Development and validation of Stability-indicating RP-UPLC Method for the Simultaneous Determination of Ivacaftor, Tezacaftor and Elexacaftor in bulk and their Formulation

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Abstract
A simple reproducible stability indicating RP-UPLC method was developed for the simultaneous determination of Ivacaftor, Tezacaftor and Elexacaftor in their combined dosage forms using HSS C18, 1.8µm, 100mm x2.1 mm i.d. column. A mobile phase of phosphate buffer (10mM) pH-4.8 and acetonitrile in the ratio of 70: 30v/v mixture was used for separation and quantification of ivacaftor, tezacaftor and elexacaftor. The present drug analytes were run at a flow-rate of 0.3ml/ min at 30°C temperature. The injection volume was 2µL and with ultraviolet detection at 270nm. Under these conditions, elexacaftor, ivacaftor and tezacaftor were eluted at 0.72min, 1.4min and 1.9min respectively with a total run time shorter than 5min.

The developed method was validated according to International Conference on Harmonization (ICH) guidelines. The developed RP-UPLC method was applied successfully for quality control assay of Ivacaftor, Tezacaftor and Elexacaftor in their combination drug product.

Keywords: Ivacaftor, Tezacaftor, Elexacaftor, UPLC.

Introduction

Ivacaftor [1] *N*-(2,4-di*tert*-butyl-5-hydroxyphenyl)-4-oxo-1*H*-quinoline-3-carboxamide [Fig.1(a)] (also known as Kalydeco or VX-770) is a drug used for the management of cystic fibrosis (CF). Ivacaftor is administered as a monotherapy and also administered in combination with other drugs for the management of cystic fibrosis. Cystic fibrosis is an autosomal recessive disorder caused by one of several different mutations in the gene for the cystic fibrosis.

Tezacaftor [2] [Fig.1(b)] (R)-1-(2,2-difluorobenzo[d][1,3] dioxol-5-yl)-N-(1-(2,3-dihydroxypropyl)-6-fluoro-2-(1-hydroxy-2-methylpropan-2-yl)-1H-indol-5-yl)cyclopropanecarboxamide is used to manage cystic fibrosis. Elexacaftor [3] *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-trimethyl pyrrolidin-1-yl]pyridine-3-carboxamide [Fig.1(c)]. (previously VX-445) is a small molecule, next-generation corrector of the cystic fibrosis transmembrane conductance regulator (CFTR) protein.

These three drugs are marketed as combined dose tablet in the brand name of Trikafta (Label claim 75mg, 50mg, 100mg ivacaftor, tezacaftor and elexacaftor) designated for the treatment of cystic fibrosis. At the time this examination showing RP-UPLC strategy was accounted for the measure of ivacaftor, tezacaftor and elexacaftor in combinational structure. It was in this way thought worth-while to build up dependability showing chromatographic strategy for the concurrent examination of these three medications in their joined structures. The present research paper describes the development and validation of selective reverse phase UPLC assay procedure for the analysis of ivacaftor, tezacaftor and elexacaftor in combined dosage formulation in accordance with the ICH guidelines.

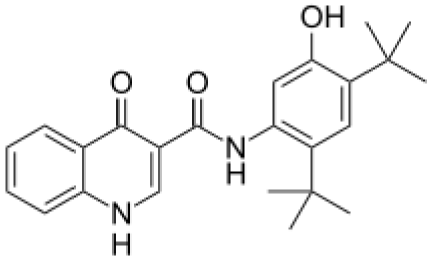

Fig. 1(a): Chemical Structure of Ivacaftor

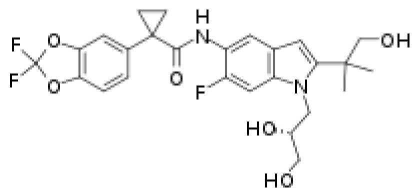

Fig. 1(b): Chemical Structure of Tezacaftor

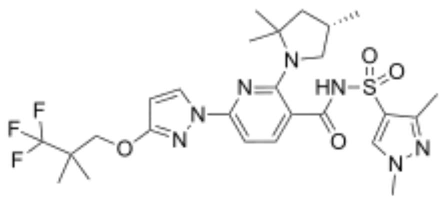

Fig. 1(c): Chemical Structure of Elexacaftor

Material and Methods

Instrumentation: The UPLC method of development and validation procedures was performed on The Waters UPLC system consisting of UV detector. The chromatographic separations were performed using HSS C18, 1.8µm, 100mm x2.1 mm I.D. column maintained at 30°C temperature. Data integration was performed using Empower-1 software.

Reagents and Chemicals: Pure samples of ivacaftor, tezacaftor and elexacaftor were made available by Spectrum Pharma Labs, Hyderabad. Acetonitrile were of HPLC grade purchased from Merck Chemicals Ltd., India. Ortho-phosphoric acid and potassium dihyrogen ortho phosphate were obtained from Rankem (India). Commercially available dosage form in the brand name of trikafta (Label claim 75mg, 50mg, 100mg ivacaftor, tezacaftor and elexacaftor) was procured from local pharmacy store.

All chemicals and reagents used were of HPLC grade and were purchased from Merck Chemicals, India. Milli-Q water was used throughout the analysis. All solvents and solutions were filtered through a membrane filter (Millipore Millex - HV filter units, 0.45 µm pore size; nylon) and degassed before use.

Preparation of mobile phase: The mobile phase used was a mixture of phosphate buffer (10mM) pH-4.8 and acetonitrile in the ratio of 70: 30%v/v in isocratic mode at a flow rate of 0.3ml/min respectively. The buffer was prepared by weighing accurately 1.36gms of KH_2PO_4 and dissolved with 1000ml of HPLC grade water, then adjust the pH: 4.8 with ortho-phosphoric acid or sodium hydroxide prior to the assay. The mobile phase components were filtered through 0.45µ membrane filter prior to use.

Preparation of diluent: The same mobile phase prepared above was used as diluent in the present study.

Preparation of standard solutions: Accurately weigh and transfer 18.75 mg of ivacaftor working standard and 12.5mg of tezacaftor and 25mg elexacaftor working standard powders (pure) into 50ml volumetric flask individually containing 10ml of diluent and sonicated to dissolve completely. Make the volume up to the mark with the diluent. Further, pipette out 5.0ml from the above stock solution into 50ml volumetric flask and dilute up to the mark with diluent.

Preparation of sample solution [assay of pharmaceutical dosage form]: Twenty tablets of trikafta (Label claim 75mg, 50mg, 100mg ivacaftor, tezacaftor and elexacaftor) procured from local pharmacy store were weighed to get the average weight and finely powdered in a mortar. An amount of powder equivalent to 18.75mg, 12.5mg, 25mg of ivacaftor, tezacaftor and elexacaftor tablet powder was accurately weighed and transferred into a 50ml volumetric flask containing diluent and sonicated to dissolve it completely and filter the solution through 0.45µ filter paper and make the volume up to the mark with the same diluent. Further, pipette 5.0ml of above stock solution into a 50ml volumetric flask and then dilute up to the mark with diluent.

Results and Discussion

Method Development: Various chromatographic parameters were optimized to develop UPLC method for simultaneous estimation of ivacaftor, tezacaftor and elexacaftor with acceptable resolution respectively. Initially, studies were made by selecting appropriate column. For this purpose, the authors used CSS C18 (100mm x 2.1mm, 1.7µm), Hibar C18(1.7µm, 100mm x2.1 mm) and SB C18(100x2.1) mm, 1.7µ columns. Out of these, the UPLC columns, HSS C18, 1.7µm, 100mm x 2.1 mm I.D. column was found to be better as it gave the peaks with better Gaussian shape for the three drugs.

To improve the shape and width of the obtained peaks for the above column, a suitable mobile phase was examined using mobile phase mixtures of different polarity. Various combinations of mobile phases were screened and finally, the mobile phase consisting of phosphate buffer (10mM) pH-4.8, acetonitrile in the ratio of 70: 30%v/v was preferred to obtain symmetric peaks of ivacaftor, tezacaftor and elexacaftor respectively.

The best sensitivity and selectivity were obtained by online wavelength switching at 270nm which allowed the analysis of these three drugs in a single run as the isoabsortive point of ivacaftor, tezacaftor and elexacaftor selected was 270nm and this wavelength was chosen for further analysis. Further, the flow rates of the mobile phase between 0.3 and 0.5ml/min were studied.

From these studies, it is revealed that the flow rate of 0.0ml/min gave an optimal signal to noise ratio with a reasonable separation time of ivacaftor, tezacaftor and elexacaftor respectively.

Finally, the simultaneous determination of Ivacaftor, tezacaftor and elexacaftor was carried out by isocratic elution with a flow rate of 0.3 mL/min on HSS C18, 1.7µm, 100mm x 2.1 mm at 30 C temperature. The standard chromatogram so obtained was shown in figure 2. The system suitability parameters were shown in table 2. The retention times of ivacaftor, tezacaftor and elexacaftor were found to be 1.4min, 1.9min and 0.7min respectively.

Forced Degradation Studies: Intentional degradation was attempted to stress conditions exposing it to acid (2N Hydrochloric acid), alkali (2N NaOH), hydrogen peroxide (20%), photolytic, neutral and heat (105°C) to evaluate the ability of the proposed method to separate ivacaftor, tezacaftor and elexacaftor from degradation products.

Preparation of stock solution: An amount of tablet powder equivalent to 18.75mg, 1205mg and 25mg of ivacaftor, tezacaftor and elexacaftor tablet powder respectively was added into a 50 ml volumetric flask individually, add diluent and sonicate to dissolve it completely and make volume up to the mark with the same diluent.

Acid degradation condition: Pipette 5.0ml of the above stock solution into a 50ml volumetric flask and 5.0ml of 2N HCl was added.

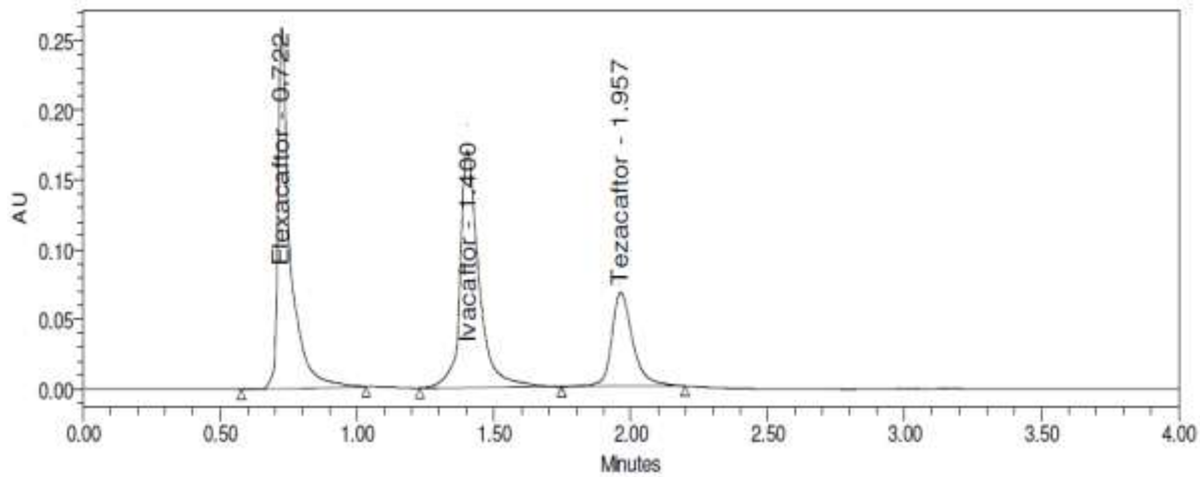

Fig. 2: Standard chromatogram

Table 1
Results of % degradation studies of Ivacaftor, Tezacaftor and elexacaftor

Degradation Conditions	%Total Degradation		
Drug Name	**Ivacaftor**	**Tezacaftor**	**Elexacaftor**
Acid	6.15%	6.44%	5.94%
Base	5.4%	5.20%	5.03%
Peroxide	4.99%	3.78%	4.32%
Thermal	2.74%	2.91%	2.56%
UV/LUX	1.71%	1.67%	1.48%
Neutral	1.01%	0.82%	0.58%

Table 2
System suitability results of Ivacaftor, Tezacaftor and elexacaftor

Parameter	Elexacaftor	Ivacaftor	Tezacaftor
Retention Time	0.72	1.38	1.95
USP Tailing	1.37	1.32	1.34
USP Resolution	----	7.4	4.6
Area%RSD	1.3	1.1	0.9

Then, the volumetric flask was kept at 60ºC for 30 minutes and then neutralized with 2 N NaOH and make up to 50ml with diluent. Filter the solution with 0.45 microns syringe filters and place in vials.

Alkali degradation condition: Pipette 5.0 ml of the above stock solution into a 50ml volumetric flask and add 5.0ml of 2N NaOH in 50 ml of volumetric flask.

Then, the volumetric flask was kept at 60ºC for 30minutes and then neutralized with 2N HCl and made up to 50ml with diluent. Filter the solution with 0.45 microns syringe filters and place in vials.

Oxidative degradation: Pipette 5.0ml of the above stock solution into a 50ml volumetric flask, 5.0ml of 20.0% w/v of hydrogen peroxide was 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 60°C for 30 min. Filter the solution with 0.45 microns syringe filters and place in vials.

Thermal degradation: The sample was taken in Petri dish and kept in hot air oven at 105ºC for 6 hours. Then, the sample was taken and diluted with diluents and injected into the above described UPLC system and analysed.

Photolytic studies: The sample was taken in Petri dish and kept under UV and Lux Light Chamber 200 watt hours and 1.2 million Lux hours in photo stability chamber. The sample was taken and diluted with diluents and injected into the above described UPLC system and analysed.

Neutral studies: The sample was studied by refluxing in water for 6hrs at a temperature of 60ºC, the sample was taken and diluted with diluents and injected into the above described UPLC system and analysed. The degradation chromatograms are shown in fig. 3a – 3f.

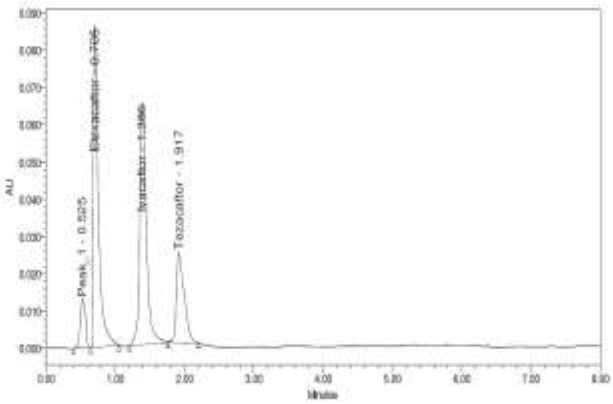

Fig. 3a: Chromatogram of acid degradation

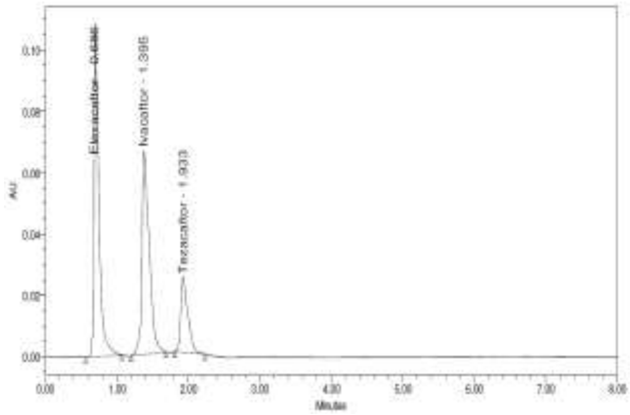

Fig. 3b: Chromatogram of alkali degradation

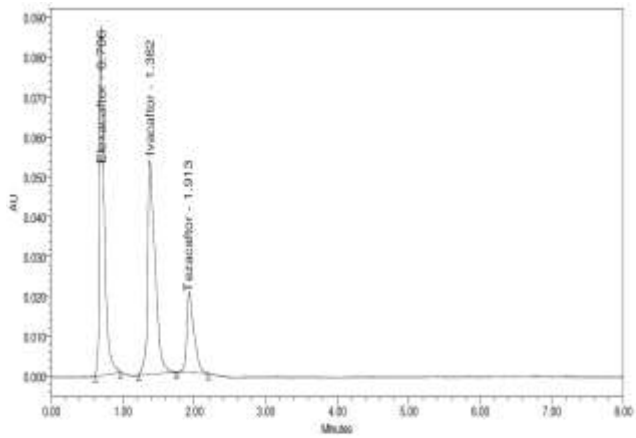

Fig. 3c: Chromatogram of oxidative degradation

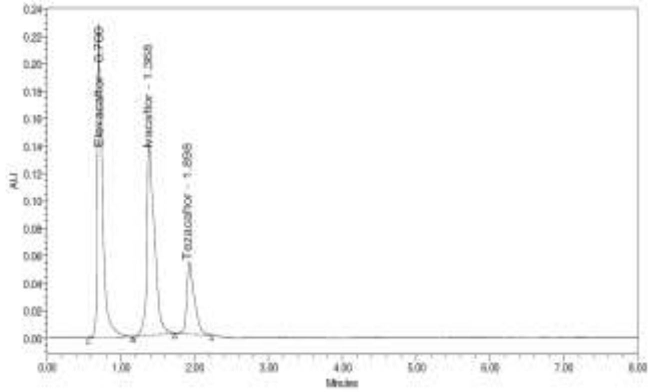

Fig. 3d: Chromatogram of thermal degradation

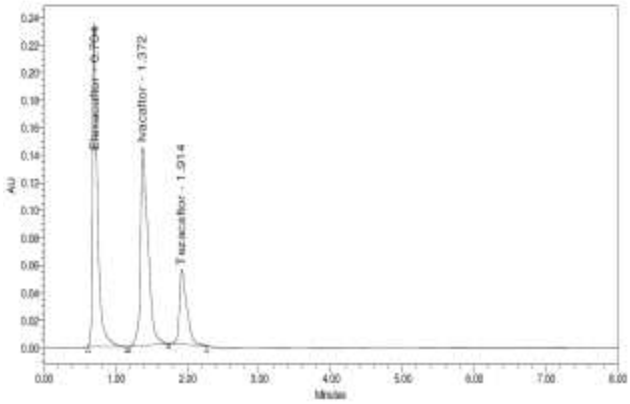

Fig. 3e: Chromatogram of photo degradation

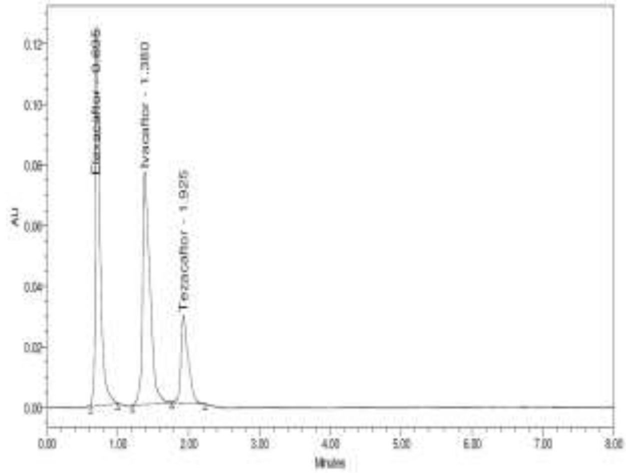

Fig. 3f: Chromatogram of neutral degradation

From the results of degradation studies, considerable degradation was observed under the prescribed different stress conditions such as acid (2N NaOH), base (2N HCl), hydrogen peroxide (20.0%) and thermal degradation confirming the stability indicating nature of the developed RP-UPLC method. The summary of forced degradation studies is given in table 1.

Method Validation: The proposed UPLC method was validated for parameters such as system suitability, specificity and linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision, robustness and ruggedness according to International Conference on Harmonization (ICH) guidelines.

System Suitability: After optimization of the proposed method, the efficiency of a chromatographic separation was monitored by applying the following system suitability tests: tailing factor and theoretical plates and resolution (Table 2).

Selectivity: The selectivity of the proposed method was evaluated by preparing solution of analytical blank and other solutions that were run in the instrument one after another. The results of these tests proved that the components other than the drug did not produce any detectable signal at the retention time of ivacaftor, tezacaftor and elexacaftor which confirmed the specificity of the method.

Linearity: Linearity for ivacaftor, tezacaftor and elexacaftor was established by analyzing six working standard concentrations ranging from 9.375-56.25µg/ml for ivacaftor, 6-25-37.5µg/ml for tezacaftor and 12.5-75µg/ml for elexacaftor prepared and injected in triplicate into the LC system and their respective chromatograms were recorded respectively.

The calibration graphs for the above three drugs were obtained by plotting peak area vs the concentration data. The linearity plots of ivacaftor, tezacaftor and elexacaftor were depicted in figures 4(a), (b) and (c) and the linearity results of both the drugs are given in table 3.

Excellent linearity was obtained for compounds between the peak areas and concentrations of 9.375-56.25µg/ml with r2 = 0.999 for ivacaftor, 6-25-37.5µg/ml with r2 = 0.999 for tezacaftor and 12.5-75µg/ml with r2 = 0.999 for elexacaftor respectively revealing the good linearity of the proposed RP-UPLC method.

LOD and LOQ: The LOD and LOQ value were found to be 0.14µg/ml and 0.43µg/ml for ivacaftor, 0.10µg/ml and 0.29µg/ml for tezacaftor and 0.47µg/ml and 1.42µg/ml for elexacaftor respectively which indicated the good sensitivity of the proposed method for ivacaftor, tezacaftor and elexacaftor respectively (Table 3).

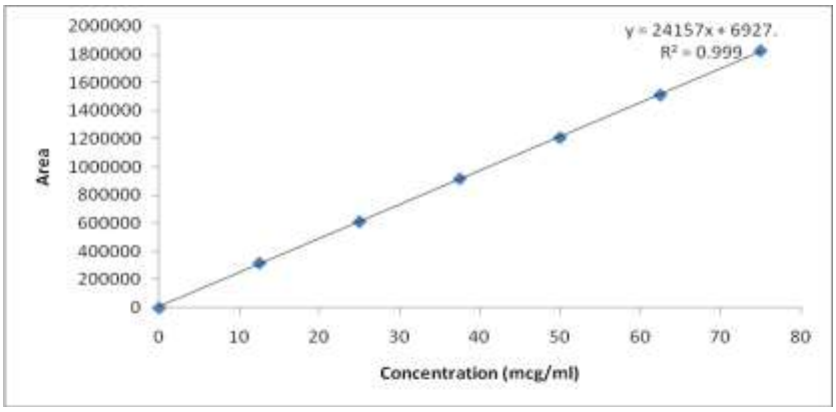

Fig. 4(a): Linearity plot of Elexacaftor

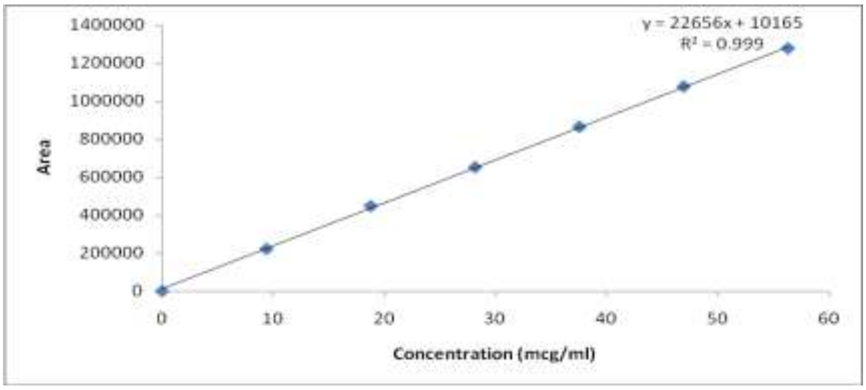

Fig. 4(b): Linearity plot of Ivacaftor

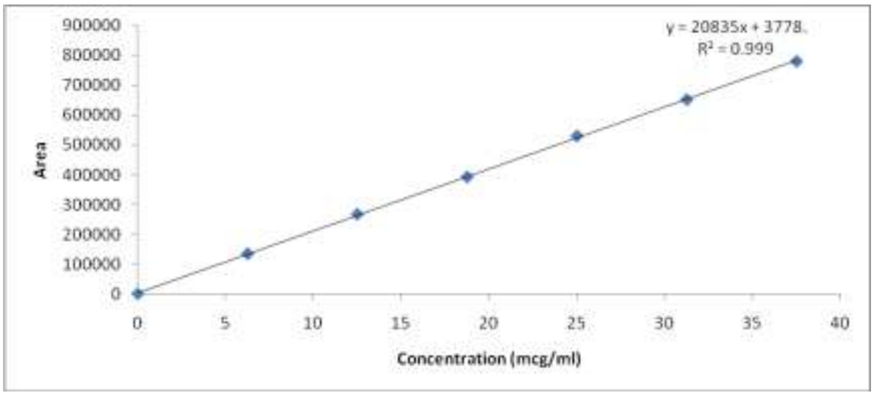

Fig. 4(c): Linearity plot of Tezacaftor

Table 3
Results of linearity studies of Ivacaftor, Tezacaftor and elexacaftor

Parameter	Ivacaftor	Tezacaftor	Elexacaftor
concentration range (µg/ml)	9.375-56.25	6-25-37.5	12.5-75
correlation coefficient	0.999	0.999	0.999
intercept	10165.5	3778.4	6927.5
slope	22656.3	20879	24156.7
LOD(µg/ml)	0.14	0.10	0.47
LOQ(µg/ml)	0.43	0.29	1.42

Precision: In the present study, the authors carried out repeatability that was carried out for short time interval under the same chromatographic conditions for ivacaftor, tezacaftor and elexacaftor. Homogeneous sample solutions of ivacaftor, tezacaftor and elexacaftor at 100% concentration were injected for 6 preparations into the above said column under controlled experimental conditions and the corresponding assay for all the six preparations was calculated and the mean and % relative standard deviation (%RSD) were calculated and the results are shown in table 4 respectively.

The results of above precision studies [repeatability] for ivacaftor, tezacaftor and elexacaftor were found to be precise as %RSD value for repeatability was within acceptable limits (RSD <2).

Accuracy: The accuracy of the present developed method was determined by standard addition method which was performed at three concentration levels of 50%, 100% and 150%. For this a known amount of standard drug powder was added to the fixed amount of pre-analyzed tablet solution and analyzed in triplicate at each level as per the proposed method and the % recovery at each level was calculated and presented in table 5. The results showed the best recovery i.e. 99.32-100.97% for ivacaftor, 99.32-101.53% for tezacaftor and 98.343-100.61% for elexacaftor respectively indicating that developed RP-UPLC method was accurate.

Table 4
Results of method precision studies (repeatability) of Ivacaftor, Tezacaftor and elexacaftor

S.N.	Tezacaftor	Ivacaftor	Elexacaftor
1.	100.60	99.97	98.95
2.	100.48	100.93	100.25
3.	100.74	101.34	99.88
4.	99.85	100.23	99.64
5.	98.84	100.52	99.03
6.	100.99	100.56	99.58
Average	100.25	100.59	99.56
Standard Deviation	0.79	0.49	0.50
*% RSD	0.80	0.50	0.50

Table 5
Results of accuracy studies of Ivacaftor, Tezacaftor and Elexacaftor

Spiked Level	Elexacaftor				
	Sample amount added (mcg/ml)	Sample amount recovered (mcg/ml)	% recovery	mean % recovery	%RSD
50%	25	25.0471	100.19	99.39	0.95
50%	25	24.5871	98.35		
50%	25	24.9058	99.62		
100%	50	49.5502	99.10	99.80	0.76
100%	50	50.304	100.61		
100%	50	49.8512	99.70		
150%	75	73.7583	98.34	99.40	0.93
150%	75	75.037	100.05		
150%	75	74.8566	99.81		

Spiked Level	Ivacaftor				
	Sample amount added (mcg/ml)	Sample amount recovered (mcg/ml)	% recovery	mean % recovery	%RSD
50%	18.75	18.7245	99.86	99.87	0.06
50%	18.75	18.7373	99.93		
50%	18.75	18.7165	99.82		
100%	37.5	37.5377	100.10	100.15	0.79
100%	37.5	37.2662	99.38		
100%	37.5	37.8622	100.97		
150%	56.25	55.8535	99.30	99.53	0.24
150%	56.25	56.1269	99.78		
150%	56.25	55.9788	99.52		

Spiked Level	Tezacaftor				
	Sample amount added (mcg/ml)	Sample amount recovered (mcg/ml)	% recovery	mean % recovery	%RSD
50%	12.5	12.5665	100.53	100.39	1.0
50%	12.5	12.6644	101.32		
50%	12.5	12.4145	99.32		
100%	25	25.0924	100.37	99.54	0.75
100%	25	24.8373	99.35		
100%	25	24.7287	98.91		
150%	37.5	37.2581	99.35	100.45	1.02
150%	37.5	37.734	100.62		
150%	37.5	38.018	101.38		

Table 6
Results of assay of Ivacaftor, Tezacaftor and Elexacaftor

Drugs	Label Claim	*% Assay
Elexacaftor	100mg	99.56
Ivacaftor	75mg	100.59
Tezacaftor	50mg	100.25

*Average of six determinations

Robustness: The robustness study of the proposed method was performed by slight modification in flow rate of the mobile phase and detection wavelength. The change was made in the flow rate ±0.05mL and detection wavelength by ±5nm. It was found that there were no significant changes in the chromatographic patterns for ivacaftor, tezacaftor and elexacaftor concluding that the proposed RP-UPLC method was robust.

Ruggedness: The ruggedness of the UPLC method was evaluated by carrying out the analysis using the standard working solution of ivacaftor, tezacaftor and elexacaftor with the same chromatographic system and the same column on different days and within the limits indicating that the developed RP-UPLC method was found to be rugged.

Assay of tablet dosage forms: Analysis of marketed tablets trikafta (Label claim 75mg, 50mg, 100mg of ivacaftor, tezacaftor and elexacaftor) was carried out using the proposed RP-UPLC method using the optimized UPLC conditions. The experimental results of the amount of ivacaftor, tezacaftor and elexacaftor in dosage forms were expressed as a percentage of label claim (99.56 % for Elexacaftor, 100.59 % for ivacaftor and 100.25 % for tezacaftor) and were in good agreement with the label claims thereby suggesting that there is no interference from any of the excipients which are normally present and the results are reported in table 6.

Conclusion
The proposed RP-UPLC strategy was seen as straightforward, explicit, exact, precise and affordable for synchronous estimation of ivacaftor, tezacaftor and elexacaftor in tablet dose structure. RP-UPLC technique is excellent for synchronous assurance of ivacaftor, tezacaftor and elexacaftor in their blend tranquilized item.

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