Development & Validation of Stability Indicating Rp HPLC Method for Simultaneous Estimation of Tazacafter and Ivacafter in Combined Dosage Form

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Abstract **- A simple, accurate, precise method was developed for the simultaneous estimation of the ivacaftor and tezacaftor in bulk and tablet dosage form. Chromatogram was run through discovery 250 x 4.6 mm, 5µ. Mobile phase containing buffer 0.01N potassium dihydrogen ortho phosphate: acetonotrile taken in the ratio 50:50 was pumped through column at a flow rate of 1 ml/min. Buffer used in this method was 0.01N potassium dihydrogen buffer. Temperature was maintained at 30°C. Optimized wavelength selected was 290 nm. Retention time of ivacaftor and tezacaftor were found to be 2.373 min and 2.967. %RSD of method precision of ivacaftor and tezacaftor were found to be 0.8 and 0.8, respectively. %Recovery was obtained as 100.69% and 100.81% for ivacaftor and tezacaftor respectively. LOD & LOQ values obtained from regression equations of ivacaftor and tezacaftor were found to be 0.22, 0.66 and 0.15, 0.46, respectively. Regression equation of ivacaftor is y = 31543x + 76309, and tezacaftor is y = 24409x + 4287. Retention times were decreased and that run time was decreased, so the developed method was simple and economical that can be adopted in regular quality control test in Industries.**

Index Terms **- Ivacaftor, Tezacaftor, RP-HPLC**

INTRODUCTION

Ivacaftor (also known as Kalydeco or VX-770) is a drug used for the management of cystic fibrosis (CF) in patients aged 2 years and older. Cystic fibrosis is an autosomal recessive disorder caused by one of several different mutations in the gene for the cystic fibrosis transmembrane conductance regulator (CFTR) protein, an ion channel involved in the transport of chloride and sodium ions across cell membranes. CFTR is active in epithelial cells of organs such as of the lungs, pancreas, liver, digestive system, and reproductive tract. Alterations in the CFTR gene result in altered production, misfolding, or function of the protein and consequently abnormal fluid and ion transport across cell membranes(1,2). As a result, CF patients produce thick, sticky mucus that clogs the ducts of organs where it is produced making patients more susceptible to complications such as infections, lung damage, pancreatic insufficiency, and malnutrition.

Tezacaftor is a small molecule that can be used as a corrector of the cystic fibrosis transmembrane conductance regulator (CFTR) gene function. It was developed by vertex pharmaceuticals and FDA approved in combination with ivacaftor; a CFTR potentiator that allows the proteins at the cell surface to open longer and improve nutrient transport.

MATERIALS AND REAGENTS

Tezacaftor and ivacaftor pure drugs (API), distilled water, acetonitrile, phosphate buffer, methanol, potassium dihydrogen ortho phosphate buffer, ortho-phosphoric acid. All the above chemicals and solvents are from Rankem

Chromatographic Conditions and Equipments

Electronic Balance-Denver, pH meter -BVK enterprises, India, Ultrasonicator-BVK enterprises, WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and Auto sampler integrated with Empower 2 Software. UV-VIS Spectrophotometer PG Instruments T60 with special bandwidth of 2 mm and 10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbances of tezacaftor and ivacaftor solutions. The mobile phase used was 0.01N potassium dihydrogen ortho phosphate and acetonitrile in the gradient mode employing flow rate at 1.2ml/min. The analytical column inspires C18 (4.6

x 250 mm, 5 µm). The detection was carried out at a wavelength of 290 nm with a run time of 10 min. Water and acetonitrile in the ratio of 50:50 v/v used as diluent

Standard Solution Preparation

Accurately weighed and transferred 25mg & 37.5mg of ivacaftor and tezacaftor pure drugs into 25mL clean dry volumetric flasks separately, add 3/4th volume of diluent, sonicated for 10 minutes and made up to the final volume with diluent. From the above each stock solution, 1 mL was pipette out in to a 10mL volumetric flask and then made up to final volume with diluents, mixed well and filtered through 0.45µm filter

Sample Solution Preparation

About 5 tablets were weighed and calculated the average weight of each tablet then the tablet powder weight equivalent to one tablet was transferred into a 100mL volumetric flask, 3/4th volume of diluent, sonicated for 25 minutes and made up to the final volume with diluent. From this 1mL was pipetted out into a 10 mL volumetric flask and made up to 10 mL with diluent.

RESULTS AND DISCUSSION

Method Development and Optimization

Prime objective of an RP-HPLC method development for determination of ivacaftor and tezacaftor in Pharmaceutical dosage form was that the method should be able to determine assay of drug and should be precise, accurate, reproducible, specific, and stability indicating. All degradation products from stress conditions should be well separated. Method should be simple so that it can be useful in analytical research and quality control laboratory for routine use.

Mobile Phase and Chromatographic Conditions Optimization

Method development was tried initially with ascentics C18 150 × 4.6 mm, 5µm column, as stationary phase and ortho phosphoric acid: methanol (50:50 v/v) as mobile phase at a flow rate 1.2 mL/min, no peak of ivacaftor was found under this condition in a run time of 10 min. Further trials were carried out with different mobile phase compositions by keeping rest of the chromatographic conditions were unchanged. Good separation with asymmetry of the peak was obtained by using buffer: acetonitrile (50:50 %v/v). Finally, well resolved peak with a good symmetry was obtained at 2.37 min (Ivacaftor) & 2.96 min (Tezacaftor) by replacing the column with Discovery C18 (250 × 4.6 mm, 5µm) with mobile phase composition of buffer:acetonitrile at 50:50 ratio (Figure 3). The developed method was validated as per ICH guidelines and after complete validation applicability of the method has been done for pharmaceutical dosage form. The amount of drug present in the marketed formulation was calculated and the % assay was found to be 100.69% for Ivacaftor and 100.81% forTezacaftor

Validation of method

Analytical method validation was carried out by means of system suitability, linearity, precision, accuracy, robustness, and forced degradation studies.

Linearity

Different aliquots of Ivacaftor and Tezacaftor standard stock solutions were prepared and injected into chromatographic system. The linearity of the method was demonstrated over six linear concentrations of Ivacaftor (37.5-225 µg/ml) and Tezacaftor (25-150 µg/ml) are prepared and injected. Regression equation of the Ivacaftor and Tezacaftor are found to be, y = 31543x+76309, and y = 24409x+4287 and regression co-efficient was 0.999.Calibration curve of Ivacaftor and Tezacaftor was constructed by plotting peak area vs. concentration as shown in (Figure 4 & 5).The results are represented in Table 1.

Precision

The precision of the method was demonstrated by system precision and method precision. In system precision, standard solution of Ivacaftor and Tezacaftor was injected six times and the % RSD of peak area was found to be less than 1.5. In the method precision, the pre-analyzed sample solution was injected in triplicate within a day and on three different days and the %RSD was found to be less than 2, which indicates that the method is more precise. The results were incorporated in Table 2.

Accuracy

Accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the pre-analyzed sample solution at 3

different concentrations i.e 50%, 100% and 150%. The resulting solutions were analyzed in triplicate at each level as per the ICH guidelines. The mean recoveries of Ivacaftor and Tezacaftor were found to be in the range of 100.69 – 100.81%. The % recovery results are represented in Table 3.

LOD and LOQ

The LOD and LOQ were determined based on the standard deviation of response of the calibration curve. The residual standard deviation of the regression line and slope of the calibration curve were used to calculate the LOD and LOQ. The values of LOD were found to be 0.22µg/mL and 0.15µg/mL, for Ivacaftor and Tezacaftor and the values of LOQ were found to be 0.66µg/mL and 0.46µg/mL, respectively.

Robustness

As defined by the ICH, the robustness of an analytical procedure describes its capability to remain unaffected by small and deliberate variations in method parameters. The robustness as a measure of method capacity to remain unaffected by small but deliberate changes in chromatographic conditions was studied by testing influence of small changes in flow rate (mL/min), change in column oven temperature (^{0}C), and change in mobile phase composition with ±0.2 %. There is no significant changes in the results, thus the method is more robust. The results were incorporated in Table 4.

Forced Degradation Studies

Oxidation:

To 1 mL of stock solution of Ivacaftor &Tezacaftor, 1 mL of 20% hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 30 min at 60°C. For HPLC study, the resultant solution was diluted to obtain 100µg/mL& 150 µg/mL solution and 10 µL were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies:

To 1 mL of stock solutions of Ivacaftor &Tezacaftor, 1 mL of 2N Hydrochloric acid was added and refluxed for 30 min at 60°C.The resultant solution was diluted to obtain 100 µg/mL& 150µg/mL solution and 10 µL solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies:

To 1 mL of stock solution Ivacaftor &Tezacaftor, 1 mL of 2N sodium hydroxide was added and refluxed for 30mins at 600c. The resultant solution was diluted to obtain 100µg/mL& 150µg/mL solution and 10 µL were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies:

The standard drug solution was placed in oven at 105°C for 6 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 100µg/mL& 150µg/mL solution and10µL were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Photo Stability studies:

The photochemical stability of the drug was also studied by exposing the 1000µg/mL&1500 µg/mL solution to UV Light by keeping the beaker in UV Chamber for 1days or 200-Watt hours/m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 100µg/mL &100µg/mL solutions and 10 µL were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Degradation Studies:

Stress testing under neutral conditions was studied by refluxing the drug in water for 1hrs at a temperature of 60ºC. For HPLC study, the resultant solution was diluted to 100 µg/mL &150µg/mL solution and 10 µL were injected into the system and the chromatograms were recorded to assess the stability of the sample.

In above all the conditions, both the drugs were degraded (fig 6, 7, 8 and9) and the % of degradation was incorporated in Table 5.

CONCLUSION

The proposed method was used successfully to determine Ivacaftor and Tezacaftor in raw material and tablets even in presence of degradation products. The results of validation studies shown that the stability indicating HPLC method is accurate, significantly linear and precise the stability tests results showed that Ivacaftor and Tezacaftortablets are more prone to acidic and oxidative degradation than other stress conditions and the current method has

resolved the degraded products from the main peak. Thus, the proposed HPLC method is useful for routine quality control analysis of Ivacaftor and Tezacaftor in bulk and its formulations.

ACKNOWLEDGEMENT

The authors would like to thank Spectrum Pharma Research Solutions, Hyderabad for supplying gift sample of Ivacaftor and Tezacaftor and special thanks to Hindu College of Pharmacy, Guntur for good support and amenities.

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Table 1: Calibration data of ivacaftor and tezacaftor

Ivacaftor		Tezacaftor	
Conc (µg/ml)	Peakarea	Conc (µg/ml)	Peakarea
0	0	0	0
37.5	1383092	25	623529
75	2459475	50	1195600
112.5	3607415	75	1833775
150	4743670	100	2475043
187.5	5940125	125	3096407
225	7240736	150	3620356

Table 2: Repeatability Results

S.No	Areaof Ivacaftor	Areaof Tezacaftor

1.	4270052	2541098
2.	4289162	2517093
3.	4291789	2497923
4.	4300614	2489937
5.	4327186	2503087
6.	4314452	2529554
Mean	4298876	2513115
S.D	20112.2	19686.0
% RSD	0.5	0.8

Table 3: Accuracy Results (n=3)

Sample	% Level	% Recovery	% RSD
Ivacaftor	50	101.41	0.8
	100	99.73	
	150	100.63	
Tezacaftor	50	100.63	0.8
	100	101.16	
	150	100.62	

Table 4:Robustness Results

S.no	Condition	%RSD of Ivacaftor	%RSD of Tezacaftor
1	Flow rate (-) 0.9ml/min	0.4	0.4
2	Flow rate (+) 1.1ml/min	1.2	1.5
3	Mobile phase (-) 55B:45A	1.4	0.2
4	Mobile phase (+) 45B:55A	0.8	0.1
5	Temperature(-)25°C	0.3	0.7
6	Temperature(+)35°C	1.3	0.8

Table 5: Degradation data in all stress conditions

Type of degradation	Ivacaftor		Tezacaftor	
	%RECOVERED	%DEGRADED	%RECOVERED	%DEGRADED
Acid	93.92	6.08	93.80	6.20
Base	95.47	4.53	95.28	4.72
Peroxide	95.86	4.14	95.51	4.49
Thermal	98.25	1.75	97.66	2.34
UV	98.63	1.37	99.40	0.60
Water	99.56	0.44	99.20	0.80

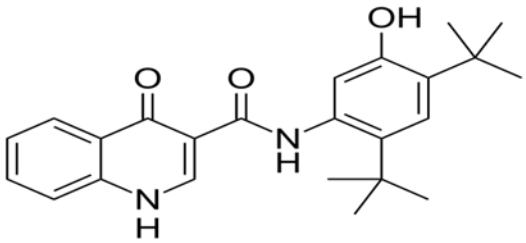

Figure 1: Chemical structure of ivacaftor

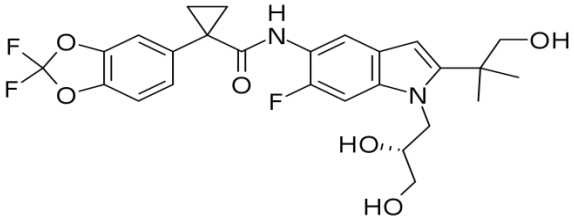

Figure 2: Chemical structure of tezacafter

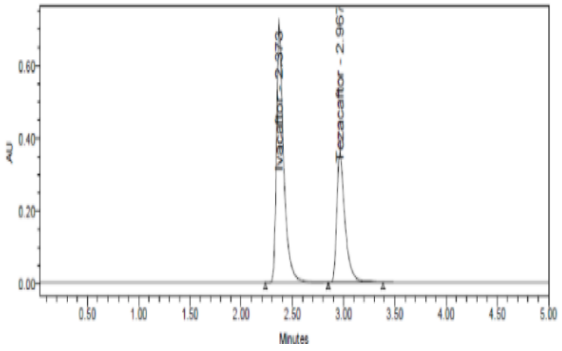

Figure 3: Standard chromatogram

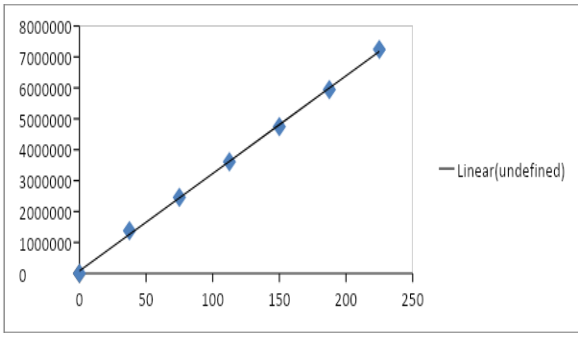

Figure 4: Linearity graph for ivacaftor

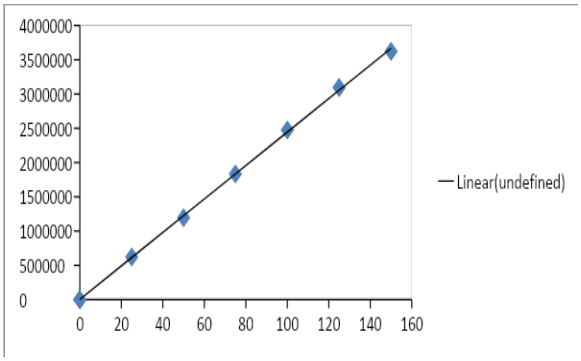

Figure 5: Linearity graph for tezacaftor

Degradation chromatograms

Acid degradation chromatogram

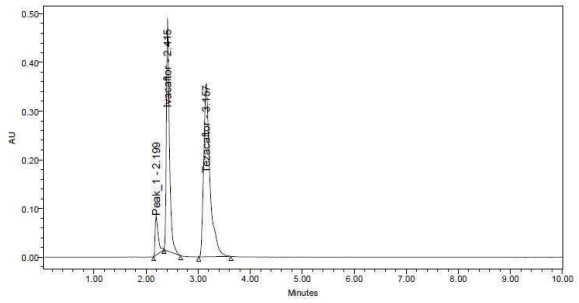

Fig.6 acid

Base degradation chromatogram

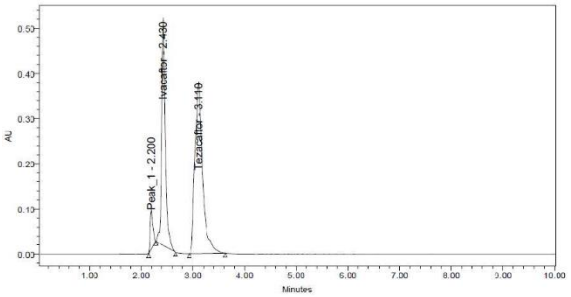

Fig.7 base

Peroxide degradation chromatogram

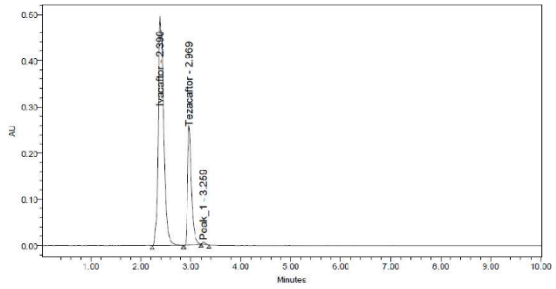

Fig 8 peroxide

Thermal degradation chromatogram

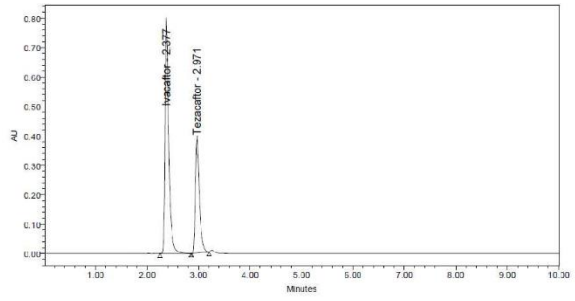

Fig.9 thermal