Development and validation of stability indicating method for simultaneous estimation of Tezacaftor, Ivacaftor and Elexacaftor in tablet dosage form

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ABSTRACT: This study aimed to optimize and validate methods for the analysis of Tezacaftor, Ivacaftor, and Elexacaftor. Method is optimized with X- Bridge phenyl (150x 4.6mm, 3.5µ) column at 210nm wavelength using Acetonitrile and 0.1% Formic acid 80:20 at 1 mL/min in isocratic mode. The injection volume is 10µL at 25□ C. According to ICH guidelines, the approach was validated for system suitability, specificity, linearity, robustness, precision, and accuracy. The limit of detection (µg/mL) for Tezacaftor, Ivacaftor, and Elexacaftor is found to be 0.060, 0.098, and 0.13 respectively. The limit of quantification (µg/mL) is found to be 0.21, 0.33, and 0.37 respectively. Forced degradation studies using various conditions like acid, base, peroxide, thermal, U.V, reduction, control, and hydrolysis were carried out and in all cases and the peak purity passes. Thus the method is found as stability indicating as per ICH guidelines.

Keywords: Tezacaftor, Ivacaftor, and Elexacaftor, RP-HPLC, Validation, Forced degradation.

1. Drug profile

1.1. Tezacaftor [1]

Tezacaftor is a medication used to treat homozygous or heterozygous F508del mutation cystic fibrosis

Chemical structure:

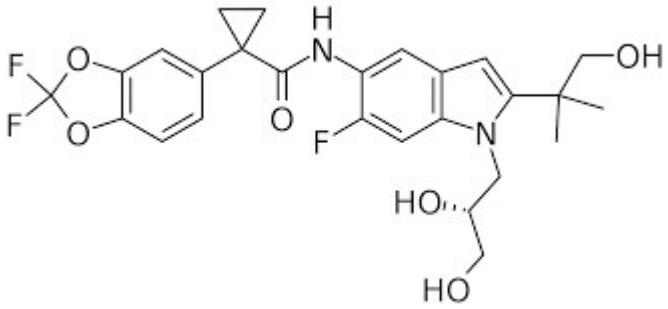

Figure 1: Chemical structure of Tezacaftor IUPAC name:

1-(2,2-difluoro-2H-1,3-benzodioxol-5-yl)-N-{1-[(2R)-2,3-dihydroxypropyl]-6-fluoro-2-(1-hydroxy-2methylpropan-2-yl)-1H-indol-5-yl}cyclopropane-1-carboxamide

Molecular formula: $C_{26}H_{27}F_3N_2O_6$

Molecular weight: 520.505g/mol

Appearance: Solid powder

Solubility: Soluble in DMSO, insoluble in water **pKa:**

13.99

CAS number: 1152311-62-0

Pharmacodynamics: Tezacaftor does not induce clinically significant QT prolongation. When given with ivacaftor, tezacaftor can lead to liver transaminase elevations.

Mechanism of action: CFTR (cystic fibrosis transmembrane regulator) correctors such as tezacaftor aim to repair F508del cellular misprocessing. This is done by modulating the position of the CFTR protein on cell surface to the correct position, allowing for adequate ion channel formation and increased in water and salt movement through the cell membrane [2]. **Absorption:** Reaches its maximum plasma concentration in about 2-6 hours

Half –Life: 57.2 hrs

Route of elimination: 72% excreted in feces and 14% excreted in urine

1.2. Ivacaftor [3]

Ivacaftor is in a class of medications called cystic fibrosis transmembrane conductance regulator (CFTR) potentiators. It works by improving the function of a protein in the body to decrease the build-up of thick mucus in the lungs and improving other symptoms of cystic fibrosis.

Chemical structure:

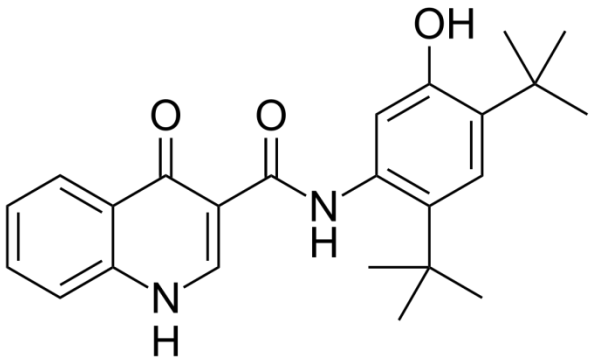

Figure 2: Chemical structure of Ivacaftor

IUPAC name: N-(2,4-di-tert-butyl-5-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide

Molecular formula: $C_{24}H_{28}N_2O_3$

Molecular weight: 392.49 g/mol

Appearance: White to off-white powder

Solubility: Freely soluble in methyl ethyl ketone/water mixture, soluble in 2-methyl THF and PEG 400, slightly soluble in methanol, acetone, and ethanol and practically insoluble in water **pKa:** 11.08

CAS number: 873054-44-5

Pharmacodynamics: Ivacaftor is a "potentiator" of CFTR, meaning it increases the probability that the defective channel will be open and allow chloride ions pass through the channel pore.

Mechanism of action: The CFTR protein is a chloride channel present at the surface of epithelial cells in multiple organs. Ivacaftor facilitates increased chloride transport by potentiating the channelopen probability (or gating) of the CFTR protein [4]**.**

Absorption: Reaches its maximum plasma concentration in about 4 hours

Elimination Half- Life: 12hrs

Route of elimination: Faeces (87.8%) and traces in urine

1.3. Elexacaftor [5]

Elexacaftor is a small molecule CFTR corrector used in combination with tezacaftor and ivacaftor for the treatment of cystic fibrosis patients with one F508del-CFTR mutation **Chemical Structure:**

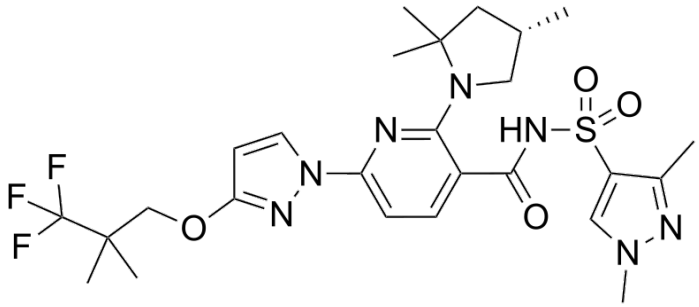

Figure 3: Chemical structure of Elexacaftor

IUPAC name: *N*-(1,3-dimethylpyrazol-4-yl)sulfonyl-6-[3-(3,3,3-trifluoro-2,2 dimethylpropoxy)pyrazol-1-yl]-2-[(4*S*)-2,2,4-trimethylpyrrolidin-1-yl]pyridine-3-carboxamide

Molecular formula: $C_{26}H_{34}F_3N_7O_4S$

Molecular weight: 597.70g/mol

Appearance: White to off-white powder

Solubility: Insoluble in water, soluble in

DMSO **CAS number:** 2216712-66-0 **pKa:** 4.1

Pharmacodynamics: As a CFTR corrector, elexacaftor works to increase the amount of mature CFTR proteins present on the surface of cells. When used in combination with CFTR potentiators, which enhance the function of cell-surface CFTR proteins, drugs like elexacaftor help to improve a variety of multi-organ cystic fibrosis symptoms, including lung function, nutritional status, and overall quality of life

Mechanism of action: The CFTR proteins produced by the CFTR gene are transmembrane ion channels that move sodium and chloride across cell membranes - water follows the flow of chloride ions to the cell surface, which consequently helps to hydrate the surface of the cell and thin the secretions (i.e. mucous) around the cell [6]

Absorption: maximum concentration at T_{max} of 4 hours post-dose

Half-Life: 24.7 hrs

Routes of elimination : 87.3% excreted in feces and 0.23% excreted in urine

1.4. Combined dosage form:

Trikafta is a fixed-dose combination tablet for oral administration. Each tablet contains 100 mg of elexacaftor, 50mg of tezacaftor, and 75mg of ivacaftor.

2. Literature review

An extensive literature study is done to have an idea of various works carried out for the simultaneous estimation of Elexacaftor, Ivacaftor and Tezacaftor in its pharmaceutical dosage form [7-10].

3. Materials and methods

3.1. Chemicals and Solvents:

HPLC grade Acetonitrile was purchased from Ramkem Haryana, India. HPLC-grade water was prepared by using Millipore Milli- Q water purification system used throughout the process. Analytical grade chemicals include sodium hydroxide, hydrochloric acid, 20% hydrogen peroxide, ortho phosphoric acid, methanol and formic acid were purchased from E. Merck Limited, Mumbai, India.

3.2. References drugs and pharmaceutical dosage form:

The References samples of Tezacaftor, Ivacaftor, and Elexacaftor were provided as a gift samples from Shree Icon Pharma laboratory, Vijayawada. **Trikafta** tablet labeled to contain 100 mg of elexacaftor, 50mg of tezacaftor, and 75mg of ivacaftor and purchased from local pharmacy store.

3.3. Instrumentation:

Waters HPLC e 2695 separation module equipped with a waters 1525 binary HPLC pump, Waters 2998 photodiode array detector, and Waters 2707 auto sampler. The data were acquired and processed using Windows Empower-2 software. Electronic balance Sartorius was used for weighing the drugs. Digital pH meter make by Mestar company was used for all pH measurements. Ultrasonic bath from Unichrome was used for sonication of the samples. Hot air oven was used to carry out thermal degradation studies. UV cross linker, with series of 23400 model UV chamber, equipped with a UV fluorescence lamp with the wavelength range between 200 & 300 nm was used for photo degradation studies

3.4. Preparation of Mobile Phase:

Mobile phase was prepared by mixing 0.1% formic acid and ACN taken in the ratio 20:80. It was filtered through 0.45µ membrane filter to remove the impurities which may interfere in the final chromatogram. Mobile phase is used as diluent.

3.5. Preparation of standard stock solution :

Accurately weighed and transfered 75mg of Ivacaftor, 50 mg of Tezacaftor and 100mg of Elexacaftor into a 100 mL clean dry volumetric flask. Diluent was added and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stock solution). Further pipetted 5 mL of the

above stock solution into a 50 mL volumetric flask and diluted up to the mark with diluent (75ppm of Ivacaftor, 50ppm of Tezacaftor and 100ppm of Elexacaftor).

3.6. Preparation of Sample Solution:

10 tablets were weighed, powdered and accurately weighed and transfer 279mg of sample into a 100mL clean dry volumetric flask. Diluent was added and sonicated it up to 30 mins to dissolve it completely and made volume up to the mark with the same solvent. Then it is filtered through 0.45 micron Injection filter. (Stock solution). Further pipetted 5 mL of the above stock solution into a 50mL volumetric flask and diluted up to the mark with diluent (75ppm of Ivacaftor, 50ppm of Tezacaftor and 100ppm of Elexacaftor).

3.7. Preparation of solutions for linearity:

From the above stock solution, 0.5, 1.25, 2.5, 5.0, 6.25, and 7.5mL aliquots individually pipetted into 50mL volumetric flasks and final volumes made upto the mark with diluent. The least square regression analysis of the peak area to concentration ratio was calculated. The concentration of the unknown can be determined either using the corresponding calibration curve or regression equation.

3.8. Preparation of sample solutions for accuracy:

From the powdered sample, accurately weighed 139.5, 279, and 418.5mg respectively into three 100 mL clean dry volumetric flasks and added the diluent. Then it was sonicated to dissolve it completely and made volume up to the mark with the diluent. Further pipetted 5 mL of the above prepared solutions into three separate 50mL volumetric flasks and diluted up to the mark with diluent to obtain 50%, 100%, and 150% solutions respectively with respect to target assay concentrations.

3.9. Forced Degradation Studies

A stress study was conducted to demonstrate the effective separation of degradation from the main analyte peaks of the sample when exposed to the following stress conditions. All the stressed samples were suitability diluted to required concentration with diluents and injected twice into the HPLC system by using optimized chromatographic conditions and the chromatograms were recorded and evaluated for the peak purity and the % degradation were calculated.

3.9.1. Oxidation:

Pipetted 5 mL of stock solution into a 50mL volumetric flask, 1 mL of 3% w/v of hydrogen peroxide added in 50 mL of volumetric flask and the volume was made up to the mark with diluent. The volumetric flask was then kept at room temperature for 15 min. The solution was filtered the solution with 0.45 microns syringe filters and placed in vials.

3.9.2. Acid Degradation Studies:

Pipetted 5 mL of stock solution into a 50mL volumetric flask and 1 mL of 1N HCl was added. Then, the volumetric flask was kept at 60ºC for 6 hours and then neutralized with 1 N NaOH and made up to 10mL with diluent. Filtered the solution with 0.45 microns syringe filters and placed in vials.

3.9.3. Alkali Degradation Studies:

Pipetted 5 mL of stock solution into a 50mL volumetric flask and 1mL of 1N NaOH was added. Then, the volumetric flask was kept at 60ºC for 6 hours and then neutralized with 1N HCl and made up to 10mL with diluent. The solution was filtered with 0.45 microns syringe filters and placed in vials.

3.9.4. Dry Heat Degradation Studies:

Tezacaftor, Ivacaftor and Elexacftor sample was taken in petridish and kept in Hot air oven at 110^0 C for 24 hours. Then the sample was taken and diluted with diluents and injected into HPLC and analyzed.

3.9.5. Photo Stability Studies:

The photochemical stability of the drug studied by exposing the prepared stock solution to UV light by keeping the beaker in UV chamber for 3days or 200Watt hours/m^2 in photo stability chamber.

Then the sample was injected into HPLC and analyzed.

3.9.6. Neutral Degradation Studies:

Pipette 5 mL of stock solution into a 50mL volumetric flask, added 1 mL of water into 50 mL of volumetric flask and the volume was made up to the mark with diluent. The volumetric flask was then kept at room temperature for 15 min. Filter the solution with 0.45 microns syringe filters and injected into HPLC.

4. Results and discussion

4.1. Method development:

Method is optimized with X- Bridge phenyl (150x 4.6mm, 3.5µ) column at 210nm wavelength using Acetonitrile and 0.1% Formic acid 80:20 at 1 mL/min in isocratic mode.

The injection volume is 10µL at 25□ C.

4.2. Analytical method validation:

The method was validated for its linearity range, accuracy, precision, and specificity. Method validation was carried out as per ICH [11] guidelines

4.2.1. System suitability: All the system suitability parameters were within the range and satisfactory as per ICH guidelines.

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

S.No	Parameter	Tezacaftor	Ivacaftor	Elexacaftor
1	Retention time	2.900	4.716	7.830
2	Plate count	5810	4416	10909
3	Tailing factor	1.27	1.14	1.06
4	Resolution		8.38	10.69
5	%RSD	0.40	0.91	0.47

4.2.2. Specificity:

RT of Tezacaftor, Ivacaftor and Elexacaftor were 2.900, 4.716 and 7.830 min respectively. No interfering peaks found in blank and placebo. So, this method was specific.

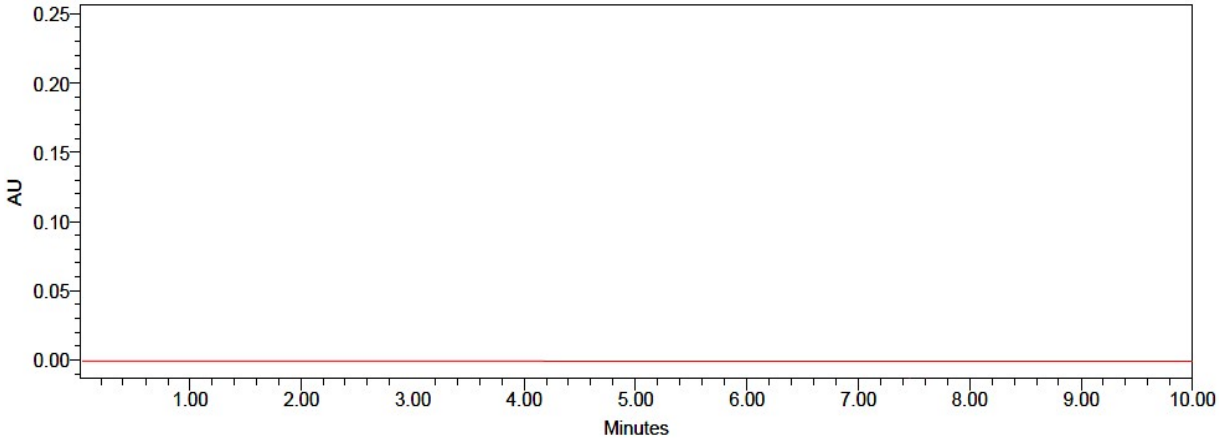

Figure 4: Chromatogram of blank

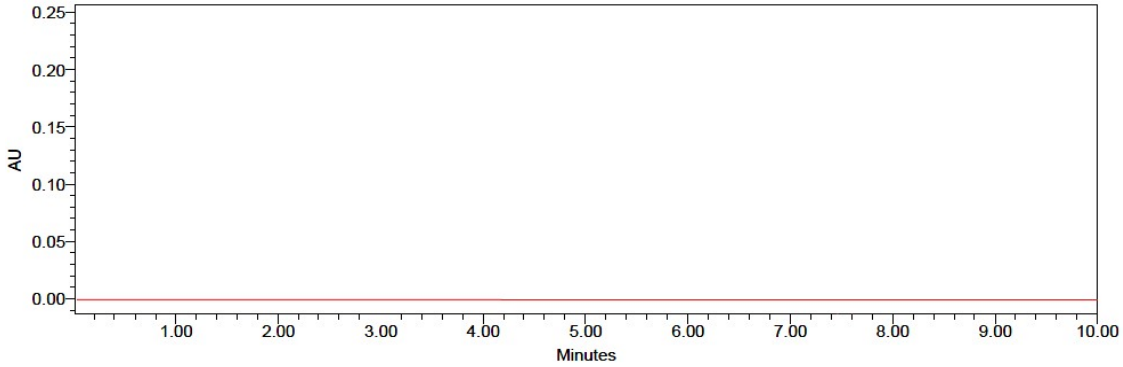

Figure 5: Chromatogram of placebo

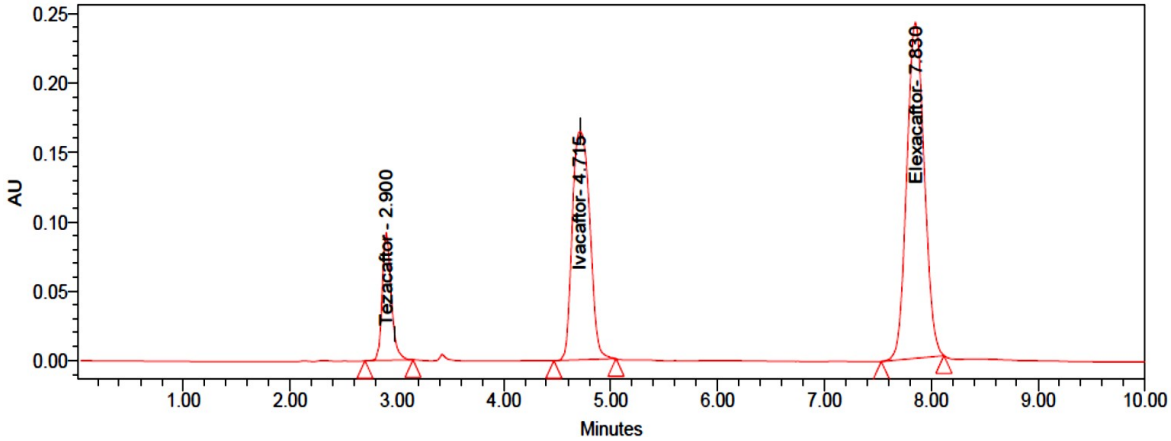

Figure 6: Optimized chromatogram

4.2.3. Linearity:

Table 3: Results of linearity for Tezacaftor, Ivacaftor and Elexacaftor

S.NO	Tezacaftor		Ivacaftor		Elexacaftor	
	Conc.(µg/mL)	Peak area	Conc.(µg/mL)	Peak area	Conc.(µg/mL)	Peak area
1	5.00	54315	7.50	190320	10.00	268415
2	12.50	149302	18.75	487956	25.00	677456
3	25.00	278541	37.50	954786	50.00	1358742
4	50.00	536210	75.00	1823654	100.00	2786284
5	62.50	685634	93.75	2278462	125.00	3468745
6	75.00	793658	112.50	2654810	150.00	4054810
Regression equation	y = 6908.92x + 34317.67		y = 5327.34x +2767.45		y = 9589.95x+37318.96	
Slope	6908.92		23729.08		9589.95	
Intercept	10647.46		29065.27		485.63	
R^2	0.99956		0.99960		0.9997	

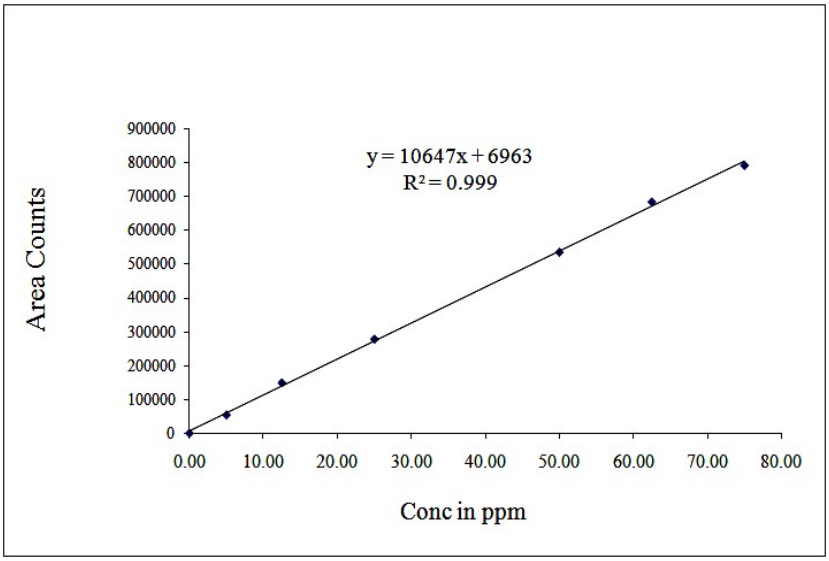

Figure 7: Calibration curve for Tezacaftor

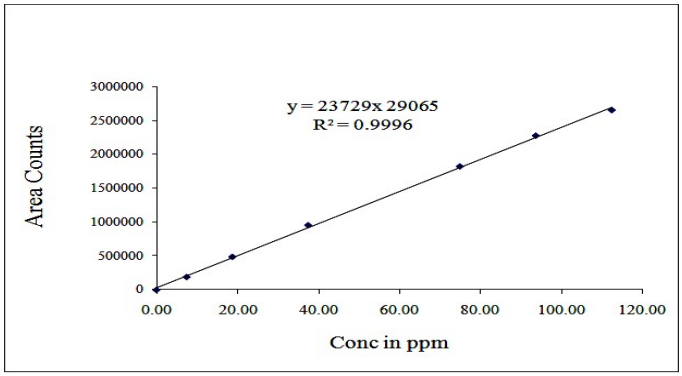

Figure 8: Calibration curve for Ivacaftor

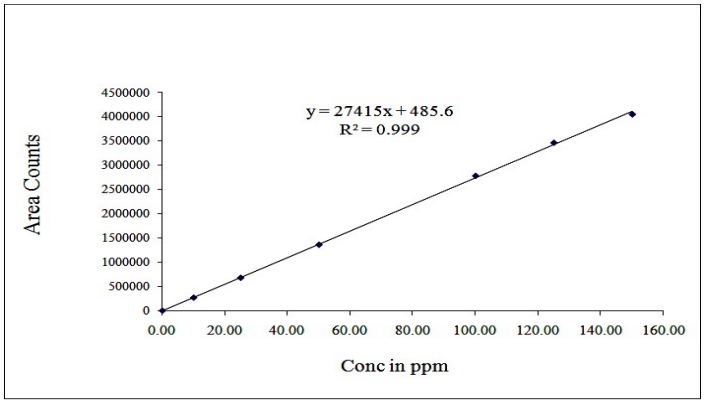

Figure 9: Calibration curve for Elexacftor

4.2.4. Precision:

a) System Precision:

Table 4: System precision table of Tezacaftor, Ivacaftor and Elexacaftor

S. No	Conc. of Tezacaftor (µg/mL)	Area of Tezacaftor	Conc. of Ivacaftor (µg/mL)	Area of Ivacaftor	Conc. of Elexacaftor (µg/mL)	Area of Elexacaftor
1.	50	533713	75	1876617	100	2751325
2.	50	539183	75	1878148	100	2791471
3.	50	536732	75	1854574	100	2765880
4.	50	533350	75	1842918	100	2763501
5.	50	535926	75	1842984	100	2765497
6.	50	535475	75	1843448	100	2767840
Mean		535730		1856448		2767586
S.D		2133.7		16819.81		13106.79
%RSD		0.398		0.906		0.474

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b) Repeatability:

Table 5: Method Precision for Tezacaftor, Ivacaftor and Elexacaftor

S. No.	Area for Tezacaftor	Area for Ivacaftor	Area for Elexacaftor
1	537450	1871153	2789315
2	531665	1880820	2788941
3	539553	1822189	2764650
4	536337	1872461	2790843

5	537450	1871153	2789315
6	535149	1862189	2789421
Average	536267	1863327	2785414
Std dev	2684.39	21002.66	10193.62
%RSD	0.50	1.13	0.37

c) Intermediate precision (Day_ Day Precision):

Six replicate injections of same concentration were analysed on system 1 by analyst 1 on day 1. Six replicate injections of same concentration were analysed on system 2 by analyst 2 on day 2. The results are summarized in table 6 and are within the limits.

Table 6: Intermediate Precision data

S.NO	**Day-1 Analyst-1**	**Day-2 Analyst-2**	**Day-1 Analyst-1**	**Day-2 Analyst-2**	**Day-1 Analyst-1**	**Day-2 Analyst-2**
	Tezacaftor		**Ivacaftor**		**Elexacaftor**	
1	526958	533713	1850853	1871153	2751325	2789315
2	525540	539183	1878148	1880820	2791471	2788941
3	529954	536732	1856443	1822189	2765880	2764650
4	532326	533350	1842918	1872461	2763501	2790843
5	526742	535926	1832645	1871153	2765497	2789315
6	527752	535475	1843448	1862189	2767840	2789421
Mean	528212	535730	1850742	1863327	2767586	2785414
SD	2491.75	2133.7	15655.81	21002.66	13106.79	10193.62
% RSD	0.47	0.398	0.85	1.13	0.474	0.37

Discussion: % RSD were found to be within acceptable limit of ≤ 2. Hence the method is precise, reproducible and rugged for 48 hours' study

4.2.5. Accuracy:

Table 7: Accuracy results of Tezacaftor

%Conc.	**Area**	**Amt. Added (mg)**	**Amt. Found (mg)**	**% Recovery**	**Mean Recovery**
50%	264967	25	24.73	98.9	99.3

100%	537450	50	50.16	100.3	
150%	793703	75	74.08	98.8	

Table 8: Accuracy results for Ivacaftor

%Conc.	Area	Amt. Added (mg)	Amt. Found (mg)	% Recovery	Mean Recovery
50%	914105	37.5	36.93	98.5	99.8
100%	1871153	75	75.59	100.8	
150%	2787220	112.5	112.6	100.1	

Table 9: Accuracy results for Elexacaftor

%Conc.	Area	Amt. Added (mg)	Amt. Found (mg)	% Recovery	Mean Recovery
50%	1383585	50	49.99	100.0	100.6
100%	2789315	100	100.79	100.8	
150%	4189106	150	151.36	100.9	

Discussion: The mean % recovery was obtained as 99.3%, 99.8% and 100.6% for Tezacaftor, Ivacaftor and Elexacaftor respectively and was accurate.

4.2.6. Robustness:

Table 10: Robustness results of Tezacaftor

Parameter	Condition	RT (min)	Peak area	Resolution	Tailing	Count
Flow rate Change(mL/min)	Less flow(0.8mL)	3.471	556341		1.44	7083
	Actual(1mL)	2.900	533713		1.27	5810
	More flow(1.2mL)	2.370	476300		1.40	4617

Organic Phase change	Less Org (72:28)	2.573	483793		1.22	11353
	Actual(80:20)	2.901	539183		1.29	5809
	More Org(88:12)	2.592	569642		1.38	2842

Table 11: Robustness results of Ivacaftor

Parameter	**Condition**	**RT (min)**	**Peak area**	**Resolution**	**Tailing**	**Count**
Flow rate Change(mL/min)	Less flow(0.8mL)	5.288	1694368	7.91	1.20	5484
	Actual(1mL)	4.715	1876617	8.38	1.14	4416
	More flow(1.2mL)	4.160	1971064	8.43	1.16	3306
Organic Phase change	Less Org (72:28)	4.126	1775233	9.27	0.93	3079
	Actual (80:20)	4.712	1878148	8.39	1.15	4354
	More Org(88:12)	4.142	2246039	7.63	1.25	6511

Table 12: Robustness results of Elexacaftor

Parameter		**RT**	**Peak area**	**Resolution**	**Tailing**	**Count**
	Condition	**(min)**				
Flow rate Change (mL/min)	Less flow(0.8mL)	9.139	2346748	7.90	1.12	2985
	Actual(1mL)	7.830	2751325	10.69	1.06	10909
	More flow(1.2mL)	6.960	2555432	9.98	1.41	10292

Organic Phase change	Less Org (72:28)	6.015	2626585	12.33	1.06	3816
	Actual (80:20)	7.825	2791471	10.63	1.08	10698
	More Org (88:12)	6.035	2804690	8.93	1.15	12727

Discussion: Results were found to be within acceptable limit with effect of change in parameters volume. Hence the study is proposed to be robust. **4.2.7. Limit of detection and Limit of quantification:**

Table 13: Limit of Detection and Limit of Quantification data

S.No	Parameter	Measured values (µg/mL)		
		Tezacaftor	Ivacaftor	Elexacaftor
1	LOD	0.060	0.098	0.13
2	LOQ	0.21	0.33	0.43

Discussion: The lowest values of LOD and LOQ indicates method is sensitive.

4.2.8. Assay:

Table 14: Assay of Tezacaftor, Ivacaftor and Elexacaftor

Brand	Drug	Avg sample area (n=5)	Std. wt (mg)	Sample wt. (mg)	Label amount (mg)	Std purity	Amount found (µg/mL)	% assay
Trikafta	Tezacaftor	531736	50	279	50	99.9	49.84	99.2
	Ivacaftor	1865025	75	279	75	99.7	75.07	100.3
	Elexacaftor	2774254	100	279	100	99.8	100.03	100.2

4.2.9. Forced degradation studies:

Degradation studies were carried as per ICH [12] guidelines.

Table 15: Forced Degradation results for Tezacaftor, Ivacaftor and Elexacaftor

Results: %	Tezacaftor	Ivacaftor	Elexacaftor

Degradation results	Area	% Degradation	Area	% Degradation	Area	% Degradation
Control	534980	0.1	1858342	0	2765482	0.1
Acid	457965	14.4	1549733	16.6	2342278	15.3
Alkali	454471	15.1	1594604	14.2	2365307	14.4
Peroxide	456705	14.7	1589441	14.5	2335681	15.5
Reduction	462101	13.7	1615562	13.1	2388404	13.6
Thermal	464705	13.2	1629449	12.4	2355681	14.8
Photo	451815	15.6	1636226	12	2427402	12.2
Hydrolysis	466797	12.8	1600030	13.9	2446025	11.5

Conclusion

From the obtained results it is concluded that the proposed stability indicating RP-HPLC method was found to be simple, sensitive, reliable, accurate, precise, robust and the linear over the concentrations range used. No interfering peaks in chromatograms run for formulation samples. The short analytical run time shows the speed of analysis which enables more number of samples analyzed per unit time. More over the process is found to be stability indicating. Therefore, it is concluded that this method can be applied for regular analysis of Tezacaftor, Ivacaftor, and Elexacaftor in pharmaceutical formulations.

5. References

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