Stability Indicating Rp-Hplc Method Development and Validation for the Simultaneous Estimation of Tezacaftor and Ivacaftor in Bulk and Marketed Formulation

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ABSTRACT :
An Analytical, rapid and precise, accurate reverse phase - high performance liquid chromatographic method has been developed for the validated of Tezacaftor and Ivacaftor, in its pure form as well as in marketed pharmaceutical dosage form. This Chromatography was carried out on Kromasil, C_{18}, ODS (4.6mm×250mm) 5µm particle size Column using a mixture of Methanol and Phosphate buffer (pH-2.8) (30:70% v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 238nm. The retention times of the Tezacaftor and Ivacaftorwas found to be 2.133, 3.692± 0.02min respectively. The developed method produce linear responses in the concentration range of 20-60µg/ml of Tezacaftor and 10-30µg/ml of Ivacaftor respectively. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and marketed pharmaceutical formulations.
Keywords: Tezacaftor and Ivacaftor, RP-HPLC, Accuracy, Precision, Validation.

I. INTRODUCTION

Ivacaftor, Lumacaftor and Tezacaftor are orally available potentiators or correctors of the cystic fibrosis Trans membrane conductance regulator (CFTR) that are used to treat patients with cystic fibrosis with specific mutations of the CFTR[1]. Ivacaftor alone or in combination with Lumacaftor or Tezacaftor has been associated with transient serum enzyme elevations during treatment, but neither agent has been convincingly implicated in cases of clinically apparent acute liver injury with jaundice[2].This product is used to treat cystic fibrosis in certain people (those with an abnormal "CFTR" gene). It may help to improve breathing, reduce the risk of lung infections, and improve weight gain[3]. The IUPAC Name of Tezacaftor is 1-(2,2-difluoro-1,3-benzodioxol-5-yl)-N-[1-[(2R)-2,3-dihydroxypropyl]-6-fluoro-2-(1-hydroxy-2-methylpropan-2-yl)indol-5-yl]cyclopropane-1-carboxamide. The Chemical Structure of Tezacaftor is shown in following figure-1.

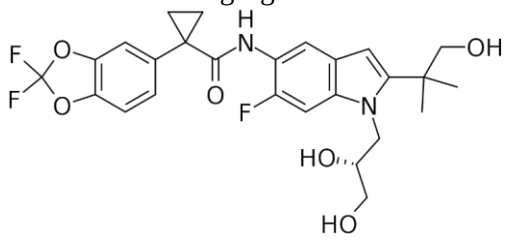

Fig-1: Chemical Structure of Tezacaftor

Ivacaftor (also known as Kalydeco or VX-770) is a drug used for the management of Cystic Fibrosis (CF). It is manufactured and distributed by Vertex Pharmaceuticals[4]. It was approved by the Food and Drug Administration on January 31, 201213, and by Health Canada in late 2012.15 Ivacaftor is administered as a monotherapy and also administered in combination with other drugs for the management of CF[5].Ivacaftor is used to treat certain types of cystic fibrosis (an inborn disease that causes problems with breathing, digestion, and reproduction) in adults and children 4 months of age and older. Ivacaftor should be used only in people with a certain genetic make-up[6]. The IUPAC name of Ivacaftor is N-(2,4-ditert-butyl-5-hydroxyphenyl)-4-oxo-1H-quinoline-3-carboxamide. The Chemical Structure of Ivacaftor is shown in following figure-2.

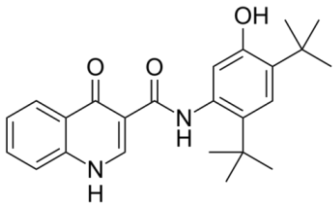

Fig-2: Chemical Structure of Ivacaftor

II. EXPERIMENTAL WORK

Instruments Used:

Table-1: Instruments used

S.No.	Instruments and Glass wares	Model
1	HPLC	WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA detector.
2	pH meter	Lab India
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital Ultra Sonicator	Labman

Chemicals Used:

Table-2: Chemicals Used

S.No.	Chemical	Brand Names
1	Tezacaftor (Pure)	Synpharma Research Lab, Hyderabad
2	Ivacaftor (Pure)	Synpharma Research Lab, Hyderabad
3	Water and Methanol for HPLC	LICHROSOLV (MERCK)
4	Acetonitrile for HPLC	Merck
5	Acetic Acid	Merck

HPLC Method Development:

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Tezacaftor and Ivacaftor working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.4ml of Tezacaftor and 0.2ml of Ivacaftor from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines[7-10].

Mobile Phase Optimization:

Initially the mobile phase tried was Methanol: Water and ACN: Water with varying proportions. Finally, the mobile phase was optimized to Methanol and Phosphate buffer (pH-2.8) in proportion 30:70% v/v respectively.

Optimization of Column:

The method was performed with various C18 columns like Symmetry, X terra and ODS column. Kromasil, C_{18}, ODS (4.6mm×250mm) 5µm particle size Column was found to be ideal as it gave good peak shape and resolution at 1ml/min flow[11].

Preparation of Mobile Phase:

Accurately measured 300ml of Methanol (30%) of and 700ml of Phosphate buffer (70%) were mixed and degassed in a digital ultra sonicater for 20 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:
The Mobile phase was used as the diluent.

Method Validation Parameters
System Suitability
Accurately weigh and transfer 10 mg of Tezacaftor and Ivacaftor working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent[12]. (Stock solution)
Further pipette out 0.4ml of Tezacaftor and 0.2ml of Ivacaftor from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Procedure:
The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Specificity:
Preparation of Standard Solution:
Accurately weigh and transfer 10 mg of Tezacaftor and Ivacaftor working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette out 0.4ml of Tezacaftor and 0.2ml of Ivacaftor from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Preparation of Sample Solution:
Take average weight of Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight ofTezacaftor and Ivacaftor sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Filter the sample solution by using injection filter which contains 0.45µ pore size[13].
Further pipette out 0.4ml of Tezacaftor and 0.2ml of Ivacaftor Sample solution from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Procedure:
Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula[14]:

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

Linearity:
Accurately weigh and transfer 10 mg of Tezacaftor and Ivacaftor working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (20ppm of Tezacaftor and 10ppm of Ivacaftor):
Pipette out 0.2ml of Tezacaftor and 0.1ml of Ivacaftor in to a 10ml volumetric flask and make the volume upto mark by using diluent and sonicate for air entrapment.

Preparation of Level – II (30ppm of Tezacaftor and 15ppm of Ivacaftor):
Pipette out 0.3ml of Tezacaftor and 0.15ml of Ivacaftor in to a 10ml volumetric flask and make the volume upto mark by using diluent and sonicate for air entrapment.

Preparation of Level – III (40ppm of Tezacaftor and 20ppm of Ivacaftor):
Pipette out 0.4ml of Tezacaftor and 0.2ml of Ivacaftor in to a 10ml volumetric flask and make the volume upto mark by using diluent and sonicate for air entrapment.

Preparation of Level – IV (50ppm of Tezacaftor and 25ppm of Ivacaftor):
Pipette out 0.5ml of Tezacaftor and 0.25ml of Ivacaftor in to a 10ml volumetric flask and make the volume upto mark by using diluent and sonicate for air entrapment [15].

Preparation of Level – V (60ppm of Tezacaftor and 30ppm of Ivacaftor):
Pipette out 0.6ml of Tezacaftor and 0.3ml of Ivacaftor in to a 10ml volumetric flask and make the volume up to mark by using diluent and sonicate for air entrapment.
Procedure:

Inject each level into the chromatographic system and measure the peak area.
Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient[16].

Precision
Repeatability
Preparation of Tezacaftor and Ivacaftor Product Solution for Precision:
Accurately weigh and transfer 10 mg of Tezacaftor and Ivacaftor working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette out 0.4 ml of Tezacaftor and 0.2ml of Ivacaftor from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.
The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits[17].

Intermediate Precision:
To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure:
Day 1:
The standard solution was injected for Six times and measured the area for all Six injections in HPLC. The %RSD for the area of Six replicate injections was found to be within the specified limits.
Day 2:
The standard solution was injected for Six times and measured the area for all Six injections in HPLC. The %RSD for the area of Six replicate injections was found to be within the specified limits.

Accuracy:
For preparation of 50% Standard stock solution:
Accurately weigh and transfer 10mg of Tezacaftor and Ivacaftor working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette out 0.2ml of Tezacaftor and 0.1ml of Ivacaftor from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent[18].

For preparation of 100% Standard stock solution:
Accurately weigh and transfer 10 mg of Tezacaftor and Ivacaftor working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette out 0.4ml of Tezacaftor and 0.2ml of Ivacaftor from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

For preparation of 150% Standard stock solution:
Accurately weigh and transfer 10 mg of Tezacaftor and Ivacaftor working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette out 0.6ml of Tezacaftor and 0.3ml of Ivacaftor from the above stock solutions into a 10ml

volumetric flask and dilute up to the mark with Diluent.

Procedure:

Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Tezacaftor and Ivacaftor and calculate the individual recovery and mean recovery values[19].

Robustness:

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results.

For preparation of Standard solution:

Accurately weigh and transfer 10 mg of Tezacaftor and Ivacaftor working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent[20]. (Stock solution)

Further pipette out 0.4ml of Tezacaftor and 0.2ml of Ivacaftor from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Effect of Variation of Flow Conditions:

The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 20µl of the above sample was injected and chromatograms were recorded[21-24].

Effect of Variation of Mobile Phase Organic Composition:

The sample was analyzed by variation of mobile phase i.e. Methanol: Phosphate buffer was taken in the ratio 35:65 and 25:75 instead of 30:70 remaining conditions are same. 20µl of the above sample was injected and chromatograms were recorded.

III. RESULTS AND DISCUSSION

Method Development:

Optimized Chromatographic Conditions:

Mobile phase ratio	: Methanol and Phosphate buffer (pH-2.8) (30:70% v/v)
Column	: Kromasil, C_{18}, ODS (4.6mm×250mm) 5µm particle size
Column temperature	: Ambient
Wavelength	: 238nm
Flow rate	: 1.0ml/min
Injection volume	: 20µl
Run time	: 6.0minutes

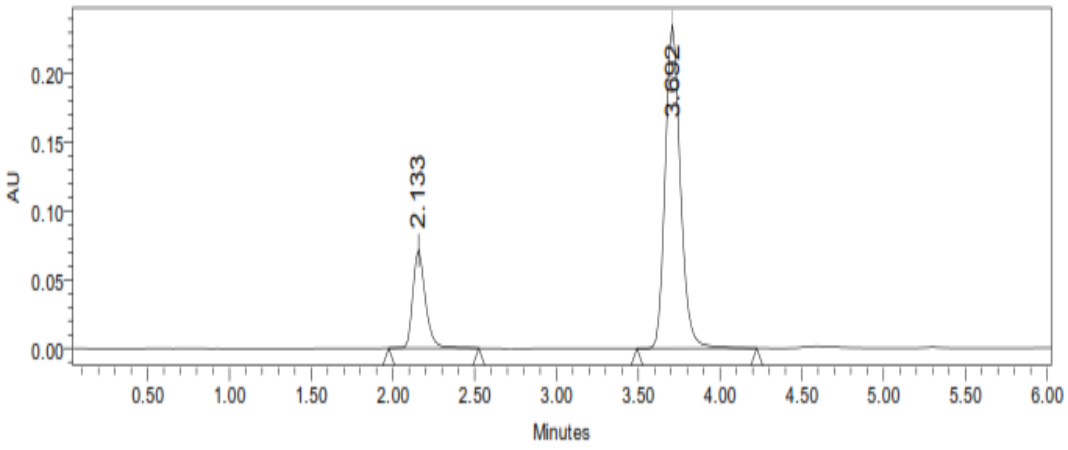

Fig-3: Optimized Chromatogram

Method Validation:

System Suitability[25]:

Table-3: Results of System Suitability for Tezacaftor

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Tezacaftor	2.152	526358	86598	5695	1.56
2	Tezacaftor	2.157	526548	86254	5652	1.57
3	Tezacaftor	2.141	526854	86598	5627	1.56
4	Tezacaftor	2.133	526598	86245	5692	1.57
5	Tezacaftor	2.166	524874	86521	5641	1.56
Mean			**526246.4**			
Std.Dev			**787.353**			
%RSD			**0.149617**			

Table-4: Results of System Suitability for Ivacaftor

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing	Resolution
1	Ivacaftor	3.674	1682821	1686958	8659	1.56	9.8
2	Ivacaftor	3.631	1682726	1685745	8675	1.57	9.9
3	Ivacaftor	3.625	1687361	1685421	8692	1.56	9.8
4	Ivacaftor	3.692	1682811	1685242	8642	1.57	9.8
5	Ivacaftor	3.629	1683816	1685364	8635	1.58	9.8
Mean			**1683907**				
Std.Dev			**1982.03**				
%RSD			**0.117704**				

Specificity

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. Analytical method was tested for specificity to measure accurately quantitatesTezacaftor and Ivacaftor in drug product[26].

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Tezacaftor and Ivacaftor in pharmaceutical dosage form was found to be 99.89%

Linearity:

Table-5: Chromatographic Data for Linearity Study of Tezacaftor

Concentration µg/ml	Average Peak Area
20	272897
30	402986
40	526389
50	649785
60	769287

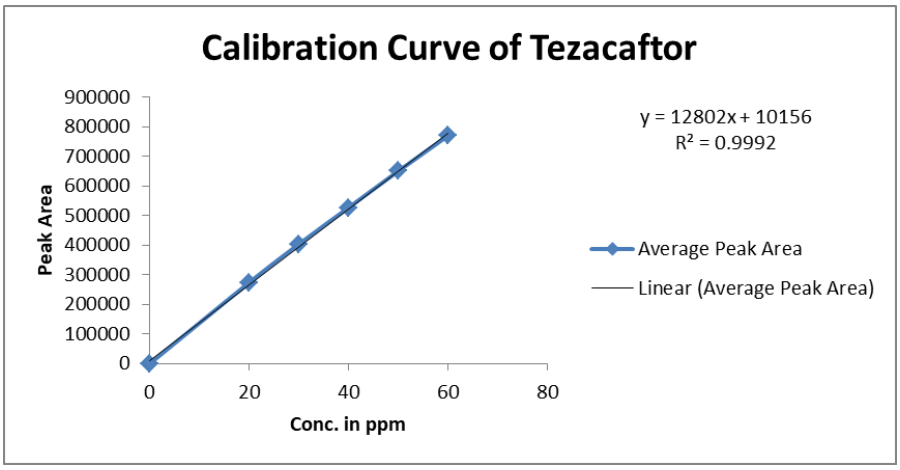

Fig-4: Calibration Curve of Tezacaftor

Linearity Plot: The plot of Concentration (x) versus the Average Peak Area (y) data of Tezacaftor is a straight line.

Y = mx + c
Slope (m) =12802
Intercept (c) = 10156
Correlation Coefficient (r) = 0.99

Validation Criteria: The response linearity is verified if the Correlation Coefficient is 0.99 or greater[27].
Conclusion: Correlation Coefficient (r) is 0.99, and the intercept is 10156. These values meet the validation criteria.
Chromatographic Data for Linearity Study of Ivacaftor:

Table-6: Chromatographic Data for Linearity Study of Ivacaftor

Concentration µg/ml	Average Peak Area
10	1000237
15	1448768
20	1887285
25	2365897
30	2826845

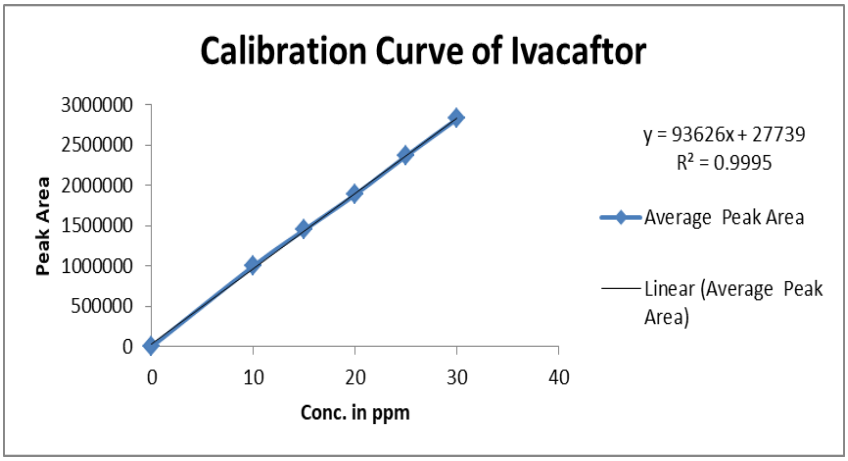

Fig-5: Calibration Curve of Ivacaftor

Linearity Plot: The plot of Concentration (x) versus the Average Peak Area (y) data of Ivacaftor is a straight line.

Y = mx + c
Slope (m) = 93626

Intercept (c) = 27739
Correlation Coefficient (r) = 0.99

Validation Criteria: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.
Conclusion: Correlation Coefficient (r) is 0.99, and the intercept is 27739. These values meet the validation criteria.

Precision:

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions[28].

Repeatability: Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

Table-7: Results of repeatability for Tezacaftor

S. No.	Peak Name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Tezacaftor	2.157	526358	86598	5689	1.56
2	Tezacaftor	2.159	524856	86542	5687	1.57
3	Tezacaftor	2.186	526985	86578	5684	1.56
4	Tezacaftor	2.160	528654	86354	5689	1.56
5	Tezacaftor	2.170	528457	86958	5639	1.56
Mean			527062			
Std. Dev			1569.114			
%RSD			0.297709			

Table-8: Results of Repeatability for Ivacaftor

S. No.	Peak Name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Ivacaftor	3.603	1687589	367859	8659	1.79
2	Ivacaftor	3.608	1685987	368547	8679	1.80
3	Ivacaftor	3.600	1685987	367985	8645	1.80
4	Ivacaftor	3.696	1685754	365874	8695	1.79
5	Ivacaftor	3.629	1685985	364589	8625	1.79
Mean			1686260			
Std. Dev			749.493			
%RSD			0.044447			

Intermediate Precision:

Day 1:

Table-9: Results of Intermediate Precision for Tezacaftor

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	%Assay
1	Tezacaftor	2.198	546585	87589	5898	1.58	100%
2	Tezacaftor	2.196	548758	87985	5879	1.59	100%
3	Tezacaftor	2.160	549854	87452	5868	1.58	100%
4	Tezacaftor	2.160	548798	87421	5847	1.59	100%
5	Tezacaftor	2.160	542659	87963	5896	1.58	100%
6	Tezacaftor	2.186	548754	87254	5874	1.59	100%
Mean			547568				

Std.Dev.			2631.576				
%RSD			0.480593				

Table-10: Results of Intermediate Precision for Ivacaftor

S.No.	Peak Name	Rt	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	Resolution
1	Ivacaftor	3.623	1698587	385482	8789	1.81	9.8
2	Ivacaftor	3.611	1698574	385698	8759	1.80	9.8
3	Ivacaftor	3.696	1698532	385748	8754	1.81	9.9
4	Ivacaftor	3.696	1698574	386958	8754	1.81	10.01
5	Ivacaftor	3.696	1698532	385755	5798	1.80	9.98
6	Ivacaftor	3.642	1698547	386558	8762	1.80	10.02
Mean			1698558				
Std.Dev.			23.77113				
%RSD			0.001399				

Day 2:

Table-11: Results of Intermediate Precision Day 2 for Tezacaftor

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Tezacaftor	2.198	536854	8758	5789	1.58
2	Tezacaftor	2.196	536985	8795	5726	1.59
3	Tezacaftor	2.178	536587	8746	5742	1.58
4	Tezacaftor	2.142	532546	8754	5746	1.59
5	Tezacaftor	2.177	534587	8725	5798	1.58
6	Tezacaftor	2.177	538598	8726	5785	1.59
Mean			536026.2			
Std.Dev.			2131.492			
%RSD			0.397647			

Table-12: Results of Intermediate Precision Day 2 for Ivacaftor

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	Resolution
1	Ivacaftor	3.611	1678598	356875	8875	1.82	9.9
2	Ivacaftor	3.623	1678985	358985	8856	1.83	10.01
3	Ivacaftor	3.684	1678984	358754	8862	1.82	9.9
4	Ivacaftor	3.697	1678985	352412	8849	1.83	10.01
5	Ivacaftor	3.684	1678549	358987	8873	1.82	9.9
6	Ivacaftor	3.684	1678984	358986	8842	1.83	10.01
Mean			1678848				
Std.Dev.			212.8048				
%RSD			0.012676				

Accuracy: Accuracy at different concentrations (50%, 100%, and 150%) was prepared and the % recovery was calculated[29].

Table-13: The Accuracy Results for Tezacaftor

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	267011.3	20	20.063	100.315%	100.28%
100%	523752.3	40	40.118	100.295%	
150%	778457.3	60	60.133	100.221%	

Table-14: The Accuracy Results for Ivacaftor

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	972876.3	10	10.094	100.94%	100.48%
100%	1900122	20	19.998	99.99%	
150%	2851152	30	30.156	100.52%	

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Limit of Detection

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value[30].

$$LOD = 3.3 \times \sigma / s$$

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

TEZACAFTOR

Result:

= 1.04µg/ml

IVACAFTOR

Result:

= 3.12µg/ml

Quantitation Limit

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

$$LOQ = 10 \times \sigma / S$$

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

TEZACAFTOR

Result:

=2.1µg/ml

IVACAFTOR

Result:

=6.3µg/ml

Robustness: Tezacaftor and Ivacaftor were tested for resilience at flow rates ranging from 0.9 to 1.1 ml/min and mobile phase ratios varying from more to less organic phase. Only at low flow rates is the technique robust, and it is also resistant to a 10% shift in the mobile phase. The standard and samples of Tezacaftor and Ivacaftor were injected using a variety of chromatographic settings. The resolution, tailing factor, and plate count properties remained unchanged[31].

Table-15: Results for Robustness Tezacaftor

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	526389	2.133	5679	1.56
Less Flow rate of 0.9 mL/min	542685	2.210	5264	1.54
More Flow rate of 1.1 mL/min	526483	2.184	5426	1.52

Less organic phase	516854	2.200	5163	1.57
More Organic phase	506898	2.172	5098	1.51

Table-16: Results for Robustness Ivacaftor

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	1687285	3.692	8685	1.79
Less Flow rate of 0.9 mL/min	1725468	4.498	8265	1.68
More Flow rate of 1.1 mL/min	1652847	3.505	8415	1.59
Less organic phase	1687485	4.504	8326	1.62
More organic phase	1674524	3.512	8415	1.63

Stability Studies:

The specificity of the method can be demonstrated by applying stress conditions using acid, alkaline, peroxide, thermal, UV, water degradations. The sample was exposed to these conditions the main peak of the drug was studied for peak purity that indicating the method effectively separated the degradation products from the pure active ingredient[32].

Table-17: Results of Forced Degradation Studies

S.No.	Stress Condition	Peak Area	% of Degraded Amount	% of Active Amount	Total % of Amount
1	Standard	526389	0	100%	100%
2	Acidic	371683.27	29.39	70.61	100%
3	Basic	411794.11	21.77	78.23	100%
4	Oxidative	480645.79	8.69	91.31	100%
5	Thermal	327045.48	37.87	62.13	100%
6	Photolytic	477118.99	9.36	90.64	100%

Table-18: Results of Forced Degradation Studies

S.No.	Stress Condition	Peak Area	% of Degraded Amount	% of Active Amount	Total % of Amount
1	Standard	1687285	0	100%	100%
2	Acidic	1359614.25	19.42	80.58	100%
3	Basic	1445497.05	14.33	85.67	100%
4	Oxidative	1644427.96	2.54	97.46	100%
5	Thermal	1297353.43	23.11	76.89	100%
6	Photolytic	1632954.42	3.22	96.78	100%

CONCLUSION

Summary of Validation data for Tezacaftor:

Table-19: Summary of Validation data for Tezacaftor

S.No.	Parameter	Observation	Acceptance Criteria
1	**System suitability** Theoretical plates Tailing %RSD	 5679 1.56 0.14	 Not less than 2000 Not more than 2 Not more than 2.0%
2	**Specificity** %Assay	 99.89%	 98-102%
3	**Method Precision** (%RSD)	0.29	Not more than 2.0%

4	**Linearity** Slope Correlation coefficient (r^2)	20-60 µg/ml 12802 0.999	 ≤0.99
5	**Accuracy** Mean % recovery	 100.28%	 98 - 102%
6	**Robustness** a) Flow rate variation b) Organic phase variation	All the system suitability parameters are within the limits.	

Summary of validation data for Ivacaftor:

Table-20: Summary of validation data for Ivacaftor

S.No	Parameter	Observation	Acceptance criteria
1	**System suitability** Theoretical plates Tailing %RSD	 8685 1.79 0.11	 Not less than 2000 Not more than 2 Not more than 2.0%
2	**Specificity** %Assay	 99.89%	 98-102%
3	**Method Precision**(%RSD)	0.044	Not more than 2.0%
4	**Linearity** Slope Correlation coefficient(r^2)	10-30 µg/ml 93626 0.999	 ≤0.99
5	**Accuracy** Mean % recovery	 100.48%	 98 - 102%
6	**Robustness** a) Flow rate variation b) Organic phase variation	All the system suitability parameters are within the limits.	

Stability study correspondingly confirmed the specificity of the method. As a part of peak purity study, peak threshold was found to be higher than angle and no flag for both the analytes was observed. Degradation study revealed that Tezacaftor and Ivacaftor were degraded in acidic and thermal condition only.

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