A New RP-HPLC Method for The Determination of Tezacaftor and Ivacaftor in Bulk Form and Marketed Pharmaceutical Dosage Form

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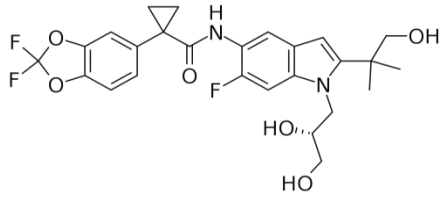
Abstract— The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 260 nm and the peak purity was excellent. Injection volume was selected to be 10µl which gave a good peak area. The column used for study was Symmetry C18 because it was giving good peak. Ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time. Mobile phase is Methanol: TEA pH 4.2 (40:60) was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study. Run time was selected to be 5 min because analyze gave peak around 2.781, 4.048 ±0.02 min respectively and also to reduce the total run time. The percent recovery was found to be 98.0-102 was linear and precise over the same range. Both system and method precision was found to be accurate and well within range. The analytical method was found linearity over the range 37.5-187.5µg/ml of Ivacaftor and 25-125 µg/ml of Tezacaftor of the target concentration. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

Index Terms- Develosil ODS HG-5 RP C_{18}column, Tezacaftor and Ivacaftor, RP-HPLC.

I. INTRODUCTION

Combination therapy that combines more than one method of treatment. Also called multimodality therapy and multimodality treatment[1-3]. Tezacaftor is a drug of the cystic fibrosis transmembrane conductance regulator (CFTR) potentiator class. It was developed by Vertex Pharmaceuticals and FDA approved in combination with Ivacaftor to manage cystic fibrosis. This drug was approved by the FDA on February 12, 2018. 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. It was approved by the Food and Drug Administration on January 31, 2012, and by Health Canada in late 2012.16 Ivacaftor is administered as a monotherapy and also administered in combination with other drugs for the management of CF. Various HPLC estimations have been reported in the literature for the determination of Tezacaftor and Ivacaftor present in pharmaceutical dosage forms. This method was validated according to ICH guidelines for specificity, LOD, LOQ, Precision, Accuracy, and Linearity[4-7]. The method showed good reproducibility and recovery with %RSD less than 2. Hence we had made an attempt to develop a simple accurate and precise RP HPLC method for the simultaneous estimation of Tezacaftor and Ivacaftor in bulk and in tablet dosage form.

Drug Profile[8-13]:
Name: Tezacaftor
Structure:

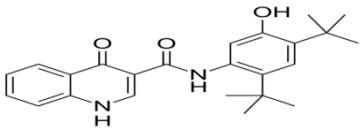


IUPAC Name: 1-(2, 2-difluoro-2H-1, 3-benzodioxol-5-yl)-N-{1-[(2R)-2, 3-dihydroxypropyl]-6-fluoro-2-(1-hydroxy-2-methylpropan-2-yl)-1H-indol-5-yl} cyclopropane-1-carboxamide

Description: Tezacaftor is a drug of the cystic fibrosis transmembrane conductance regulator (CFTR) potentiator class. It was developed by Vertex Pharmaceuticals and FDA approved in combination with Ivacaftor to manage cystic fibrosis.

Name: Ivacaftor

Structure:



IUPAC Name: N-(2,4-di-tert-butyl-5-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide.

Description: 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 Pharmaceutical

II. MATERIALS AND METHODS

Instruments used:

Table No 1. Instruments used

S.No.	Instruments And Glass wares	Model
1	HPLC	WATERS, software: Empower 2, Alliance 2695 separation module. 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	Enertech

Chemicals used:

Table No2. Chemicals used

S.No.	Chemical	Brand names
1	Ivacaftor	Sura labs
2	Tezacaftor	Sura labs
3	Water and Methanol for HPLC	LICHROSOLV (MERCK)
4	Acetonitrile for HPLC	Merck
5	Triethyl amine	Sura labs

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 0.75 ml of Tezacaftor and 1.125 ml of Ivacaftor from the above stock solutions into a 10 ml volumetric flask and dilute up to the mark with diluents.

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.

Mobile Phase Optimization:

Initially the mobile phase tried was methanol: water with varying proportions. Finally, the mobile phase was optimized to methanol: TEA Buffer in proportion 40:60 v/v respectively.

Optimization of Column:

The method was performed with various columns like C18 column, Symmetry and X-Bridge. Symmetry C18 (4.6×150mm, 5µ) was found to be ideal as it gave good peak shape and resolution at 1 ml/min flow.

Optimized chromatographic conditions:

Instrument used : Waters HPLC with auto sampler and PDA Detector 996 model.
Temperature : 40ºC
Column : Symmetry C18 (4.6×150mm, 5µ)
PH : 4.2
Mobile phase : Methanol: TEA buffer pH 4.2 (40:60v/v)
Flow rate : 1ml/min
Wavelength : 260 nm
Injection volume : 10 µl
Run time : 6 min

Chromatographic trial for simultaneous estimation of Tezacaftor and Ivacaftor by RP- HPLC

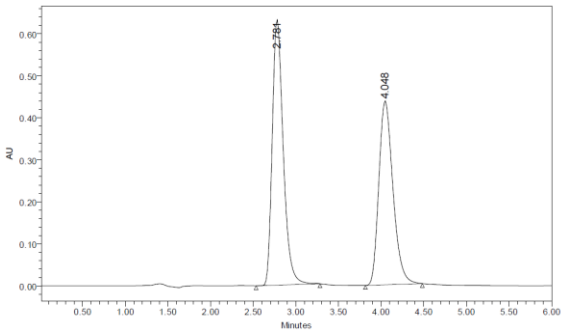


Fig.No.1 Optimized chromatographic conditions

III. RESULTS AND DISCUSSIONS

Method validation[14-20]:

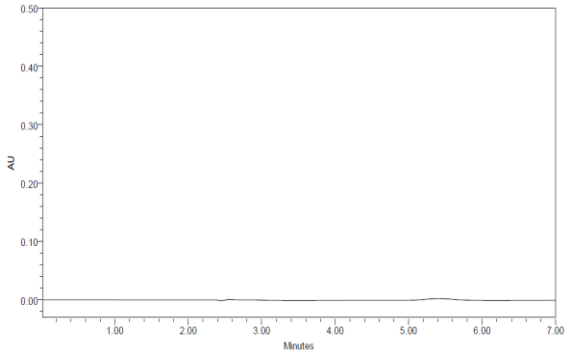


Fig No 2. Chromatogram showing blank (mobile phase preparation)

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 quantitates the drugs in drug product.

Assay calculation for Tezacaftor and Ivacaftor:

The assay study was performed for the Tezacaftor and Ivacaftor The chromatograms area unit shown in Fig. No.3 and results area unit tabulated in Table. No.3

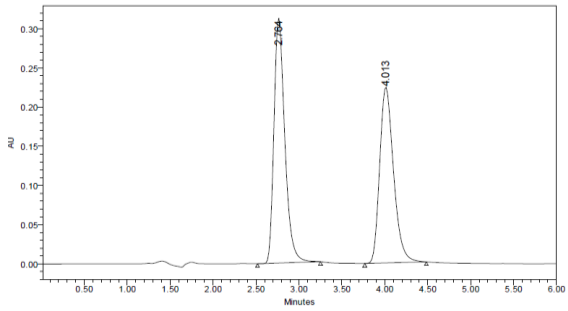


Fig.No.3. Chromatogram showing assay of sample injection-1, 2

Table.No.3. Showing assay results:

S. No.	Name	RT	Area	Height	USP Resolution	USP Tailing	USP Plate Count	Injection
5	Ivacaftor	2.764	2744776	312684	4.6	1.3	6329	1
6	Tezacaftor	4.013	2515628	206571	4.6	1.3	5990	2

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

The % purity of Tezacaftor and Ivacaftor in pharmaceutical dosage form was found to be 100. 9%, 100. 7%. The specificity check was performed for Tezacaftor and Ivacaftor. It had been found that there was no interference of impurities in retention time of analytical peak.

Linearity:

The one-dimensionality study was performed for the concentration of 37.5 µg/ml to 187.5 µg/ml for Ivacaftor and 25ppm to 125ppm for Tezacaftor. The results area unit tabulated in Table. No.4-5. Standardization graph area unit shown in Fig.No.4- 5.

Table No.4 Linearity Results for Ivacaftor

Concentration µg/ml	Average Peak Area
37.5	892464
75	1866364
112.5	2777423

150	3709213
187.5	4601317

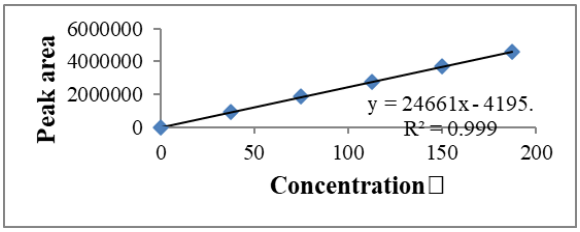


Fig.No.4. Showing calibration graph for Ivacaftor

Table No.5 Linearity Results for Tezacaftor

Concentration µg/ml	Average Peak Area
25	920032
50	1752782
75	2521426
100	3326009
125	4217393

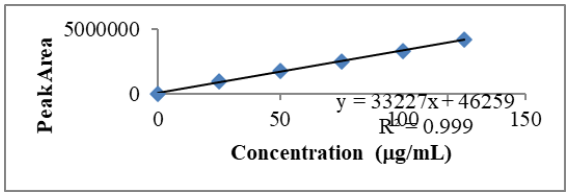


Fig.No.5. Showing calibration graph for Tezacaftor

The response linearity is verified if the Correlation Coefficient is 0.99 or greater. Correlation Coefficient (r) is 0.99, and the intercept is 46259. 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.

i) Repeatability:

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

Table No 6. Results of repeatability for Ivacaftor

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Ivacaftor	2.766	2766870	294578	6684	1.3
2	Ivacaftor	2.774	2771971	286541	6347	1.3
3	Ivacaftor	2.770	2771958	302657	6674	1.3
4	Ivacaftor	2.772	2780299	293412	6451	1.3
5	Ivacaftor	2.771	2789695	283154	6678	1.3
Mean			2776159			
Std. Dev			8969.6			
% RSD			0.32			

Table No 7. Results of method repeatability for Tezacaftor

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Tezacaftor	4.025	2534539	193240	5761	1.3
2	Tezacaftor	4.040	2539247	201647	5489	1.3
3	Tezacaftor	4.032	2544661	193472	5367	1.3
4	Tezacaftor	4.041	2548839	196475	5845	1.3
5	Tezacaftor	4.036	2558822	201394	5347	1.3
Mean			2545221			
Std.			9330.0			

Dev						
% RSD			0.37			

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

ii) Intermediate Precision:

Table No 8. Results of Intermediate precision for Ivacaftor

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Ivacaftor	2.781	2715421	294651	6647	1.3
2	Ivacaftor	2.780	2778540	284123	6781	1.3
3	Ivacaftor	2.782	2754247	274561	6984	1.3
4	Ivacaftor	2.780	2780545	281241	6475	1.3
5	Ivacaftor	2.782	2777021	286471	6647	1.3
6	Ivacaftor	2.774	2780254	294512	6489	1.3
Mean			2764338			
Std. Dev			25974			
% RSD			0.9			

Table No 9. Results of Intermediate precision for Tezacaftor

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Tezacaftor	4.015	2536301	211541	5495	1.4
2	Tezacaftor	4.007	2541972	206141	5694	1.4
3	Tezacaftor	4.323	2521259	198641	5785	1.4
4	Tezacaftor	4.065	2537081	206741	5947	1.4
5	Tezacaftor	4.020	2549869	209487	5742	1.4
6	Tezacaftor	4.015	2536301	193481	5914	1.4
Mean			2537131			
Std. Dev			93700.087			
% RSD			0.36			

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

Accuracy:

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

Table No 10. The accuracy results for Ivacaftor

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery

50%	1382603	56.25	55.05	99. 9	99.8 %
100%	2777270	112.5	112.4	99. 9	
150%	41448756	225	224.6	99.6	

Table No 11. The accuracy results for Tezacaftor

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	1306990	37.5	37.5	100	99.4 %
100%	2510628	75	74.8	98.6	
150%	3777999	150	149.96	99.8	

The percentage recovery of both drugs 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.

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.

LOD= 3.3 × σ / s

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

The LOD was performed for Ivacaftor and Tezacaftor was found to be 0.8µg/ml and 0.7µg/ml respectively.

Limit of Quantitation:

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

LOQ=10×σ/S

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

The LOQ was performed for Ivacaftor and Tezacaftor was found to be 2.4µg/ml and 2.19 µg/ml respectively.

Robustness:

The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Ivacaftor and Tezacaftor. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±10%. The standard and samples of Ivacaftor and Tezacaftor 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.

Table No 12. Results for Robustness Ivacaftor

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical	Tailing factor
Actual Flow rate of 1.0 mL/min	2774027	2.781	6314	1.2
Less Flow rate of 0.9 mL/min	2884521	3.327	6199	1.4
More Flow rate	2542	2.516	6234	1.4
Less organic phase	2888515	3.326	6298	1.4
More organic phase	2541550	2.416	6287	1.2

Table No 12. Results for Robustness Tezacaftor

Parameter used for sample analysis	Peak Area	Retentio	Theoretical plates	Tailing
Actual Flow rate of 1.0 mL/min	2533532	4.048	5521	1.3
Less Flow rate of 0.9 mL/min	2750214	5.319	5643	1.6
More Flow rate of	225410	3.64	5782	1.5
Less organic phase	2754017	5.318	5309	1.4
More organic phase	2215870	3.233	5580	1.51

Acceptance criteria the tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

SUMMARY AND CONCLUSION

The developed method was successfully applied for simultaneous estimation of Ivacaftor and Tezacaftor in

compound tablet formulation. The proposed method was found to be simple, accurate and precise. There is no analytical method available to determine the same combination of drugs. Therefore, this method may be useful for routine analysis of Ivacaftor and Tezacaftor in API drugs and pharmaceutical dosage forms.

ACKNOWLEDGEMENT

We take pleasure to express our sincere thanks to our beloved Dr.DVN. Bikshapathi.M.Pharma,Ph.D Principal, Teegala Ram Reddy college of Pharmacy for his undivided attention, valuable suggestion, and endeavor throughout the during project work. Special thanks to all faculty members of Teegala ram reddy college of pharmacy for their help, support and encouragement during project work.

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