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STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF IVACAFTOR AND TEZACAFTOR IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A new simple, accurate, rapid and precise method was developed and validated for the simultaneous estimation of Ivacaftor and Tezacaftor in pharmaceutical dosage form. Chromatogram was run through Inertsil ODS C_{18} (250 x 4.6 mm, 5µ) Column. Mobile phase contains Phosphate buffer and Acetonitrile taken in the ratio 40:60 was pumped through column at a flow rate of 1 ml/min. The pH was adjusted to 3.2 with Orthophosphoric acid. Temperature was maintained at 25°C. Optimized wavelength selected was 259 nm. The retention times were found to be 3.285 and 4.635 minutes for Ivacaftor and Tezacaftor. % RSD of the Ivacaftor and Tezacaftor were found to be 0.1 and 0.7 respectively. % Recovery was obtained as 99.96% and 99.98% for Ivacaftor and Tezacaftor respectively. Signal to Noise ratio for LOD, LOQ values obtained from regression equations of Ivacaftor and Tezacaftor were found to be 3, 9.98 and 3.02, 10 respectively. Retention times were decreased and run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

KEYWORDS: Ivacaftor, Tezacaftor, RP-HPLC.

INTRODUCTION

Development of simple and reproducible analytical methods for estimation of multicomponent drugs is very important part of quality control and for social awareness which is established in present work.[1]

Ivacaftor[2] exerts its effect by acting as a potentiate of the Cystic Fibrosis Trans membrane Regulator protein, an ion channel involved in the transport of chloride and sodium ions across cell membranes of the lungs, pancreas, and other organs.

Tezacaftor[3] exerts its effect by modulating the position of the Cystic Fibrosis Trans membrane Regulator protein on the cell surface to the correct position, allowing for adequate ion channel formation and increased in water and salt movement through the cell membrane. The concomitant use of Ivacaftor is intended to maintain an open channel, increasing the transport of chloride.

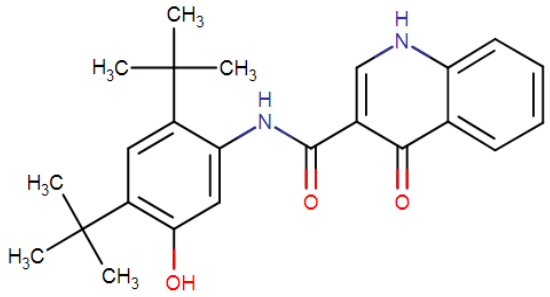

Fig. 1: Chemical Structure of Ivacaftor.

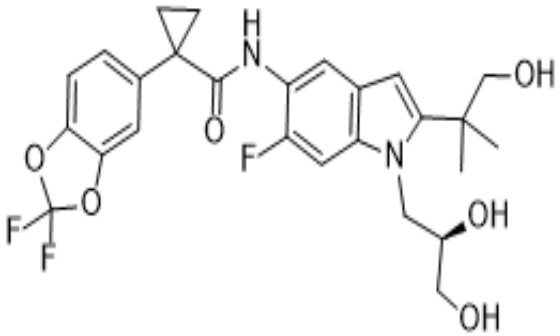

Fig. 2: Chemical Structure of Tezacaftor.

MATERIALS AND METHODS

Chemical and Reagents

Ivacaftor and Tezacaftor were kindly gifted by Sodum Drugs & Pharmaceuticals pvt ltd certified to contain 99.94% and 99.96% purity respectively. The drugs were used without further purification. All the solvents used in analysis were of HPLC grade. Symdeko Tablets of Vertex Pharmaceuticals was used in analysis.

HPLC method

Instrument

LC system used consists of Waters HPLC having Empower Software with 2695 separation module having PDA detector with universal loop injector of injection capacity 20µL. The column used was Inertsil OD Standard discovery C_{18} (250x4.6mm) 5µ at ambient temperature. Different mobile phases were tested in order to find the best conditions for separating both the drugs simultaneously.

Optimized Chromatographic conditions

The mobile phase having Acetonitrile and Phosphate buffer (60: 40) v/v was selected because it was found that it ideally resolve the peaks. The retention time was found to be 3.285 and 4.635 minutes for Ivacaftor and Tezacaftor respectively. Wavelength was selected by scanning all standard drugs over a wide range of wavelength 200nm to 350nm. Both the components showed reasonably good response at 259 nm.

Preparation of Phosphate Buffer

Weigh accurately 3.4g of KH_2PO_4 and dissolve in 1000 ml of HPLC water and pH was adjusted with Sodium Hydroxide up to 6.8. final solution was filtered through 0.44 m Membrane filter and sonicate it for 10 min.

Preparation of Mobile Phase

Accurately measured 400 ml (40%) of above buffer and 600 ml of Acetonitrile HPLC (60%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45µ filter under vacuum filtration.

Diluent Preparation: The Mobile phase was used as the diluent.

Standard Solution Preparation

Accurately weigh and transfer 15 mg of Ivacaftor and 10 mg of Tezacaftor working standard into a 10 ml clean dry volumetric flask and add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 1ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Sample Solution Preparation

Accurately weigh 10 tablets crush in mortar and pestle and transfer equivalent to 15 mg of Ivacaftor and 10 mg of Tezacaftor sample into a 10 mL clean dry volumetric flask add about 7 mL of Diluent and sonicate it up to 15 min to dissolve it completely and make volume up to the mark with the same solvent. Then it is filtered through 0.45 micron Injection filter. Further pipette 1ml of Ivacaftor and Tezacaftor from the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Recovery studies

To check the accuracy of sample by the developed methods and to study the interference of formulation additives, analytical recovery experiments were carried out by standard addition method at 50, 100 and 150% level. From the total amount of drug found, the percentage recovery was calculated.

RESULTS AND DISCUSSION

HPLC Method Validation

As per the ICH guidelines, the method validation parameters checked were linearity, accuracy, Specificity, precision, limit of detection, limit of quantitation.

Specificity: The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The specificity was performed by Injecting blank.

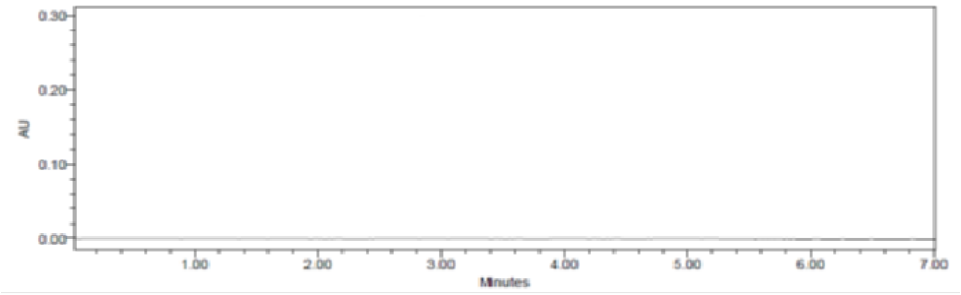

Fig. 3: Blank Chromatogram.

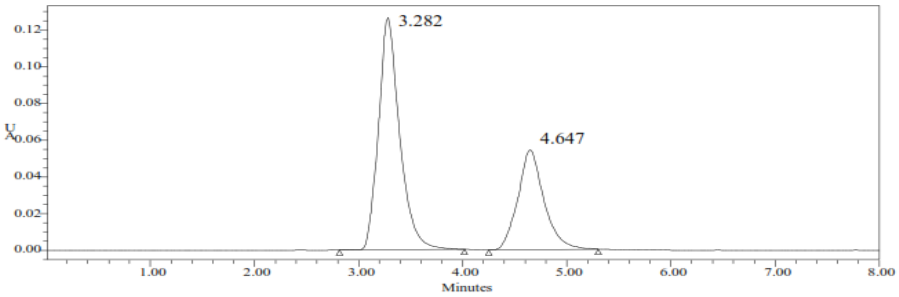

Fig. 4: Standard Chromatogram of Ivacaftor and Tezacaftor.

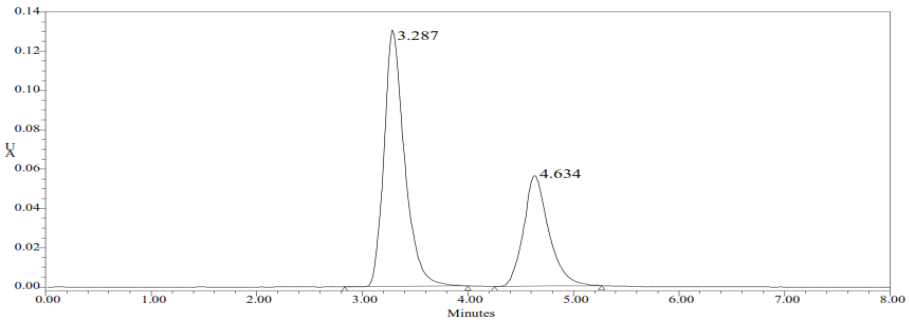

Fig 5: Sample Chromatogram of Ivacaftor and Tezacaftor.

Linearity

The linearity of the proposed RP-HPLC method for determination of Ivacaftor and Tezacaftor was evaluated by analysing a series of different concentrations of standard drug.

In this study five concentrations were chosen, ranging between 50-250 µg/ml of Ivacaftor and 30-160 µg/ml of Tezacaftor. Each concentration was repeated three times. The linearity of the calibration graphs was validated by the high value of correlation coefficient, slope and the intercept value. A linear relationship was obtained for Ivacaftor in the range of 50-250 µg/ml and Tezacaftor in the range of 30-150 µg/ml respectively.

Table 1: Linearity Table of Ivacaftor and Tezacaftor.

S. No.	Ivacaftor		Tezcaftor	
	Concentration (µg/ml)	Area	Concentration (µg/ml)	Area
1	50	244841	30	29672
2	100	525756	60	68336
3	150	856654	90	113345
4	200	1150925	120	159680
5	250	1435608	150	204473

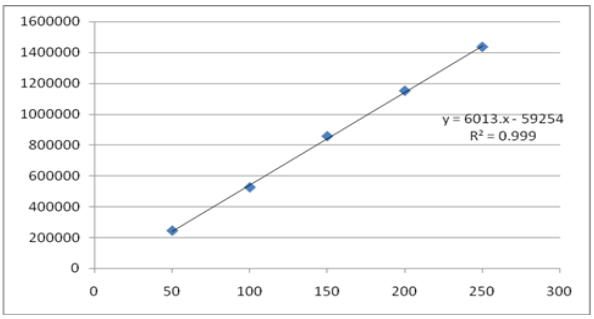

Fig. 6: Calibration curve of Ivacaftor.

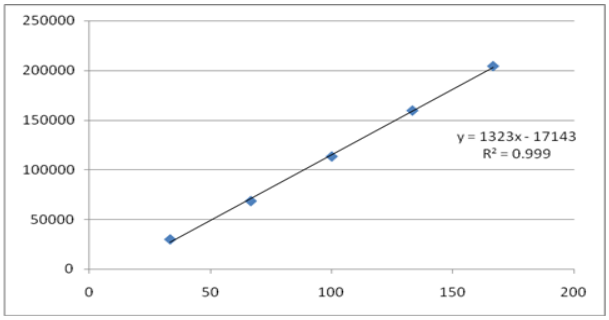

Fig. 7: Calibration curve of Tezacaftor.

Precision

Precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample. Precision was demonstrated by repeatability and intermediate precision measurements of peak area and peak symmetry parameters of RP- HPLC method for each title ingredients. The repeatability (within-day in triplicates) and intermediate precision (for 2 days) were carried out at five concentration levels for each compound. Triplicate injections were made and the obtained results within and between the days of trials were in acceptable range. The %RSD values for Ivacaftor and Tezacaftor were found to be less than 2 indicate that the developed method was precise.

Table 2: Precision Data for Ivacaftor and Tezacaftor.

S. No	Area of Ivacaftor	Area of Tezacaftor
1.	852828	111368
2.	852337	112717
3.	858355	112655
4.	852839	113939
5.	858513	111251
6.	857582	112282
Mean	855409.0	112662.3
S.D	12.524.5	845.7
%RSD	0.4	0.8

Accuracy

Accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The accuracy of an analytical method should be established across its linearity range. Accuracy was performed in three different levels, each level in triplicate for Ivacaftor and Tezacaftor using standards at 50%, 100% and 150%. Each sample was analysed in triplicate for each level. The mean recoveries were found in the range of 98-102 % by which we can say the method was accurate.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

It is calculated according to ICH recommendations where the approach is based on the signal to-noise ratio. Chromatogram signals obtained with known low concentrations of analytes were compared with the signals of blank samples. A signal-to-noise ratio 3:1 and 10:1 was considered for calculating LOD and LOQ respectively.

Table 3: Sensitivity Tables of Ivacaftor and Tezacaftor.

Drug name	Baseline noise (µV)	Signal obtained (µV)	S/N ratio
Ivacaftor	66	198	3.00
Tezcaftor	66	199	3.02

Drug name	Baseline noise (µV)	Signal obtained (µV)	S/N ratio
Ivacaftor	66	659	9.98
Tezcaftor	66	660	10.00

Degradation Studies

Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation.

Table 4: Degradation data of Ivacaftor.

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	4.79	0.318	0.378
2	Alkali	2.67	0.234	0.258
3	Oxidation	1.35	0.318	0.328
4	Thermal	0.67	0.234	0.218
5	UV	0.46	0.229	0.267
6	Water	0.67	0.050	0.239

Table 5: Degradation data of Tezacaftor.

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	4.84	0.132	0.346
2	Alkali	2.87	0.123	0.263
3	Oxidation	1.94	0.118	0.326
4	Thermal	0.61	0.117	0.283
5	UV	0.85	0.124	0.276
6	Water	0.93	0.118	0.265

CONCLUSION

The methods described for simultaneous estimation of Ivacaftor and Tezacaftor are found to be simple, sensitive, accurate, precise, rapid and economical. Hence method could be successfully employed for routine analysis of method development and validation for simultaneous estimation of Ivacaftor and Tezacaftor in combined pharmaceutical dosage form.

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