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STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF TAZACAFTOR AND IVACAFTOR IN ITS PURE AND PHARMACEUTICAL DOSAGE FORM

Sanapala Srinivasa Rao*[1] , Shravya[2], Dr.V.Jagadeesh Babu[3]

[1,2,3]Pulla Reddy Institute of Pharmacy, Domadugu village, Gummadidala mandal, Sangareddy district, Hyderabad, Telangana, India

*Corresponding Author E-mail: sanjayswamy317@gmail.com

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ABSTRACT

A new, simple, precise, accurate and reproducible Rp-Hplc method for simultaneous estimation of its pure form and Pharmaceutical formulations. Separation of Tazacaftor and Ivacaftor was successfully achieved by using column like Agilent Eclipse column (4.6 x 150mm, 5µm)or equivalent in an isocratic mode utilizing 0.1% TEA: Methanol (30: 70) at a flow rate of 1.0ml/min and eluate was monitored at 298nm with a retention time of 2.461min and 4.387 min for Tazacaftor and Ivacaftor respectively. The values of the correlation coefficient were found to 0.999 for Tazacaftor and 1 for Ivacaftor. The LOD and LOQ for Tazacaftor were found to be 3.00 and 10.02 respectively. The LOD and LOQ for Ivacaftor were found to be 3.02 and 10.07 respectively. This method was found to be good percentage recovery which indicates that the proposed method is highly accurate. This method was extensively validated according to ICH guidelines for accuracy, precision, linearity, robustness and system suitability.

INTRODUCTION

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. 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.

Ivacaftor are orally available potentiators or correctors of the cystic fibrosis transmembrane conductance regulator (CFTR) that are used to treat patients with cystic fibrosis with specific mutations of the CFTR. Ivacaftor alone or in combination with 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.

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.

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.

Preparation of 0.1% TEA

Take 1ml Triethylamine in 1000ml volumetric flask and make up with HPLC water and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45µ 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.

Diluent Preparation

The Mobile phase was used as the diluent.

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.

METHOD VALIDATION

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.

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.

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)

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.

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

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

Table 1(a) : 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 1(b) : 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.

Table 2(a) : 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.

Table 3(a) : 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

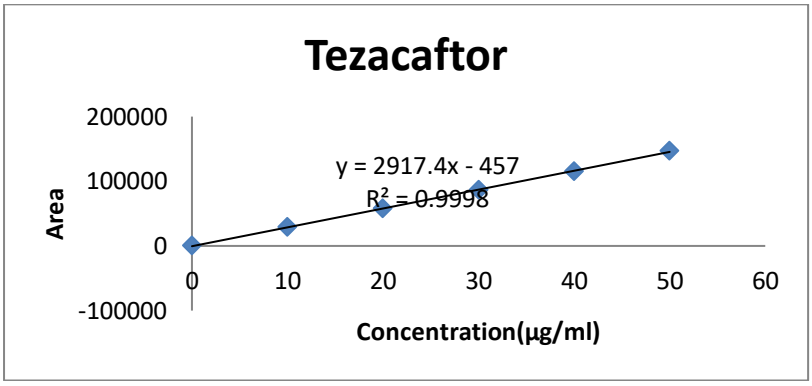

Fig.1(a) : Linearity plot of Tezacaftor

Table 3(b) : 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

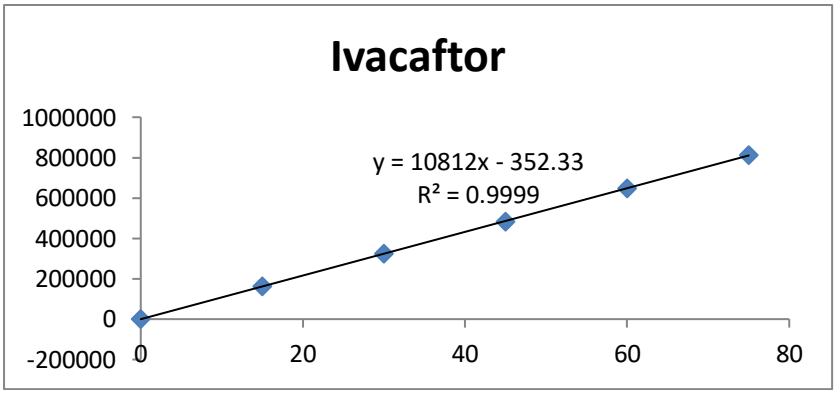

Fig.1(b) : Linearity plot of Ivacaftor

Table 3(c) : 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 0.999The correlation coefficient obtained was 0.999 which is in the acceptance limit.

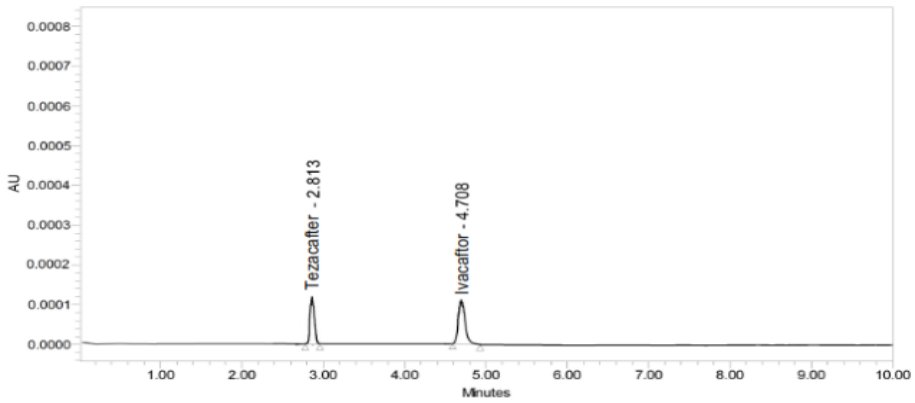

Fig.2(a) : Chromatogram of Tezacaftor, Ivacaftor showing LOD

Table 4(a) : 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..

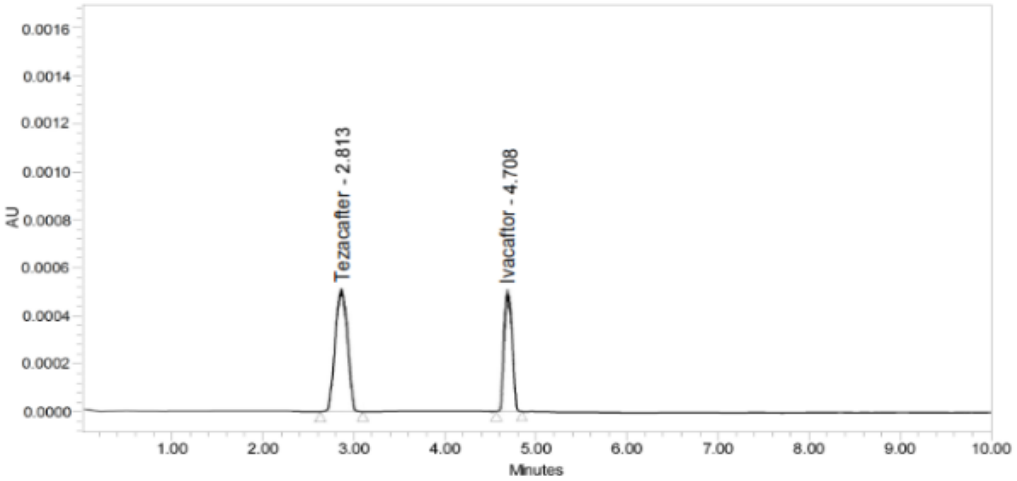

Fig.2(b) : Chromatogram of Tezacaftor, Ivacaftor showing LOQ

Table 4(b) : 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.

DEGRADATION STUDIES:

Table 5 : Degradation results for Tezacaftor and Ivacaftor

	Tezacaftor		Ivacaftor	
Sample Name	**Area**	**% Degraded**	**Area**	**% Degraded**
Standard	86350		487070.3	
Acid	81637	5.46	448922	7.83
Base	83562	3.23	452928	7.01
Peroxide	82081	4.94	459177	5.73
Thermal	84017	2.70	462638	5.02
Photo	84261	2.42	465877	4.35

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 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 accuracy limit is the percentage recovery should be in the range of 97.0% - 103.0%. 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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