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Analytical Method Development and Validation for The Quantitative Estimation of Elexacaftor, Ivacaftor and Tezacaftor in Bulk and Tablet Formulation by RP – HPLC and Its Application in Dissolution Studies

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ABSTRACT

This study displayed method development and its validation by RP-HPLC in combined assessment process to assay ivacaftor (IVR), elexacaftor (EXR) & tezacaftor (TZR) contents in Trikafta pills using 150 mm C18 Inertsil column with 60:40 volume/volume proportionality, KH_2PO_4 (0.1M) & methanol blend mobile phase. IVR, EXR & TZR were spotted and accurately assessed at a wavenumber of 262 nm. The R_T minutes are 2.055 min (TZR), 2.640 (IVR) & 3.272 min (EXR). With concentrations tend to range from 37.5 – 112.50µg/ml (IVR), 50-150µg/ml (EXR) and 25 – 75µg/ml (TZR), linear responses are received. LODs for IVR, EXR & TZR are 0.259µg/ml, 0.241µg/ml & 0.167µg/ml, respectively. LOQs for IVR, EXR & TZR are 0.863µg/ml, 0.804µg/ml & 0.556µg/ml, respectively. Precision for IVR, EXR & TZR are 0.278%RSD, 0.214%RSD & 0.625%RSD, respectively. Accuracy for IVR, EXR & TZR is 98.82%recovery, 99.29%recovery & 98.08%recovery. The RP-HPLC combined assessment process is also proficiently implemented for dissolution study of IVR, EXR, & TZR.

Keywords: RP-HPLC, Dissolution Study, Ivacaftor, Tezacaftor, Elexacaftor

CYSTIC FIBROSIS

Cystic fibrosis (CYFI) is a malady that is passed down through the generations. It mostly attacks the lungs as well as digestive system; however, it can also evolve to deadly complications such as diabetes & liver problems [1, 2]. The gene that induces CYFI generates stiffer, stickier mucus unlike usual, which is difficult to evacuate from the lungs via cough, resulting in breathing difficulties and serious lung infections.

The pancreas functioning is also disrupted by the mucus, which prevents the enzymes from disintegrating the meal, creating digestive problems and malnutrition. In men, mucus hardening causes infertility because the vas deferens, or channel delivering sperms out from testes towards the urethra, becomes obstructed [3].

CYFI is a severe infection with a significant risk of life-threatening outcomes. Respiratory failure is the most prevalent cause of death in patients with CYFI.

CAUSE OF CYSTIC FIBROSIS:

CYFI is an illness that is passed down through both parents. The faulty gene produces a protein that inhibits salt and water passage outside of organs such as the lungs and pancreas [7-9].

In sufferers with CYFI, the salt balance is disrupted, resulting in very little salt content plus water from outside cells, resulting in thicker mucus than in healthy people.

Carriers are individuals who have only single copy of a faulty gene but do not show any symptoms. Only if both parents are carriers does the sickness impact the child.

If two carriers produce a kid, the disease's chances increase:

- Either 25 /100, or 1 / 4, to have CYFI
- Either 50 /100, or 1 / 2, when child acts as a carrier but not possess CYFI
- Either 25 /100, or 1 / 4, child will act as neither a carrier nor have CYFI

MEDICINES:

The medications are [13 -18]

- Antibiotics for medicating & dodge lung infections
- Anti-inflammatory for medicating that diminishes bulge in airways to lungs
- Drugs that induce mucus-thinning to cough out phlegm increasing competence of lung functioning
- Medicines like inhalers named bronchodilators favours to open airways by comforting bronchial tubes muscles.
- Oral pancreatic enzymes supporting the digestive tract in absorption of nutrients.

Ivacaftor [19], a relatively new drug, is indicated for CYFI patients with certain gene mutations to help improve lung function & weight while lowering salt levels in sweat. The dose is determined by the patient's age and weight.

Liver function tests & eye screenings are conducted before to the medication of ivacaftor to screen for adverse effects such as abnormal liver function & cataracts.

Another medicine with even a mixture of ivacaftor and tezacaftor [20] is recommended for persons aged 12 and older who have a particular gene mutation. This combination might help to optimize lung function, lowering the likelihood of exacerbations. However, some people may experience adverse effects such as chest tightness, shortness of breath, and high blood pressure when taking the prescription.

RP-HPLC

In conventional chromatography, the stationary phase is really a polar phase, although the mobile phase may be a non-polar organic solvent. Nonpolar materials are eluted just with solvent, whereas polar additives ingested in the mobile phase are attracted to polar stationary phase. Delineation is centered on this basis.

This kind of chromatography is known as standard phase chromatography [23, 24]. A variety of biological attributes were employed to isolate ions, spanning from ion transport to biological sensitivity.

EXPERIMENTATION

DRUG PROFILE

A 3 drug combination of ivacaftor, tezacaftor and elexacaftor was chosen under the brand name of Trikafta.

IVACAFTOR

IVR acts as enhancer of CyFiTCR protein. This CyFiTCR protein forms the ion canals through which Cl- & Na+ ions are transmitted by lungs, pancreas, as well as further organs cell system membranes [28-30]. In protein development with changed processing, misfolding & work, CyFiTCR gene alteration impacts.

TEZACAFTOR

TZR operate as corrector for CyFiTCR protein. This CyFiTCR protein forms the ion canals through whom Cl- & Na+ ions are transmitted by lungs, pancreas, as well as further organs cell system membranes. In protein development with changed processing, misfolding & work, CyFiTCR gene alteration impacts.

ELEXACAFTOR

Elexacaftor is a CyFiTCR corrector, modulating CyFiTCR proteins enabling easier cell surface trafficking to be integrated through into cell membrane [38-40]. The ultimate consequence is a boost in the numbers and improvement of ion transportation and CYFI symptomatology in matured CyFiTCR proteins on cell system surface.

TRIKAFTA TABLET:

Ivacaftor (IVR), Elexacaftor (EXR), and tezacaftor are the three medications that make up Trikafta (TZR). Trikafta has been authorized by the FDA as the first triple combination treatment for individuals with the most frequent CYFI mutation [21].
Trikafta is authorized for cystic fibrosis patients aged 12 and above who have at minimum single F508del mutation in the “cystic fibrosis transmembrane conductance regulator” gene, which accounts for about 90% of the CYFI population.

MATERIALS AND METHODS

Chemicals and reagents: HPLC grade water, methanol, potassium dihydrogen phosphate, sodium dihydrogen phosphate, phosphoric acid, sodium hydroxide, hydrochloric acid were used during the study. Drug samples were obtained from rainbow pharma training lab.

INSTRUMENTS

Waters HPLC 2695 series with empower software and quaternary pumps, Photo Diode array detector, inertsil C18 column and ultrasonicator.

Preparation of stock and working solutions

I. Mobile phase

600 ml of KH_2PO_4 buffer and 400 ml of Methanol were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration. Adjusted the pH to 3.6

II. Stock IVR, EXR & TZR Solution

75 mg IVR, 100 mg EXR, and 50 mg TZR were balanced accurately in a flask (100 ml), subsequently diluted fittingly in diluting fluid (with a 60:40 volume/volume proportionality, pH 3.6 KH_2PO_4 (0.1M) & methanol are combined) to prepare stock IVR, EXR, and TZR solution. Concentration: 750 µg/ml - IVR, 1000 µg/ml - EXR & 500 µg/ml - TZR.

III. Working IVR, EXR & TZR Solutions

Working IVR, EXR & TZR Solutions were made from stock IVR, EXR, & TZR solution (750 µg/ml IVR, 1000 µg/ml EXR & 500 µg/ml TZR) with precise dilution in diluting fluid (with a 60:40 volume/volume proportionality, pH 3.6 KH_2PO_4 (0.1M) & methanol are combined). Concentration: 75 µg/ml - IVR, 100 µg/ml - EXR & 50 µg/ml - TZR.

IV. Tablet IVR, EXR & TZR Solution

Ten Trikafta pills were weighted and shredded. The aggregate weight was estimated and then leveraged to generate the tablet stock IVR, EXR, and TZR solutions. The 225 mg Trikafta pill powder equal to 75 mg IVR, 100 mg EXR, and 50 mg TZR was were balanced accurately in a flask (100 ml), subsequently diluted fittingly in diluting fluid (with a 60:40 volume/volume proportionality, pH 3.6 KH2PO4 (0.1M) & methanol are combined) to prepare Trikafta stock IVR, EXR, and TZR solution. Concentration: 750 µg/ml - IVR, 1000 µg/ml - EXR & 500 µg/ml - TZR.

Trikafta working IVR, EXR & TZR Solutions were made from 2.5 ml Trikafta stock IVR, EXR, & TZR solution (750 µg/ml IVR, 1000 µg/ml EXR & 500 µg/ml TZR) with precise dilution in 222.5 ml diluting fluid (with a 60:40 volume/volume proportionality, pH 3.6 KH2PO4 (0.1M) & methanol are combined). Concentration theoretically: 75 µg/ml - IVR, 100µg/ml - EXR & 50 µg/ml - TZR.

OPTIMIZED CONDITIONS

Mobile phase: KH_2PO_4 : Methanol, 60 : 40 v/v

Column: Inertsil, C18 150x4.6mm, 5µm

Flow rate: 1.0ml/min

Temperature: $25^{O}c$

Injection volume: 10µl

Runtime: 6 minutes

Wavelength: 262

pH: 3.6

Observation: Tezacaftar, Ivacaftar and Elexacaftar were eluted at 2.055 min, 2.640min and 3.272min respectively with good resolution. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated.

Method validation

Method validation was carried out according to the ICH guidelines. The validation parameters include linearity, precision, accuracy, recovery, selectivity, assay, robustness, LOD and LOQ.

RESULTS AND DISCUSSIONS

SYSTEM SUITABILITY

The system suitability parameters were determined by preparing standard solutions of Tezacaftar, Ivacaftar and Elexacaftar and the solutions were injected five times and the parameters like peak tailing, resolution and USP plate count were determined. According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits. The results were show in table

LINEARITY

Analysed five differing concentrations of IVR, EXR & TZR, stretching from 37.5 – 112.50 µg/ml (IVR), 50-150 µg/ml (EXR) and 25 – 75 µg/ml (TZR) by applying chromatographic configurations as per at section: "CONDITIONS: RP-HPLC COMBINED ASSESSMENT PROCESS FOR IVR, EXR & TZR". The IVR, EXR, & TZR peak response were weighed up. The IVR, EXR, & TZR peak response set against the typical IVR, EXR, & TZR concentrations was established through exploitation of least square process.

PRECISION

The precision was examined by applying chromatographic configurations as per at section: "CONDITIONS: RP-HPLC COMBINED ASSESSMENT PROCESS FOR IVR, EXR & TZR" via peak response measurements (n=6) of working IVR, EXR, & TZR solution (75 g/ml IVR, 100 g/ml EXR, and 50 g/ml TZR) in one day. Mean IVR, EXR, & TZR peak response, %RSD and SD of IVR, EXR, & TZR peak response were weighed up.

ACCURACY

The accuracy was examined by applying chromatographic configurations as per at section: "CONDITIONS: RP-HPLC COMBINED ASSESSMENT PROCESS FOR IVR, EXR & TZR" via peak response measurements (n=6) of working IVR, EXR, & TZR solution (75 µg/ml IVR, 100 µg/ml EXR, and 50 µg/ml TZR) in one day. Mean assay of IVR, EXR, & TZR content were weighed up.

RECOVERY

The accuracy further was experimented on recoveries, appraised following spiking Trikafta working IVR, EXR, & TZR solution (75 µg/ml IVR, 100µg/ml EXR, and 50 µg/ml TZR) with IVR, EXR, & TZR standards at contrasting dissimilar levels:

- IVR: 37.125 µg/ml, EXR: 49.5 µg/ml and TZR: 24.5 µg/ml - 50% recovery level.
- IVR: 74.25 µg/ml, EXR: 99.0 µg/ml and TZR: 49.0 µg/ml - 100% recovery level.

- IVR: 111.375 µg/ml, EXR: 148.5 µg/ml and TZR: 73.5 µg/ml - 150 % recovery level.

Recovery percent's of IVR, EXR, & TZR were weighed up in spiked Trikafta working IVR, EXR, & TZR solution applying chromatographic configurations as per at section: "CONDITIONS: RP-HPLC COMBINED ASSESSMENT PROCESS FOR IVR, EXR & TZR".

SELECTIVITY

Analysed blank (with a 60:40 volume/volume proportionality, pH 3.6 KH2PO4 (0.1M) & methanol are combined), working IVR, EXR, & TZR solution (75 g/ml IVR, 100 g/ml EXR, and 50 g/ml TZR) and Trikafta working IVR, EXR, & TZR solution (75 g/ml IVR, 100 g/ml EXR, and 50 g/ml TZR) by applying chromatographic configurations as per at section: "CONDITIONS: RP-HPLC COMBINED ASSESSMENT PROCESS FOR IVR, EXR & TZR". The IVR, EXR, & TZR peak response and IVR, EXR, & TZR RTs were weighed up and compared

In blank (with a 60:40 volume/volume proportionality, pH 3.6 KH_2PO_4 (0.1M) & methanol are combined), the IVR, EXR, and TZR signals were not identifiable.

ROBUSTNESS:

The robustness of the procedure model was examined by looking at the effects of small variations in the analytical circumstances. The major modification made includes variation in methanol ratio, flow rate, temperature, wavelength and pH

LOD

The LOD of IVR, EXR, & TZR is the quantity of IVR, EXR, & TZR bestowing a signal-to-noise relationship of near here 3:1.

IVR's LOD – 0.259µg/ml; IVR's signal-to-noise relationship – 3.6

EXR's LOD – 0.241µg/ml; EXR's signal-to-noise relationship – 3.8

TZR's LOD – 0.167µg/ml; TZR's signal-to-noise relationship – 3.0

LOQ

The LOQ of IVR, EXR, & TZR is the quantity of IVR, EXR, & TZR bestowing a signal-to-noise relationship of near here 10:1.

IVR's LOQ – 0.863µg/ml; IVR's signal-to-noise relationship – 10.2

EXR's LOQ – 0.804µg/ml; EXR's signal-to-noise relationship – 10.5

TZR's LOQ – 0.556µg/ml; TZR's signal-to-noise relationship – 10.2

ASSAY

A process of analyzing the amount of drug present in the formulation by using the formula given below:

Assay in % =

$$\frac{\text{Area of Sample}}{\text{Area of Standard}} \text{ x } \frac{\text{Weight of Standard}}{\text{Dilution of Standard}} \text{ x } \frac{\text{Dilution of Sample}}{\text{Weight of Sample}} \text{ x } \frac{\text{Average Weight}}{\text{Label Claim}} \text{ x Purity of Standard}$$

DISSOLUTION STUDY

V. Dissolution Investigation on IVR, EXR, & TZR

MEDIA: 0.9gm NaOH plus 13.609gm KH_2SO_4 were perfectly weighed in a 1000 ml container and dissolved precisely with 1000 ml dilution fluid (water). Fix pH using HCl if needed. To expel air bubbles, degas liquid media over 41°c for ten min. After that, pour the degassed liquid media into the 6 distinct bowls, choose the relevant program for the Trikafta IVR, EXR, & TZR tablets, and waited again until bowl gets 37° C. Clamp the device and instantly put the Trikafta IVR, EXR, and TZR tablets and start the tester when all bowls have reached the necessary temperature.

VI. Dissolution Test Conditions

Dissolution Media: 6.5 pH Phosphate buffer

Dissolution Medium Quantity: 900ml

Dissolution Bath Temperature: 38° c

Dissolution Bowl Temperature: 37° c

Dissolution Apparatus: USP type 2 (paddle apparatus)

RPM: 50

Sample Collection Time Interval: 30min

Sample Collection Volume: 10ml

Rinse Volume: 3ml

Replenish: No

Trikafta dissolution IVR, EXR, & TZR solution prepared as per at section "DISSOLUTION TEST CONDITIONS" was evaluated by applying chromatographic configurations as per at section: "CONDITIONS: RP-HPLC COMBINED ASSESSMENT PROCESS FOR IVR, EXR & TZR". Recovery percent's of IVR, EXR, & TZR were weighed up in Trikafta dissolution IVR, EXR, & TZR solution.

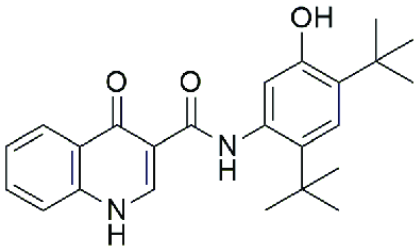

Fig No. 1: Chemical Structure of Ivacaftor

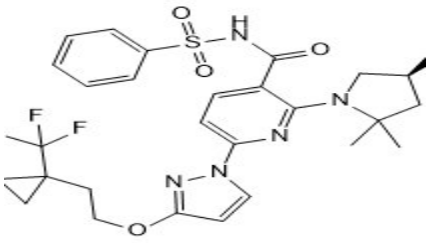

Fig No. 2: Chemical Structure of Elexacaftor

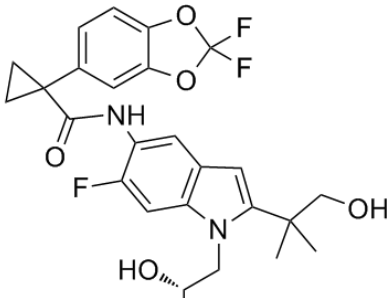

Fig No. 3: Chemical Structure of Tezacaftor

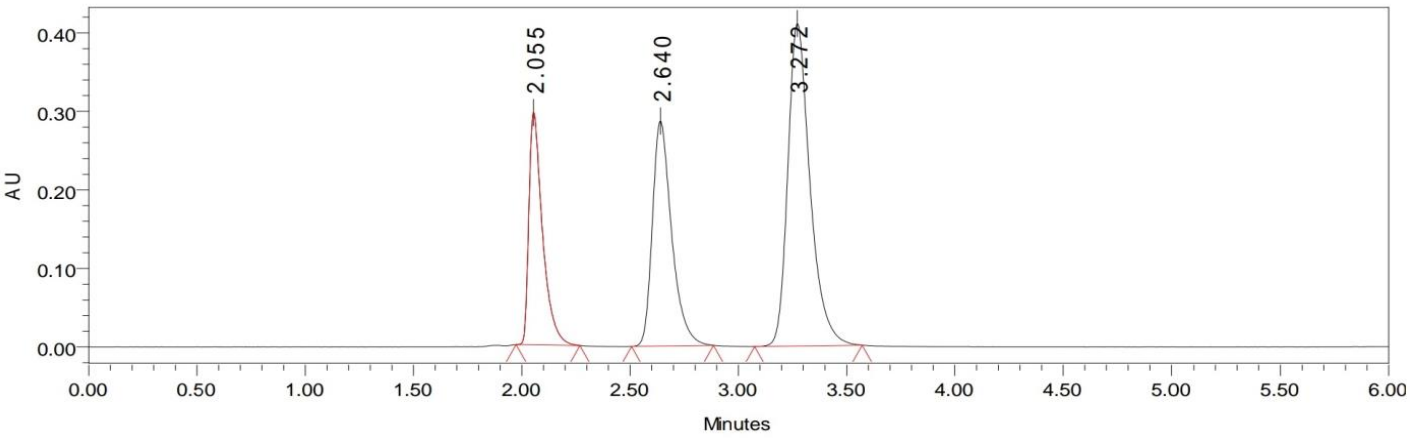

Fig No. 4: Optimized Conditions Chromatogram of Ivacaftor, Elexacaftor And Tezacaftor

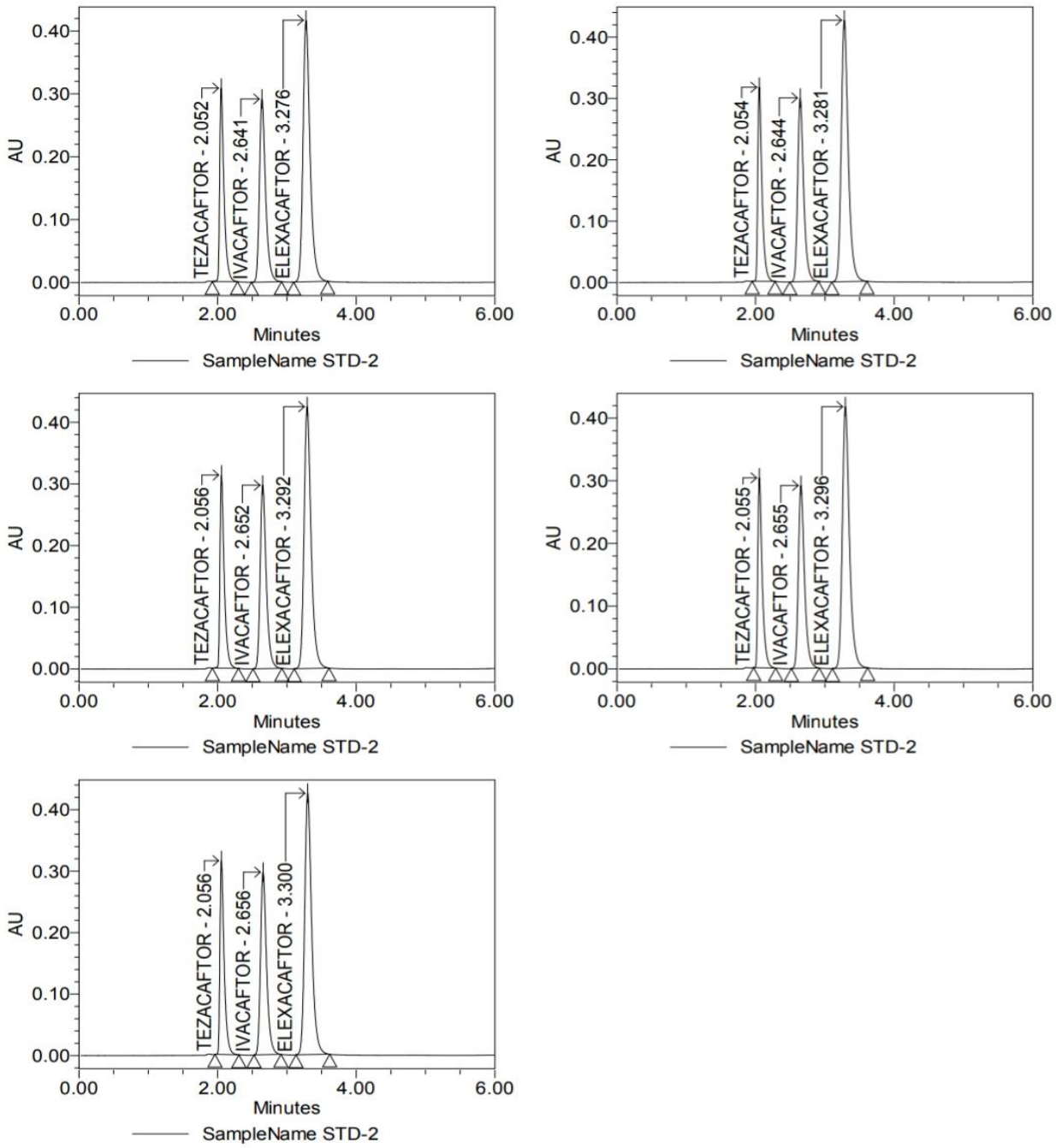

Fig No. 5: System Suitability Chromatogram of Ivacaftor, Elexacaftor and Tezacaftor

Table No. 1: System Suitability Parameters of Tezacaftor

S.No	Sample Name	Peak Name	R_T	Area	USP Plate Count	USP Tailing
1	STD 2	Tezacaftor	2.052	1315149	5728	1.85
2	STD 2	Tezacaftor	2.054	1327664	5854	1.83
3	STD 2	Tezacaftor	2.056	1332643	5751	1.82
4	STD 2	Tezacaftor	2.055	1321309	5439	1.84
5	STD 2	Tezacaftor	2.056	1329410	5845	1.81
Mean				1325235.1		
% RSD				0.5		

Table No. 2: System Suitability Parameters of Ivacaftor

S.No	Sample Name	Peak Name	R_T	Area	USP Resolution	USP Plate Count	USP Tailing
1	STD 2	Ivacaftor	2.641	179859	4.26	4474	1.38
2	STD 2	Ivacaftor	2.644	1799929	4.35	4683	1.36
3	STD 2	Ivacaftor	2.652	1803870	4.35	4612	1.36
4	STD 2	Ivacaftor	2.655	1790868	4.29	4527	1.38
5	STD 2	Ivacaftor	2.656	1790527	4.41	4704	1.35
Mean				1795810.5			
% RSD				0.3			

Table No. 3: System Suitability Parameters of Elexacaftor

S.No	Sample Name	Peak Name	R_T	Area	USP Resolution	USP Plate Count	USP Tailing
1	STD 2	Elexacaftor	3.276	29332230	3.64	5276	1.33
2	STD 2	Elexacaftor	3.281	2981262	3.70	5357	1.31
3	STD 2	Elexacaftor	3.292	2983106	3.69	5343	1.31
4	STD 2	Elexacaftor	3.296	2958761	3.67	5290	1.33
5	STD 2	Elexacaftor	3.300	2964467	3.744	5450	1.30
Mean				2964160.0			
% RSD				0.7			

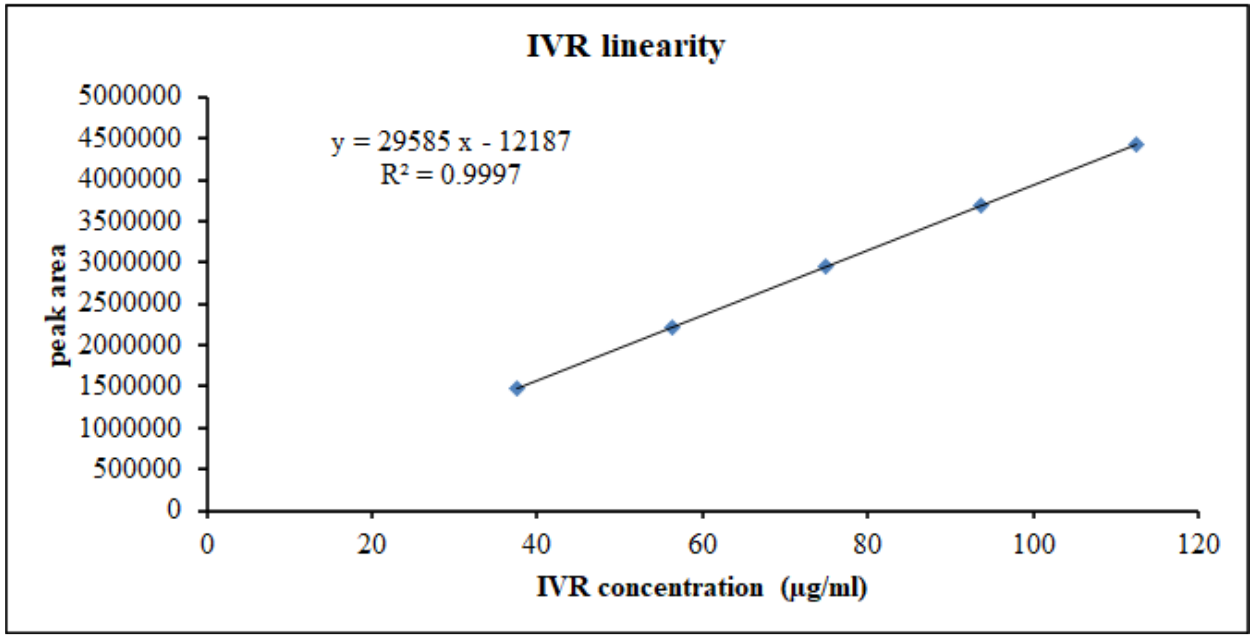

Fig No. 6: Linearity Chromatogram of Ivacaftor

Table No. 4: Linearity Table of Ivacaftor

µg/ml Quantity IVR	IVR Peak Response
37.5	1466006
56.25	2209721
75	2945996
93.75	3681525
112.50	4428199

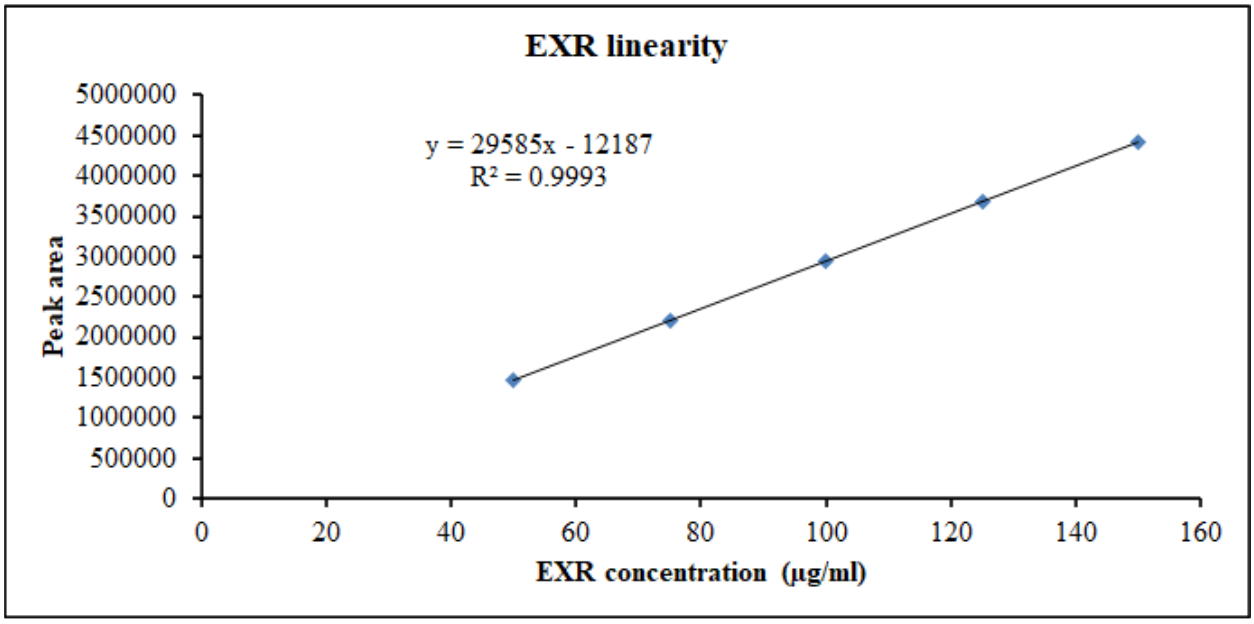

Fig No. 7: Linearity Chromatogram of Elexacaftor

Table No. 5: Linearity Table of Elexacaftor

µg/ml Quantity EXR	EXR Peak Response
50	1466006
75.00	2209721
100.00	2945996
125	3681525
150	4428199

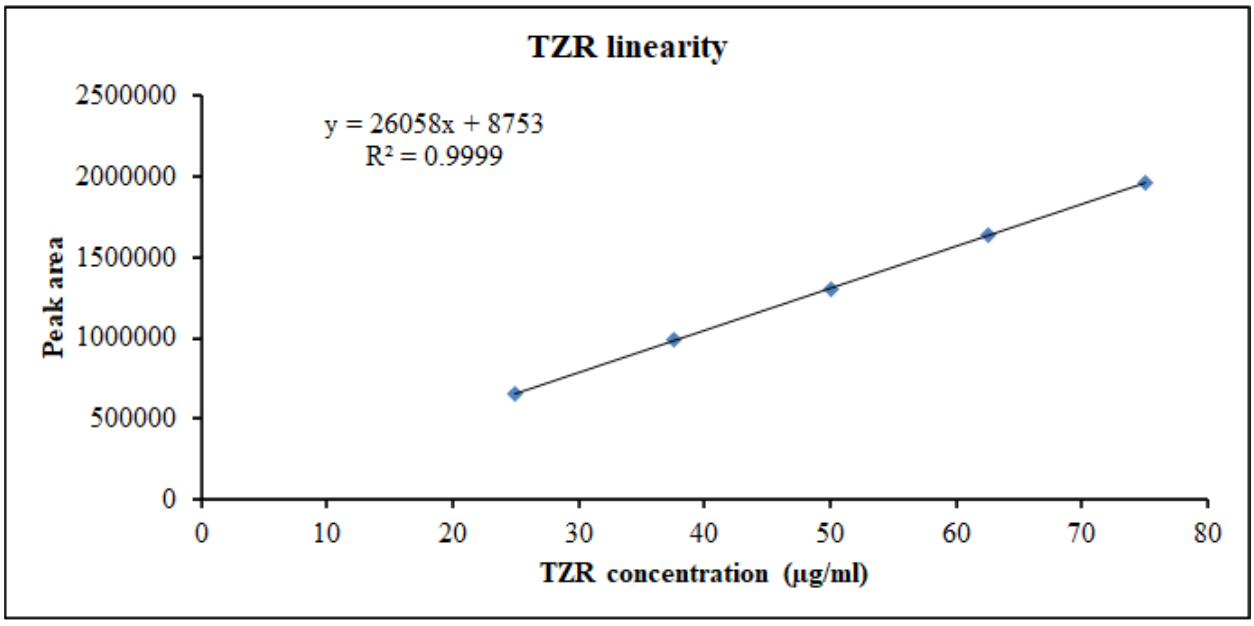

Fig No. 8: Linearity Chromatogram of Tezacaftor

Table No. 6: Linearity Table of Tezacaftor

µg/ml Quantity TZR	TZR Peak Response
25	660457
37.50	991082
50.00	1305325
62.5	1633622
75	1967823

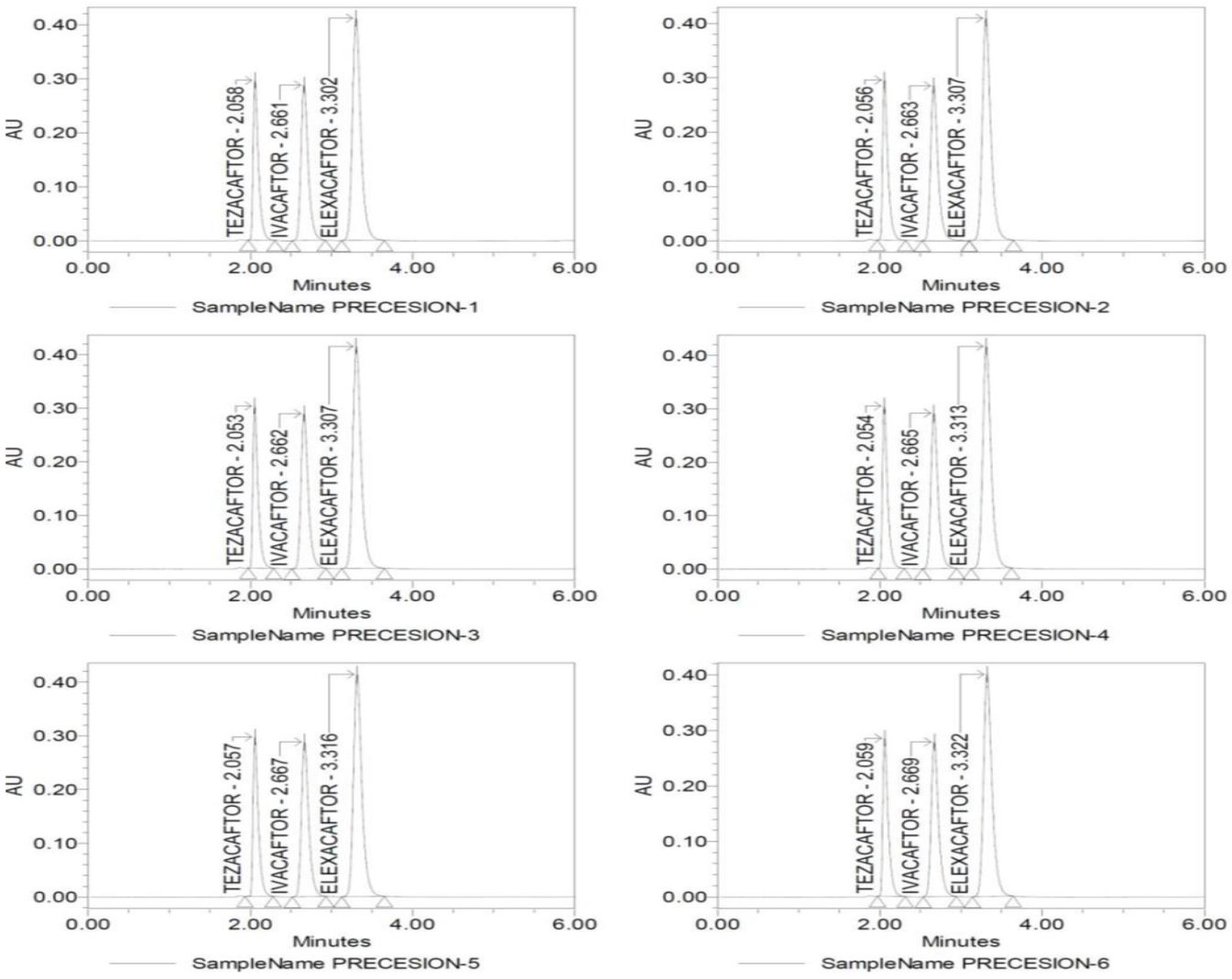

Fig No. 9: Precision Chromatogram of Ivacaftor, Elexacaftor and Tezacaftor

Table No. 7: Precision of IVR

S.no.	IVR Peak Response
1	1775215
2	1779618
3	1782517
4	1778181
5	1788541
6	1775889
Mid peak response	1779994
SD peak response	4950.4535
RSD peak response	**0.278**

Table No. 8: Precision of Elexacaftor

S.no.	EXR Peak Response
1	2956698
2	2946201
3	2956854
4	2942478
5	2947393
6	2943999
Mid peak response	2948937
SD peak response	6306.8878
RSD peak response	**0.214**

Table No. 9: Precision of Tezacaftor

S.no.	TZR Peak Response
1	1301754
2	1319221
3	1314005
4	1300272
5	1301455
6	1301204
Mid peak response	1306319
SD peak response	8517.9820
RSD peak response	**0.625**

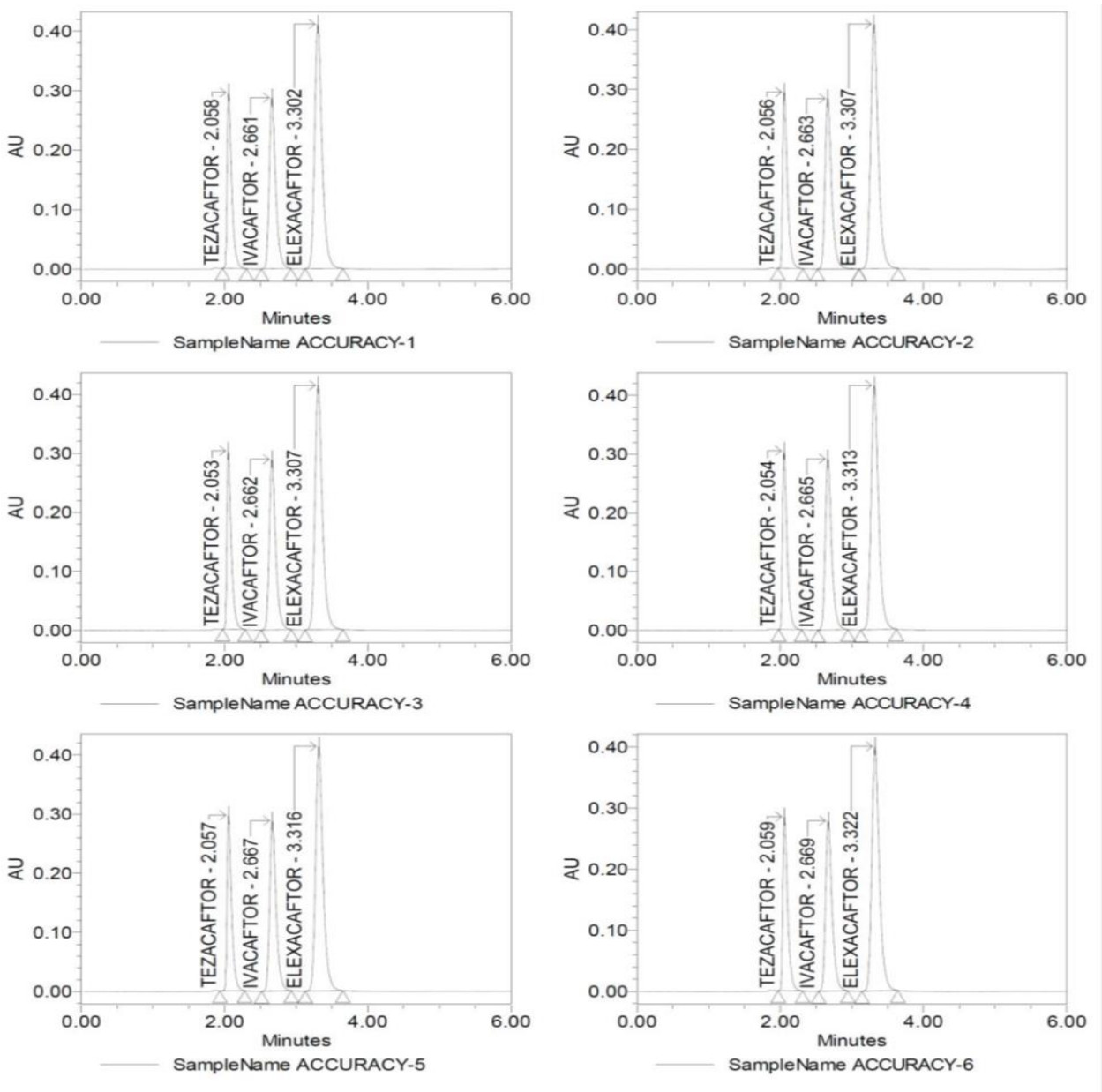

Fig No. 10: Accuracy Chromatogram of Ivacaftor, Elexacaftor and Tezacaftor

Table No. 10: Accuracy of Ivacaftor

Analyzed Value (µg/ml)	Determined Value (µg/ml)	Assaying Value (%)	Mean Value (%)
75	73.92	98.56	98.82%
75	74.10	98.8	
75	74.22	98.96	
75	74.04	98.72	
75	74.48	99.3	
75	73.94	98.59	

Table No. 11: Accuracy of Elexacaftor

Analyzed Value (µg/ml)	Determined Value (µg/ml)	Assaying Value (%)	Mean Value (%)
100	99.55	99.55	99.29%
100	99.20	99.20	
100	99.55	99.55	
100	99.07	99.07	
100	99.24	99.24	
100	99.12	99.12	

Table No. 12: Accuracy of Tezacaftor

Analyzed Value (µg/ml)	Determined Value	Analyzed Value (µg/ml)	Determined Value
50	48.87	97.74	98.08%
50	49.53	99.05	
50	49.33	98.66	
50	48.82	97.63	
50	48.86	97.71	
50	48.85	97.70	

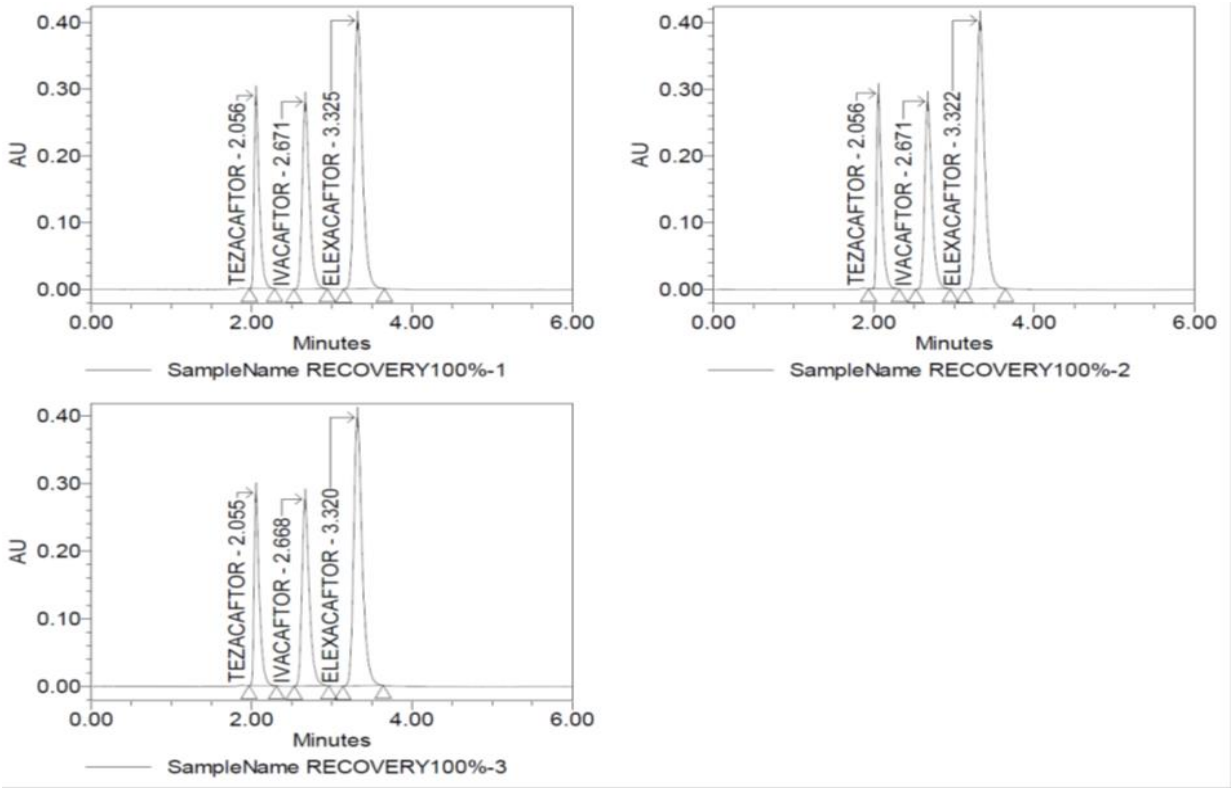

Fig no.13: 150% Recovery Level of TZR, IVR and EXR

Table no.14: IVR Recovery

Value added (µg/ml)	Determined value (µg/ml)	Assaying value (%)	Mean value (%)
50% recovering level			
37.125	37.29	100.45	100.47%
37.125	37.30	100.48	
37.125	37.30	100.48	
100% recovering level			
74.250	73.91	99.54	99.70%
74.250	74.10	99.80	
74.250	74.08	99.77	
150% recovering level			
111.375	111.58	100.18	

111.375	111.54	100.15	100.06%
111.375	111.21	99.85	

Table no.15: EXR Recovery

Value added (µg/ml)	Determined value (µg/ml)	Assaying value (%)	Mean value (%)
50% recovering level			
49.500	49.14	99.27	99.55%
49.500	49.44	99.89	
49.500	49.25	99.50	
100% recovering level			
99.000	99.13	100.13	100.58%
99.000	99.25	100.25	
99.000	99.15	100.16	
150% recovering level			
148.500	149.02	100.35	100.31%
148.500	148.94	100.30	
148.500	148.91	100.28	

Table no.15: TZR Recovery

Value added (µg/ml)	Determined value (µg/ml)	Assaying value (%)	Mean value (%)
50% recovering level			
24.500	24.78	101.16	101.19%
24.500	24.79	101.19	
24.500	24.80	101.23	
100% recovering level			
49.000	49.04	100.09	100.24%
49.000	49.14	100.29	
49.000	49.16	100.32	
150% recovering level			
73.500	73.82	100.44	100.27%
73.500	73.59	100.13	
73.500	73.67	100.24	

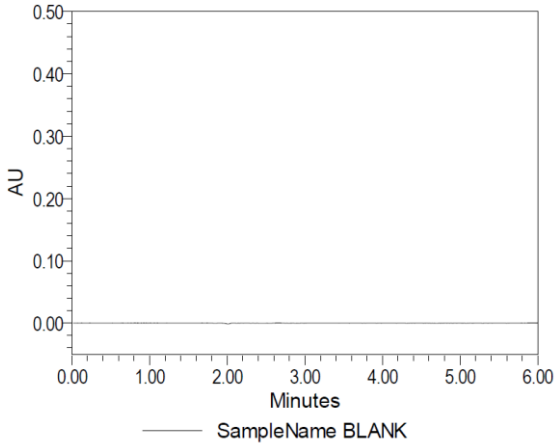

Fig no.14: Blank Solution Selectivity Chromatograph

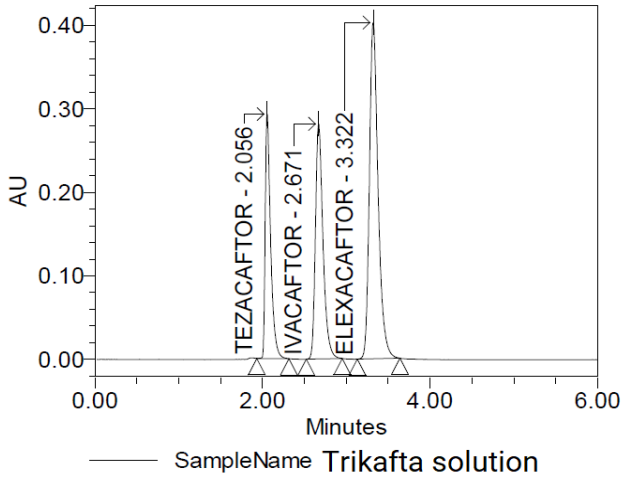

Fig no.15: Trikafta Solution Selectivity Chromatograph

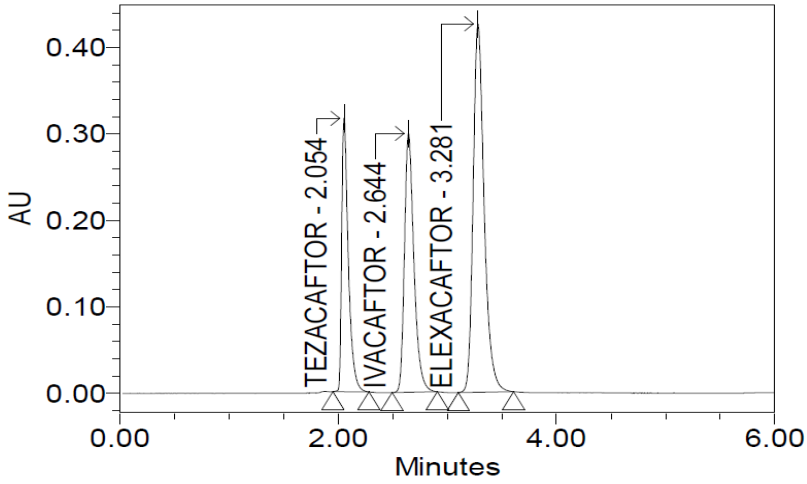

Fig no.16: Working Solution Selectivity

Table no.16: Varied Conditions for Testing Robustness

Conditions	Methanol Ratio (%)	Flow Rate (ml/min)	Temperature (^{O}C)	Wavelength (nm)	pH (units)
1	45	1.1	27	264	3.8
2	35	0.9	23	260	3.4

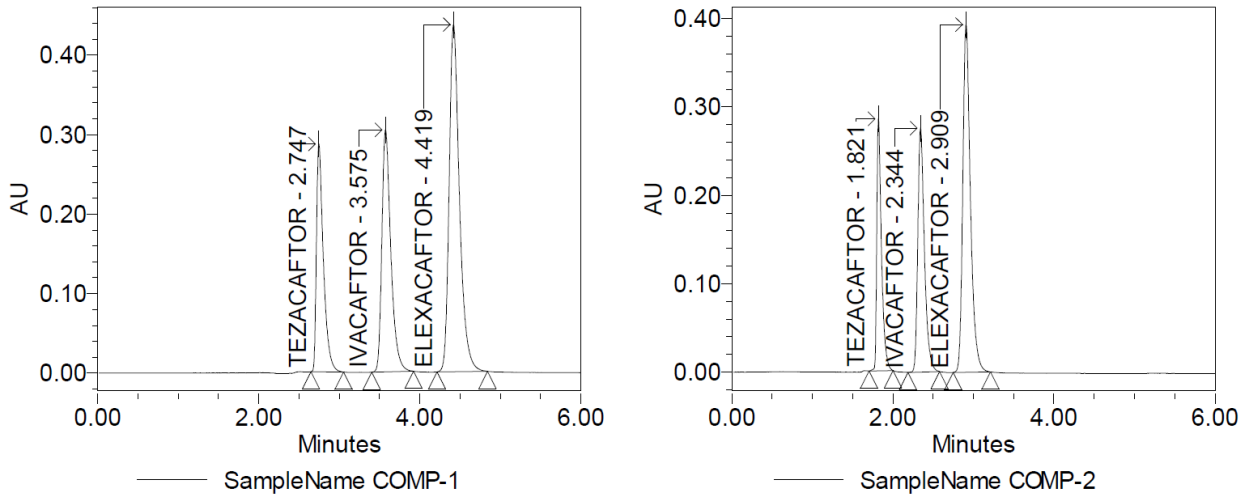

Fig no.17: Robustness Chromatographs of Variation in Methanol Ratio

Table no.17: Robustness - EXR

S.No	Sample Name	Peak Name	R_T	Area (µv*sec)	USP Resolution	USP Plate Count	USP Tailing
1	Comp-1	Elexacaftor	4.419	3878584	3.84	6039	1.38
2	Comp-2	Elexacaftor	2.909	2594333	3.47	4743	1.35

Table no.18: Robustness - IVR

S.No	Sample Name	Peak Name	R_T	Area (µv*sec)	USP Resolution	USP Plate Count	USP Tailing
1	Comp-1	Ivacaftor	3.575	2348847	4.50	5203	1.44
2	Comp-2	Ivacaftor	2.344	1569794	4.04	4112	1.40

Table no.19: Robustness - TZR

S.No	Sample Name	Peak Name	R_T	Area (µv*sec)	USP Plate Count	USP Tailing
1	Comp-1	Tezacaftor	2.747	1720703	4988	1.81
2	Comp-2	tezacaftor	1.821	1147071	5054	1.80

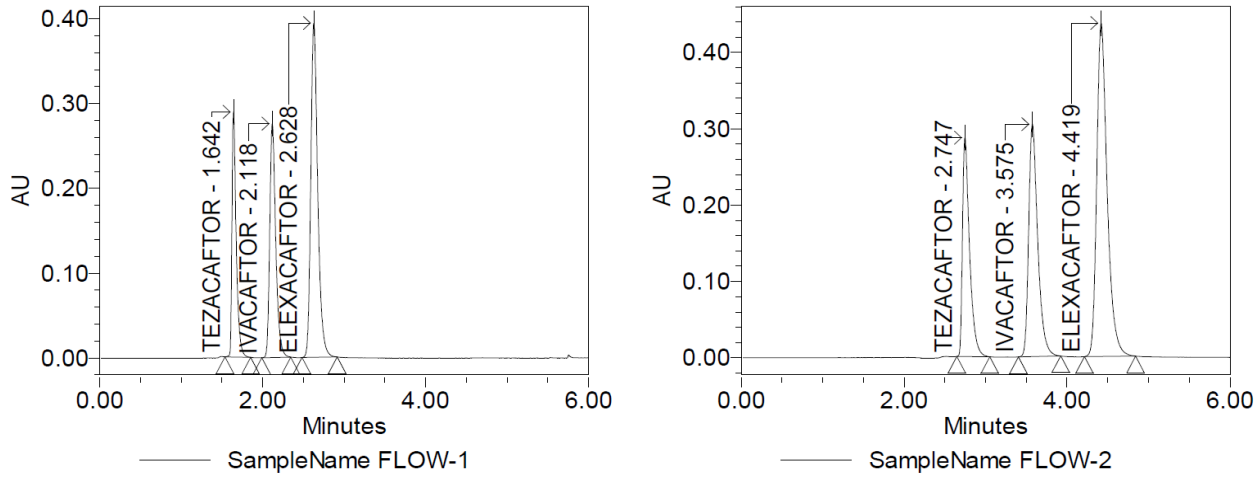

Fig no.18: Robustness Chromatographs for Variations in Flow Rate

Table no.20: Robustness - TZR

S.No	Sample Name	Peak Name	R_T	Area (µv*sec)	USP Plate Count	USP Tailing
1	Flow-1	Tezacaftor	1.642	1039385	5172	1.74
2	Flow-2	Tezacaftor	2.747	1720703	4988	1.81

Table no.21: Robustness - IVR

S.No	Sample Name	Peak Name	R_T	Area (µv*sec)	USP Resolution	USP Plate Count	USP Tailing
1	Flow-1	Ivacaftor	2.118	1406898	4.13	4150	1.35
2	Flow-2	Ivacaftor	3.575	2348847	4.50	5203	1.44

Table no.22: Robustness - EXR

S.No	Sample Name	Peak Name	R_T	Area (µv*sec)	USP Resolution	USP Plate Count	USP Tailing
1	Flow-1	Elexacaftor	2.628	2325083	3.49	4798	1.31
2	Flow-2	elexacaftor	4.419	3878584	3.84	6039	1.38

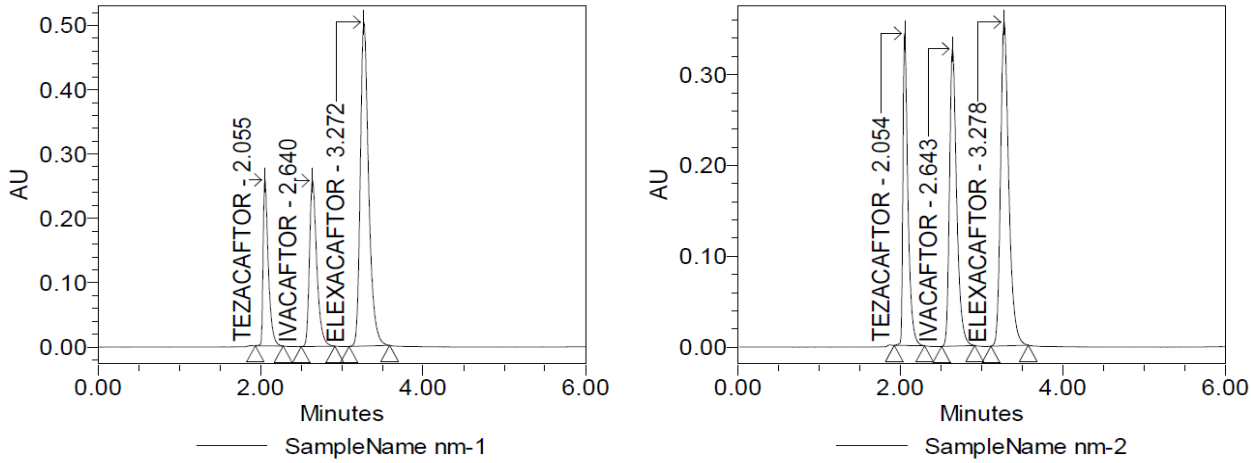

Fig no.19: Robustness Chromatographs for Variation in Wavelength

Table no.23: Robustness - TZR

S.No	Sample Name	Peak Name	R_T	Area (µv*sec)	USP Plate Count	USP Tailing
1	Nm-1	Tezacaftor	2.055	1138210	5326	1.83
2	Nm-2	tezacaftor	2.054	1472678	5732	1.86

Table no.24: Robustness - IVR

S.No	Sample Name	Peak Name	R_T	Area (µv*sec)	USP Resolution	USP Plate Count	USP Tailing
1	Nm-1	ivacaftor	2.640	1605123	4.13	4367	1.40
2	Nm-2	ivacaftor	2.643	2012799	4.26	4508	1.38

Table no.25: Robustness - EXR

S.No	Sample Name	Peak Name	R_T	Area (µv*sec)	USP Resolution	USP Plate Count	USP Tailing
1	Nm-1	Elexacaftor	3.272	3607152	3.56	5078	1.35
2	Nm-1	Elexacaftor	3.278	2503613	3.64	5290	1.34

Table no.26: Robustness - TZR

S.No	Sample Name	Peak Name	R_T	Area (µv*sec)	USP Plate Count	USP Tailing
1	pH-1	Tezacaftor	2.058	1321754	5129	1.83
2	pH-2	tezacaftor	2.056	1319221	5177	1.84

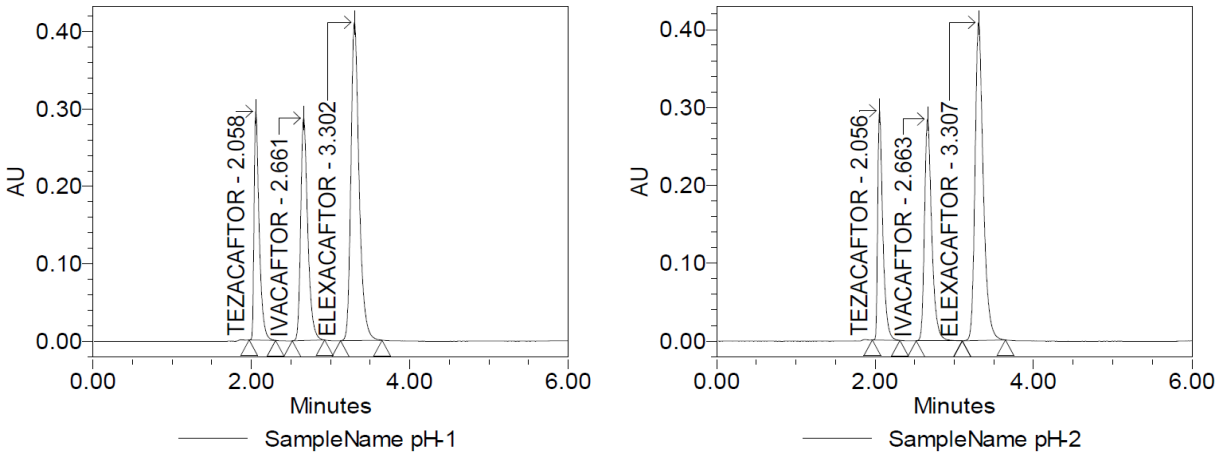

Fig no.20: Robustness Chromatographs for Variation in pH units

Table no.26: Robustness - IVR

S.No	Sample Name	Peak Name	R_T	Area (µv*sec)	USP Resolution	USP Plate Count	USP Tailing
1	pH-1	Ivacaftor	2.661	1775215	4.26	4479	1.37
2	pH-2	Ivacaftor	2.663	1799618	4.24	4346	1.39

Table no.27: Robustness - EXR

S.No	Sample Name	Peak Name	R_T	Area (µv*sec)	USP Resolution	USP Plate Count	USP Tailing
1	pH-1	Elexacaftor	3.302	2956698	3.63	5148	1.33
2	pH-2	elexacaftor	3.307	2956201	3.60	5107	1.33

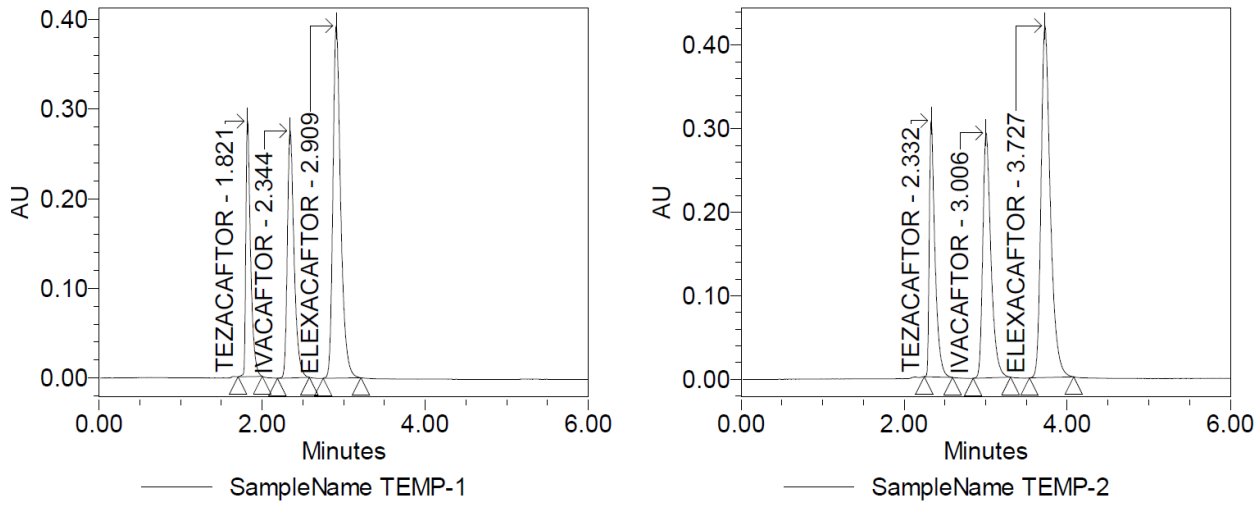

Fig no.21: Robustness Chromatographs for Variation in Temperature

Table no.28: Robustness - TZR

S.No	Sample Name	Peak Name	R_T	Area (µv*sec)	USP Resolution	USP Plate Count	USP Tailing
1	Temp-1	Elexacaftor	2.909	2594333	3.47	4743	1.35
2	Temp-2	elexacaftor	3.727	3343499	3.66	5392	1.38

Table no.29: Robustness - IVR

S.No	Sample Name	Peak Name	R_T	Area (µv*sec)	USP Resolution	USP Plate Count	USP Tailing
1	Temp-1	Ivacaftor	2.344	1569794	4.04	4112	1.40
2	Temp-2	Ivacaftor	3.006	2030577	4.29	4567	1.43

Table no.30: Robustness – EXR

S.No	Sample Name	Peak Name	R_T	Area (µv*sec)	USP Plate Count	USP Tailing
1	Temp-1	Tezacaftor	1.821	1147071	5054	1.80
2	Temp-2	tezacaftor	2.332	1478933	5736	1.95

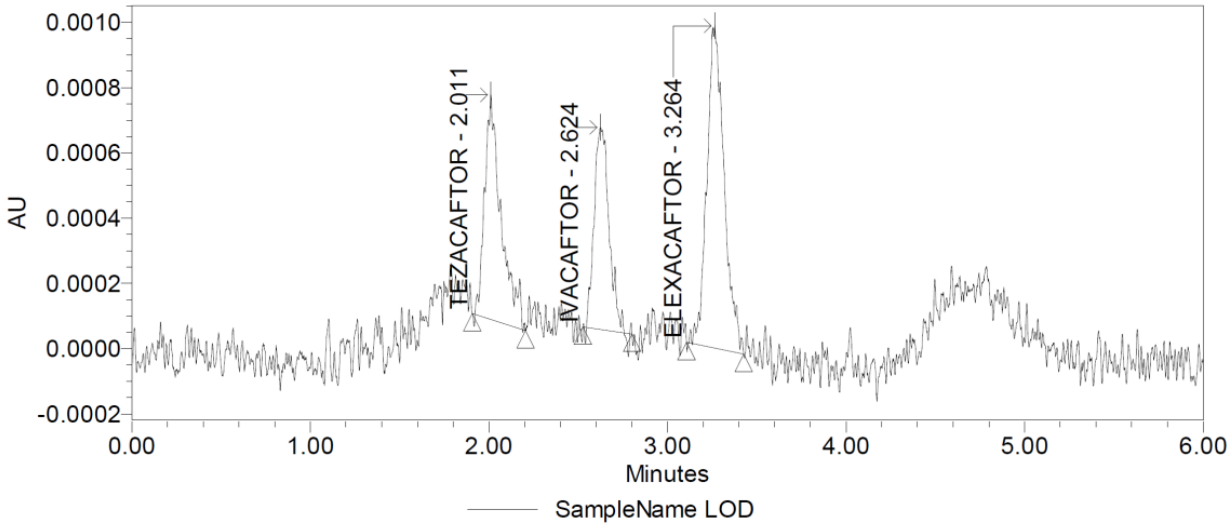

Fig no.22: LOD Chromatograph of TZR, IVR AND EXR

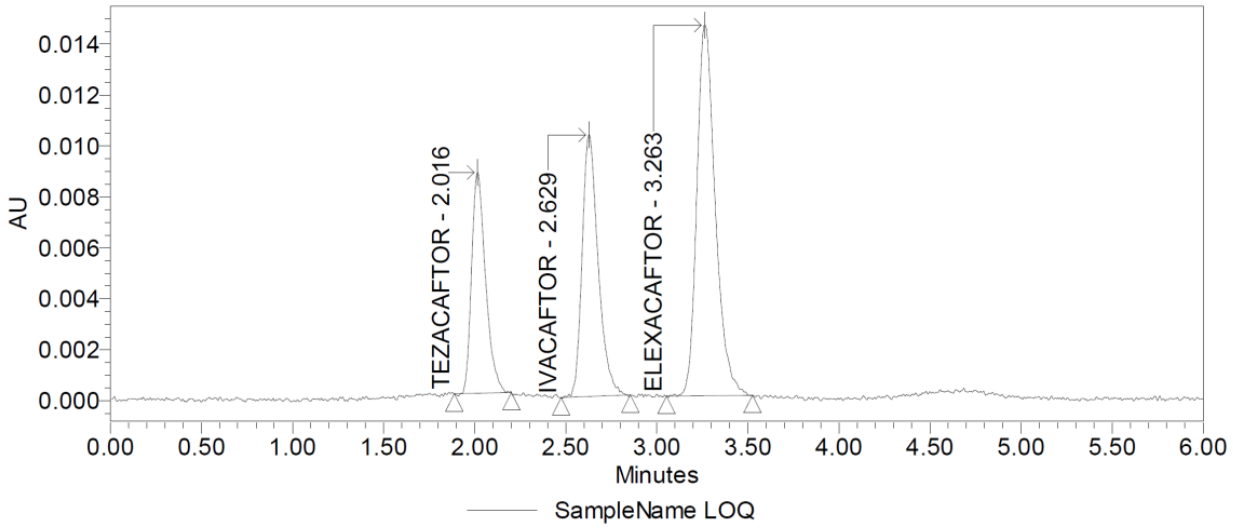

Fig no.23: LOQ chromatograph of TZR, IVR and EXR

Table no.31: % Assay

	Ivacaftor	Elexacaftor	Tezacaftor
Mean Area	1779994	2948937	1306319
Label Claim	75mg	100mg	50mg
Average Weight	225	225	225
Potency of STD	99.5	99.8	99.5
Weight of STD	75	100	50

Table no.32: % Assay of IVR, EXR and TZR

S.No	Sample Weight	IVR Area	EXR Area	TZR Area	IVR %Assay	EXR %Assay	TZR %Assay
1	225	1775215	2956698	1301754	98.56	99.55	97.74
2	225	1779618	2946201	1319221	98.8	99.2	99.05
3	225	1782517	2956854	1314005	98.96	99.55	98.66
4	225	1778181	2942478	1300272	98.72	99.07	97.63
5	225	1788541	2947393	1301455	99.3	99.24	97.71
6	225	1775889	2943999	1301204	98.59	99.12	97.7
Average assay					98.82	99.28	98.08
SD					0.27	0.21	0.61
%RSD					0.27	0.21	0.62

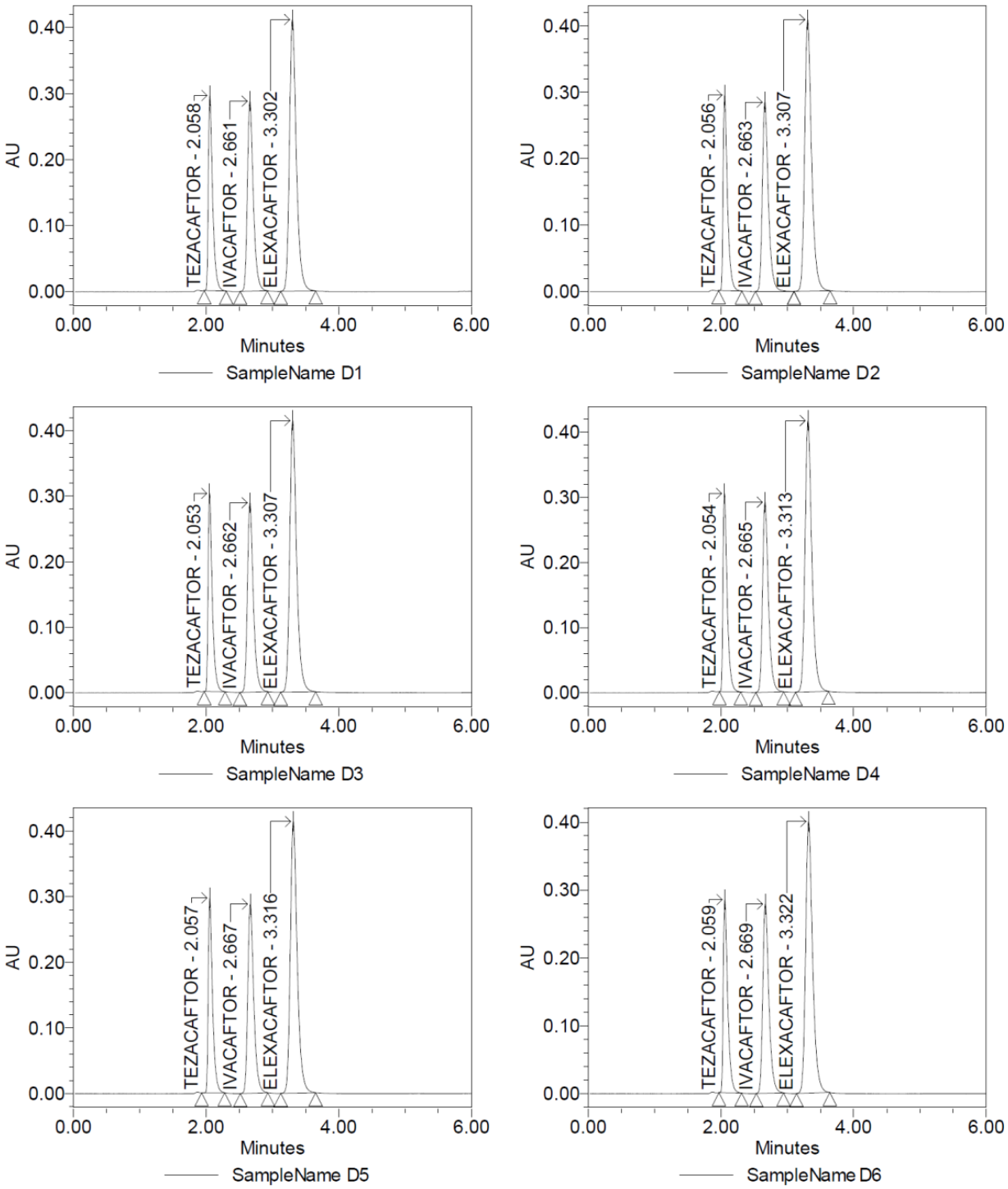

Fig no.24: Dissolution Chromatographs of TZR, IVR and EXR

Table no.33: Dissolution Test of IVR, EXR & TZR

Sample	IVR (%) Assayed	EXR (%) Assayed	TZR (%) Assayed
1	98.56	99.55	97.74
2	98.80	99.20	99.05
3	98.96	99.55	98.66
4	98.72	99.07	97.63
5	99.30	99.24	97.71
6	98.59	99.12	97.70

CONCLUSION

For combined IVR, EXR & TZR assessments, an RP-HPLC assay system was developed. Validation of RP-HPLC combined assessment process for IVR, EXR & TZR. In terms of linearity, accuracy, selectivity, specificity, precision, robustness, and accuracy, the RP-HPLC combined assessment process for IVR, EXR & TZR has indeed being verified and proved to fulfill the ICH endorsements. The RP-HPLC combined assessment process was implemented for the quantitation of IVR, EXR & TZR in tablets (Trikafta), and dissolution of Trikafta samples proving the competence of this combined assessment process to be operated for quality regular control analysis of IVR, EXR & TZR and dissolution analysis of IVR, EXR & TZR. The method developed for combined assessment of IVR, EXR and TZR produced retention time lower than the retention time of studies/methods discussed in the literature review.

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