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Stability Indicating Rp- Hplc Method For Simultaneous Estimation Of Tezacaftor And Evacaftor In Tablet Dosage Form

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Abstract:

A simple, Accurate, precise method was developed for the simultaneous estimation of the Ivacaftor and Tezacaftor in Tablet dosage form. Chromatogram was run through Zodiacil C18 (150 x 4.6 mm, 3.5µ) Mobile phase containing 0.01N KH_2PO_4: Acetonitrile taken in the ratio 55:45 was pumped through column at a flow rate of 1.0 ml/min. Temperature was maintained at 30°C. Optimized wavelength selected was 292.0 nm. Retention time of Ivacaftor and Tezacaftor were found to be 2.269 min and 3.164 min. %RSD of the Ivacaftor and Tezacaftor were and found to be 0.5 and 1.0 respectively. %Recovery was obtained as 100.14% and 100.07% for Ivacaftor and Tezacaftor respectively. LOD, LOQ values obtained from regression equations of Ivacaftor and Tezacaftor were 0.56, 1.71 µg/ml and 0.07, 0.11 µg/ml respectively. Regression equation of Ivacaftor is y = 14394x + 3350, and y = 6134.x + 432.1. of Tezacaftor. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

Key words: Tezacaftor, Evacaftor, stability indicating, RP- HPLC

1. INTRODUCTION

Ivacaftor [N-(2,4-di-tert-butyl-5-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide] is used for the management of Cystic Fibrosis (CF) in patients aged 2 years and older. Ivacaftor exerts its effect by acting as a potentiator of the CFTR protein, an ion channel involved in the transport of chloride and sodium ions across cell membranes of the lungs, pancreas, and other organs. Tezacaftor is indicated for the treatment of cystic fibrosis in people aged 12 years or older who have at least one mutation in the CFTR gene. HPLC methods for estimation of Ivacaftor and Tezacafor were published individually and in combination with other drugs like lumacaftor[1- 4]. Literature review revealed that there are no methods published till date for the simultaneous estimation Ivacaftor and Tezacaftor hence there is a scope for development of a new method for the simultaneous estimation of both the drugs in combined dosage forms. The main objective of present study is to develop a simple, accurate, precise, sensitive, selective, reproducible and rapid analytical technique for simultaneous estimation of Ivacaftor, Tezacaftor in bulk ant tablet dosage form.

2. MATERIALS AND METHODS

Ivacaftor and Tezacaftor pure drugs (API) received from Aurobindo pharma Ltd.
Combined form of Ivacaftor and Tezacaftor Injection (**Symdeko**). Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem

2.1. Solutions:

2.1.1. Stock solutions:

Accurately weighed 150 mg of Ivacaftor and 100 mg of Tezacaftor and transferred to 100 ml volumetric flask. And 3/4 th of diluents was added to these flask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (1500 µg/ml of Ivacaftor and 1000µg/ml of Tezacaftor). 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (60µg/ml Ivacaftor of and 40µg/ml of Tezacaftor). This is used as working standard solution (100 %).

2.1.2. Samples Preparation

10 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (1500µg/ml of Ivacaftor and 1000µg/ml of Tezacaftor). 0.4ml of filtered sample stock solution was transferred to 10 ml volumetric flask and made up with diluent. (100µg/ml of Ivacaftor and 100µg/ml of Tezacaftor)

2.1.3. Cc standards;

Calibration curve standards were prepared by pipetting suitable aliquots from stock solution (600µg/ml of Ivacaftor and 400µg/ml of Tezacaftor) into separate 10 ml volumetric flasks and the volume was made up to the mark with diluent to obtain the CC standards in the range of 15 to 90 µg/ ml concentrations.

2.2. Preparation of diluent: Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50 %v/v. It was ultra-sonicated then for 5min. The prepared solution was stored under room temperature at (20 ±5 °C), used within a time period of 7days from the date of preparation.

2.3. Chromatographic conditions:

The new HPLC method for estimation of Ivacaftor and Tezacaftor was developed and validated using Zodiacil C18, 150 x 4.6mm column. 45 volumes of HPLC grade Acetonitrile and 55 volumes of 0.01N Potassium phosphate buffer adjusted to pH 4.8 (45: 55% v/v) as mobile phase. Separation was achieved through isocratic elution mode at 1.0 mL/min flow rate.

2.4. System suitability:

The system suitability parameters were determined by preparing standard solutions of Ivacaftor (60ppm) and Tezacaftor (40ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

2.5. Method validation

The method validation was performed in accordance with ICH guidelines

2.5.1. Linearity

Prepared standard dilutions of Ivacaftor (15-90µg/ml) and Tezacaftor (10-60µg/ml) were injected and chromatogram was recorded in duplicate. A calibration curve was constructed by taking concentration on X- axis and average peak area on Y- axis.

2.5.2. Accuracy

Accuracy was determined by the recovery studies of the analyte. It is determined by standard addition method where the test solution of known quantity is spiked with standard solutions at three levels i.e., 50%, 100% & 150% in triplicate. Mean percentage recoveries at all the levels were calculated.

2.5.3. Precision

Precision of the method was established at two levels i.e., system precision and method precision. System precision was performed by injecting the working standard solution. six injections were given from the same standard solution and the peak areas were obtained. Average area, standard deviation and % RSD were calculated for two drugs. Method precision was established by taking multiple sampling from a sample stock solution. Six working sample solutions of same concentrations were prepared, single injection from each working sample solution was given. Average peak area, standard deviation and % RSD were calculated for two drugs

2.5.4. Robustness

Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made. Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus(35°C) was maintained and samples were injected in sextet. System suitability parameters are evaluated by making the deliberate changes.

2.5.5. Specificity:

The specificity was established by performing stress degradation studies for the analyte under various conditions like acid degradation, alkali degradation, oxidative degradation, thermal degradation and photo degradation.

Acid Degradation Studies:

To 10 ml of stock solution of Ivacaftor and Tezacaftor, 10 ml of 2N Hydrochloric acid was added and refluxed for 30 mins at 60^0C .The resultant solution was diluted to obtain 60 µg/ml & 40µg/ml solutions. 10 µl of solution was injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies:

To 10 ml stock solution of Ivacaftor and Tezacaftor, 10 ml of 2N sodium hydroxide was added and refluxed for 30mins at 60^0C. The resultant solution was diluted to obtain 6 0 µg/ml & 40µg/ml solution. 10 µl was injected into the system and the chromatograms were recorded to assess the stability of sample.

Thermal Degradation Studies:

The standard drug solution was placed in oven at 105°C for 1 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 60 µg/ ml & 40 µg/ ml solution. 10µl was 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 600 µg/ ml & 400 µg/ ml solution under UV Light by keeping the beaker in UV Chamber for 1day in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 60 µg/ ml & 40 µg/ ml solutions. 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Oxidative degradation:

To 1 ml of stock solution of Ivacaftor and Tezacaftor, 1 ml of 20% hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 30 min at 60^0C. For HPLC study, the resultant solution was diluted to obtain 60 µg/ ml & 40 µg/ ml solution. 10 µl was injected into the system and the chromatograms were recorded to assess the stability of sample.

3. RESULTS AND DISCUSSION

3.1 Assay of formulation:

Assay of the formulation is performed as per the given procedure. This was done in triplicate. The amount of drug present in the formulation was calculated from standard graph. The % assay of Ivacaftor and Tezacaftor obtained was 99.37 and 99.84% respectively. Representative chromatograms for standard, test and blank was given in figures 3,4 &5. Results were given in the table 1.

Table 1. Assay Data of Ivacaftor

S.no	Peak area of Ivacaftor	Peak area of Tezacaftor
1	864776	248081
2	859825	245316
3	853467	248964
4	858138	252481
5	859540	250841
6	862664	249412
Avg	859735	249183

Regression equation	y = 14394x + 3350.	y = 6134.x + 432.1
% Assay	100.06%	100.14%

3.2 **System suitability**

System suitability parameters were determined according to ICH guidelines. Plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits. The results showing system suitability parameters were given in table no. 2

Table 2: System suitability parameters for Ivacaftor and Tezacaftor

S no	**Ivacaftor**			**Tezacaftor**			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resolution
1	2.269	6702	1.39	3.164	10541	1.39	7.1
2	2.271	6304	1.39	3.164	9793	1.49	7.4
3	2.271	7287	1.41	3.165	9670	1.45	7.5
4	2.272	7023	1.36	**3.167**	10088	1.39	7.3
5	2.272	7328	1.32	3.168	10121	1.41	7.3
6	2.274	7115	1.30	3.168	10158	1.39	7.1

3.3 **Validation**

3.3.1. **Linearity**

The linearity was determined at six concentration in the range of 15-90 µg/ ml for Ivacaftor and 10-60 µg/ ml for Tezacaftor. The Peak areas against concentration were plotted and the calibration curve was constructed. The calibration curve was illustrated in Figure 3. The Correlation coefficient (r^2) was greater than 0.99 within the concentration range for both the drugs. The results for linearity were given in the table 3.

3.3.2. **Accuracy**

Accuracy of the method was established at three levels of concentrations by standard addition method. Triplicate injections were given at each level of accuracy and percentage recoveries

were calculated. The mean % Recovery was obtained was 100.14 % and 100.07 % for Ivacaftor and Tezacaftor respectively. The results for accuracy was given in the table 4.

Table 3: Linearity data of Ivacaftor and Tezacaftor.

Ivacaftor		Tezacaftor	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
0	0	0	0
15	217913	10	59455
30	436958	20	125470
45	656524	30	185389
60	865591	40	246389
75	1088946	50	307984
90	1291564	60	366543

3.3.3. Precision:

The precision of the method was studied by considering system precision and method precision. System precision was studied by taking six replicate injections from same homogenous standard solution and peak areas were determined. Average area, standard deviation and % RSD were calculated for two drugs. % RSD obtained as 0.4% and 0.8% respectively for Ivacaftor and Tezacaftor. Method precision was studied by preparing six test solutions of same concentration and injected once from each solution and peak areas were determined. Average area, standard deviation and % RSD were calculated for two drugs and obtained as 0.5% and 1.0% respectively for Ivacaftor and Tezacaftor. The results for precision were given in the table 5

3.3.4. Robustness:

Robustness of the method was studied by making deliberate changes in flow rate, mobile phase ratio and column oven temperature. After making each change in the conditions, chromatograms were recorded by injecting the standard solutions in six replicates. System suitability parameters were checked at each level. System suitability parameters were not much affected and all the parameters were passed. % RSD was within the limit. Results were given in the table 6.

Table 4: Accuracy table of Tezacaftor

% Level N=3	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery
Ivacaftor			
50 %	20	20.26	101.32
100%	40	39.68	99.20
150 %	60	59.84	99.74
Tezacaftor			
50 %	30	30.17	100.56
100%	60	60.17	100.29
150 %	90	89.05	98.95

Table 5: Precision data of Ivacaftor and Tezacaftor

	System precision		Method precision	
S. No	**Area of Ivacaftor**	**Area of Tezacaftor**	**Area of Ivacaftor**	**Area of Tezacaftor**
1.	857200	247748	864776	248081
2.	866094	250826	859825	245316
3.	864830	249381	853467	248964
4.	863721	246000	858138	252481
5.	861746	250748	859540	250841
6.	867254	249782	862664	249412
Mean	863474	249081	859735	249183
S.D	3616.7	1880.1	3895.5	2445.2
%RSD	0.4	0.8	0.5	1.0

Table 6: Robustness data for Ivacaftor and Tezacaftor.

S.no	Condition	%RSD of Ivacaftor	%RSD of Tezacaftor
1	Flow rate (-) 0.9ml/min	0.3	0.8
2	Flow rate (+) 1.1ml/min	1.1	0.7
3	Mobile phase (-) 60B:40A	0.6	0.8
4	Mobile phase (+) 50B:50A	0.5	0.9
5	Temperature (-) 25°C	0.8	0.7
6	Temperature (+) 35°C	0.5	0.4

Table 7: Degradation data showing specificity

Type of degradation	Ivacaftor			Tezacaftor		
	area	%recovered	% degraded	area	%recovered	% degraded
Acid	827320	95.62	4.38	236197	94.64	5.36
Base	803986	92.92	7.08	207754	83.24	16.76
Peroxide	814804	94.17	5.83	228696	91.63	8.37
Thermal	830881	96.03	3.97	242450	97.14	2.86
Uv	847962	98.01	1.99	246679	98.84	1.16
Water	860915	98.01	1.99	247465	99.15	0.85

3.3.5. Specificity:

Specificity of the method was established by performing the degradation studies under the stress conditions like acid, alkali, oxidation, photo and thermal degradation. The study was conducted as mentioned for the period of 30 minutes. the chromatograms were recorded after subjecting the drugs under stress and the percentage recoveries were calculated. The drugs were found stable under these conditions. The results were given in the table 7

4. CONCLUSION

A simple, Accurate, precise method was developed for the simultaneous estimation of the Ivacaftor and Tezacaftor in injection dosage form. Retention time of Ivacaftor and Tezacaftor were found to be 2.269 min and 3.164 min. %RSD of the Ivacaftor and Tezacaftor were and found to be 0.5and 1.0 respectively. %Recovery was obtained as 100.14% and 100.07% for

Ivacaftor and Tezacaftor respectively. LOD, LOQ values obtained from regression equations of Ivacaftor and Tezacaftor were 0.56, 1.17 µg/ml and 0.07, 0.21µg/ml respectively. Regression equation of Ivacaftor is y = 14394x + 3350., and y = 6134.x + 432.1 of Tezacaftor. The method was found specific for the drugs without having interference form the degradants. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

5. REFERENCES:

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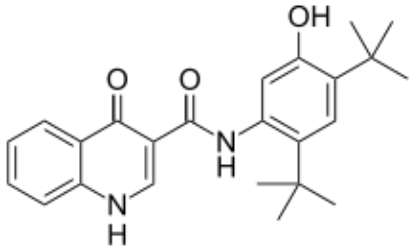

Fig 1: Structure of Ivacaftor

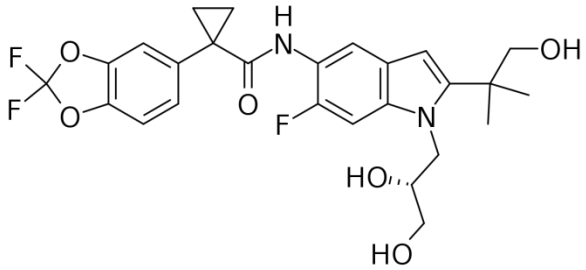

Fig.2: Structure of Tezacaftor

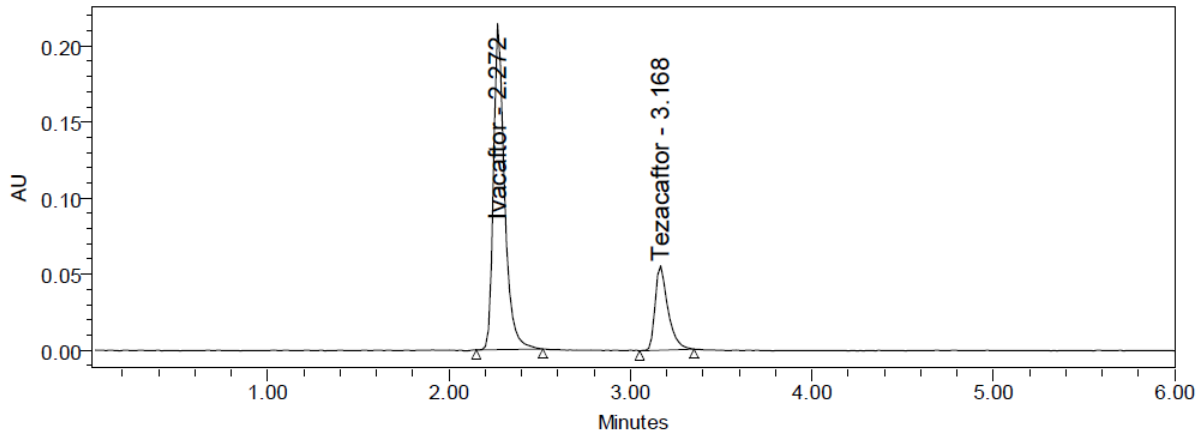

Fig 3: Representative Chromatogram of working standard solution

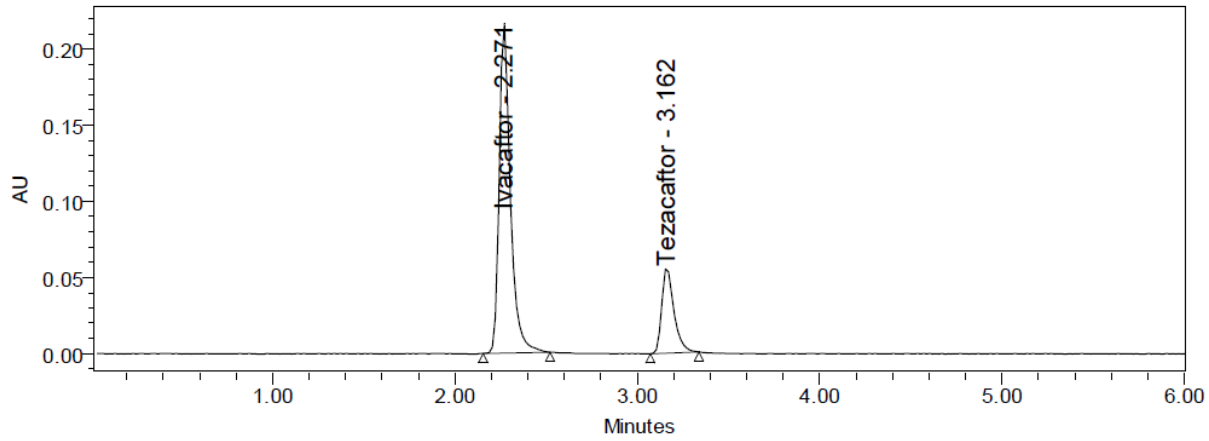

Fig 4: Representative Chromatogram of working sample solution

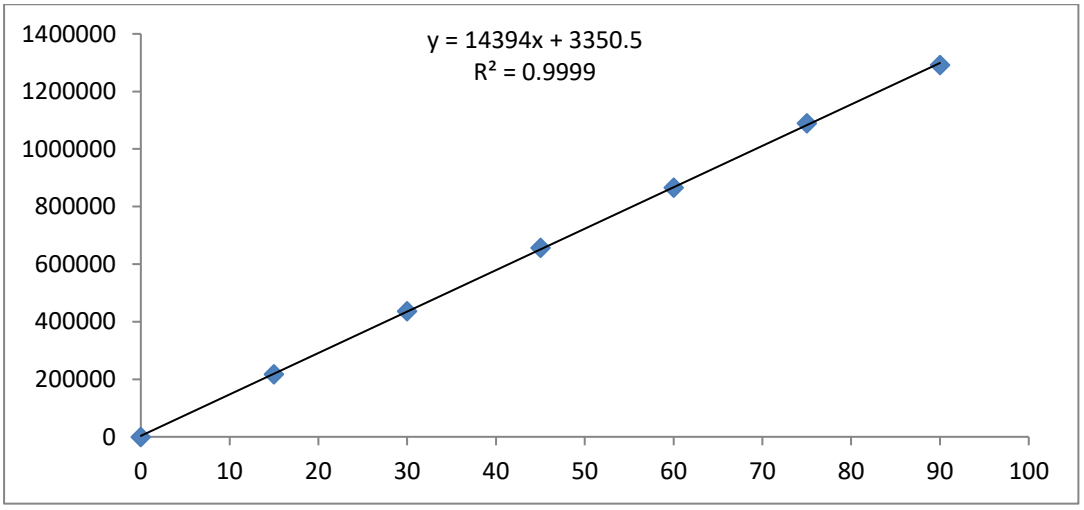

Fig 5: Calibration curve of Ivacaftor

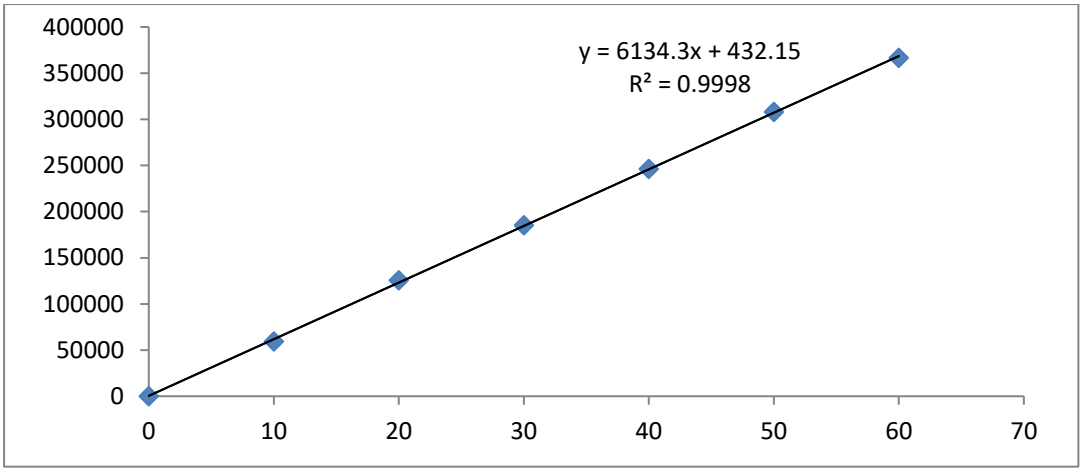

Fig 6: Calibration curve of Tezacaftor