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DEVELOPMENT OF VALIDATED STABILITY INDICATING HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ASSAY METHOD FOR THE SIMULTANEOUS ESTIMATION OF IVACAFTOR AND TEZACAFTOR IN BULK AND PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

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ABSTRACT

Objective: Ivacaftor and Tezacaftor are used in treatment of cystic fibrosis in certain people. The aim of the present study was to develop and validate a rapid, simple, sensitive, precise and accurate reverse phase high performance liquid chromatographic (RP-HPLC) method for simultaneous determination of Ivacaftor and Tezacaftor in bulk and tablet dosage form. **Methods:** Chromatographic separation of these two drugs was achieved on Kromosil C18 column (250 mm x 2.1 mm, 1.7 µm) as stationary phase with a mobile phase of acetonitrile: water (55:45 v/v) at a flow rate of 0.9 ml/min isocratically and photo diode array (PDA) detection at 292 nm. The retention times of Ivacaftor and Tezacaftor were found to be 2.212 min and 2.752 min respectively. **Results and Discussion:** The proposed method was validated for system suitability, linearity, accuracy, precision, LOD, LOQ and robustness. The calibration curves were linear in the concentration range of 15-90 to 10-60 µg/ml of the working concentration (r^2 = 0.999) for both the drugs in binary mixture. The LOD was found to be 0.07 µg/ml and 0.01 µg/ml and LOQ was found to be 0.22 µg/ml and 0.03 µg/ml for Ivacaftor and Tezacaftor respectively. **Conclusion:** Hence the proposed RP-HPLC method can be used in routine analysis of drugs in bulk as well as in tablets containing Ivacaftor and Tezacaftor.

INTRODUCTION

Ivacaftor is chemically N-(2, 4-di-tert-butyl-5-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide. Tezacaftor is chemically 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. A combination of above drugs is used in the management of cystic fibrosis. Extensive literature survey revealed that there were analytical methods for the estimation of Ivacaftor with Lumacaftor [1-3]. A bioanalytical method has been proposed for the estimation of Ivacaftor and Lumacaftor [4]. Two UPLC methods has been published for the estimation of Ivacaftor and Tezacaftor [5,6]. An ultra violet spectroscopic method has been established for the estimation of Ivacaftor and Tezacaftor [7]. An RP-HPLC method has been proposed for the estimation of Ivacaftor alone and in combination with Tezacaftor [8-11]. In the proposed methods

for the simultaneous estimation of Ivacaftor and Tezacaftor, the retention times of drugs were more and used buffers as mobile phases for the chromatographic analysis which decreases the life of the column and increases analysis time. In the present study a rapid and accurate RP-HPLC method for simultaneous determination of Ivacaftor and Tezacaftor in bulk and pharmaceutical formulation using a simple mobile phase.

MATERIALS AND METHODS

Materials: Ivacaftor and Tezacaftor pure drugs and formulation containing 150 mg of Ivacaftor and 100 mg of Tezacaftor were obtained from Spectrum Pharma Research Solutions, Hyderabad. Distilled water and acetonitrile were from Rankem.

Chromatographic conditions: Chromatographic separation was performed on reverse phase Kromosil C18 (250 x 2.1 mm, 1.7 µm) column. The mobile phase consisted of acetonitrile and water was pumped n the ratio of 55:45 v/v with photo diode array detection at 292 nm. The flow rate was set at 0.9 ml/min for simultaneous determination of Ivacaftor and Tezacaftor.

Diluent: Acetonitrile and water in the ratio of 55:45 %v/v was used as diluent.

Standard solution preparation: Accurately 15 mg of Ivacaftor and 10 mg of Tezacaftor standards were accurately weighed and transferred into a 25 ml clean dry volumetric flask, 15 ml of diluent was added, sonicated for 10 min and made up to the final volume with diluent. Further 1 ml from the above stock solution was taken into a 10 ml volumetric flask and made up to 10 ml with diluent so as to get final concentration of 60 µg/ml of Ivacaftor and 40 µg/ml of Tezacaftor.

Sample solution preparation: 20 tablets were weighed and crushed. A powder equivalent to 15 mg of Ivacaftor and 10 mg of Tezacaftor was taken and then transferred into a 25 ml clean dry volumetric flask, 15 ml of diluent was added, sonicated for 25 min and made up to the final volume with diluent. Further 1 ml from the above stock solution was taken into a 10 ml volumetric flask and made up to 10 ml with diluent.

Forced degradation samples preparation: The drugs were subjected to various stress conditions like acid, alkali, oxidative, photolytic and thermal conditions recommended by ICH guidelines. The degraded products were generated and analysed. For acid degradation study, 1 ml of 2 N HCl was added to 1 ml of stock solution and refluxed for 30 min at 60 ˚C. To this 1 ml of 2 N NaOH was added to neutralize the resultant solution and was diluted to 10 ml with diluent. For alkali degradation a study, 1 ml of 2 N NaOH was added to 1 ml of 2 N NaOH was added. To this 1 ml of 2 N HCl was added to neutralize the resultant solution and was diluted to 10 ml with diluent. For oxidative degradation, 1 ml of 20 % hydrogen peroxide was added to 1 ml of standard stock solution. The solution was kept for 30 min at 60 °C. The resultant solution was diluted to 10 ml with diluent. For thermal degradation, the standard stock solution was taken in a beaker and placed in oven at 105 ˚C for 1 hr and 1 ml from the resultant solution was diluted to 10 ml with diluent. For photo stability studies, the standard drug solution to UV light by keeping the beaker in UV chamber for 1 day, from the resultant solution 1 ml was taken and diluted up to 10 ml with diluent.

Assay of formulation: The standard preparations were made from the reference standard at target concentration level and sample preparations were made from formulation. Both sample and standards are injected six times into the chromatographic system. Amount of drug present in the formulation was estimated by taking the standard as the reference. The percentage assay was calculated.

Method Development: Many chromatographic conditions were tried for better separation and resolution. Kromosil column was found satisfactory. Peak purity of Ivacaftor and Tezacaftor as checked using photo diode array detector and 292 nm was considered satisfactory for detecting both the drugs with adequate sensitivity. A typical RP-HPLC chromatogram for simultaneous determination of Ivacaftor and Tezacaftor from standard preparation and from pharmaceutical formulation was shown in Fig.1 and 2.

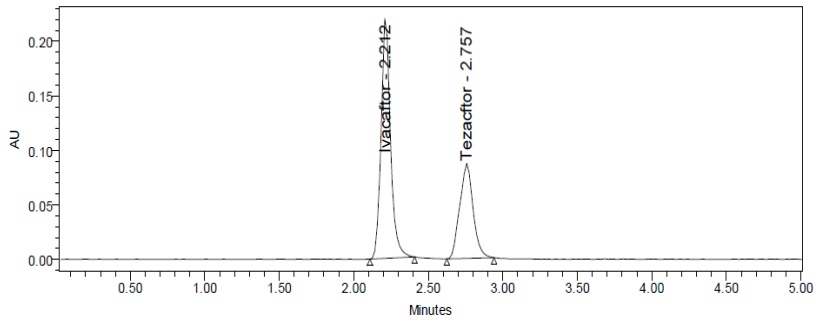

FIG. 1: Chromatogram of Ivacaftor and Tezacaftor in standard preparation

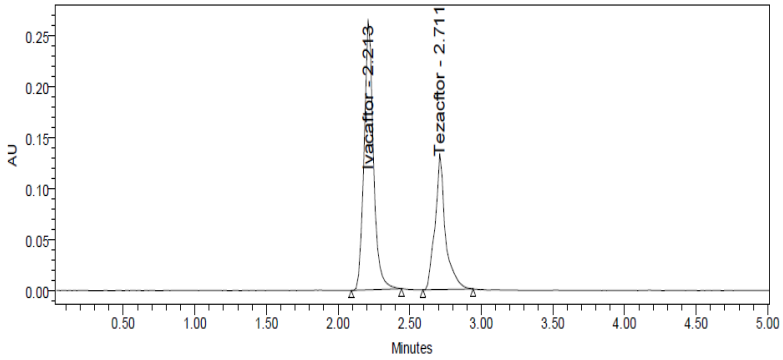

FIG. 2: Chromatogram of Ivacaftor and Tezacaftor in sample preparation

Table 1: Results of System Suitability

Analytes	Retention Times (min)	Resolution	Theoritical Plates	Tailing Factor	Peak Area
Ivacaftor	2.212	-	6038	1.18	1065059
Tezacaftor	2.757	3.7	3689	1.08	560664

#Min: Minutes

Table 2: Results Of Method Precision

N	Ivacaftor		Tezacaftor	
	Rt (min)	Peak Area	Rt (min)	Peak Area
Injection 1	2.213	1057350	2.702	569597
Injection 2	2.214	1050225	2.705	566187
Injection 3	2.216	1058503	2.709	567325
Injection 4	2.216	1060109	2.711	566441
Injection 5	2.216	1058971	2.711	566080
Injection 6	2.216	1052585	2.711	564759
Average	2.218	**1056291**		**566732**
SD		**3957**		**1628**
RSD(%)		**0.4**		**0.3**

Rt: Retention Times, # N: Number of Injections=6

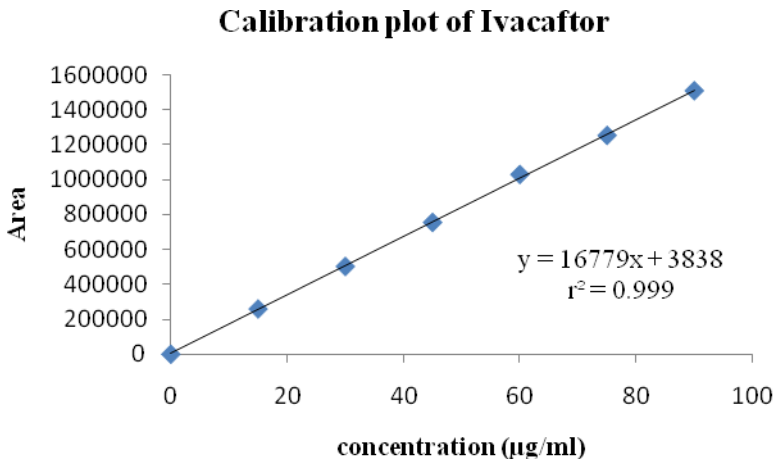

Fig. 3: Linearity Chart of Ivacaftor

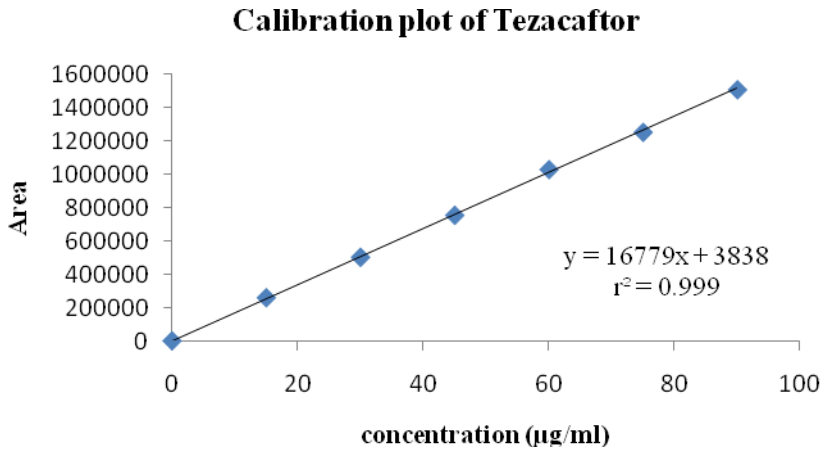

Fig. 4: Linearity Chart of Tezacaftor

Table 3: Results Of Linearity

Analytes	Correlation Coefficient (R^2)
Ivacaftor	0.999
Tezacaftor	0.999

Table 4: Results of Accuracy

Analytes	Pre-Analysed Sample Conc. (µg/ml)	Amount added (µg/ml)	Average Amount Found* (µg/ml)	Mean Recovery* (%)
Ivacaftor	60	30	29.97	99.89
	60	60	59.83	99.73
	60	90	90.13	100.46
Tezacaftor	40	20	20.09	100.52
	40	40	39.80	99.52
	40	60	60.04	100.07

* Represents mean of triplicate observations

Table 5: Results of Robustness

Parameter	Ivacaftor		Tezacaftor	
	Tailing*	Plate Count*	Tailing*	Plate Count
Less Flow Rate (0.8 Ml/Min)	1.23	4771	1.26	6505
More Flow Rate (1.0 Ml/Min)	1.24	4574	1.24	4455
Less Column Temperature (28º C)	1.26	4281	1.20	6188
More Column Temperature (32º C)	1.26	4321	1.21	1835
Less Organic Phase (45:55)	1.26	4506	1.30	2600
More Organic Phase (35:65)	1.30	4691	1.19	6559

* Represents mean of triplicate observations

Table 6: Results of Forced Degradation Studies

Type Of Degradation	Ivacaftor		Tezacaftor	
	Purity Angle	Purity Threshold	Purity Angle	Purity Threshold
Acid	0.213	0.335	0.282	0.462
Base	0.673	0.709	0.175	0.339
Peroxide	1.084	1.137	0.397	0.417
Thermal	0.999	1.499	0.226	0.332
Uv	0.350	1.186	0.338	0.560

Table 7: Results of Assay Formulation

S. No.	Ivacaftor % Assay	Tezacaftor % Assay
1	99.80	100.53
2	99.13	99.93
3	99.91	100.13
4	100.06	99.97
5	99.95	99.91
6	99.35	99.68
Mean	99.70	100.03

RESULTS AND DISCUSSION

Method Validation: The developed RP-HPLC method was validated for parameters like specificity, linearity, accuracy, precision, LOD, LOQ and robustness according to ICH guidelines [12].

System suitability: Standard solutions were prepared as per the test method and injected into the chromatographic system. The system suitability parameters like peak tailing, resolution, plate count were evaluated. The system suitability parameters were tabulated in table 1. All the parameters were found to be within the limits.

Precision:

The precision of the analytical method was verified by repeatability method. The standard solution was prepared at working concentration and analysis was carried out at replicates. The standard solutions of Ivacaftor and Tezacaftor were prepared as per the test method and injected 6 times into the column. The results of precision were tabulated in table 2. The average was taken; % RSD was calculated and reported. % RSD values were found within the limits, indicating the developed method was precise.

Linearity: The linearity of the test solutions for the assay method was prepared from standard stock solution at five concentration levels from 25% to 150% of assay concentration. The peak area versus concentration data was treated by least-squares linear regression analysis (fig. 3 and 4). The results tabulated in table 3 have shown an excellent correlation between peak areas and concentration within the concentration range of 15–90 µg/ml for Ivacaftor, 10-60 µg/ml for Tezacaftor. The correlation coefficients were found to be 0.999 for both the drugs, which meet the method validation acceptance criteria and hence the method was said to be linear for both the drugs.

Accuracy: To demonstrate accuracy recovery studies were carried out by standard addition method. A known quantity of pure drug was added to pre-analyzed sample and contents were reanalyzed by the proposed method and the percent recovery was reported. The results were given in table 4.

Limit of Detection and Limit of Quantitation: The limit of detection (LOD) and limit of quantitation (LOQ) were established by formula method from standard deviation of y-intercept and slope of linearity chart. The LOD and LOQ of Ivacaftor and Tezacaftor were experimentally determined by injecting six injections of each drug. The LOD of Ivacaftor and Tezacaftor was found to be 0.07µg/ml and 0.01µg/ml respectively. The LOQ of Ivacaftor and Tezacaftor was found to be 0.22µg/ml and 0.03µg/ml respectively.

Robustness: Robustness of the method was verified by altering the chromatographic conditions like mobile phase composition, column temperature, flow rate and the system suitability parameters were reported. Small changes in the operational conditions were allowed and the extent to which the method was robust was determined. A deviation of ± 2ºC in the column temperature and ± 0.1 ml/min in the flow rate and 5 % deviation in mobile phase were tried individually. A solution of target test concentration with the specified conditions was injected to the instrument in triplicate. The system suitability parameters were reported in the table 5 and found to be within the limits.

Forced degradation studies: The degradation behaviour of Ivacaftor and Tezacaftor was studied by subjecting the drugs to various stress conditions like acid, alkali, oxidative, photolytic, thermal and neutral analysis recommended by ICH. The purity angle of the analyte components under various stress conditions was less than the threshold values, indicating spectral homogeneity across the peak. The results of forced degradation studies were presented in table 6. From the findings of degradation studies show that the drugs have undergone extensive degradation under acidic, alkaline and peroxide conditions. The method shows that there was no degradants interference at the retention times of analyte peaks. Hence the method has successfully resolved the degraded component peaks.

Assay of formulation: Amount of drug present in the formulation was estimated by taking the standard as the reference. The average % assay was calculated and found to be 99.70 % and 100.03 % for tablet form of Ivacaftor and Tezacaftor respectively. Hence the method was successfully employed for assay of available formulation.

CONCLUSION

The proposed RP-HPLC method was found to be simple, accurate, precise, robust, rapid and economical. This method gives good resolution between two compounds with a short analysis time. Hence this method can be used in quality control departments with respect to routine analysis for the assay of the tablets containing Ivacaftor and Tezacaftor.

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Conflict of interests: Authors declare that no conflicts of interest exist in this research work.

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