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A novel rp-hplc method for the estimation of ivacaftor and tezacaftor in bulk and pharmaceutical dosage formulations

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ABSTRACT

A selective and sensitive reverse phase high performance liquid chromatography (RP-HPLC) has been developed for the separation and quantification of and Ivacaftor and Tezacaftor in tablet dosage form and validated .The determination was carried out using Symmetry C18 column (250 mm ×4.6 mm id) as a stationary phase and mobile phase comprised of Methanol: TEA buffer pH 4.2 (40:60v/v) with pH adjusted to 4.2±0.5 by using Ortho phosphoric acid. The flow rate was 1.0ml/min and the eluent was monitored at 260 nm. The retention time of and Ivacaftor and r were 2.773 ±0.018 min and 4.065±0.024 min respectively. The Coefficient of correlation and percentage recoveries of Ivacaftor and Tezacaftor were 0.9986 and 100.0l % and 0.9994 and 99.98% respectively. The method is validated for accuracy, Precision, ruggedness and Robustness. The proposed method is successfully applied for the simultaneous determination of both drugs in commercial tablet preparation. The results of the analysis have been validated statistically and by recovery studies.

Keywords: Ivacaftor, Tezacaftor, RP-HPLC, Robustness and ICH Guidelines.

INTRODUCTION

Ivacaftor, (*N*-(2,4-di*tert*-butyl-5-hydroxyphenyl)-4-oxo-1*H*-quinoline-3-carboxamide) (Figure.1) with molecular formula $C_{24}H_{28}N_2O_3$ is used to treat cystic fibrosis (CF). It is only used for patients who have the following mutations in their CF transmembrane conductance regulator (CFTR) gene: G551D, G1244E, G1349D, G178R, G551S, R117H, S1251N, S1255P, S549N, or S549R mutations. Your doctor will test for the presence of the mutation before you receive the medicine. Ivacaftor is a CFTR potentiator, and works by moving more chloride into the cells of your body.[1]

Tezacaftor , 1-(2, 2-difluoro-1, 3-benzodioxol-5-yl)-N-[1-[(2R)-2, 3-dihydroxypropyl]-6-fluoro-2-(1-hydroxy-2-methylpropan-2-yl) indol-5-yl] cyclopropane-1-carboxamide (Figure1.) with molecular formula $C_{26}H_{27}F_3N_2O_6$, is used as a corrector of the cystic fibrosis (CF) trans membrane conductance regulator (CFTR) gene function. [2]

The clinical studies done with Ivacaftor and Tezacaftor combination for the treatment of cystic fibrosis exhibited significant improvement in lung function of the patients. The FDA approved SYMDEKO (a combination formulation of Ivacaftor and Tezacaftor in 2018 for the treatment of cystic fibrosis in the patients aged 12 years or above,

followed by the approval for the treatment of underlying cause of CF in children aged between 6 and 11 years in 2019.[3-5]

The literature survey revealed that there is no simple isocratic elution reversed-phase high-performance liquid chromatography (RP-HPLC)-based simple buffer method for the concomitant determination of Ivacaftor and Tezacaftor in combination dosage form.

Therefore, an attempt was made to develop a novel, simple, accurate and precise method for the simultaneous estimation of Ivacaftor and Tezacaftor in combined pharmaceutical dosage form. This manuscript describes the development and validation of RP-HPLC method for simultaneous estimation of these drugs as per ICH guidelines.

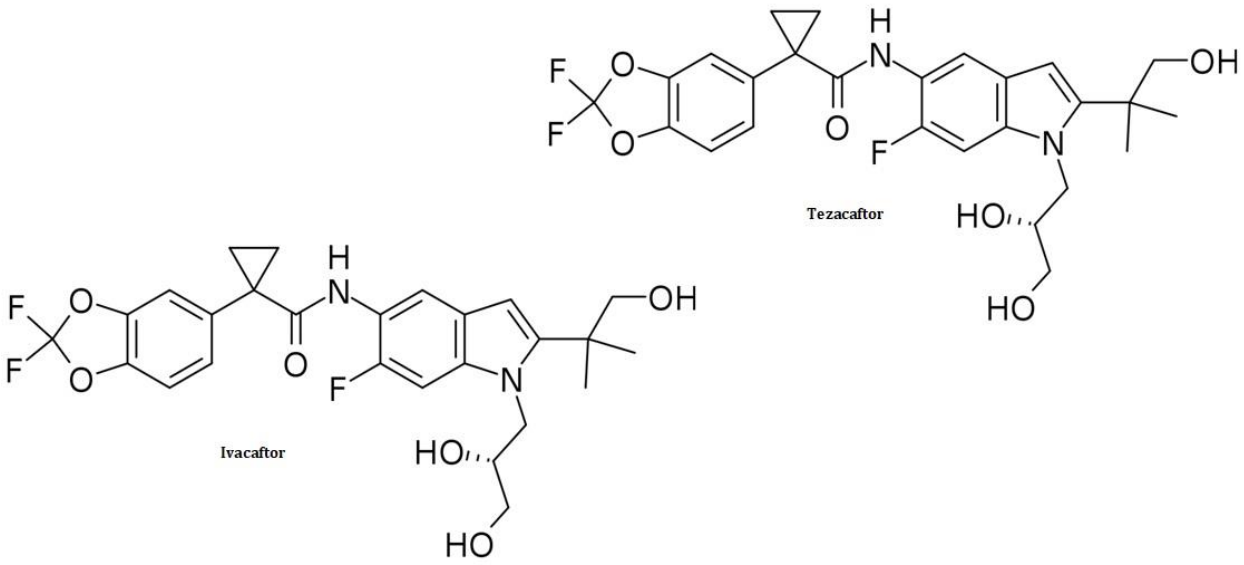

Figure1.Structure of Ivacaftor and Tezacaftor.

Experimentation

Equipment

Chromatographic separation was performed on Waters HPLC system consist of model 2695 having PDA detector and Rheodyne injector with 20µl loop volume. Waters Empower software was applied for data collecting and processing.

Reagents and chemicals

Acetonitrile, Methanol and water of HPLC grade were procured from Rankem lab ltd. working standard of Ivacaftor and was provided by Orchid pharmaceuticals Chennai and Tezacaftor was provided by Emcure Pharmaceutical Ltd, Pune. Sodium acetate and orthophosphoricacid were A.R grade from Merck chemicals Mumbai, India. Tablets two different brands were purchased from Indian market, containing 150 mg/75mg of Ivacaftor and 100 mg/50mg of Tezacaftor per tablet.

Optimized chromatographic Condition

A Symmetry C18 column (25cm×4.6mm, 5µ) column was used as the stationary phase. A mixture of Methanol: TEA buffer pH 4.2 (40:60v/v) was used as a mobile phase and P^H 4.2±0.5adjusted with orthophosphoric acid. It was filtered through 0.45µ membrane filter and degassed. The mobile phase was pumped at 1 ml/min. The eluents were monitored at 260nm.The injection volumes of samples and standard were 20µl.

Optimized chromatographic conditions

Instrument used	:	Waters HPLC with auto sampler and PDA Detector 2695 model.
Temperature	:	40ºC
Column	:	Symmetry C18 (4.6×150mm, 5µ)
pH	:	4.2
Mobile phase	:	Methanol: TEA buffer pH 4.2 (40:60v/v)
Flow rate	:	1ml/min
Wavelength	:	260 nm
Injection volume :	:	20 µl
Run time	:	6 min

Standard preparation

Preparation of standard solution:

10 mg of Ivacaftor and 10mg of Tezacaftor were accurately weighed and transferred into a 10 ml clean dry volumetric flask, about 7 ml of diluent was added, sonicated to dissolve it completely and the volume was made up to the mark with the same solvent to give a concentration of 1000 µg/ml. (Stock solution) . The working standard solutions were prepared and further diluted in mobile phase to Ivacaftor and Tezacaftor contain a mixture of in over the linearity ranges from 37.5-187.5 µg/ml and 25-125 µg/ml.

Sample preparation

Twenty tablets Symdeko [Rx] were weighed and finely powdered. A quantity of powder equivalent to 100 mg of Tezacaftor and 150mg of Ivacaftor was weighed and transferred to a 25 ml volumetric standard flask and added 10 ml of mobile phase. The sample was kept in an ultrasonic bath for 20 min and further diluted using mobile phase to get 150µg/ml of Ivacaftor and 100µg/ml of Tezacaftor. Then it is filtered through 0.22µ membrane filter paper.20µl of this solution was injected in to HPLC system and chromatograms were recorded. Concentrations of Ivacaftor and Tezacaftor in the tablet formulation were calculated by comparing area of the sample with that of standard. The percentage assay of individual drug was calculated and presented in Table1.

Table1: Table for Assay

Tablet formulation	Drug	Amount present mg	Amount found* (mg/tab)	% label claim*
T1	Ivacaftor	150	149.98	99.99
	Tezacaftor	100	99.97	99.97
T2	Ivacaftor	75	74.91	99.74
	Tezacaftor	50	49.98	99.98

T1 and T2 are two different brands of tablet formulations.*Each value is average of six determinations.

RESULTS AND DISCUSSION

The proposed HPLC method required fewer reagents and materials and it is simple and less time consuming. This method could be used in quality control test in Pharmaceutical industries. The chromatograms sample and standard solution of Ivacaftor and Tezacaftor were shown in (Figure.1) and (Figure.2). There was clear resolution between Ivacaftor and Tezacaftor with retention time of 2.795and4.067 minutes respectively.

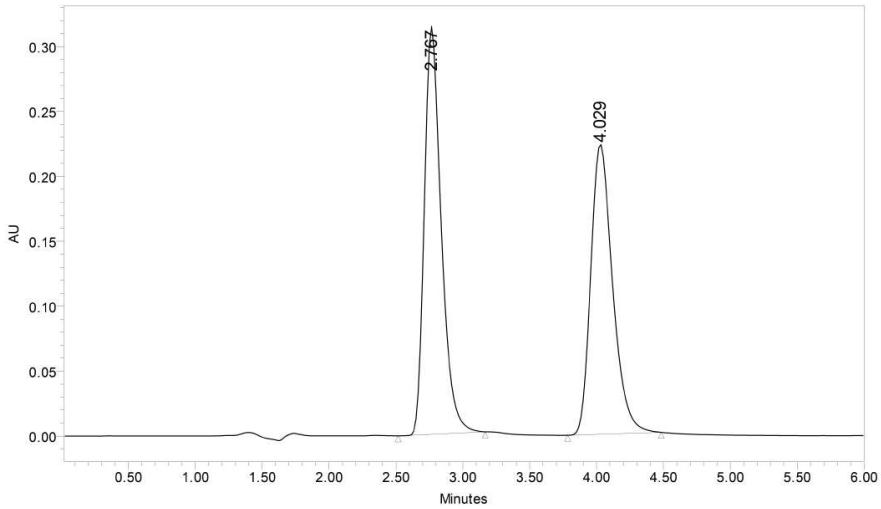

Figure 1: Typical Chromatogram of standard solution of Ivacaftor and Tezacaftor

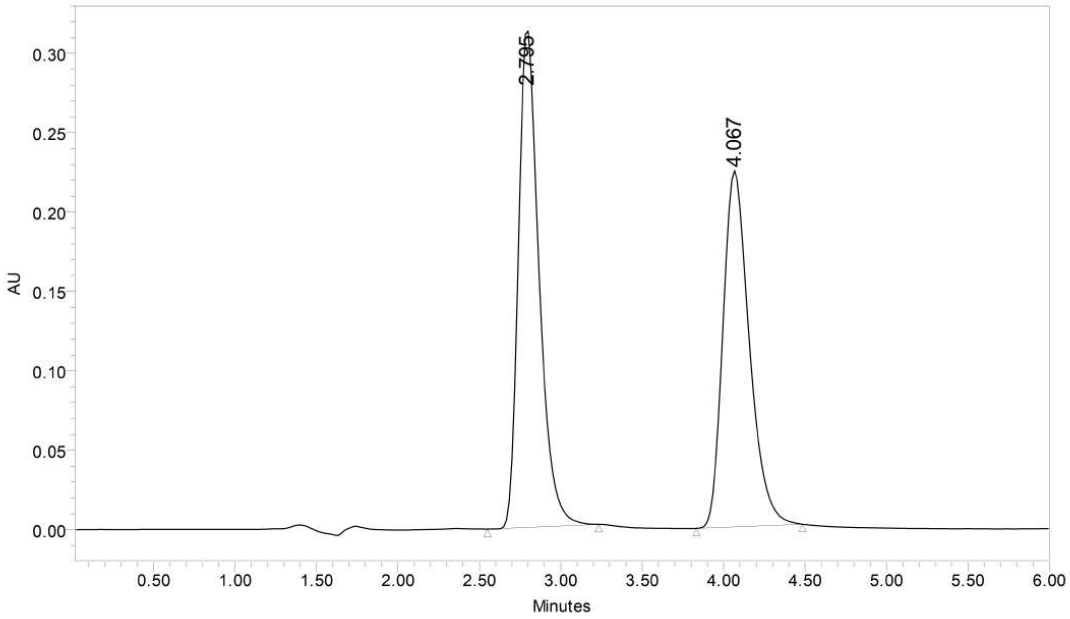

Figure 2: Typical Chromatogram of sample solution of Ivacaftor and Tezacaftor

Validation of the method

System suitability

The column efficiency, resolution and peak symmetry were calculated for the standard solutions (Table.2). The values obtained demonstrated the suitability of the system for the analysis of this drug combination and the system suitability parameters fall with in±3% standard deviation range during performance of the method. Here tailing factor for peaks of Ivacaftor and Tezacaftor was less than 2% and resolution was satisfactory. The peaks obtained for Ivacaftor and Tezacaftor were sharp and have clear base line separation.

Table 2: System Suitability

S.No	Parameters	Ivacaftor	Tezacaftor
1	Capacity factor	1	1
2	Theoretical plate	5623	5589
3	Asymmetry of the peak	0.89	0.79
4	Retention time (min)	2.795	4.067
5	Resolution	4.6	

Linearity

The response for the detector was determined to be linear over the range of 37.5-187.5 µg/ml (37.5,75,112.5,150,187) of Ivacaftor and 25-125µg/ml (25,50,75,100,125) for Tezacaftor. Each of this concentration was injected in six times to get reproducible response. The calibration curve was plotted as concentration of the respective drug versus the response at each level.(Figure 3& Figure 4.) The proposed method was evaluated by its correlation coefficient and intercept value calculated in the statistical study. The results show that an excellent correlation exits between response factor and concentration of drugs within the concentration range indicated above. (Table 3)

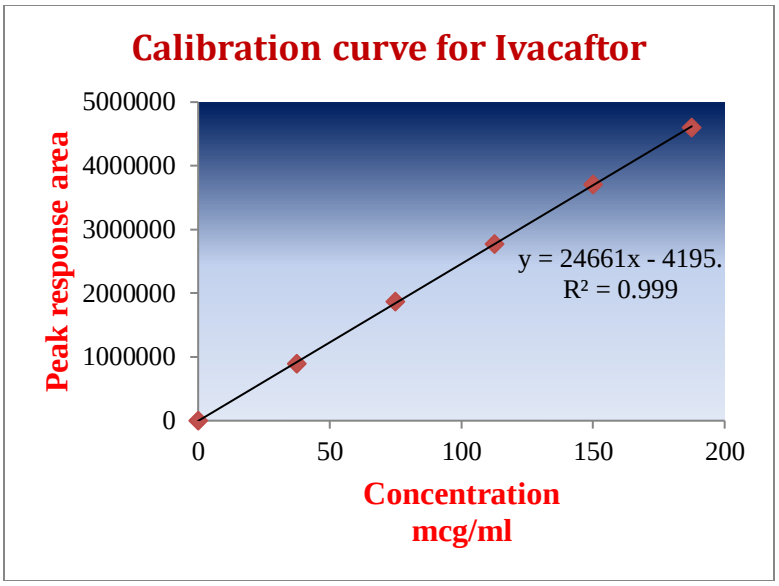

Figure 3. calibration graph for Ivacaftor

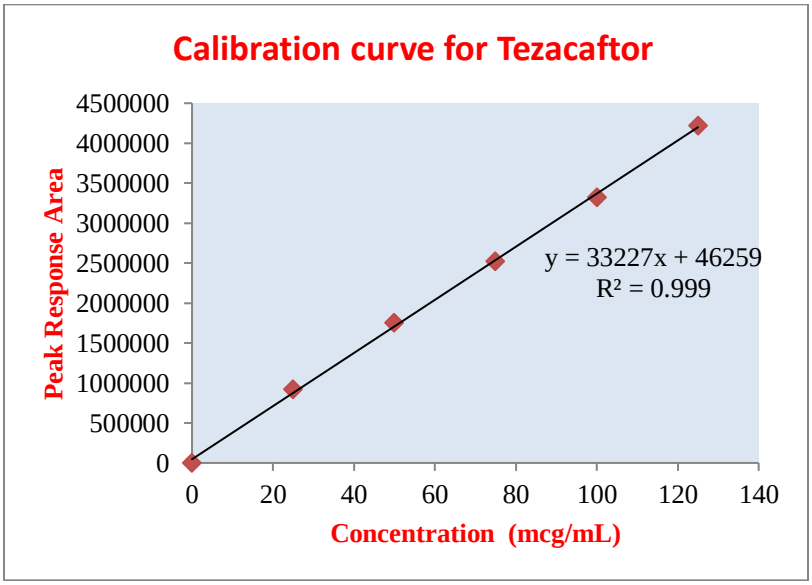

Figure 4: calibration graph for Tezacaftor

Table 3: Summary of analytical method validation

S.No	Parameters	Acceptance criteria	Ivacaftor	Tezacaftor
1	Linearity	r^2=0.995 to1.0	0.9999	0.9999
2	Specificity	No interference with placebo	specific	specific
3	Accuracy(Recovery studies)	Recovery 98.0-102.0%	99.97%	100.02%
4	**Precision**			
	Intraday	RSD NMT 2.0%	0.1252	0.3622
	Interday	RSD NMT 2.0%	0.5526	0.4723

5	**Robustness**			
	Change inflow rate	NMT±1%	0.2%	0.3%
	Change in mobile phase ratio	NMT±1%	0.2%	0.3%
	Change in p^H	NMT±1%	0.3%	0.4%
6	Limit of detection µg/ml	----------	0.5µg/ml	1µg/ml
	Limit of Quantification µg/ml	----------	1.5µg/ml	3µg/ml

Precision and Accuracy

Recovery studies were carried out by applying the standard addition method. A known amount of standard Ivacaftor and Tezacaftor corresponding to 80%, 100%, and 120% of the label claim was added to pre analyze sample of tablet dosage form separately. The recovery studies were carried out six times at each level of recovery. From the data obtained, recoveries of standard drugs were found to be accurate (Table.3.). The %RSD of interday and intraday precision obtained was less than2%for both the drugs. The intraday and interday precision of Ivacaftor was 0.1252 and 0.5526 and Tezacaftor was 0.3622and 0.4723 respectively. From the data obtained, the developed HPLC method was found to be precise and accurate.

Specificity of the method

The specificity of the method was checked for the interference of impurities in the analysis of a blank solution (without any sample) and then a drug solution of 10 µg/ml was injected into the column, under optimized chromatographic conditions, to demonstrate the separation of both Ivacaftor and Tezacaftor from any of the impurities, if present. As there was no interference of impurities and also no change in the retention time, the method was found to be specific and also confirmed with the results of analysis of formulation.

LOD and LOQ

Limit of detection (LOD) and limit of quantification (LOQ) were calculated as 3.3 ∂/S and 10 ∂/S, respectively as per ICH guidelines, where ∂ is the standard deviation of the response (*y*-intercept) and *S* is the slope of the calibration plot. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3).The LOD for Ivacaftor and Tezacaftor was found to be 0.8µg/ml and 0.7µg/ml, respectively. The LOQ is the smallest concentration of the analyte which gives response that can be accurately quantified (signal to noise ratio of 10).The LOQ was 2.41µg/ml and 2.19µg/ml for Ivacaftor and Tezacaftor respectively.(Table3)

Ruggedness and Robustness:

The ruggedness of the method was determined by carrying out the experiment on different instrument like Waters HPLC and Agilent HPLC by different operators using different columns of similar type like HypersilC18, ZorbaxC18 column. Robustness of the method was determined by making slight changes in the experimental conditions such as the composition of the mobile phase, pH of the mobile phase, and flow rate of the mobile phase and the chromatographic characteristics were evaluated. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed, are rugged and robust.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Ivacaftor and Tezacaftor in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Methanol: TEA pH 4.2 (40:60) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectro photometric methods. This method can be used for the routine determination of Tezacaftor and Ivacaftor in bulk drug and in Pharmaceutical dosage forms.

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