S.NO	Degradation Condition	Area	% Area Recovery	% Drug Degraded
1	Acid	1641326	97.69	2.31
2	Alkali	1563353	93.05	6.95
3	Oxidation	1650964	98.26	1.74
4	Thermal	1660847	98.85	1.15
5	UV	1661622	98.90	1.10
6	Water	1676538	99.79	0.21

Assay: The label claim Tezacaftor 75mg, Ivacaftor 50mg and Elexacaftor 100mg per unit formulation. Assay was performed with the above formulation. Average % assay for Tezacaftor, Ivacaftor and Elexacaftor obtained were 99.44%, 100.03% and 100.15% respectively.

Table 12: Assay Data of Tezacaftor

S.No.	Standard Area	Sample area	% Assay
1	241552	243899	100.40
2	238037	240258	98.90
3	243849	239092	98.42
4	241828	239856	98.74
5	243505	245050	100.87
6	244992	241265	99.32
Avg	242442	241570	99.44
Std Dev	2451.7	2384.4	0.982
%RSD	1.0	1.0	1.0

Table 13: Assay Data of Ivacaftor

S.No.	Standard Area	Sample area	% Assay
1	695730	694009	100.14
2	693416	692897	99.98
3	685418	689369	99.47
4	694133	691419	99.76
5	689646	696462	100.49
6	691669	695636	100.37
Avg	4146.8	693299	100.03
Std Dev	0.6	2649.9	0.38
%RSD	695730	0.4	0.4

Table 14: Assay Data of Elexacaftor

S.No.	Standard Area	Sample area	% Assay
1	1649897	1710706	101.82
2	1662332	1666432	99.19
3	1666767	1655676	98.54
4	1716345	1695007	100.89
5	1687564	1684358	100.25
6	1687753	1684057	100.23
Avg	1678443	1682706	100.15
Std Dev	23736.9	19674.0	1.171
%RSD	1.4	1.2	1.2

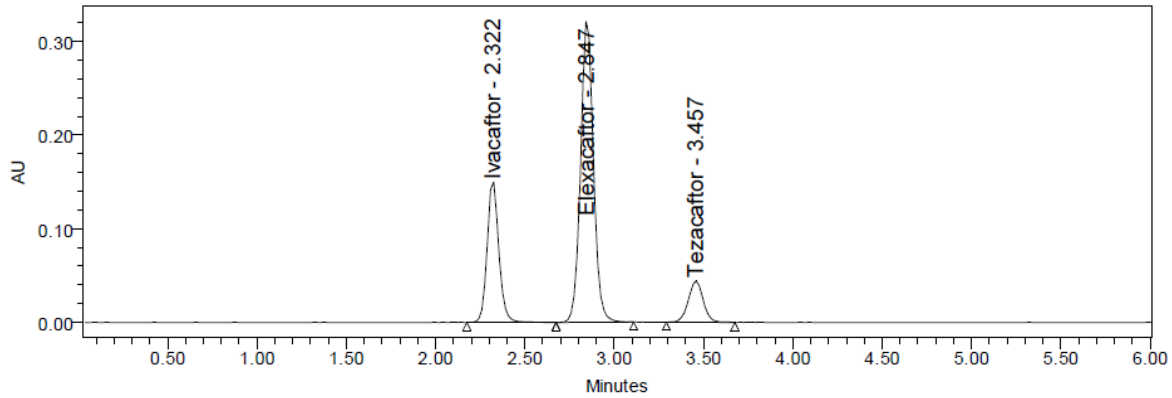

Fig 35: Chromatogram of working standard solution

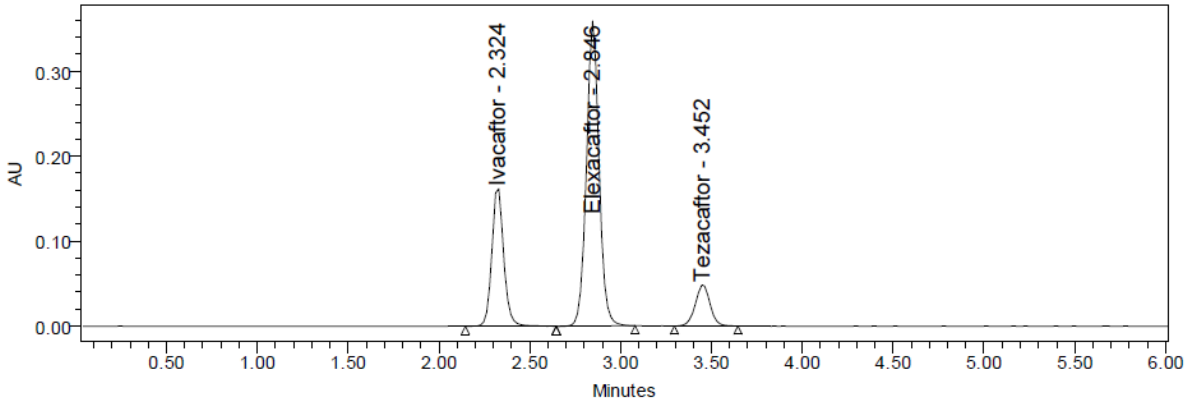

Fig 36: Chromatogram of Working sample solution

CONCLUSION

A simple, accurate, precise method was developed for the simultaneous estimation of Tezacaftor, Ivacaftor and Elexacaftor in tablet dosage form. Retention time of Tezacaftor, Ivacaftor and Elexacaftor was found to be 3.468 min, 2.322 min and 2,855 min, % RSD of system precision for Tezacaftor, Ivacaftor and Elexacaftor was found to be 1.0,0.6 and 1.4 respectively. % RSD of method precision for Tezacaftor, Ivacaftor and Elexacaftor 1.0, 0.4 and 1.2 respectively. % Recovery was obtained as 99.44%, 100.03% and 100.15% for Tezacaftor, Ivacaftor and Elexacaftor respectively. LOD, LOQ values are obtained from regression equations of Tezacaftor, Ivacaftor and Elexacaftor was 0.07 ppm and 0.21 ppm, 0.29 ppm and 0.88 ppm ,0.40 ppm and 1.20 ppm respectively. Regression equation of Tezacaftor was y=10491x + 863.8, Ivacaftor was y= 18876x + 7683 and Elexacaftor was y= 33896x + 19526. Retention times are decreased, so; the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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