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METHOD DEVELOPMENT AND VALIDATION AND STABILITY STUDIES FOR THE SIMULTANIOUS ESTIMATION OF TEZACAFTOR AND IVACAFTOR IN TABLET DOSAGE FORM BY RP-HPLC METHOD

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ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Tezacaftor and Ivacaftorin Tablet dosage form. Chromatogram was run through Std BDS 150 x 4.6 mm, 5µ. Mobile phase containing NaH_2PO_4 Buffer: Acetonitrile taken in the ratio 50:50at 1ml/min flow rate. Optimized wavelength selected was 292nmRetention time of Tezacaftor and Ivacaftor were found to be 2.088 min and2.482 min. %RSD of the Tezacaftor and Ivacaftor were and found to be 0.3 and 0.4singly. LOD, LOQ values obtained from regression equations of Tezacaftor and Ivacaftor were 0.16, 0.49 and 0.33, 1.00singly. Regression equation of Tezacaftor is y = 14384x + 2974, and y = 26897x + 4791of Ivacaftor. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

INTRODUCTION [1-11]

It is the study of compound and mixture identification (qualitative analysis) or the assessment of constituent purity (quantitative analysis). Titration, rainfall, spectroscopy, chromatography, etc. are the widely used method.

Chromatography is a versatile method of separation commonly used to acquire pure mixture compounds. All chromatographic methods rely on a stationary phase that passes through a mobile phase, generally a gas or liquid, generally a finely split solid or covered solid.

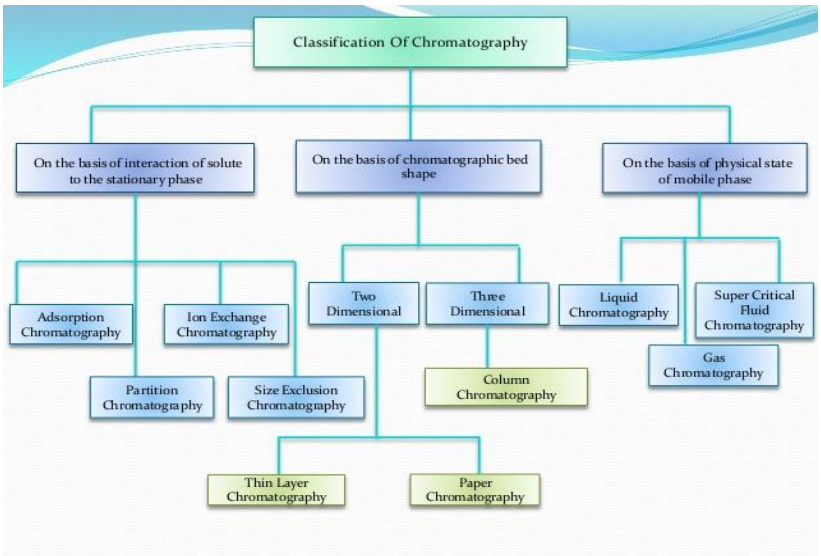


FIG-1. Diagram showing the classification of Chromatography.

High performance liquid chromatography (HPLC): [4-10] HPLC is a procedure in analytical chemistry utilized to isolate, distinguish, and evaluate every element in a blend**.**

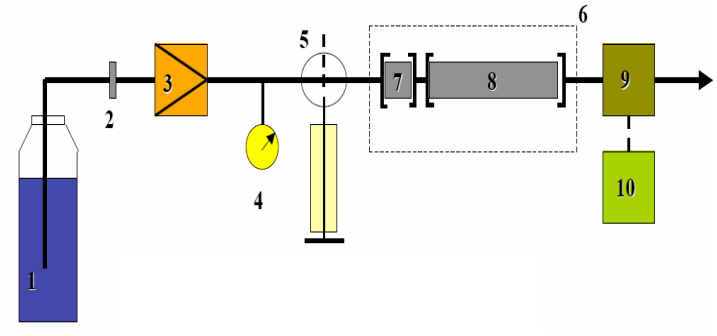
Types of phases	Normal Phase	RP-HPLC
Mobile phase	Non polar	polar
Stationary phase	polar	Non polar

Adsorption chromatography	Partition chromatography
Based on adsorption	Separation on the stationary phase occurs by partition
Solid -liquid phases	Liquid- Liquid phases

Types of chromatography	Description
Size exclusion chromatography	This includes a solid stationary stage with controlled shaft measure. Solids are split indicated to atomic size, with the enormous molecule unfit to enter the pores eluted first.
Ion exchange chromatography (IEC):	It is a chromatography procedure that isolates particles and polar atoms dependent on their partiality to the particle exchanger
Size exclusion chromatography (SEC):	It is otherwise called atomic sifter chromatography,[1] is a chromatographic strategy where particles in arrangement are isolated by their size, and now and again sub-atomic weight.

Table 1. Classification of HPLC.

Instrumentation of HPLC



1. Eluent reservoir
2. Filter
3. High pressure pump
4. Pressure gauge
5. Sample injection valve with syringe
6. Column oven
7. Guard column
8. Column
9. Detector
10. Recorder (integrator, PC etc

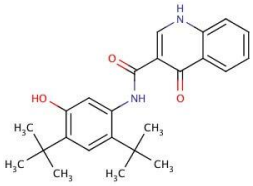
Applications of HPLC in pharmaceutical research: [6-10]

- Separation
- Identification
- Quantification
- Isolation

Drug Profile:

Ivacaftor: [12-17]

Ivacaftor is an aromatic amide produced by formal carboxy group condensation of 4-oxo-1,4-dihydroquinoline-3-carboxylic acid with 5-amino-2,4-di-tert-butylphenol amino group. Used for cystic fibrosis therapy. It is a potential CFTR and an orphan drug.



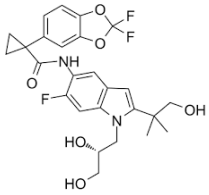
Mechanism of action: By potentiating the channel-open probability (or gating) of the G551D-CFTR protein, Ivacaftor facilitates improved chloride transport. Cystic fibrosis is caused by mutations in a gene that encodes ion (such as chloride) and water transport in the body for the CFTR protein.

Uses:

This medication is used to treat cystic fibrosis in certain people

Tezacaftor:

Tezacaftor is a small molecule that can be used as a corrector of the cystic fibrosis transmembrane conductance regulator (CFTR) gene function. A CFTR potentiator that allows the proteins at the cell surface to open longer and improve nutrient transport.



Mechanism of action: The objective of Tezacaftor is to repair cellular F508del misprocessing This is achieved by modulating the CFTR protein's position on the cell surface to the right place, enabling appropriate ion channel formation and enhanced movement of water and salt through the cell membrane. The simultaneous use of ivacaftor is designed to keep an open channel, increase chloride transport and reduce the production of dense mucus.

Uses:

This medication is used to treat homozygous or heterozygous F508del mutation cystic fibrosis.

MATERIALS

Chemicals: Ivacaftor and Tezacaftor pure drug (API) received from Aurobindo pharma Ltd, Ivacaftor and Tezacaftor tablets (Symdeko). Distilled water, Acetonitrile, Phosphate buffer, methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid from Rankem.

Instruments: Electronic Balance (Denver),P^H meter (BVK enterprises), Ultrasonicator (BVK enterprises), Acuity UPLC system equipped with quaternary pumps, Acquity TUV detector and Auto sampler integrated with Empower 2 software, UV-VIS spectrophotometer PG instruments T60 with special bandwidth of 2mmand 10mm and matched quartz cells integrated with UV win6 software was used for measuring absorbances of Simeprevir and Sofosbuvir solutions.

METHODS [18-23]

Preparation of Standard stock solutions: Accurately weighed Ivacaftor 15mg and Tezacaftor 10 mg and moved to 25ml volumetric flask and 20 ml of diluents was added to this flask and sonicated for 10 minutes. Flask was composed of diluents and marked as the standard solution for inventory (Ivacaftor 600ug/ml & Tezacaftor 400ug/ml). 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made with diluent (60ug/ml of Ivacaftor and 40 ug/ml of Tezacaftor).

Preparation of Sample stock solutions: 10 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, 50 ml of diluent and filtered by HPLC filter (1500 ug/ml of Ivacaftor & 100ug/ml of Tezacaftor) 0.4 ml of filtered sample stock solution was transferred to 10 ml volumetric flask and made up with diluent (60 ug/ml of Ivacaftor and 40ug/ml of Tezacaftor).

Method validation: Method validation was carried on according to ICH guidelines Q2R1. The validation parameters include system suitability, specificity, linearity, accuracy, precision, LOD & LOQ and robustness.

System suitability parameters: The system suitability parameters were determined by preparing standard solutions of Tezacaftor (40 µg/ml), Ivacaftor (15 µg/ml) and the solutions were injected six times. The parameters like peak tailing, resolution and USP plate count were determined. USP Plate count was more than 2000 and tailing factor was less than 2 for 2 drugs in combination. All the system suitable parameters were passed and were within the limits. The results were shown in (Table 3).

Specificity: Checking the interference in the optimized technique. In this technique, we should not discover interfering peaks in blank and placebo at retention moments of these drugs. This technique was said to be particular.

Precision: Precision can be described as "the degree of agreement between individual test outcomes when the method is continuously applied to various homogeneous sample samples." The International Conference on Harmonization (ICH) has suggested a more extensive definition. divides precision into three types: Repeatability, Intermediate precision and Reproducibility. From the formulation same six preparations are prepared for precision.

Linearity: A method's linearity is a measure of how well a reaction vs. concentration calibration plot approximates a straight line. From the standard stock preparations are prepared for precision.

S.No	Pipetted from stock (mL)	Volume of flask (mL)	Concentration in ppm (Tez)	Concentration in ppm (IVA)	% Linearity Level
1	0.25	10	15	10	25
2	0.5	10	30	20	50
3	0.75	10	45	30	75
4	1	10	60	40	100
5	0.25	10	75	50	125
6	0.50	10	90	60	150

Table 2. Stock preparations.

Accuracy: From the sample and standard stock solution the following preparations were prepared.0

Preparation of 50% spiked solution: 0.5 ml 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: 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: 1.5 ml of sample stock solution was taken into a 10ml volumetric.

Robustness: Small deliberate changes in methods like flow rate, mobile phase ration and temperature are made but there was no recognized changes in the result and are within range as per ICH guide lines.

LOD & LOQ preparation: All the concentrations were prepared from linearity curve method. LOD's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (s). The LOD was calculated according to the formula: [LOD = 3.3 ×SD/s], LOQ's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (s) according to the formula: [LOD = 10 × SD/s].

Degradation studies:

Oxidation: To 1ml of stock solution of Ivacaftor and Tezacaftor, 1ml of 20% hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 30min at 60ºC. For HPLC study, the resultant solution was diluted to obtain 60ug/ml and 40 ug/ml solution and 10ul were injected into system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies: To 1ml of stock solution of Ivacaftor and Tezacaftor, 1ml of 2N Hydrochloric acid was added and refluxed for 30 mins at 60ºC. The resultant solution was diluted to obtain 60ug/ml and 40ug/ml solution and 10ul solution were injected into the system and the chromatograms were recorded to assess the stability of sample.

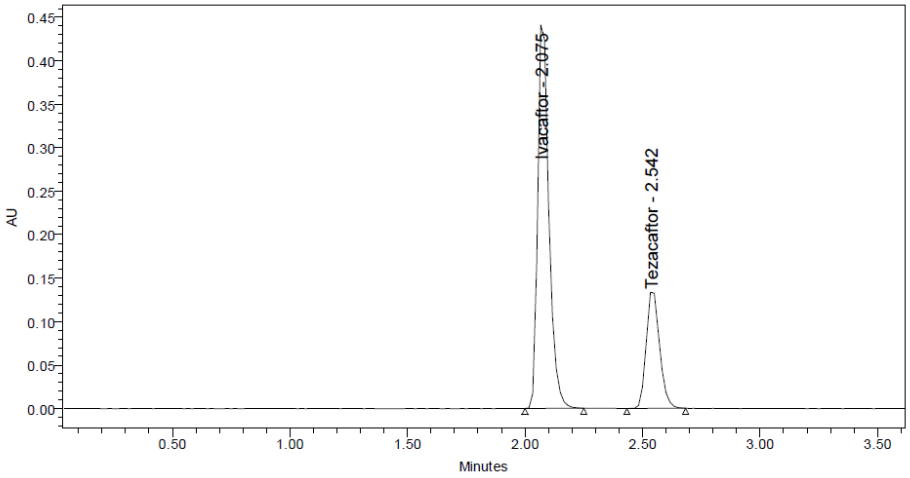
Photo Stability studies: The photochemical stability of the drug was also studied by exposing the 600ug/ml and 400ug/ml solution to UV light by keeping the beaker in UV Chamber for 1 day or 200-Watt hours/m^2 in photo stability chamber for HPLC study, the resultant solution was diluted to obtain 60ug/ml and 40ug/ml solutions and 10ul 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 for1hrs at a temperature of 60ºC. For HPLC study, the resultant solution was diluted to 60ug/ml and 40ug/ml solution and 10 ul were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Alkali Degradation studies: To 1ml of stock solution Ivacaftor and Tezacaftor, 1ml of 2N sodium hydroxide was added and refluxed for 30mins at 60ºC. The resultant solution was diluted to obtain 60ug/ml and 40ug/ml solution and 10ul 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 105ºC for 1h to study dry heat degradation. For HPLC study, the resultant solution was diluted t 60 ug/ml and 40ug/ml solution and 10ul were injected into the system and the chromatograms were recorded to assess the stability of the sample.

RESULTS AND DISCUSSION



Observation: Tezacafter and Ivacafter were eluted at 2.075 min and 2.542 min respectively with good resolution. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated.

Discussion: The Tezacaftor and Ivacaftor retention times were 2.075 minutes and 2.542 minutes one-to-one. In this method, we did not find and interfere with peaks of these drugs in blank and placebo at retention times. So, it has been said that this method is specific.

FIG-3. Optimized Chromatogram of Tezacafter and Ivacafter.

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

S.no	Ivacaftor			Tezacaftor			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resolution
1	2.087	7144	1.22	2.482	9944	1.11	3.9
2	2.087	7235	1.23	2.482	9575	1.11	3.9
3	2.087	7272	1.22	2.487	10360	1.19	4.1
4	2.088	7175	1.21	2.498	10088	1.12	4.1
5	2.088	7317	1.22	2.505	10231	1.18	4.2
6	2.089	6797	1.15	2.508	9997	1.12	4.1

Table 3. System suitability parameters for Tezacaftor and Ivacaftor.

Validation:

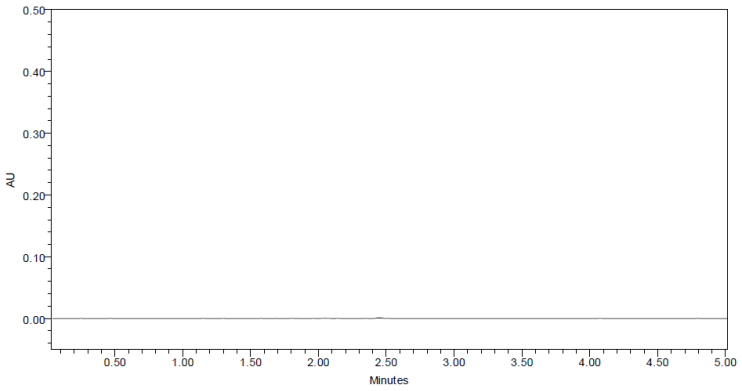


FIG-4. Chromatogram of blank.

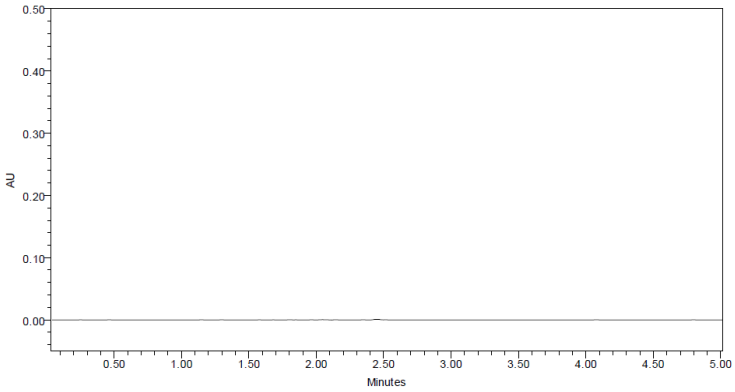


FIG-5. Chromatogram of placebo.

Linearity:

Tezacaftor		Ivacaftor	
Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area
0	0	0	0
10	146447	15	409597
20	294048	30	804040
30	434965	45	1241010
40	580783	60	1600869
50	722135	75	2032439
60	863179	90	2418022

Table 4. Linearity table for Tezacaftor and Ivacaftor.

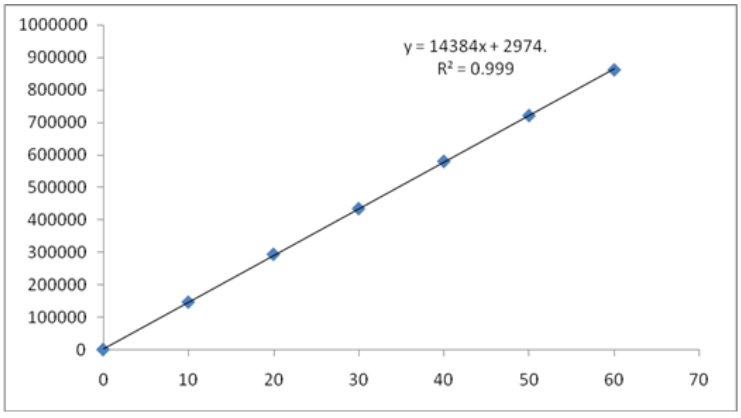


FIG-5. Calibration curve of Tezacaftor.

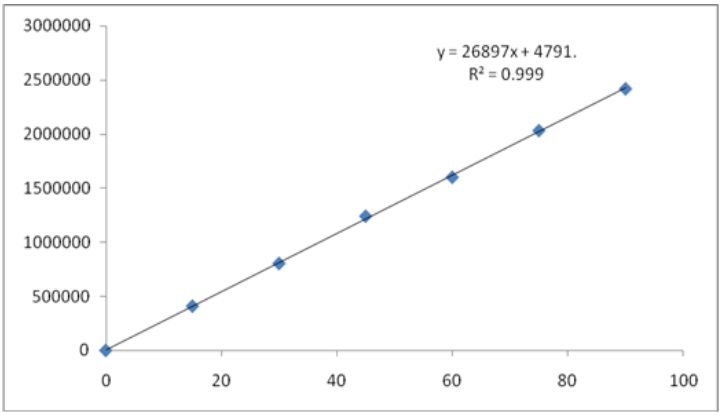


FIG-6. Calibration curve of Ivacaftor.

Discussion: Six linear concentrations of Tezacafter (10-60ug/ml) and Ivacafter (15-90ug/ml) were injected I na duplicate manner. Average areas were mentioned above and linearity equations obtained for Tezacafter was y=14384x + 2974 and of Ivacafter was y= 26897x+4791 correlation coefficient obtained was 0.999 for the two drugs.

Precision:

System Precision:

S. No	Area of Tezacaftor	Area of Ivacaftor
1.	588425	1612621
2.	587693	1605128
3.	585274	1617454
4.	584351	1605003
5.	587645	1600081
6.	585220	1612093
Mean	586435	1608730
S.D	1683.7	6391.1
%RSD	0.3	0.4

Discussion: 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 two drugs. % RSD obtained as 0.6% and 0.3% respectively for Tezacafter and Ivacafter. As the limit of precision was <"2" the system precision was passed in this method.

Table 5. System precision table of Tezacaftor and Ivacaftor.

Repeatability:

S. No	Area of Tezacaftor	Area of Ivacaftor
1.	582247	1601878
2.	581258	1612233
3.	586698	1617098
4.	585183	1607477
5.	577131	1609540
6.	579549	1609095
Mean	582011	1609554
S.D	3536.3	5050.4
%RSD	0.6	0.3

Scrutiny: % RSD were calculated for two drugs and obtained as 0.3% and 0.4% respectively for Tezacaftor and Ivacaftor. As the limit of precision was <"2" the method passed system precision.

Table 6. Repeatability table of Tezacafter and Ivacafter.

Intermediate precision (Day_Day Precision):

S. No	Area of Tezacaftor	Area of Ivacaftor
1.	553418	1599443
2.	554441	1602128
3.	558897	1561725
4.	557728	1587543
5.	553672	1580035
6.	565075	1590947
Mean	557205	1586970
S.D	4461.8	14743.7
%RSD	0.8	0.9

Observation: % RSD were calculated for two drugs and we acquired 0.8% and 0.9% respectively for Tezacaftor and Ivacaftor. Inter Day precision was passed in this method.

Table 7. Intermediate precision table of Tezacafter and Ivacaftor.

Accuracy:

% Level	Amount Spiked	Amount recovered(µg/mL)	% Recovery	Mean % Recovery
50%	20	19.938	99.69	99.83%
	20	20.003	100.01	
	20	19.980	99.90	
100%	40	40.268	100.67	
	40	39.837	99.59	
	40	39.835	99.59	
150%	60	60.084	100.14	
	60	59.901	99.83	
	60	59.815	99.69	

Table 8. Accuracy table of Tezacaftor.

% Level	Amount Spiked (µg/mL)	Amount recovered(µg/mL)	% Recovery	Mean %Recovery
50%	30	29.81	99.35	99.95%
	30	30.03	100.11	
	30	29.79	99.30	
100%	60	60.31	100.52	
	60	60.21	100.35	
	60	60.27	100.46	
150%	90	89.54	99.49	
	90	90.69	100.76	
	90	89.30	99.22	

Table 9. Accuracy table of Ivacaftor.

Discussion: Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean % Recovery was obtained as 99.83% and 99.95% for Tezacafter and Ivacafter respectively.

Sensitivity:

Molecule	LOD	LOQ
Tezacaftor	0.16	0.49
Ivacaftor	0.33	1.00

Table 10. Sensitivity table of Tezacafter and Ivacaftor.

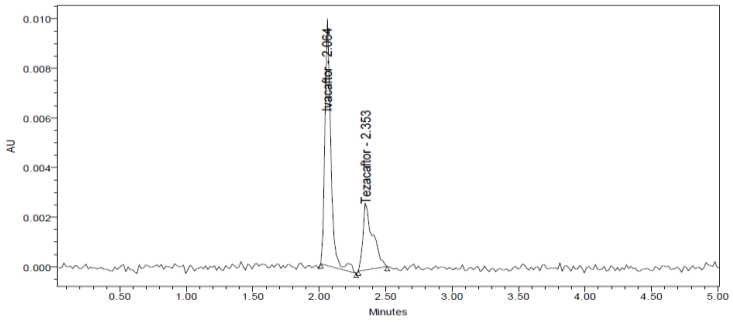


FIG-7. LOD Chromatogram of Standard.

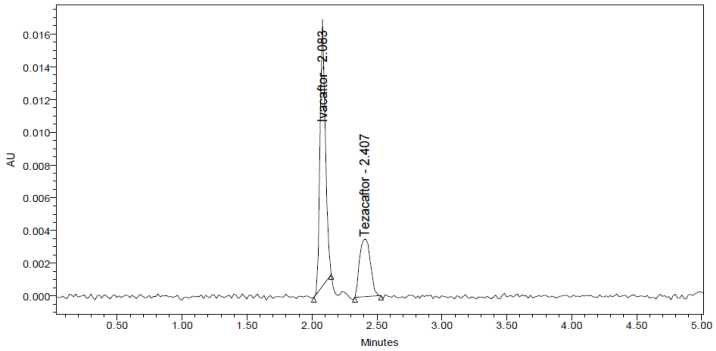


FIG-8. LOQ Chromatogram of Standard.

Robustness:

S.no	Condition	%RSD of Tezacaftor	%RSD of Ivacaftor
1	Flow rate (-) 1.1ml/min	1.0	0.4
2	Flow rate (+) 1.3ml/min	0.7	0.9
3	Mobile phase (-) 75B:25A	0.3	0.3
4	Mobile phase (+) 65B:35A	0.3	0.8
5	Temperature (-) 25℃	0.4	0.4
6	Temperature (+) 35℃	0.8	0.6

Table 11. Robustness data for Tezacafter and Ivacaftor.

Discussion: Robustness conditions like flow minus (0.9ml/min), Flow plus(1.1ml/min) mobile phase minus (75:25A), mobile phase plus (65B:35A), temperature minus(25^0C) and temperature plus (35^0C) 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.

Degradation Studies: The formulation and degraded samples were injecting degradation research. The tests were calculated and the degradation boundaries passed through all samples.

S.NO	Parameter	% Drug Degraded
1	Acid	6.00
2	Alkali	4.62
3	Oxidation	4.07
4	Thermal	3.57
5	UV	1.86
6	Water	0.78

Table 12. Degradation data of Tezacafter.

S.NO	Parameter	% Drug Degraded
1	Acid	4.97
2	Alkali	4.49
3	Oxidation	3.94
4	Thermal	3.45
5	UV	1.70
6	Water	1.70

Table 13. Degradation data of Ivacaftor.

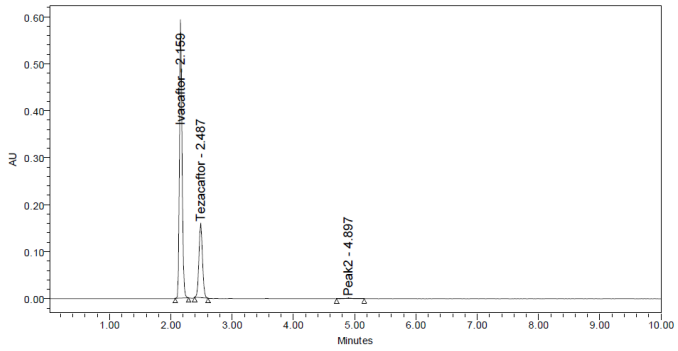


FIG-9. Acid chromatogram of Tezacaftor and Ivacaftor.

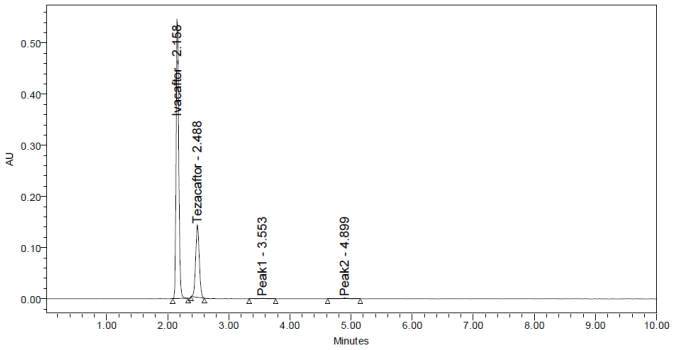


FIG-10. Base chromatogram of Tezacafter and Ivacaftor.

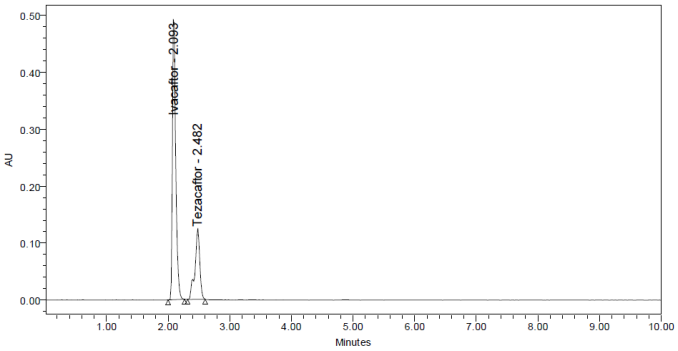


FIG-11. Peroxide chromatogram of Tezacaftor and Ivacaftor.

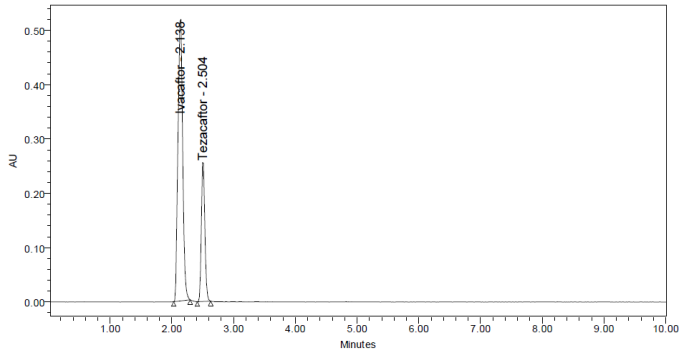


FIG-12. Thermal chromatogram of Tezacaftor and Ivacaftor.

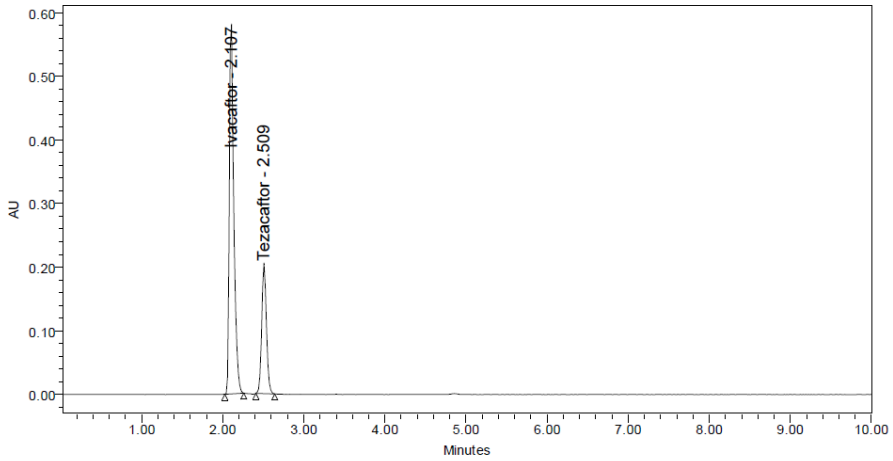


FIG-13. UV chromatogram of Tezacaftor and Ivacaftor.

Parameters		Tezacaftor	Ivacaftor	LIMIT
Linearity Range(µg/ml)		10-60µg/ml	15-90 µg/ml	R< 1
Regression coefficient		0.999	0.999	
Slope(m)		14384	6814	
Intercept(c)		2974	11844	
Regression equation (Y=mx +c)		y = 14384x + 2974	y = 26897.x + 4791	
Assay (% mean assay)		100.56%	99.75%	90-110%
Specificity		Specific	Specific	No interference of any peak
System precision % RSD		0.6	0.3	NMT 2.0%
Method precision % RSD		0.3	0.4	NMT 2.0%
Accuracy % recovery		99.83%	99.95%	98-102%
LOD		0.16	0.33	NMT 3
LOQ		0.49	1.00	NMT 10
Robustness	**FM**	1.0	0.4	%RSD NMT 2.0
	FP	0.7	0.9	
	MM	0.3	0.3	
	MP	0.3	0.8	
	TM	0.4	0.4	
	TP	0.8	0.6	

Table 14. Summary.

Conclusion: To simultaneously estimate Tezacaftor and Ivacaftor in the tablet dosage form, an easy, accurate and linear technique has been developed. Retention time of Tezacaftor and Ivacaftor were found to be 2.088 min and2.482 min. where 0.3 and 0.4 %RSD were observed. %Recovery was got as 99.83% and 99.95% for Tezacaftor and Ivacaftor singly. Regression equation of Tezacaftor is y = 14384x + 2974, and y = 26897x + 4791of Ivacaftor. Retention times have been reduced and runtime has been reduced, so the technique created has been easy and economical, which can be used in periodic quality control tests in industries.

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