DEVELOPMENT OF AN ANALYTICAL METHOD FOR SIMULTANEOUS ESTIMATION OF TEZACAFTOR AND IVACAFTOR IN TABLET DOSAGE FORM BY RP – HPLC

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Abstract:

Developed a simple, specific, accurate and precise reverse phase high pressure liquid chromatographic method for the simultaneous determination of Tezacaftor and Ivacaftor from combined dosage form by reverse phase C18 column (BDS Hypersil C18, 5µm, 4.6*250mm). The sample was analysed using Mixed 0.1% TEA: Methanol in proportion 30: 70 v/v respectively. as a mobile phase at a flow rate of 1ml/min and detection at 298 nm . The accuracy limit is the percentage recovery should be in the range of 97.0% - 103.0%. The total recovery was found to be 100.29% and 100.11% for Tezacaftor and Ivacaftor. The method can be used for estimation of combination of these drugs in combined dosage form. The earlier literature reveals the analytical methods like UV and HPLC were reproted for determination of Tezacaftor and Ivacaftor individually and other combinations. Therefore the present study has been undertaken in order to develop new, simple, rapid, efficient and reproducible method for the analysis of Tezacaftor and Ivacaftor.

INTRODUCTION:

Quality is important in every product or service, but it is vital in medicine as it involves life. Unlike other consumer goods, there can be and there is no second quality. Therefore analytical methods which are a measure of quality of the drugs play a very comprehensive role in drug development and follow up activities, to assure that a drug product meets the established standard, is a stable and will continue to meet purported quality throughout its shelflife[1].

These methods should be selective and sensitive to monitor the known and unknown impurities, have to be written in a format such that they can be produced over a period of time and from laboratory to laboratory, i.e. these methods should be validated.

Analytical methods are required to characterize drug substance and drug product composition during all phases of pharmaceutical development[2]. Early phase methods must support changes in synthetic routes and dosage form and elucidate the structures and levels of impurities. In later phases, goals change to the development of rapid and robust methods for release and stability evaluation.

Analysis includes a wide range of simple and instrumental analytical methods, but the most widely most used analytical methods for quality assurance are spectroscopy and chromatography based. Most quantitative analysis require, measuring specified components in the presence of sample matrix and /or related substances, therefore isolation or separation of the components are required preceding quantitative analysis. In such cases chromatographic techniques are used for quantitative analysis. In cases where matrix interference is not observed quantitative measurements are made using spectroscopic or titration methods directly[3].

MATERIALS AND METHODS:

Tezacaftor and Ivacaftor were purchased from Yarrow chem. products, Mumbai. Water Methanol and Acetonitrile for HPLC were purchased from Thermo Fisher Scientific India. Pvt. Ltd., Mumbai., All other chemicals and solvents were of analytical grade satisfying pharmacopeial specifications.

HPLC METHOD DEVELOPMENT:

Mobile Phase Optimization:

Initially the mobile phase tried was methanol: Ortho phosphoric acid buffer and Methanol: phosphate buffer, Acetonitrile: methanol with various combinations of pH as well as varying proportions. Finally, the mobile phase was optimized to 0.1% TEA: Methanol in proportion 30: 70 v/v respectively.

Wave length selection:

UV spectrum of 10µg/ml Tezacaftor and 10µg/ml Ivacaftor in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 298 nm. At this wavelength both the drugs show good absorbance.

Optimization of Column:

The method was performed with various columns like C18 column Phenomenex column, YMC, and Inertsil ODS column. Agilent Eclipse coloumn (4.6 x 150mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 1.0 ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

Instrument used	:	Waters HPLC with auto sampler and UV detector.
Temperature	:	Ambient(25° C)
Mode of separation	:	Isocratic mode
Column	:	Agilent Eclipse coloumn (4.6 x 150mm, 5µm)
Mobile phase	:	0.1% TEA: Methanol (30: 70)
Flow rate	:	1 ml per min
Wavelength	:	298 nm
Injection volume	:	10 µl
Run time	:	10 min.

Preparation of 0.1% TEA:

Take 1ml Tri ethyl amine in 1000ml volumetric flask and make up with HPLC water and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Preparation of mobile phase:

Accurately measured 300 ml (30%) of 0.1% TEA Buffer and 700 ml (60%) of Methanol were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

PREPARATION OF THE TEZACAFTOR & IVACAFTOR STANDARD & SAMPLE SOLUTION:

Standard Solution Preparation:

Accurately weigh and transfer 20 mg of Tezacaftor and 30 mg of Ivacaftor working standard into a 100 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Sample Solution Preparation:

Accurately weigh and transfer equivalent to 20 mg of Tezacaftor and 30 mg of Ivacaftor sample into a 100 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

Inject 20 µL of the standard, sample into the chromatographic system and measure the areas for Tezacaftor and Ivacaftor peaks and calculate the %Assay by using the formulae.

SYSTEM SUITABILITY:

Tailing factor for the peaks due to Tezacaftor and Ivacaftor in Standard solution should not be more than 2.0. Theoretical plates for the Tezacaftor and Ivacaftor peaks in Standard solution should not be less than 2000. Resolution for the Tezacaftor and Ivacaftor peaks in standard solution should not be less than 2.

SAMPLE AND STANDARD DETAILS

S. No.	Samples
1	Tezacaftor & Ivacaftor Tablets 100 mg & 150 mg
2	Tezacaftor & Ivacaftor

METHOD VALIDATION SUMMARY:

LINEARITY:

Preparation of stock solution:

Accurately weigh and transfer 20 mg of Tezacaftor and 30 mg of Ivacaftor working standard into a 100 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Various concentration solutions(05,1,1.5,2,2.5 %w/v) were prepared from the stick solutions.

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.

PRECISION:

Preparation of stock solution:

Accurately weigh and transfer 20 mg of Tezacaftor and 30 mg of Ivacaftor working standard into a 100 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

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.

INTERMEDIATE PRECISION/RUGGEDNESS:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day.

Preparation of stock solution:

Accurately weigh and transfer 20 mg of Tezacaftor and 30 mg of Ivacaftor working standard into a 100 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

The standard solutions prepared in the precision was injected on the other day, 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:

Preparation of Standard stock solution:

Accurately weigh and transfer 20 mg of Tezacaftor and 30 mg of Ivacaftor working standard into a 100 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation Sample solutions:

For preparation of 50% solution (With respect to target Assay concentration):

Accurately weigh and transfer 10 mg of Tezacaftor and 15 mg of Ivacaftor working standard into a 100 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

For preparation of 100% solution (With respect to target Assay concentration):
Accurately weigh and transfer 20 mg of Tezacaftor and 30 mg of Ivacaftor working standard into a 100 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

For preparation of 150% solution (With respect to target Assay concentration):
Accurately weigh and transfer 30 mg of Tezacaftor and 45 mg of Ivacaftor working standard into a 100 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:
Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions. Calculate the Amount found and Amount added for Tezacaftor & Ivacaftor and calculate the individual recovery and mean recovery values.

DETECTION LIMIT

LIMIT OF DETECTION: (for Tezacaftor)

Preparation of 0.38 µg/ml solution:
Accurately weigh and transfer 20 mg of Tezacaftor working standard into a 100 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent. Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Further pipette 1.25 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Acceptance Criteria:
S/N Ratio value shall be 3 for LOD solution.

LIMIT OF QUANTIFICATION: (for Tezacaftor)

Preparation of 1.24 µg/ml solution:
Accurately weigh and transfer 20 mg of Tezacaftor working standard into a 100 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent. Further pipette 1 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Further pipette 4.15 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Acceptance Criteria:
S/N Ratio value shall be 10 for LOQ solution.

LIMIT OF DETECTION: (for Ivacaftor)

Preparation of 0.14 µg/ml solution:

Accurately weigh and transfer 30 mg of Ivacaftor working standard into a 100 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make

volume up to the mark with the same solvent. (Stock solution). Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent. Further pipette 1 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents Further pipette 0.3 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Acceptance Criteria:
S/N Ratio value shall be 3 for LOD solution.

LIMIT OF QUANTIFICATION: (for Ivacaftor)

Preparation of 0.48 µg/ml solution:
Accurately weigh and transfer 30 mg of Ivacaftor working standard into a 100 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent. Further pipette 1 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Further pipette 1.1 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Acceptance Criteria:
S/N Ratio value shall be 10 for LOQ solution.

Procedure for LOD and LOQ:
The LOD and LOQ solutions was prepared injected, for three times and measured the area for all three injections in UPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

ROBUSTNESS:
As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.A. The flow rate was varied at 0.9 ml/min to 1.1ml/min.Standard solution 30 ppm of Tezacaftor & 45 ppm of Ivacaftor was prepared and analysed using the varied flow rates along with method flow rate.
On evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±10%.
B. The Organic composition in the Mobile phase was varied from ±10%.
Standard solution 30 ppm of Tezacaftor & 45 ppm of Ivacaftor was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method. On evaluation of the above results, it can be concluded that the variation in 10%. Organic composition in the mobile phase affected the method significantly. Hence it
Indicates that the method is robust even by change in the Mobile phase ±10.

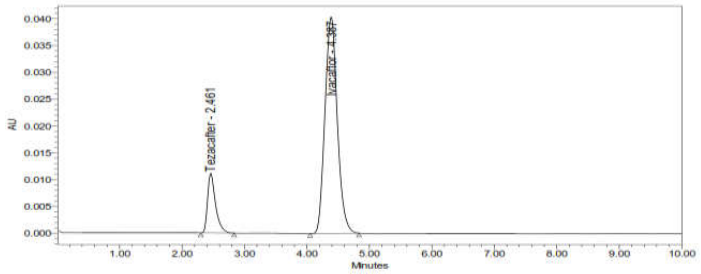
RESULTS AND DISCUSSION

METHOD DEVELOPMENT TRAIL:

Trial (optimised method):

Chromatographic conditions

Column	: Agilent Eclipse coloumn (4.6 x 150mm, 5µm)
Mobile phase ratio	: 0.1% TEA: Methanol (30: 70)
Detection wavelength	: 298 nm
Flow rate	: 1.0ml/min
Injection volume	: 20µl
Run time	: 10min



Observation: The separation was good, peak shape was good, so we conclude that there is no required for reduce the retention times of peaks, so it is taken as final method.

SYSTEM SUITABILITY:

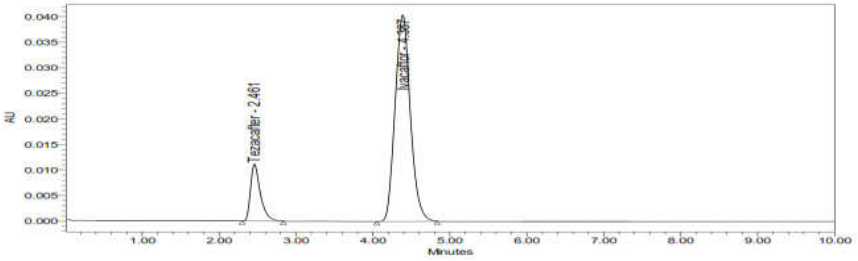


Figure 1: Chromatogram for system suitability

Table 1: Results of system suitability parameters

S. No	Name	RT(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Tezacaftor	2.461	86152	10411		1.63	4171.14
2	Ivacaftor	4.387	487733	40550	7.55	1.31	5625.95

Acceptance criteria:

- Resolution between two drugs must be not less than 2.
- Theoretical plates must be not less than 2000.
- Tailing factor must be not more than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

VALIDATION PARAMETERS:

1. ASSAY:

Standard and sample solution injected as described under experimental work. The corresponding chromatograms and results are shown below.

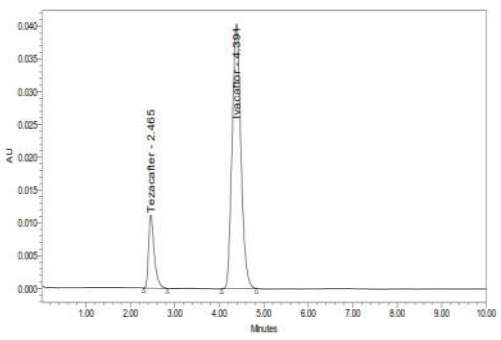
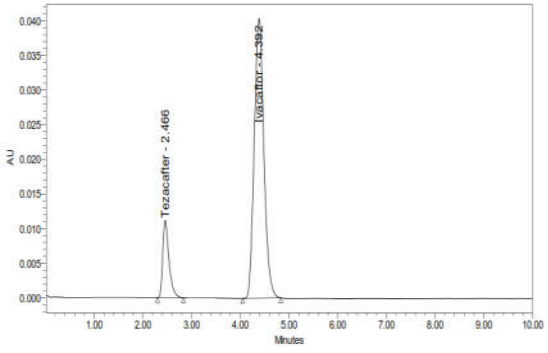


Figure 2:



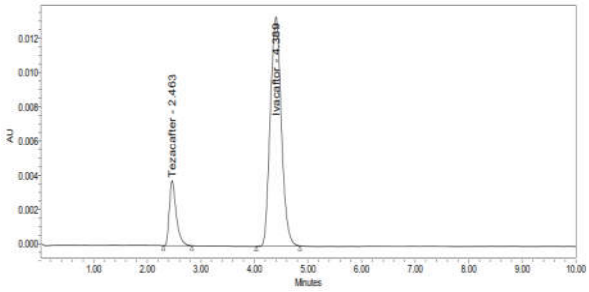
Chromatogram for Standard **Figure 3: Chromatogram for Sample**

Table 2: Results of Assay for Tezacaftor and Ivacaftor

	Label Claim (mg)	% Assay
Tezacaftor	100	100.09
Ivacaftor	150	100.92

2. LINEARITY:

The linearity range chromatograms are shown below.



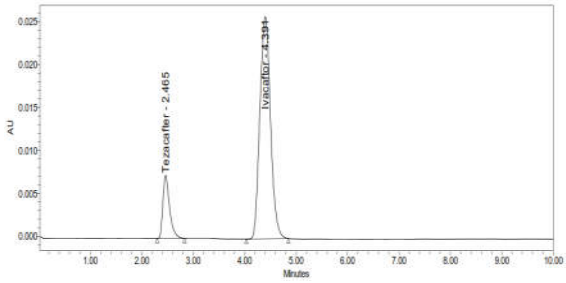
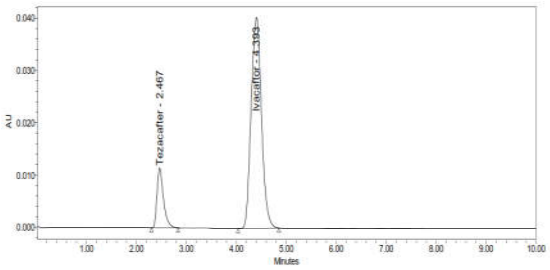


Figure 4: Chromatogram for linearity-1 **Figure 5: Chromatogram for linearity-2**



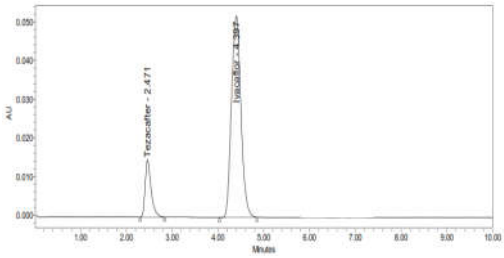


Figure 6: Chromatogram for linearity-3 **Figure 7: Chromatogram for linearity-4**

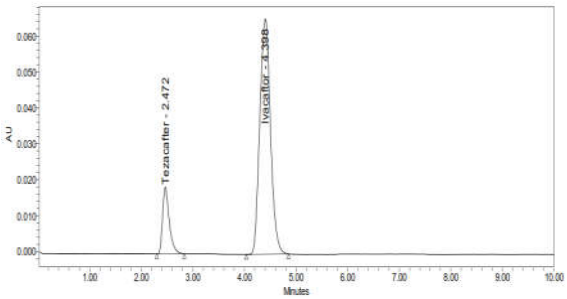


Figure 8: Chromatogram for linearity-5

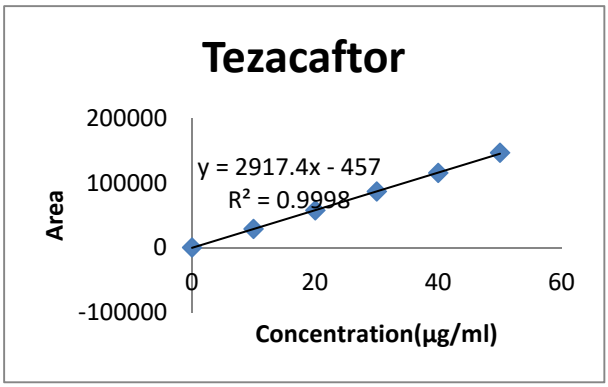
Table 3: Linearity Results: (for Tezacaftor)

S. No	Linearity Level	Concentration	Area
1	I	0	0
2	II	10	28773
3	III	20	57656
4	IV	30	86579
5	V	40	115411
6	VI	50	146452

Correlation Coefficient	0.999

Table 4: Linearity Results: (for Ivacaftor)

S. No	Linearity Level	Concentration	Area
1	I	0	0
2	II	15	162697
3	III	30	325417
4	IV	45	482354
5	V	60	646520
6	VI	75	813562
Correlation Coefficient			0.999



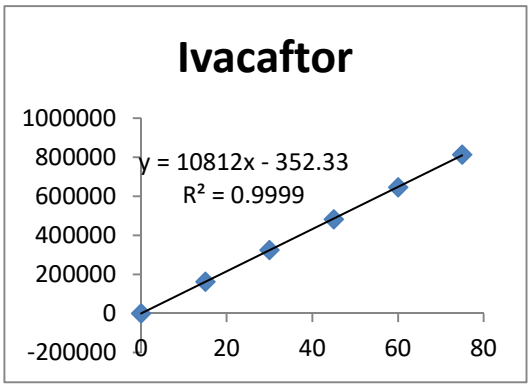


Figure 9: Calibration graph for Tezacaftor **Figure 10: Calibration graph for Ivacaftor**

Table 4: Analytical performance parameters of Tezacaftor and Ivacaftor

Parameters	Tezacaftor	Ivacaftor
Slope (m)	2917.4	10812
Intercept (c)	457	352.33
Correlation coefficient (R^2)	0.999	0.999

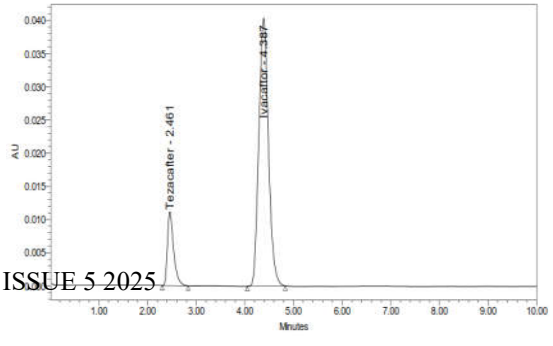
Acceptance criteria:

Correlation coefficient (R^2) should not be less than 0.999

- The correlation coefficient obtained was 0.999 which is in the acceptance limit.

3. PRECISION:

Precision of the method was carried out for both sample solutions as described under experimental work. The corresponding chromatograms and results are shown below.



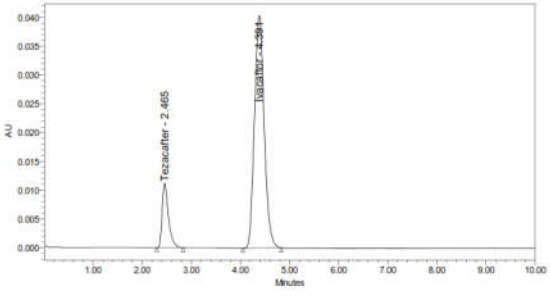
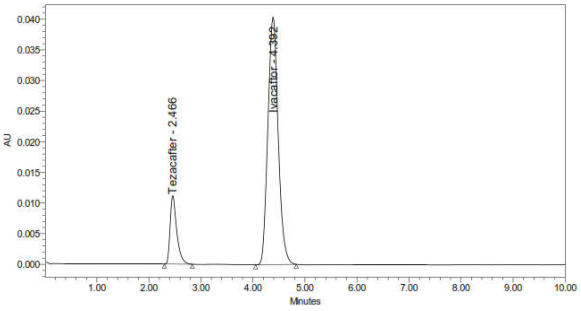


Figure 11: Chromatogram for Precision -1 **Figure 12: Chromatogram for Precision -2**



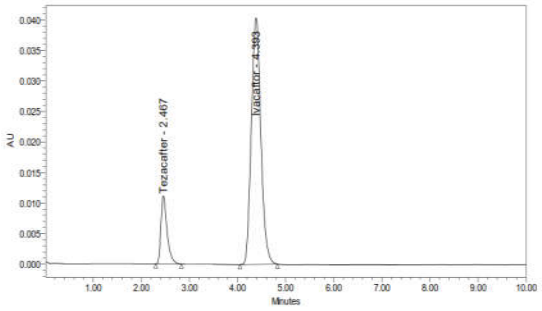
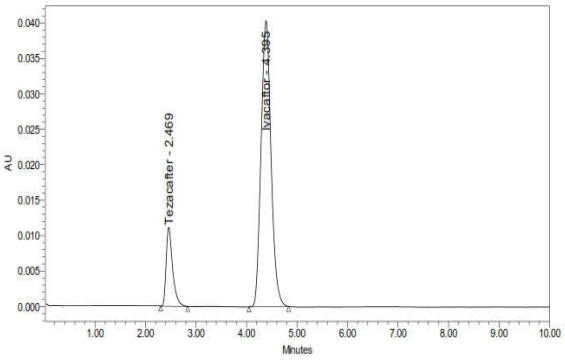


Figure 13: Chromatogram for Precision -3 **Figure 14: Chromatogram for Precision -4**



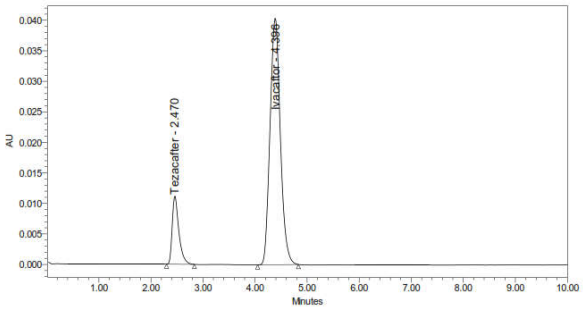


Figure 15: Chromatogram for Precision -5 **Figure 16: Chromatogram for Precision -6**

Table 5: Results showing values of Tezacaftor and Ivacaftor

Injection	Area for Tezacaftor	Area for Ivacaftor
Injection-1	86515	485692
Injection-2	86737	487526
Injection-3	86642	489964
Injection-4	86433	490536
Injection-5	86271	483951
Injection-6	86622	484285
Average	86536.7	486992.3
Standard Deviation	167.5	2826.4
%RSD	0.2	0.6

Acceptance criteria:

- %RSD for sample should be NMT 2
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

4. INTERMEDIATE PRECISION (ruggedness)

There was no significant change in assay content and system suitability parameters at different conditions of ruggedness like day to day and system to system variation.

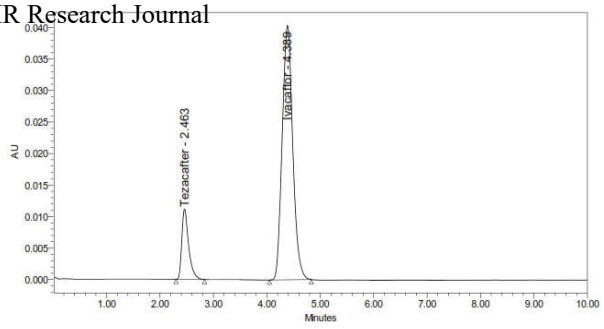


Figure 17: Chromatogram for ID Precision -1

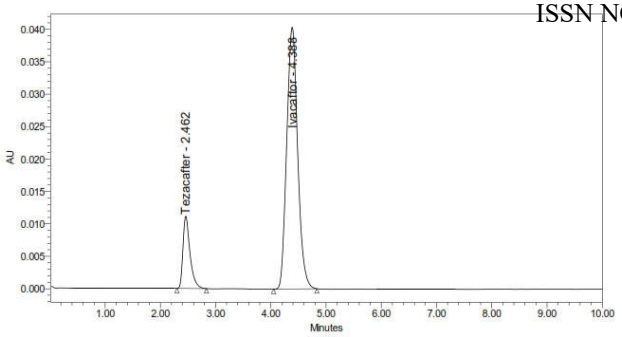


Figure 18: Chromatogram for ID Precision -2

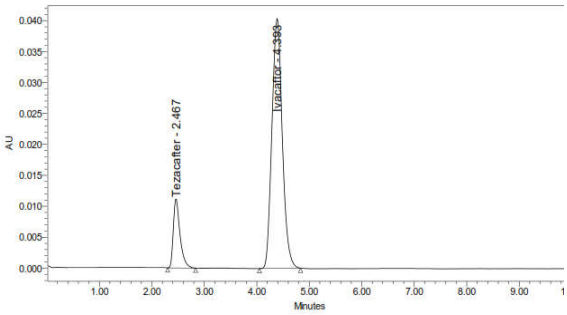


Figure 19: Chromatogram for ID Precision -3

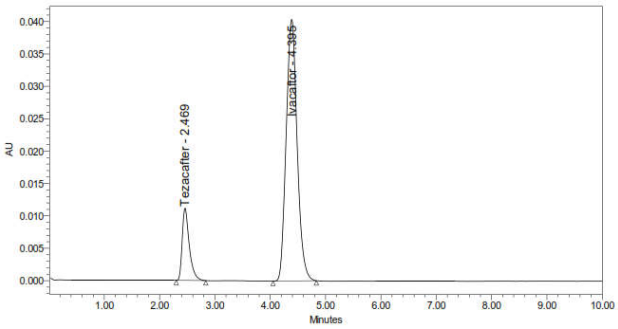


Figure 20: Chromatogram for ID Precision -4

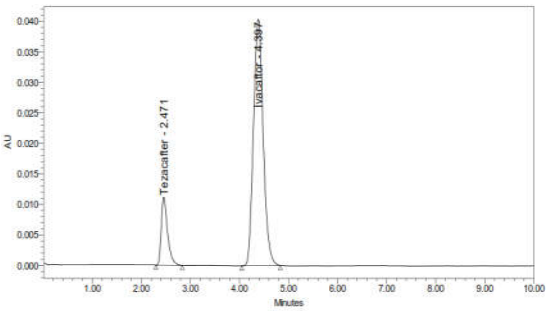


Figure 21: Chromatogram for ID Precision -5

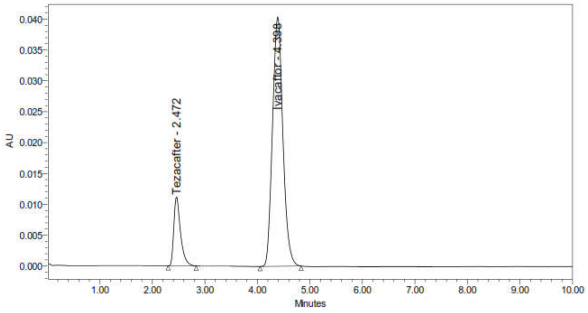


Figure 22: Chromatogram for ID Precision -6

Table 6: Results showing values of Tezacaftor and Ivacaftor

Injection	Area for Tezacaftor	Area for Ivacaftor
Injection-1	86920	484166
Injection-2	86661	489355
Injection-3	86367	484046
Injection-4	86918	486215
Injection-5	86842	488012
Injection-6	86851	486148
Average	86759.8	486323.7
Standard Deviation	214.4	2094.1
%RSD	0.2	0.4

Acceptance criteria:

- %RSD of six different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is rugged.

5. ACCURACY:

Sample solutions at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated.

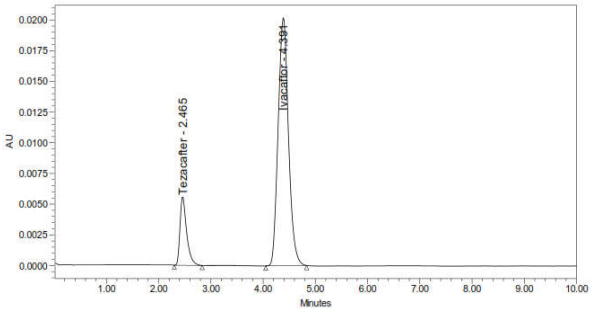


Figure 23: Chromatogram for Accuracy 50%-1

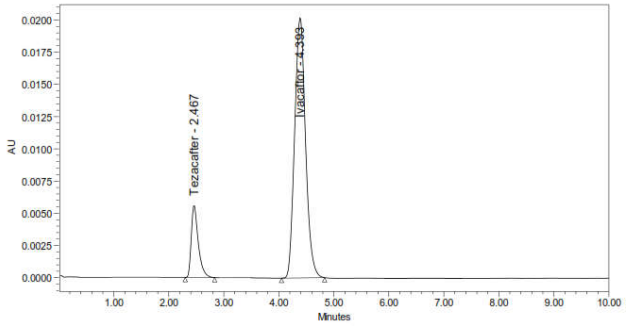


Figure 24: Chromatogram for Accuracy 50%-2

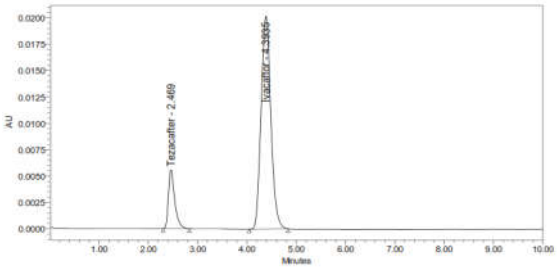


Figure 25: Chromatogram for Accuracy 50%-3

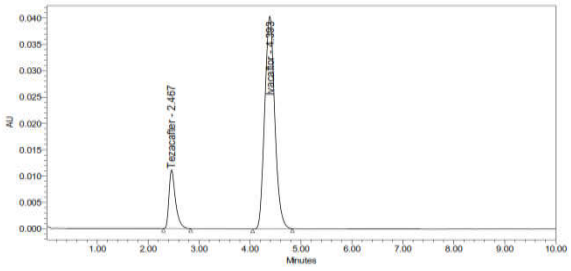


Figure 26: Chromatogram for Accuracy 100%-1

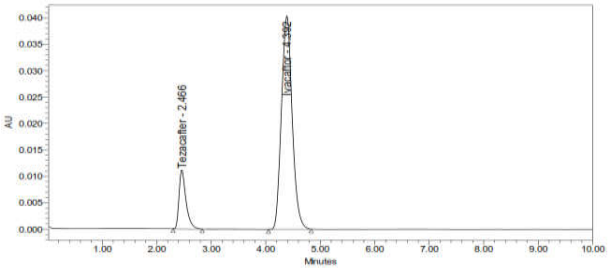


Figure 27: Chromatogram for Accuracy 100%-2

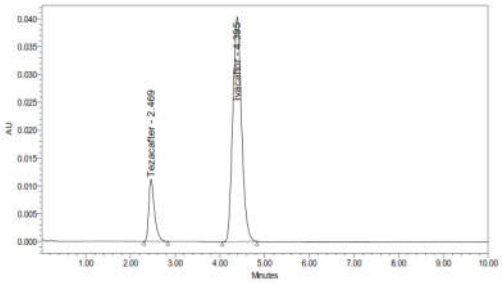


Figure 28: Chromatogram for Accuracy 100%-3

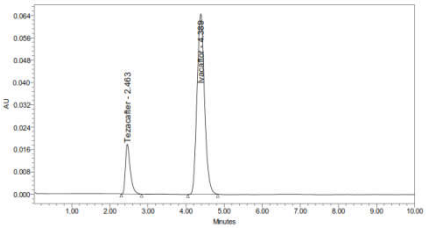


Figure 29: Chromatogram for Accuracy 150%-1

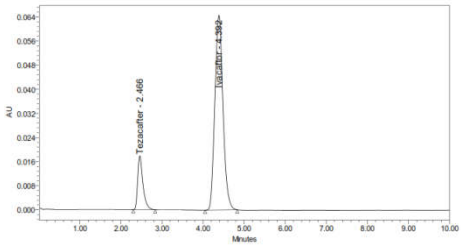


Figure 30: Chromatogram for Accuracy 150%-2

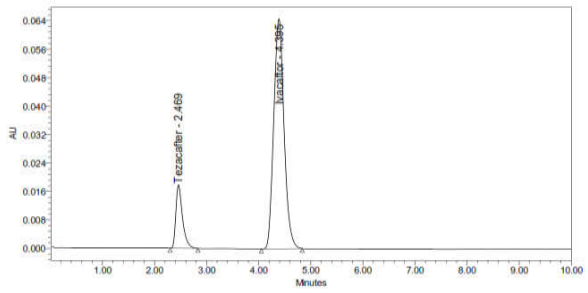


Figure 31: Chromatogram for Accuracy 150%-3

Table 7: The accuracy results for Tezacaftor

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	43621.3	10	10.08	100.83	100.29
100%	86196.3	20	19.92	99.62	
150%	130315.3	30	30.12	100.41	

Table 8: The accuracy results for Ivacaftor

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	245026.7	15	15.06	100.41	100.11
100%	485769.3	30	29.86	99.53	
150%	734871.3	45	45.17	100.38	

Acceptance Criteria:

- The percentage recovery was found to be within the limit (98-102%).

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

6. LIMIT OF DETECTION FOR TEZACAFTOR AND IVACAFTOR

The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio.

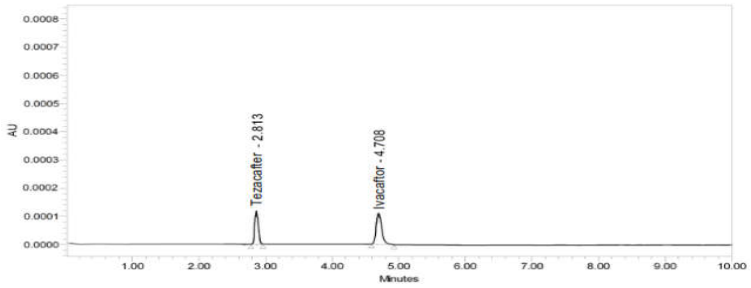


Figure 32: Chromatogram of Tezacaftor, Ivacaftor showing LOD

Table 9: Results of LOD

Drug name	Baseline noise (µV)	Signal obtained (µV)	S/N ratio
Tezacaftor	43	129	3.00
Ivacaftor	43	130	3.02

- Signal to noise ratio shall be 3 for LOD solution
- The result obtained is within the limit.

7. LIMIT OF QUANTIFICATION FOR TEZACAFTOR AND IVACAFTOR

The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio.

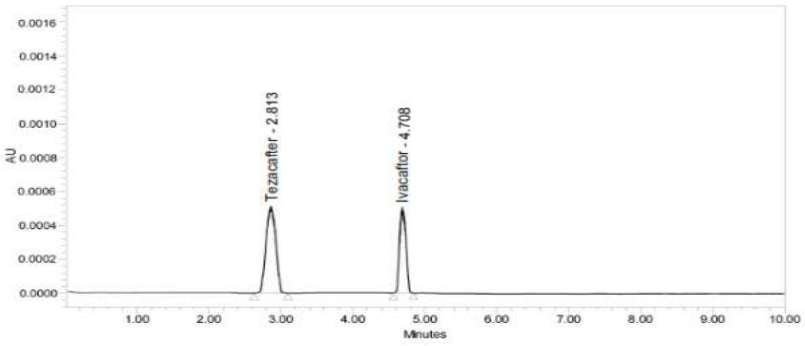


Figure 33: Chromatogram of Tezacaftor, Ivacaftor showing LOQ

Table 10: Results of LOQ

Drug name	Baseline noise (µV)	Signal obtained (µV)	S/N ratio
Tezacaftor	43	431	10.02
Ivacaftor	43	433	10.07

- Signal to noise ratio shall be 10 for LOQ solution
- The result obtained is within the limit.

8. ROBUSTNESS:

The standard and samples of Tezacaftor and Ivacaftor were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

Variation in flow

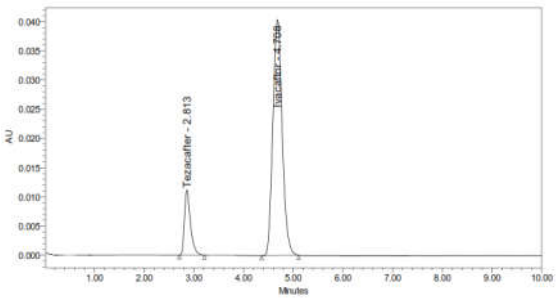


Figure 34: Chromatogram showing less flow

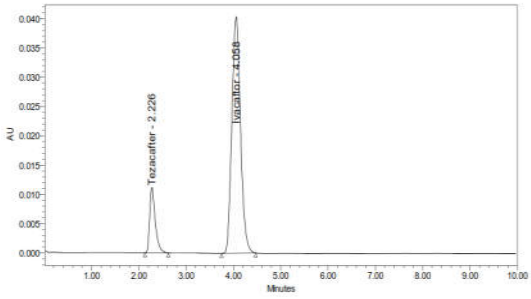
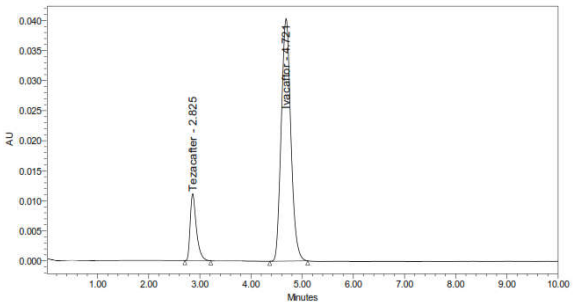
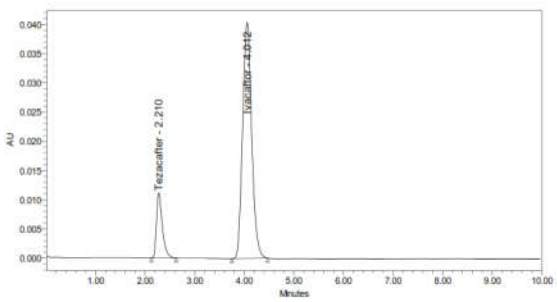


Figure 35: Chromatogram showing more flow

Variation of mobile phase organic





composition:

Figure 36: Chromatogram showing less organic composition Figure 37: Chromatogram showing more organic composition

Table 11: Results for variation in flow for Tezacaftor

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Tailing	USP Plate Count
1	0.9	1.49	4685.99
2	1.0	1.63	4171.14
3	1.1	1.49	4253.39

Table 12: Results for variation in flow for Ivacaftor:

S. No	Flow Rate (ml/min)	System Suitability Results		
		USP Resolution	USP Tailing	USP Plate Count
1	0.9	6.22	1.10	5119.35
2	1.0	7.55	1.31	5625.95
3	1.1	6.22	1.10	5262.48

***** Results for actual flow (1.0ml/min) have been considered from Assay standard.

Table 13: Results for variation in mobile phase composition for Tezacaftor

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	1.49	4253.39
2	*Actual	1.63	4171.14
3	10% more	1.49	4936.98

Table 14: Results for variation in mobile phase composition for Ivacaftor:

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results		
		USP Resolution	USP Tailing	USP Plate Count
1	10% less	6.83	1.10	5163.30
2	*Actual	7.55	1.31	5625.95
3	10% more	5.79	1.10	5867.87

* Results for actual Mobile phase composition have been considered from Accuracy standard.

Acceptance criteria:

The Retention time, USP plate count, USP tailing factor obtained for change of flow rate, variation in mobile phase was found to be within the acceptance criteria. Hence the method is robust.

SUMMARY AND CONCLUSION

The estimation of Tezacaftor and Ivacaftor was done by RP-HPLC.

The assay of Tezacaftor and Ivacaftor was performed with tablets and the % assay was found to be 100.19 and 100.92 which shows that the method is useful for routine analysis.

The linearity of Tezacaftor and Ivacaftor was found to be linear with a correlation coefficient of 0.999 and 0.999, which shows that the method is capable of producing good sensitivity.

The acceptance criteria of precision is RSD should be not more than 2.0% and the method show precision 0.2 and 0.6 for Tezacaftor and Ivacaftor which shows that the method is precise.

The acceptance criteria of intermediate precision is RSD should be not more than 2.0% and the method show precision 0.2 and 0.4 for Tezacaftor and Ivacaftor which shows that the method is repeatable when performed in different days also.

The accuracy limit is the percentage recovery should be in the range of 97.0% - 103.0%. The total recovery was found to be 100.29% and 100.11% for Tezacaftor and Ivacaftor. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy and reproducibility.

The acceptance criteria for LOD and LOQ is 3 and 10.The LOD and LOQ for Tezacaftor was found to be 3.00 and 10.02 and LOD and LOQ for Ivacaftor was found to be 3.02 and 10.07.

The robustness limit for mobile phase variation and flow rate variation are well within the limit, which shows that the method is having good system suitability and precision under given set of conditions.

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