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Research Article

RP-HPLC method development and validation for the simultaneous determination of elexacaftor, ivacator and tezacaftor in pharmaceutical dosage forms

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ABSTRACT

A simple, accurate, precise method was developed for the simultaneous estimation of the Ivacaftor, Elexacaftor and Tezacaftor in bulk and tablet dosage form. Chromatogram was run through Ascentis C18 150 x 4.6 mm, 2.4m. Mobile phase containing acetonitrile & NH2PO4 in the ratio of 60:40 v/v was pumped through column at a flow rate of 1ml/min. Temperature was maintained at 30°C. Optimized wavelength for Ivacaftor, Elexacaftor and Tezacaftor was 258.0 nm. Retention time of Ivacaftor, Elexacaftor and Tezacaftor were found to be 2.798 min, 2.137 min and 3.284 min %RSD of system precision for Ivacaftor, Elexacaftor and Tezacaftor were and found to be 0.4, 0.3 and 0.5 respectively. %RSD of method precision for Ivacaftor, Elexacaftor and Tezacaftor were and found to be 0.6,0.3, and 0.5 respectively. % recovery was obtained as 99.93%, 100.07% and 100.17% for Ivacaftor, Elexacaftor and Tezacaftor respectively. LOD, LOQ values are obtained from regression equations of Ivacaftor, Elexacaftor and Tezacaftor were 0.07 ppm, 0.09 ppm, 0.02 ppm and 0.22 ppm, 0.28 ppm, 0.7 ppm respectively. Regression equation of Tezacaftor was y = 23377x + 200.3, Ivacaftor was y = 17233x + 3180 and of Elexacaftor was y = 25892x + 1146. Retention times are decreased so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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

INTRODUCTION

Ivacaftor (also known as Kalydeco or VX- 770) is a drug used for the management of Cystic Fibrosis (CF) in patients. The approval was done on 25, 2020 by Vertex Pharmaceuticals. Cystic Fibrosis is caused by one of several different mutations in the gene for the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) protein, an ion channel involved in the transport of chloride and sodium ions across cell membrane. Alterations in the CFTR gene result in altered production,

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misfolding or function of the protein and consequently abnormal fluid and ion transport across cell membrane. As a result, CF patients produce a thick, sticky mucus that clogs the ducts of organs where it is produced making patients more susceptible to complications such infections, lung damage, pancreatic insufficiency and malnutrition.

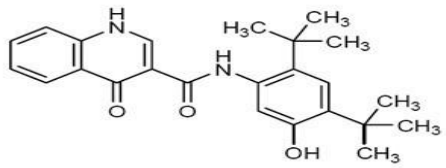

Figure-1 Structure of Ivacaftor

Tezacaftor is small molecule that can be used as a corrector of the cystic fibrosis transmembrane conductance regulator (CFTR) gene function. It was developed by Vertex Pharmaceuticals and FDA approval in combination with ivacaftor; a CFTR potentiator that allow the proteins at the cell surface to open longer and improve nutrient transport. The approval was done on February 12, 2018 to be used under prescription.

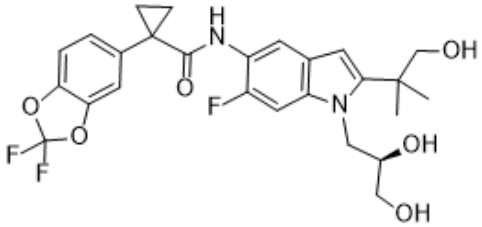

Figure-2 Structure of Tezacaftor

Elexacaftor (previously VX-445) is a small molecule, next generation corrector of the CFTR protein. It received FDA approval in October 2019 in combination with Tezacaftor and Ivacaftor as the combination product Trikafta. The triple combination product Trikafta, manufactured by Vertex Pharmaceuticals, is the first product approved for the treatment of CF in individuals who are either homo or heterozygous for the F508del-CFTR gene.

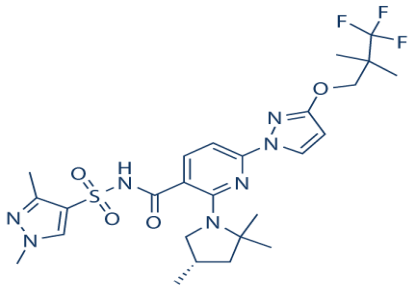

Figure-3 Structure of Elexacaftor

MATERIALS AND REAGENTS

Elexacaftor, Ivacaftor, Tezacaftor pure drugs (API), Combination Elexacaftor, Ivacaftor, Tezacaftor Pharmaceutical Dosage form. Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Ranchem

Instrumentation and chromatographic conditions: Electronic balance-Denver, pH meter-BVK enterprises India, Ultra sonicator-BVK enterprises, WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and Autosampler integrated with Empower 2 Software, Lab India UV double beam spectrophotometer with UVwin5.

Sample Processing

Diluents: Based up on the solubility of the drug diluent was selected Water: Acetonitrile (50:50 v/v)

Preparation of Standard stock solutions: Accurately weighed 5mg of Tezacaftor, 7.5mg of Ivacaftor and 10mg of Elexacaftor and transferred to three 50ml volumetric flasks separately. 10ml of diluent was added to flasks and sonicated for 20mins. Flasks were made up Diluent and labeled as Standard stock solution 1, 2 and 3. (100µg/ml of Tezacaftor, 150µg/ml of Ivacaftor and 200µg/ml of Elexacaftor)

Preparation of Standard working solutions (100% solution): 1ml from each stock solution was pipette out and taken into a 10ml volumetric flask and made up with Diluent (10µg/ml of Tezacaftor, 15µg/ml of Ivacaftor and 20µg/ml of Elexacaftor)

Preparation of Sample stock solutions: 5 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 1 tablet was transferred into a 100 mL volumetric flask, 25mL of diluent 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 0.2ml was pipette out into a 10 ml volumetric flask and made upto 10ml with diluents. (25µg/ml of Tezacaftor, 37.5µg/ml of Ivacaftor and 50µg/ml of Elexacaftor).

Preparation of buffer:
0.1% OPABuffer:1ml of Conc Ortho Phosphoric acid was diluted to 1000ml with water.

METHOD VALIDATION

As per ICH guidelines the method was validated and the parameters like Linearity, Specificity, Accuracy, Precision, Limit of Detection (LOD), Limit of Quantification (LOQ), Robustness and Stability were assessed.

System suitability parameters

The system suitability parameters were determined by preparing standard solutions of Tezacaftor, Ivacaftor and Elexacaftor and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined. The % RSD for the area of six standard injections results should not be more than 2%.

Specificity: Checking of the interference in the optimized method. We should not found interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Precision:

Preparation of Standard stock solutions

Accurately weighed 5mg of Tezacaftor, 7.5mg of Ivacaftor and 10mg of Elexacaftor and transferred to three 50ml volumetric flasks separately. 10ml of diluent was added to flasks and sonicated for 20mins. Flasks were made up Diluent and labeled as Standard stock solution 1, 2 and 3. (100µg/ml of Tezacaftor, 150µg/ml of Ivacaftor and 200µg/ml of Elexacaftor).

Preparation of Standard working solutions (100% solution): 1ml from each stock solution was pipette out and taken. into a 10ml volumetric flask and made up with Diluent (10µg/ml of Tezacaftor, 15µg/ml of Ivacaftor and 20µg/ml of Elexacaftor)

Preparation of Sample stock solutions: 5 tablets were weighed and calculate a 100 mL volumetric flask, 25mL of diluent 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 0.2ml was pipette out into a 10 ml volumetric flask and made upto 10ml with diluents. (25µg/ml of Tezacaftor, 37.5µg/ml of Ivacaftor and 50µg/ml of Elexacaftor).

Linearity:

Preparation of Standard stock solutions: Accurately weighed 5mg of Tezacaftor, 7.5mg of Ivacaftor and 10mg of Elexacaftor and transferred to three 50ml volumetric flasks separately. 10ml of diluent was added to flasks and sonicated for 20mins. Flasks were made up Diluent and labeled as Standard stock solution 1, 2 and 3. (100µg/ml of Tezacaftor, 150µg/ml of Ivacaftor and 200µg/ml of Elexacaftor).

25% Standard solution: 0.25ml each from three standard stock solutions was pipette out and made up to 10ml.

50% Standard solution: 0.5ml each from three standard stock solutions was pipette out and made up to 10ml.

75% Standard solution: 0.75ml each from three standard stock solutions was pipette out and made up to 10ml.

100% Standard solution: 1.0ml each from three standard stock solutions was pipette out and made up to 10ml.

125% Standard solution: 1.25ml each from three standard stock solutions was pipette out and made up to 10ml.

150% Standard solution: 1.5ml each from three standard stock solutions was pipette out and made up to 10ml.

Accuracy:

Preparation of Standard stock solutions: Accurately weighed 5mg of Tezacaftor, 7.5mg of Ivacaftor and 10mg of Elexacaftor and transferred to three 50ml volumetric flasks separately. 10ml of diluent was added to flasks and sonicated for 20mins. Flasks were made up Diluent and labeled as Standard stock solution 1, 2 and 3. (100µg/ml of Tezacaftor, 150µg/ml of Ivacaftor and 200µg/ml of Elexacaftor).

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

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

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

Acceptance Criteria: The % Recovery for each level should be between 98.0 to 102

Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines.

Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus(35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much effected and all the parameters were passed. %RSD was within the limit.

LOD sample Preparation: 0.25ml each from three standard stock solutions was pipette out and transferred to 3 separate 10ml volumetric flask and made up with diluents from the above solutions 0.1ml, 0.1ml and 0.1ml of Tezacaftor, Ivacaftor and Elexacaftor solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents.

LOQ sample Preparation: 0.25ml each from three standard stock solutions was pipette out and transferred to 3 separate 10ml volumetric flask and made up with diluents from the above solutions 0.3ml, 0.3ml and 0.3ml of Tezacaftor, Ivacaftor and Elexacaftor solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents.

Degradation studies: **Oxidation:**
To 1 ml of stock solutions of Tezacaftor, Ivacaftor and Elexacaftor. 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 600C. For HPLC study, the resultant solution was diluted to obtain 25µg/ml, 37.5µg/ml and 50µg/ml of all components and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid degradation Studies:
To 1 ml of stock s solution Tezacaftor, Ivacaftor and Elexacaftor, 1 ml of 2N Hydrochloric acid was added and refluxed for 30mins at 600C. The resultant solution was diluted to obtain (10µg/ml of Tezacaftor, 15µg/ml of Ivacaftor and 20 µg/ml of Elexacaftor) ml of all components and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali degradation studies:

To 1 ml of stock solution Tezacaftor, Ivacaftor and Elexacaftor, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 600C. The resultant solution was diluted to obtain (10µg/ml of Tezacaftor, 15µg/ml of Ivacaftor and 20µg/ml of Elexacaftor) of all components and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat
Degradation Studies: The standard drug solution was placed in oven at 1050c for 1 h to study dry heat degradation. For HPLC study, the resultant solution was diluted obtain 25µg/ml, 37.5µg/ml and 50µg/ml of all components and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Photo Stability studies:
The photochemical stability of the drug was also studied by exposing the 250µg/ml, 375µg/ml and 500µg/ml solution to UV Light by keeping the beaker in UV Chamber for 1days or 200 Watt hours/m2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain (10µg/ml of Tezacaftor, 15µg/ml of Ivacaftor and 20µg/ml of Elexacaftor) of all components and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Degradation Studies:
Stress testing under neutral conditions was studied by refluxing the drug in water for 6h r s at a temperature of 60ºC. For HPLC study, the resultant solution was diluted to obtain (10µg/ml of Tezacaftor, 15µg/ml of Ivacaftor and 20µg/ml of Elexacaftor) of all components and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

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

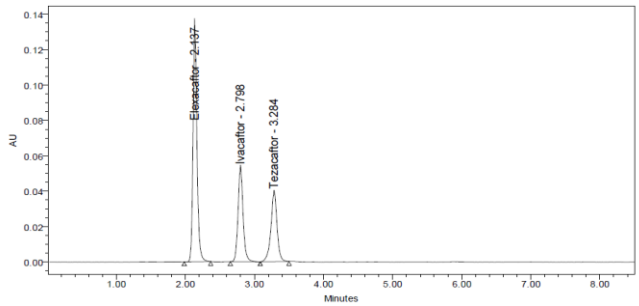

Figure-4: Optimized Chromatogram

System suitability:

Table-1: System suitability parameters for Elexacaftor, Ivacaftor, Tezacaftor

Elexacaftor			Ivacaftor				Tezacaftor			
RT(min	TP	Tailin	RT(min)	TP	Tailing	RS	RT(min)	TP	Tailing	RS
2.146	5226	1.05	2.798	4940	1.01	4.6	3.284	4719	0.92	0.92
2.151	6035	1.08	2.802	5008	1.03	4.6	3.288	4518	0.91	0.91
2.152	5583	1.05	2.804	5554	1.05	4.8	3.293	4800	0.91	0.91
2.153	5955	1.08	2.807	7003	1.1	5.3	3.295	6841	0.93	0.93
2.153	5717	1.01	2.812	5739	1.03	4.8	3.301	5336	0.92	0.92
2.154	6632	1.13	2.816	6201	1.03	4.9	3.306	5926	0.92	0.92

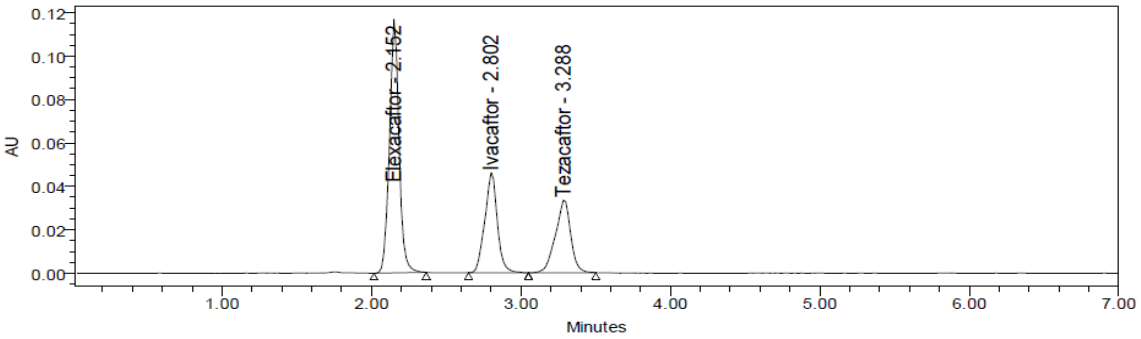

Figure-5: System suitability Chromatogram

Specificity:

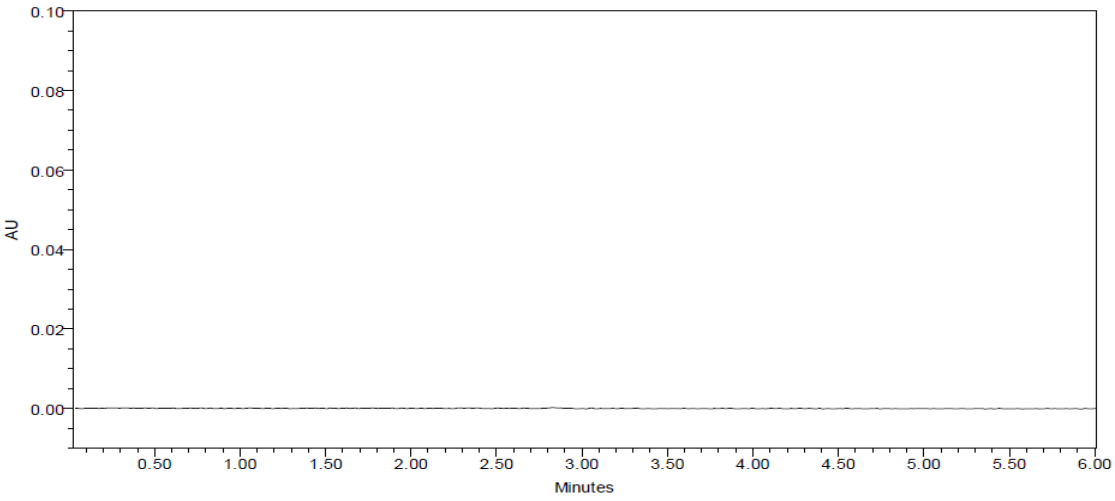

Figure-6: Blank chromatogram

Linearity:

Table-2: Linearity table for Tezacaftor, Ivacaftor, Elexacaftor

Tezacaftar		**Ivacaftar**		**Elexacaftar**	
Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area
2.5	58386	3.75	66291	5	121185
5	117663	7.5	134663	10	272082
7.5	176126	11.25	203790	15	397113
10	234977	15	263614	20	513184
12.5	288744	18.75	319911	25	643121
15	352777	22.5	391089	30	779946

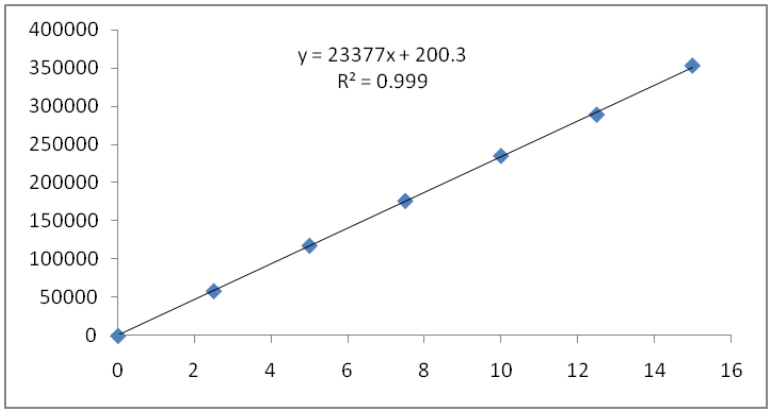

Figure-7: Calibration curve of Tezacaftor

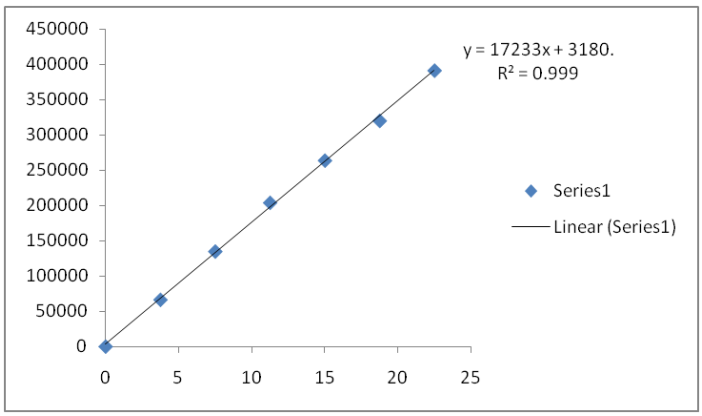

Figure-8: Calibration curve of Ivacaftor

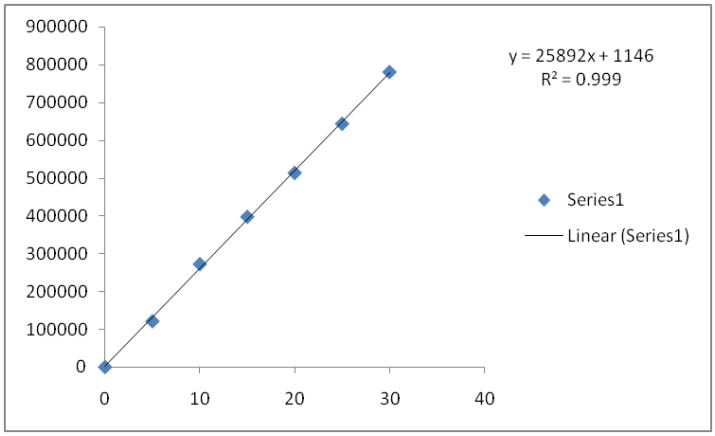

Figure-9: Calibration curve of Elexacaftor

System Precision:

Table-3 System Precision table of Tezacaftor, Ivacaftor, Elexacaftor

S. No	**Area of Tezacaftor**	**Area of Ivacaftor**	**Area of Elexacaftor**
1.	234564	264875	515005
2.	236521	267047	512313
3.	233251	264804	517095
4.	234905	266804	514879
5.	234172	266592	514561
6.	234756	266942	515950
Mean	234695	266177	514967
S.D	1073.1	1047.6	1594.5
%RSD	0.5	0.4	0.3

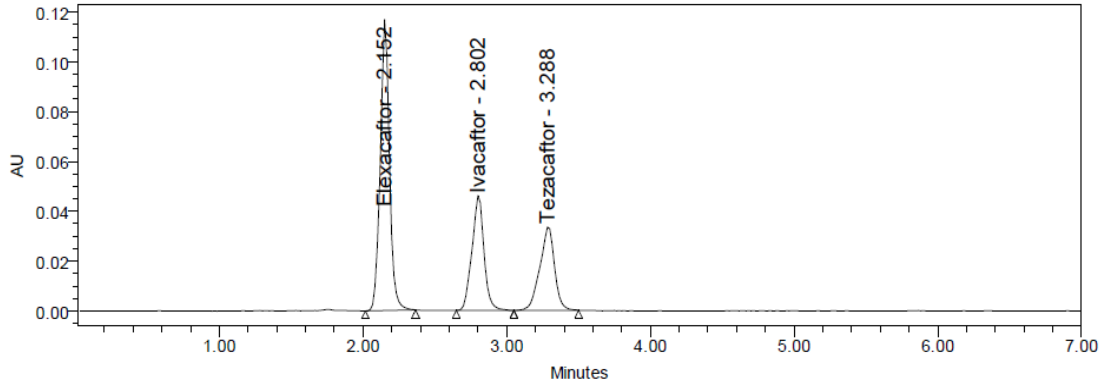

Figure-10: System Precision Chromatogram

Accuracy:

Table-4: Accuracy table of Tezacaftor

% Level	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**	**Mean %Recovery**
50%	5	5.015	100.29	100.17%
	5	4.995	99.89	
	5	4.967	99.34	
100%	10	10.082	100.82	
	10	10.083	100.83	
	10	10.072	100.72	
150%	15	14.950	99.66	
	15	14.972	99.82	
	15	15.018	100.12	

Table-5: Accuracy table of Ivacaftor:

% Level	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**	**Mean %Recovery**
50%	7.5	7.553	100.70	**99.93%**
	7.5	7.542	100.56	
	7.5	7.439	99.19	
100%	15	14.976	99.84	
	15	14.985	99.90	
	15	15.024	100.16	

150%	22.5	22.503	100.01	
	22.5	22.398	99.55	
	22.5	22.383	99.48	

Table-6: Accuracy table of Elexacaftor

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	10	10.05	100.48	100.07%
	10	10.01	100.06	
	10	10.07	100.68	
100%	20	20.05	100.27	
	20	19.93	99.66	
	20	19.90	99.52	
150%	30	30.03	100.09	
	30	29.90	99.65	
	30	30.07	100.23	

Sensitivity:

Table-7: Sensitivity table of Tezacaftor, Ivacaftor, Elexacaftor

Molecule	LOD(µg/ml)	LOQ(µg/ml)
Tezacaftor	0.02µg/ml	0.07 µg/ml
Ivacaftor	0.07 µg/ml	0.22 µg/ml
Elexacaftor	0.09 µg/ml	0.28 µg/ml

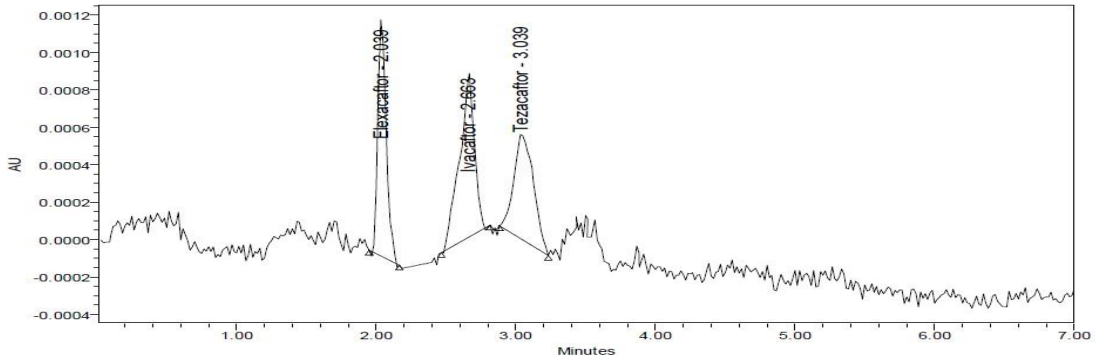

Figure-11: LOD Chromatogram of standard

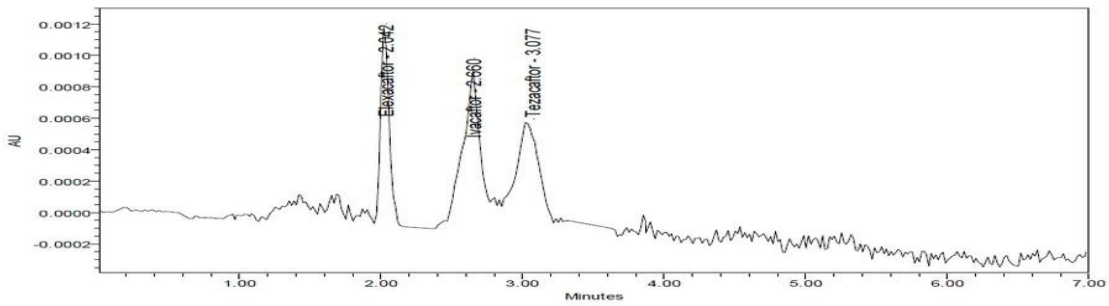

Figure-12: LOQ Chromatogram of standard

Robustness:

Table-8: Robustness table of Ivacaftor, Elexacaftor, Tezacaftor

S.no	Condition	%RSD of Ivacaftar	%RSD of Elexacaftar	%RSD of Tezacaftar.
1	Flow rate (-) 0.9ml/min	0.7	0.6	0.8
2	Flow rate (+) 1.1ml/min	1.4	0.7	0.1
3	Mobile phase (-) 55B:45A	0.6	0.5	0.6
4	Mobile phase (+)65B:35A	1.0	0.8	0.1
5	Temperature (-) 25°C	0.3	0.9	0.1
6	Temperature (+) 35°C	0.4	0.6	0.4

RESULTS AND DISCUSSION

Optimization of Chromatographic Conditions:

To develop and establish a suitable RP- HPLC method for estimation of Tezacaftor, Ivacaftor, Elexacaftor in pharmaceutical dosage form, different preliminary tests were performed and different chromatographic conditions were developed which were given in Table- 1.The final analysis was performed by using 60% Acetonitrile, 40% NH2po4 at a flow rate of 1ml/min, samples were analysed at 258.0nm detector wavelength and at an injection volume of 10 mL using Ascentic C18(150x4.6mm,2.4 m)with run time 10min. The proposed method was optimized to give sharp peak with good resolution and the optimized chromatogram was obtained.

Validation: Linearity was established at six linear concentrations of Tezacaftor (2.5- 15mL), Ivacaftor(3.75-22.5mL) and Elexacaftor(5-30mL) were injected in a triplicate manner. Linearity equation of Tezacaftor was y=23377x+200.3, Ivacaftor was y=17233x+3180 and Elexacaftor was y=25892x+1146. Correlation coefficient obtained was 0.999 for all the three drugs. The linearity calibration curves were plotted as shown in (figure-7-9) Retention time of ivacaftor was 2.798min, elexacaftor was 2.137 and tezacaftor was 3.284min no interfering peaks in blank and placebo were found in this method. So this method holds its specificity. three levels of accuracy samples 50%,100%,150% were prepared by standard addition method. Triplicate injections were given %recovery was obtained as 100.17%, 99.93% and 100.07% for tezacaftor, ivacaftor and elexacaftor respectively. %RSD for system precision for tezacaftor was 1.0%, for ivacaftor was 0.6% and elexacaftor was 1.4%. since %RSD was less then to the system precision was passed in this method shown in (table-4-6) tezacaftor ,ivacaftor and elexacaftor pure drug (API) was obtained from spectrum pharma research solutions Rhodes pharmaceuticals, bearing the label claim tezacaftor 50mg, ivacaftor 75mg,elexacaftor 100mg.assay was performed with the above formulation. Average percentage assay for tezacaftor, ivacaftor and elexacaftor obtained was 100.62%, 100.54% and 100.75% respectively.

Conclusion

A new stability indicating RP-HPLC technique was developed and validated for the simultaneous estimation of Tezacaftor, Ivacaftor, Elexacaftor in pharmaceutical dosage form. The developed method was said to be simple, precise, accurate with high resolution, shorter retention times with separated degradants and economical. Hence, this method can be used for the in- process evaluation in pharmaceutical manufacturing firms and routine quality control of these drugs in drug testing laboratories.

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