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RP-HPLC Stability Indicating Analytical Method Development and Validation For The Simultaneous Estimation Of Tezacaftor, Ivacaftor and Elexacaftor In API and Pharmaceutical Dosage Form

Mekala. Divya, K. Vinutha*, P Sridevi, M Bhagawan Raju

Department of Pharmaceutical Analysis, Sri Venkateshwara College of Pharmacy, Madhapur, Hyderabad, 500081.

ABSTRACT

We developed a straightforward, precise method for the simultaneous estimation of Ivacaftor, Elexacaftor and Tezacaftor in both bulk and tablet dosage forms. The chromatography analysis was performed using a Discovery C18 column measuring 150 x 4.6 mm with a 5µm particle size. The mobile phase, composed of Acetonitrile: Methanol: 0.1% OPA (10:35:55 v/v) was pumped at a flow rate of 0.9 ml/min, with the temperature maintained at 28°C. The optimized wavelength for detecting 3 drugs was set at 278.0 nm. Retention times were measured at 2.537 min, 2.089 min, and 3.090 min, respectively. The method precision showed low %RSD values, with 0.4 for Ivacaftor, 0.3 for Elexacaftor and 0.4 for Tezacaftor. Recovery percentages were determined as 99.79% for Ivacaftor, 99.72% for Elexacaftor and 100.05% for Tezacaftor. Furthermore, the LOD and LOQ values derived from the regression equations for Ivacaftor, Elexacaftor and Tezacaftor i.e $y = 22674x + 2799.3$, $y = 21285x + 4513.2$ and $y = 21548x + 869.63$ were found to be 0.06 ppm and 0.22 ppm, 0.18 ppm and 0.19 ppm , 0.07 ppm and 0.57 ppm respectively. With reduced retention times, this method offers simplicity and cost-effectiveness, making it suitable for routine quality control testing.

Keywords: Tezacaftor, Ivacaftor, Elexacaftor, RP-HPLC

*Corresponding Author Email: Vinuthasvcp11@gmail.com

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INTRODUCTION

Chemically, Tezacaftor can be defined as 1-(2,2-difluoro-2H-1,3-benzodioxol-5-yl)-N-{1-[(2R)-2,3-dihydroxypropyl]-6-fluoro-2-(1-hydroxy-2-methylpropan-2-yl)-1H-indol-5-yl} cyclopropane-1-carboxamide. It acts as a corrector for the cystic fibrosis transmembrane conductance regulator (CFTR) gene function [1]**.** Tezacaftor, when used in conjunction with ivacaftor, which was approved on February 12, 2018, to be used under prescription for the treatment of cystic fibrosis in individuals aged 12 years or older who possess two copies of the F508del mutation[2]**.**

Ivacaftor, characterized by the chemical formula N-(2,4-di-tert-butyl-5-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide, functions as a pharmaceutical agent employed in the treatment of cystic fibrosis in individuals with specific genetic mutations [2] **.** When administered as a monotherapy under the brand name Kalydeco, ivacaftor is approved for the management of cystic fibrosis in patients aged 2 years and older [3]

Elexacaftor, chemically represented as N-[(1,3-dimethyl-1H-pyrazol-4-yl) sulfonyl]-6-[3-(3,3,3-trifluoro-2,2-dimethylpropoxy)-1H-pyrazol-1-yl]-2-[(4S)-2,2,4-trimethylpyrrolidin-1-yl] pyridine-3-carboxamide, an advanced corrective agent for the cystic fibrosis transmembrane conductance regulator (CFTR) protein[4]. In October 2019, the FDA granted approval for Elexacaftor, in combination with Tezacaftor and Ivacaftor, as the triple combination product known as TrikaftaTM 5

From the literature survey, it was found that determination of Elexacaftor, Tezacaftor and Ivacaftor were estimated by analytical method such as novel stability indicating high performance liquid chromatographic method for determination of Ivacaftor in bulk drug and pharmaceutical dosage [6]**,** by spectrophotometric method such as UV spectrophotometer in method development and validation for simultaneous estimation of Tezacaftor and Ivacaftor in pharmaceutical dosage form [7]

As of our current understanding, there have been no reported methods for simultaneous determination of Ivacaftor, Tezacaftor and Elexacaftor. The primary objective of this study is to establish a cost-effective analytical method that is accurate, precise, sensitive, selective, reproducible, and rapid for the simultaneous estimation of Tezacaftor, Ivacaftor, and Elexacaftor in combination.

MATERIALS AND METHOD

Materials and Reagents:

The drug samples were sourced from Sri Venkateshwara College of Pharmacy and API from local market. For the study, we obtained AR grade Potassium Dihydrogen Ortho Phosphate, HPLC grade Water, HPLC grade Acetonitrile, AR grade Triethyl Amine, and AR grade Ortho Phosphoric

Acid from Ranchem. Additionally, HPLC grade water was meticulously prepared by passing reverse osmosis water through a Milli-Q System.

Instrumentation:

Chromatography analysis was conducted using the Waters HPLC 2695 series, which featured quaternary pumps, a Photo Diode Array detector, and an autosampler integrated with Empower software. The output signals were recorded with a Lab India UV double-beam spectrophotometer and continuously monitored through UVwin5. The method development involved the utilization of a Discovery C18 column measuring 150 x 4.6 mm with a 5µm particle size. The pH of the mobile phase was determined using a pH meter from BVK Enterprises in India and sample preparation was facilitated by an electronic balance from Denver and an ultrasonicator from BVK Enterprises. All measurement equipment underwent qualification procedures.

Chromatographic Conditions:

The method was established using a Discovery C18 column measuring 150 x 4.6 mm with a particle size of 5µm. The mobile phase was composed of a combination of Acetonitrile, Methanol and 0.1% OPA in the ratio of (10:35:55 v/v). The temperature was consistently maintained at 28°C.

The optimized wavelength for detecting Ivacaftor, Elexacaftor, and Tezacaftor was set at 276.0 nm. The mobile phase was delivered at a flow rate of 0.9 ml/min, and the column temperature was held at 28°C. Under these specified conditions, the total run time for the chromatograms was consistently set at 5 minutes.

Preparation of Mobile phase:

Buffer Preparation:

So as to prepare 0.1% OPA buffer, 1 ml of concentrated Ortho Phosphoric acid was diluted with water to a final volume of 1000 ml.

Diluents:

Based up on the solubility of the drug diluents was selected Water: Acetonitrile: Methanol (30:60:10 v/v)

Preparation of Standard Stock Solutions:

Precisely 10 mg of Tezacaftor, 15 mg of Ivacaftor, and 20 mg of Elexacaftor were weighed and transferred each into separate 50 ml volumetric flasks. 3/4 ml of diluent was added to each flask and sonicated for 20 minutes. Flasks are made up to the mark with diluent and labelled them as Standard Stock Solution 1 (contained of 200 µg/ml of Tezacaftor, 300 µg/ml of Ivacaftor, and 400 µg/ml of Elexacaftor).

Preparation of Standard Working Solutions (100% Solution):

1 ml from each stock solution was pipetted and transferred it into separate 10 ml volumetric flasks. The volume was made up to the mark with diluent. This resulted in solutions which contained 20 µg/ml of Tezacaftor, 30 µg/ml of Ivacaftor, and 40 µg/ml of Elexacaftor.

Preparation of Sample Stock Solutions:

5 tablets were weighed and the average weight of each tablet was calculated. The weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask. 25 ml of diluent was added and sonicated for 50 minutes. Then the volume was made up to the mark with diluent and the solution was filtered. This resulted in the solutions which contained 500 µg/ml of Tezacaftor, 750 µg/ml of Ivacaftor, and 1000 µg/ml of Elexacaftor.

Preparation of Sample Working Solutions (100% Solution):

0.4 ml from the filtered solution was pipetted and transferred it into separate 10 ml volumetric flasks. The volume was made up to the mark with diluent. This resulted in solutions which contained 20 µg/ml of Tezacaftor, 30 µg/ml of Ivacaftor, and 40 µg/ml of Elexacaftor.

Method development and selection of wavelength

Method development: Method development was done by changing various columns, mobile phase ratios, buffers and its pH etc. In this optimized method, drugs were eluted with good retention time, resolution and all the system suitable parameters like Plate count and Tailing factor were within the limits [9]. Optimized chromatogram was given below in figure 3.

Ivacaftor, Elexacaftor and Tezacaftor were eluted at 2.537 min, 2.089min and 3.090 min respectively with good resolution. Plate count and tailing factor were very satisfactory, so this method was optimized and to be validated. After scanning from 400 to 200nm in UV-VIS spectrophotometer, Tezacaftor, Ivacaftor and Elexacaftor was showed absorption maxima at 278.0 nm in diluent solution. UV spectra of drug given in figure.

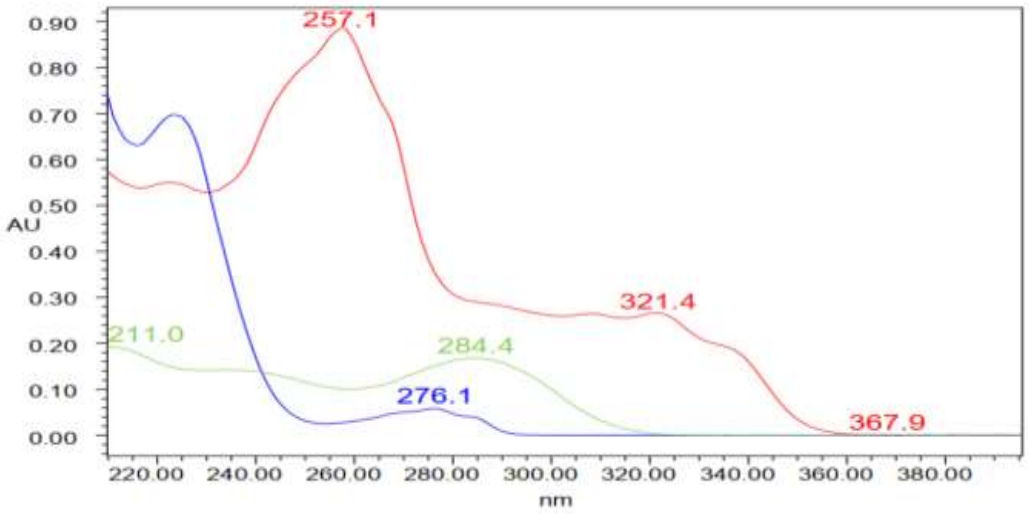

Figure: UV spectra

METHOD VALIDATION [10]:

System suitability parameters:

System suitability parameters were ascertained by preparing standard solutions of Tezacaftor, Ivacaftor, and Elexacaftor, and subsequently injecting these solutions six times. The evaluation included the determination of parameters such as peak tailing, resolution, and USP plate count. Results were given below in table 1.

Specificity:

This test was conducted to assess the presence of interference in the optimized method. If no interfering peaks were detected in the blank and placebo samples at the retention times corresponding to these drugs in the method, the method was considered specific. Results were given below in figure1, 2 and 3.

Linearity:

For the preparation of standard stock solutions:

10 mg of Tezacaftor, 15 mg of Ivacaftar, and 20 mg of Elexacaftor were accurately weighed and individually transferred into 50 ml volumetric flasks. To each flask, 3/4 ml of diluent was added, followed by sonication for 20 minutes. The flasks were then brought to volume with diluent and labeled as Standard Stock Solution 1, with concentrations of 200 µg/ml for Tezacaftor, 300 µg/ml for Ivacaftar, and 400 µg/ml for Elexacaftor.

To prepare the 25% standard solution:

0.25 ml was pipetted from the standard stock solution and made up to 10 ml, resulting in concentrations of 5 µg/ml for Tezacaftor, 7.5 µg/ml for Ivacaftar, and 10 µg/ml for Elexacaftor.

For the 50% standard solution:

0.5 ml was pipetted from the standard stock solution and made up to 10 ml, yielding concentrations of 10 µg/ml for Tezacaftor, 15 µg/ml for Ivacaftar, and 20 µg/ml for Elexacaftor.

Similarly, for the 75% standard solution:

0.75 ml was pipetted from the standard stock solution and made up to 10 ml, resulting in concentrations of 15 µg/ml for Tezacaftor, 22.5 µg/ml for Ivacaftar, and 30 µg/ml for Elexacaftor.

100% Standard Solution:

1.0 ml was withdrawn from the standard stock solutions and made up to 10 ml, resulting in concentrations of 20 µg/ml for Tezacaftor, 30 µg/ml for Ivacaftor, and 40 µg/ml for Elexacaftor.

125% Standard Solution:

For this solution, 1.25 ml was pipetted from the standard stock solutions and diluted to 10 ml, yielding concentrations of 25 µg/ml for Tezacaftor, 37.5 µg/ml for Ivacaftor, and 50 µg/ml for Elexacaftor**.**

150% Standard Solution:

1.5 ml was drawn from the standard stock solutions and made up to 10 ml, resulting in concentrations of 30 µg/ml for Tezacaftor, 45 µg/ml for Ivacaftor, and 60 µg/ml for Elexacaftor. Calibration curves and results were given below in figures 4, 5, 6 and table 2.

Precision:

For the preparation of sample stock solutions:

Five tablets were weighed, and the average weight of each tablet was calculated. The amount equivalent to one tablet was then transferred into a 100 mL volumetric flask. Subsequently, 25 mL of diluent was added, and the solution was sonicated for 50 minutes. The volume was adjusted to the mark with diluent, resulting in solutions with concentrations of 500 µg/ml for Tezacaftor, 750 µg/ml for Ivacaftor, and 1000 µg/ml for Elexacaftor.

For the preparation of sample working solutions (100% solutions):

0.4 ml of the filtered solution was pipetted into separate 10 ml volumetric flasks and made up to 10 ml with diluent, yielding solutions with concentrations of 20 µg/ml for Tezacaftor, 30 µg/ml for Ivacaftor, and 40 µg/ml for Elexacaftor. Results were given in tables 3,4,5.

Accuracy:

For the preparation of sample stock solutions:

Five tablets were weighed, and the average weight of each tablet was calculated. The amount equivalent to one tablet was then transferred into a 100 mL volumetric flask. To this, 25 mL of diluent was added and sonicated for 50 minutes. The volume was adjusted to the mark with

diluent, resulting in solutions with concentrations of 500 µg/ml for Tezacaftor, 750 µg/ml for Ivacaftor, and 1000 µg/ml for Elexacaftor.

For the preparation of standard working solutions (100% solutions):

1 ml was pipetted from the stock solution and transferred into separate 10 ml volumetric flasks, resulting in concentrations of 20 µg/ml for Tezacaftor, 30 µg/ml for Ivacaftor, and 40 µg/ml for Elexacaftor.

To prepare the 50% Spiked Solution:

0.5 ml of the sample stock solution was taken into a 10 ml volumetric flask. To this, 1.0 ml from each standard stock solution was pipetted out and made up to the mark with diluent.

Preparation of 100% Spiked Solution:

1.0ml 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:

1.5ml 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. Results were given in tables 6, 7, 8.

Robustness:

Minor deliberate adjustments were introduced to the method, including variations in flow rate, mobile phase ratio, and temperature. These modifications did not yield any discernible alterations in the results and remained within the acceptable range according to ICH guidelines. Robustness testing involved imposing conditions such as Flow Minus (0.8 ml/min), Flow Plus (0.9 ml/min), Mobile Phase Minus, Mobile Phase Plus, Temperature Minus (25°C), and Temperature Plus (35°C). Samples were injected in duplicate under these conditions. If the system suitability parameters exhibited minimal impact, all parameters were deemed to have met the specified criteria. Results were given in table 9.

Sensitivity:

LOD Sample Preparation:

To prepare the Limit of Detection (LOD) samples, 0.25 ml was pipetted from the standard stock solutions and transferred to a 10 ml volumetric flask. The volume was adjusted to the mark with diluent. Additionally, 0.3 ml of Tezacaftor, Ivacaftor, and Elexacaftor solutions, respectively, were transferred to separate 10 ml volumetric flasks and adjusted to volume with the same diluent.

LOQ Sample Preparation:

For the Limit of Quantification (LOQ) samples, 0.25 ml was pipetted from the standard stock solution and transferred to a 10 ml volumetric flask, followed by dilution with diluent. Furthermore, 0.3 ml of Tezacaftor, Ivacaftor, and Elexacaftor solutions, respectively, were transferred to individual 10 ml volumetric flasks and adjusted to volume with the same diluent. Results were given in figures 7, 8 and table 10.

Degradation Studies [11,12]

Oxidation:

For oxidation studies, 1 ml of the stock solutions of Tezacaftor, Ivacaftor, and Elexacaftor was separately combined with 1 ml of 20% hydrogen peroxide (H2O2). These solutions were then incubated at 60°C for 30 minutes. Subsequently, for HPLC analysis, the resulting solutions were diluted to achieve concentrations of 20 µg/ml, 30 µg/ml, and 40 µg/ml for all components. Ten microliters (10 µl) of each solution were injected into the system, and chromatograms were recorded to evaluate the sample's stability.

Acid Degradation Studies:

To investigate acid degradation, 1 ml of the stock solutions of Tezacaftor, Ivacaftor, and Elexacaftor was treated with 1 ml of 2N Hydrochloric acid and refluxed for 30 minutes at 60°C. The resulting solutions were then diluted to obtain concentrations of 20 µg/ml, 30 µg/ml, and 40 µg/ml for all components. Ten microliters (10 µl) of each solution were injected into the system, and chromatograms were recorded to assess the sample's stability.

Alkali Degradation Studies:

To investigate alkali degradation, 1 ml of the stock solutions of Tezacaftor, Ivacaftor, and Elexacaftor was combined with 1 ml of 2N sodium hydroxide and refluxed for 30 minutes at 60°C. The resulting solution was then diluted to achieve concentrations of 20 µg/ml, 30 µg/ml, and 40 µg/ml for all components. Ten microliters (10µl) of each solution were injected into the system, and chromatograms were recorded to evaluate the sample's stability.

Dry Heat Degradation Studies:

Dry heat degradation was studied by subjecting the standard drug solution to an oven at 105°C for 1 hour. For HPLC analysis, the resulting solution was diluted to obtain concentrations of 20 µg/ml, 30 µg/ml, and 40 µg/ml for all components. Ten microliters (10 µl) of each solution were injected into the system, and chromatograms were recorded to assess the sample's stability.

Photo Stability Studies:

The photochemical stability of the drug was examined by exposing solutions with concentrations of 200 µg/ml, 300 µg/ml, and 400 µg/ml to UV light in a UV chamber for 1 day or 200-Watt

hours/m2 in a photo stability chamber. For HPLC analysis, the resulting solution was diluted to obtain concentrations of 20 µg/ml for Tezacaftor, 30 µg/ml for Ivacaftor, and 40 µg/ml for Elexacaftor. Ten microliters (10 µl) of each solution were injected into the system, and chromatograms were recorded to assess the sample's stability.

Neutral Degradation Studies:

To study stress testing under neutral conditions, the drug was refluxed in water for 6 hours at a temperature of 60ºC. For HPLC analysis, the resulting solution was diluted to obtain concentrations of 20 µg/ml for Tezacaftor, 30 µg/ml for Ivacaftor, and 40 µg/ml for Elexacaftor. Ten microliters (10 µl) of each solution were injected into the system, and chromatograms were recorded to assess the sample's stability. Results were given in tables 14, 15, 16.

RESULTS AND DISCUSSION:

System suitability:

Blank and standard solutions were injected and recorded the chromatograms. 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 suitability parameters were passed and were within the limits.

Table 1: Results for system suitability are given as below.

Elexacaftor			**Ivacaftor**				**Tezacaftor**			
RT(min)	TP	Tailing	RT(min)	TP	Tailing	RS	RT(min)	TP	Tailing	RS
2.086	6082	1.54	2.533	7698	1.32	4.9	3.085	8284	1.37	4.2
2.087	6283	1.54	2.534	7801	1.33	4.9	3.085	8237	1.38	4.2
2.089	6057	1.6	2.535	7903	1.33	4.8	3.086	8348	1.37	4.3
2.089	6111	1.6	2.537	7626	1.39	4.8	3.090	8524	1.39	4.3
2.090	6408	1.66	2.538	7423	1.42	4.7	3.091	8585	1.39	4.3
2.099	5877	1.56	2.547	7292	1.33	4.8	3.100	8330	1.38	4.2

Specificity:

Retention times of Ivacaftor, Elexacaftor and Tezacaftor were 2.537 min, 2.089 min and 3.090min 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.

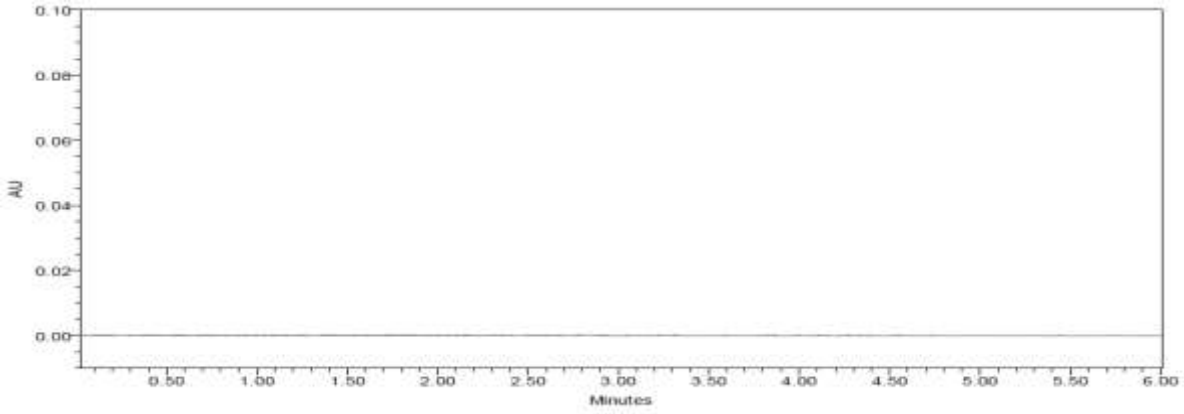

Figure 1: Blank chromatogram

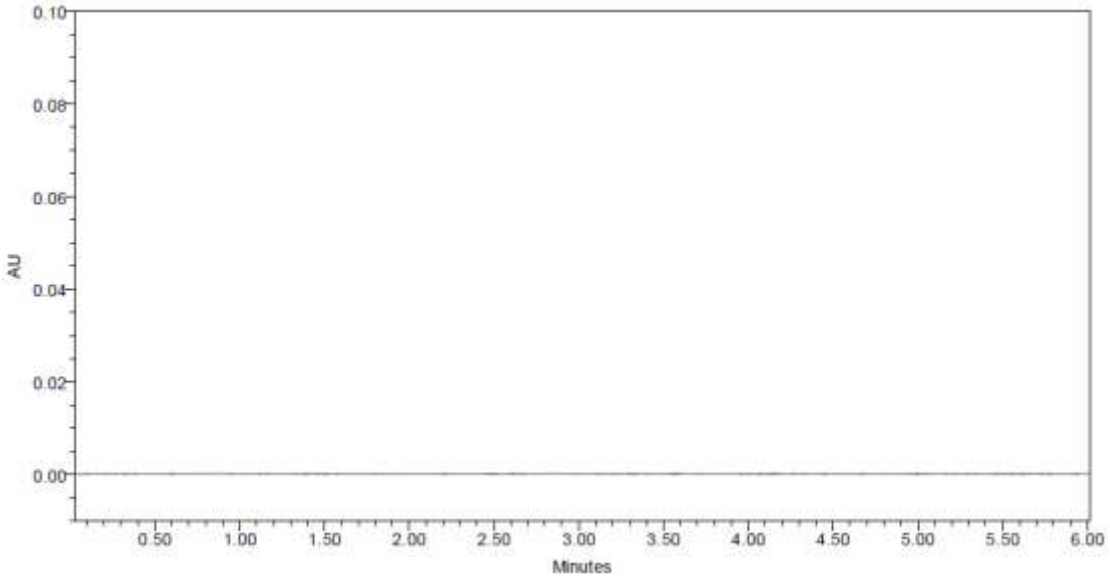

Figure 2: Placebo chromatogram

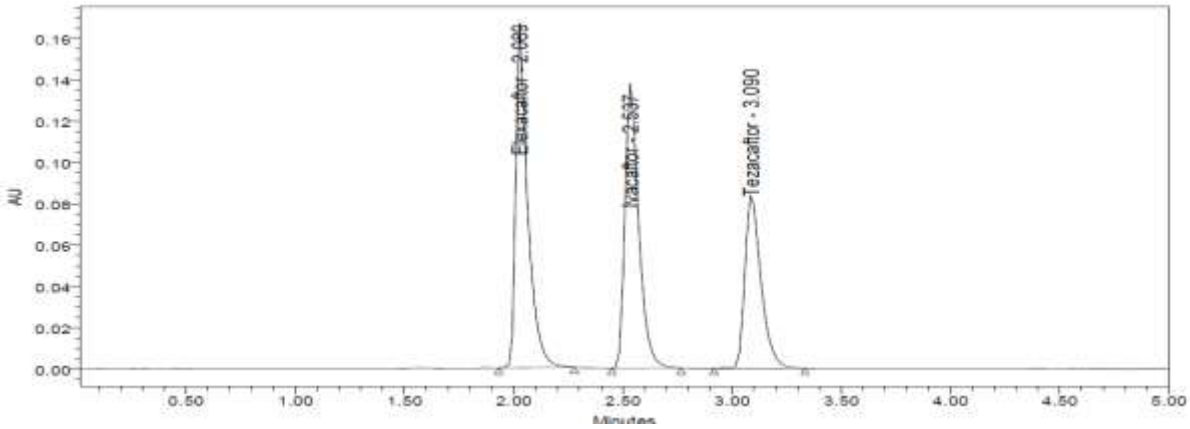

Figure 3: Optimized chromatogram

Linearity:

Six linear concentrations of Tezacaftor. (5-30µg/ml), Ivacaftor (7.5-45µg/ml) and Elexacaftor (10-60 µg /ml) were injected in a Duplicate manner. Average areas were mentioned above and linearity

equations obtained for Tezacaftor was y = 21548x + 869.63. Ivacaftor was y = 22674x + 2799.3 and of Elexacaftor was y = 21285x + 4513.2. Correlation coefficient obtained was 0.999 for all the three drugs.

Table 2: 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
5	103662	7.5	172601	10	215342
10	219398	15	345261	20	436111
15	328247	22.5	510891	30	641226
20	433108	30	689406	40	866858
25	542251	37.5	855253	50	1065838
30	641929	45	1017384	60	1275983

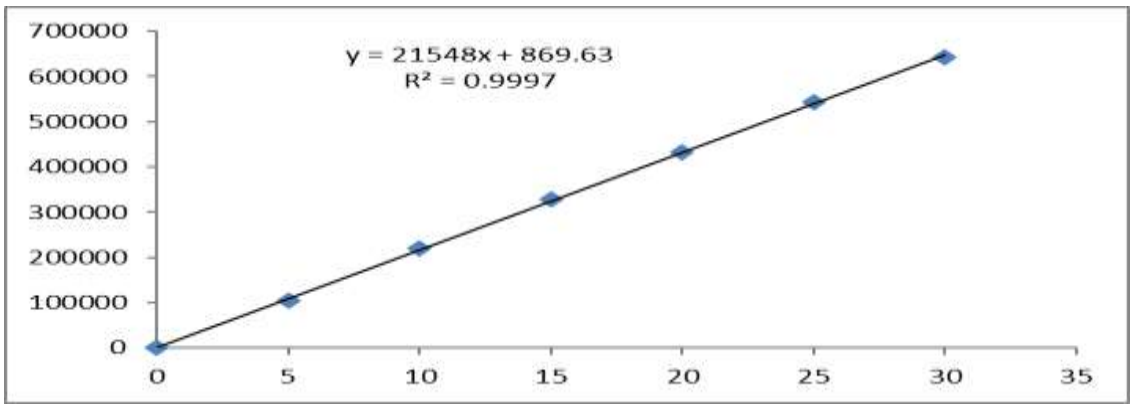

Figure 4: Calibration curve of Tezacaftor

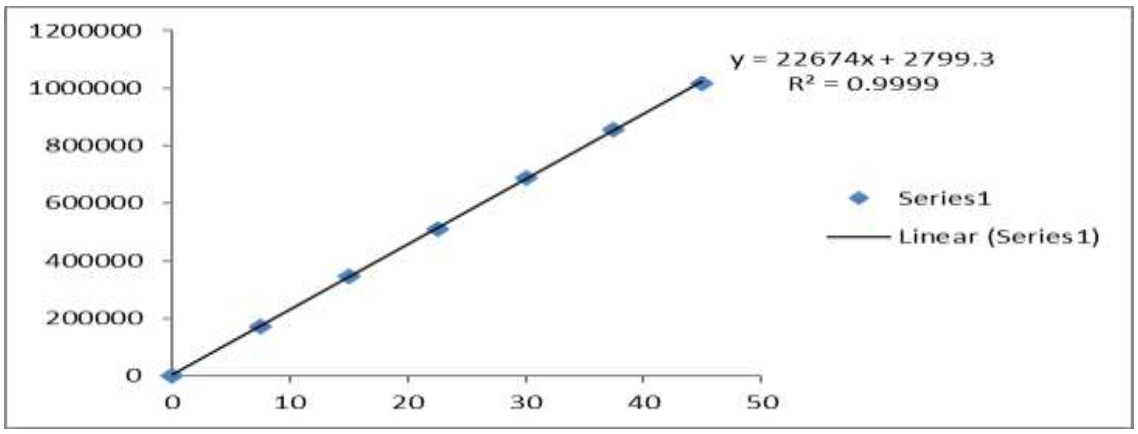

Figure 5: Calibration curve of Ivacaftor

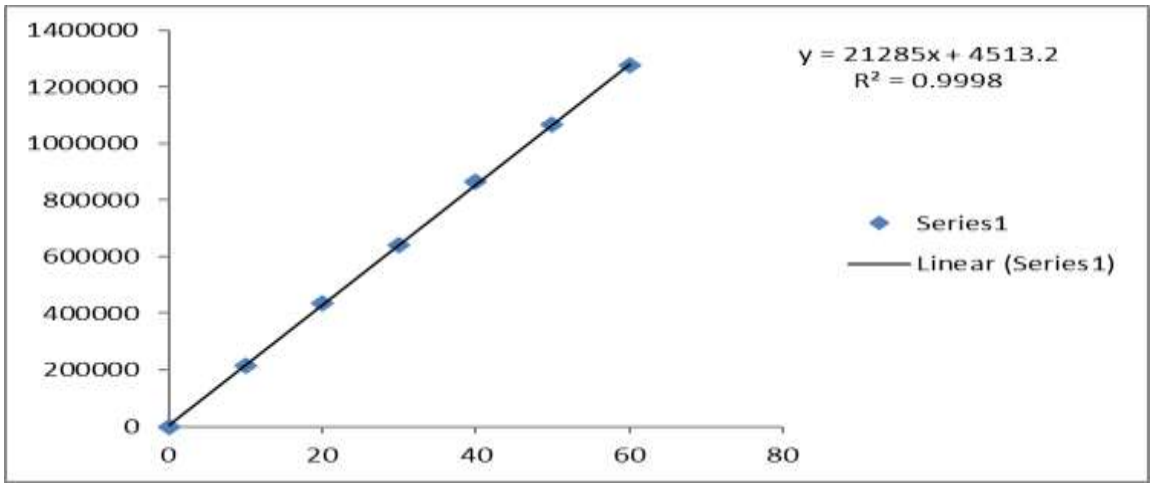

Figure 6: Calibration curve of Elexacaftor

Precision:

System Precision: 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 0.6%, 0.4% and 0.3% respectively for Tezacaftor, Ivacaftor and Elexacaftor. As the limit of Precision was less than "2" the system precision was passed in this method.

Table 3: System precision table of Tezacaftor, Ivacaftor and Elexacaftor.

S. No	Area of Tezacaftor	Area of Ivacaftor	Area of Elexacaftor
1.	438305	686055	862970
2.	436074	684208	860668
3.	432945	686220	864350
4.	432139	690942	867042
5.	431853	684779	866877
6.	436056	687505	866506
Mean	434562	686618	864736
S.D	2621.2	2414.5	2563.2
%RSD	0.6	0.4	0.3

Repeatability:

Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated for three drugs and obtained as 0.4%, 0.3% and 0.4% respectively for Tezacaftor, Ivacaftor and Elexacaftor. As the limit of Precision was less than "2" the system precision was passed in this method.

Table 4: Repeatability table of Tezacaftor, Ivacaftor and Elexacaftor.

S. No	Area of Tezacaftor.	Area of Ivacaftor	Area of Elexacaftor
1.	433928	689790	869467
2.	435245	687718	863832
3.	437317	683887	862512
4.	434161	686660	861439
5.	432373	687501	869128
6.	435884	685159	861414
Mean	434818	686786	864632
S.D	1718.5	2072.2	3722.3
%RSD	0.4	0.3	0.4

Intermediate precision (Day Precision):

Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given on the next day of the sample preparation and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated for three drugs and obtained as 1.1%, 0.3% and 0.3% respectively for Tezacaftor, Ivacaftor and Elexacaftor. As the limit of Precision was less than "2" the system precision was passed in this method.

Table 5: Intermediate precision table of Tezacaftor, Ivacaftor and Elexacaftor.

S. No	Area of Tezacaftor	Area of Ivacaftor	Area of Elexacaftor
1.	437261	689226	862635
2.	434923	685405	863018
3.	434658	683576	860598
4.	438889	688261	865159
5.	439164	684441	867118
6.	425907	684007	866104
Mean	435134	685819	864105
S.D	4904.6	2364.6	2442.4
%RSD	1.1	0.3	0.3

Accuracy:

Three levels of Accuracy sample were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 99.79%, 99.72% and 100.05% for Tezacaftor, Ivacaftor and Elexacaftor respectively.

Table 6: Accuracy table of Tezacaftor.

% Level	Amount Spiked (µg/mL)	Amount recovered(µg/mL)	% Recovery	Mean %Recovery
50%	10	10.013	100.13	99.79%
	10	9.907	99.07	
	10	9.928	99.28	

100%	20	19.808	99.04
	20	20.003	100.02
	20	20.066	100.33
150%	30	19.808	99.04
	30	20.003	100.02
	30	20.066	100.33

Table 7: Accuracy table of Ivacaftor

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	15	14.914	99.43	99.72%
	15	14.906	99.37	
	15	14.911	99.40	
100%	30	29.875	99.58	
	30	30.008	100.03	
	30	30.020	100.07	
150%	45	45.249	100.55	
	45	44.555	99.01	
	45	45.023	100.05	

Table 8: Accuracy table of Elexacaftor

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	20	20.12	100.58	100.05%
	20	19.91	99.56	
	20	20.11	100.57	
100%	40	40.19	100.48	
	40	39.93	99.82	
	40	39.78	99.45	
150%	60	59.98	99.97	
	60	59.72	99.54	
	60	60.31	100.51	

Robustness:

Robustness conditions like Flow minus (0.8ml/min), Flow plus (0.9ml/min), mobile phase minus (45B:40A:15M A), mobile phase plus (65B:25A:10A), temperature minus (26°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit**.**

Table 9: Robustness data for Tezacaftor, Ivacaftor and Elexacaftor.

S.no	Condition	%RSD of Ivacaftor	%RSD of Elexacaftor	%RSD of Tezacaftor.
1	Flow rate (-) 0.8ml/min	0.3	0.4	0.3
2	Flow rate (+) 0.9ml/min	0.6	0.5	1.3
3	Mobile phase (-) 45B:40A:15M	0.5	0.6	0.7
4	Mobile phase (+) 65B:25A:10	0.3	0.3	1.0
5	Temperature (-) 26°C	0.7	0.3	0.9

6	Temperature (+) 35°C	0.7	0.5	0.7

Sensitivity: Sensitivity table of Tezacaftor, Ivacaftor and Elexacaftor was given below

Table 10: LOD and LOQ for Tezacaftor, Ivacaftor and Elexacaftor.

Molecule	LOD(µg/ml)	LOQ(µg/ml)
Tezacaftor.	0.06µg/ml	0.18 µg/ml
Ivacaftor	0.08 µg/ml	0.23 µg/ml
Elexacaftor	0.19 µg/ml	0.57 µg/ml

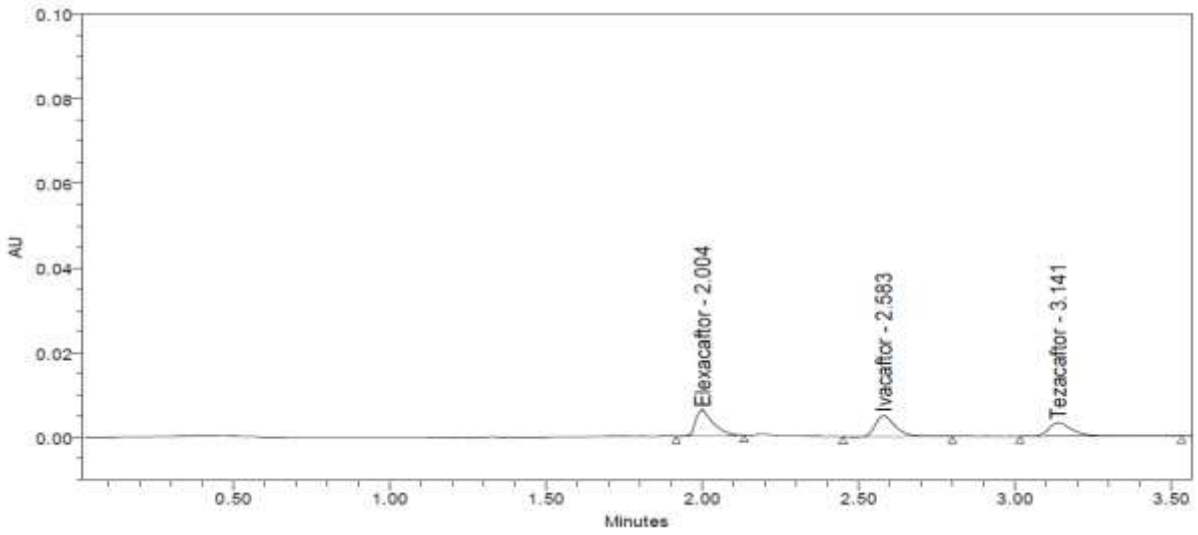

Figure 7: LOD chromatogram of standard

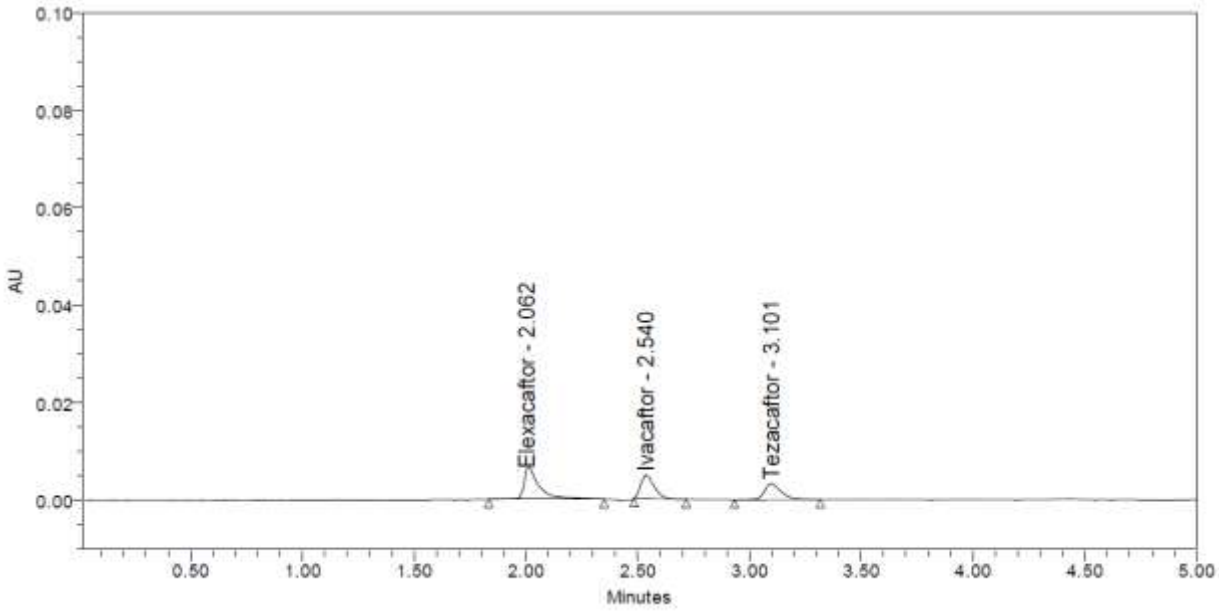

Figure 8: LOQ chromatogram of standard

Assay:

The label claim was TRIKAFTA Ivacaftor 50mg, Tezacaftor 75mg, Elexacaftor 100mg per unit formulation. Assay was performed with the above formulation.

Preparation of Sample stock solutions:

5 tablets were weighed and the average weight of each tablet was calculated. Later the weight equivalent to 1 tablet was transferred into a 100 mL volumetric flask, 25mL of diluent was added and sonicated for 50 min, further the volume made up with diluent and filtered. (500µg/ml of Tezacaftor, 750µg/ml of Ivacaftor and 1000µg/ml of Elexacaftor)

Preparation of Sample working solutions (100% solution):

From the filtered solution 0.4ml was pipetted out into a 10 ml volumetric flask and made up to 10ml with diluents. (20µg/ml of Tezacaftor, 30µg/ml of Ivacaftor and 40µg/ml of Elexacaftor)

Table 11: Assay Data of Tezacaftor.

S.no	Standard Area	Sample area	% Assay
1	438305	433928	99.65
2	436074	435245	99.96
3	432945	437317	100.43
4	432139	434161	99.71
5	431853	432373	99.30
6	436056	435884	100.10
Avg	434562	434818	99.86
St dev	2621.2	1718.5	0.395
%RSD	0.6	0.4	0.4

Table 12: Assay Data of Ivacaftor

S.no	Standard Area	Sample area	% Assay
1	686055	689790	100.26
2	684208	687718	99.96
3	686220	683887	99.40
4	690942	686660	99.81
5	684779	687501	99.93
6	687505	685159	99.59
Avg	686618	686786	99.82
St dev	2414.5	2072.2	0.30
%RSD	0.4	0.3	0.3

Table 13: Assay Data of Elexacaftor

S.no	Standard Area	Sample area	% Assay
1	862970	869467	100.45
2	860668	863832	99.80
3	864350	862512	99.64
4	867042	861439	99.52
5	866877	869128	100.41
6	866506	861414	99.52
Avg	864736	864632	99.89
St dev	2563.2	3722.3	0.430
%RSD	0.3	0.4	0.4

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

Table 14: Degradation Data of Tezacaftor.

S.NO	Degradation Condition	Area	% Area Recovery	% Drug Degraded
1	Acid	411508	94.51	5.49
2	Alkali	406508	93.36	6.64
3	Oxidation	425537	97.73	2.27
4	Thermal	229294	52.66	47.34
5	UV	406235	93.29	6.71
6	Water	433812	99.63	0.37

Table 15: Degradation Data of Ivacaftor

S.NO	Degradation Condition	Area	% Area Recovery	% Drug Degraded
1	Acid	651508	94.70	5.30
2	Alkali	641508	93.24	6.76
3	Oxidation	673537	97.90	2.10
4	Thermal	649294	94.37	5.63
5	UV	669235	97.27	2.73
6	Water	684812	99.54	0.46

Table 16: Degradation Data of Elexacaftor

S.NO	Degradation Condition	Area	% Area Recovery	% Drug Degraded
1	Acid	811259	93.72	6.28
2	Alkali	811259	93.72	6.28
3	Oxidation	839880	97.03	2.97
4	Thermal	809055	93.47	6.53
5	UV	839718	97.01	2.99
6	Water	860667	99.43	0.57

CONCLUSION:

A straightforward, precise method has been successfully developed for the simultaneous quantification of Ivacaftor, Elexacaftor, and Tezacaftor in both bulk and tablet dosage forms. The retention times for Ivacaftor, Elexacaftor, and Tezacaftor were determined to be 2.537 minutes, 2.089 minutes, and 3.090 minutes, respectively. The %RSD for system precision for Ivacaftor, Elexacaftor, and Tezacaftor was found to be 0.6, 0.4, and 0.3, respectively, while the %RSD for method precision for these components was 0.4, 0.3, and 0.4, respectively. High levels of accuracy were achieved with % recovery values of 99.79% for Ivacaftor, 99.72% for Elexacaftor, and 100.05% for Tezacaftor. The Limit of Detection (LOD) and Limit of Quantification (LOQ) values were established through regression equations, with values of 0.06 ppm and 0.18 ppm for Ivacaftor, 0.07 ppm and 0.22 ppm for Elexacaftor, and 0.19 ppm and 0.57 ppm for Tezacaftor, respectively. The regression equations for Tezacaftor, Ivacaftor, and Elexacaftor were as follows: y

= 21548x + 869.63, y = 22674x + 2799.3, and y = 21285x + 4513.2. The reduction in retention times indicates that the developed method is both straightforward and cost-effective, making it suitable for routine quality control testing.

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